Detection and quantification of ergot in cereals by near infrared hyperspectral imaging

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What is the problem?

- Reemerging of the ergot presence in cereals
  - For the farmer, yield reduction (10%)
  - For the feed/food sector, high toxicity risk for animal and human
What is ergot?

- Ergot is a sclerotium formed by the fungi *Claviceps purpurea* including ergot alkaloids a class of mycotoxins occurring in grains
- Many hosts: rye, triticale, wheat, durum, barley, oat, sorgho and several grasses
- More information on EFSA
What about the legislation?

- The concentration of ergot body in cereals is for **animal**, restricted to **1000 mg per Kg** in feedingstuffs containing unground cereals.

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Control of ergot contamination?

• **In the field:**
  – Crop rotation
  – Varietal resistance

• **In the grain industry:** detection of **ergot bodies**
  – Modern cleaning machinery
  – Microscopy method (IAG method)
  – Imaging system: CONffIDENCE

• **In the mills:** detection of **alkaloids**
  – Methods of analysis: LC-FLD and LC-MS/MS
Status of the analytical aspects?

- The existing microscopy method provides an elegant early warning tool for ergot contamination but is time-consuming.

**Method for the Determination of Ergot (Claviceps purpurea Tul.) in Animal Feedingstuff, IAG-Method A4**

International Association of Feedingstuff Analysis
Section Feedingstuff Microscopy
New project CONffIDENCE

Increasing of ergot occurrence and no rapid method available involved

new project CONffIDENCE

• One of the objectives:

Ergot detection by NIR Hyperspectral imaging

– T.4a.7 : Development of imaging method, validation and comparison with existing method

– T.4a.8 : Transfer of the imaging method to the feed sector (NUTRECO)
What is hyperspectral imaging?

Spatial information

NIR imaging instrument

Frequency information (i.e. wavelengths)

Intensity information (i.e. absorbance)
Calibration process

Data-base
Ergot – Cereals spectra

Reference values + Mathematical model

Unknown spectrum + Mathematical model → Predicted values
NIR line scan camera: instrument

SWIR ImSpector N25E spectral camera (Specim Ltd)

NIR camera

Light source

Conveyor belt (Burgermetrics)
NIR line scan camera: features

- Wavelength range: 1000-2500 nm by step of 6 nm
- 1 line = 320 pixels = 320 spectra
- Analysed surface = continuous
- Time of acquisition = 50 millisec/pixel line
Ergot detection in wheat by NIR imaging

Analytical parameters used and on-line prediction results of the PLSDA (Partial Least Squares Discriminant Analysis) model

The number of pixels counted for each class of the model is also provided.
Results of ergot bodies detection

On set of 7 laboratory samples with 0 0,01 0,05 0,1 0,15 0,5 and 1% of ergot in cleaned wheat

Corrective factor weight/area

Reference value: % weight
Predicted value: % area * 0,8
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
NIR line scan camera: instrument in demonstration at NUTRECO

NIR camera
SWIR
ImSpector
N25E
Spectra
Camera
(Specim Ltd)

Light source
Tray
Ergot detection in wheat by NIR imaging

The number of pixels counted for each class of the model and ... (object quantification)

... the distribution of groups of pixels detected as ergot are also provided (object identification)

Prediction results of the SIMCA (Soft Independent Method of Class Analogy) model
Results of ergot bodies detection

Set of 7 samples (2009-2010)
- wheat, rye

Set of 6 samples (2011)
- rye, triticale, oat

BurgerMetrics
Instrument (Pilot imaging system)

SisuChema
Instrument (Commercial imaging system)

Corrective factor weight/area
Reference value: % weight
Predicted value: % area * 0.8
Validation and transferability study of a method based on near infrared hyperspectral imaging for the detection and quantification of ergot bodies in cereals.

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Under reviewing
Further developments

- Multicontaminants detection: ergot, datura, ...

Ergot in black oat
Mixture of wheat, black oat
Rape seed, ergot and datura
Further developments

- Impurities quantification: straw, weed seeds, broken grains, germinated seeds
Benefits of the method for a feed Company

<table>
<thead>
<tr>
<th>Classical microscopy</th>
<th>NIR hyperspectral imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>High skilled personal</td>
<td>Low skilled personal</td>
</tr>
<tr>
<td>45 min / 250g</td>
<td>A few minute / 250 g</td>
</tr>
<tr>
<td>Reduced samples</td>
<td>Large samples (sampling more representative)</td>
</tr>
<tr>
<td>Dedicated to ergot</td>
<td>Multiple contaminants</td>
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</tbody>
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Thank you for your attention

More information

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