



Transfer of methodology from lab to industry for the detection of ergot



Philippe Vermeulen, Juan Antonio Fernández Pierna, Pierre Dardenne,
Vincent Baeten (CRA-W, Belgium)

Hans van Egmond (RIKILT, the Netherlands)
Albert Swinkels (NUTRECO, the Netherlands)

Prague, 5 November 2013



www.confidence.eu (2008-2012)

RAFA , Prague, 5 November 2013





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

Ergot in 1997

by Gary Munkvoid, extension plant pathologist, Department of Plant Pathology

Last year, **barley producers in northeast Iowa suffered a serious outbreak of ergot**, a fungal disease that can affect any small grain and many grass species. The fungus is called *Claviceps purpurea*, and it produces dark sclerotia (see photo). These sclerotia overwinter in or on soil and produce:

ST. PAUL, MN (June 8, 1998) — Sorghum, an extremely important cereal crop worldwide, has developed a serious enemy, ergot. This fungal disease has plant pathologists working intensely to accumulate information and develop strategies to combat the disease which can cause severe crop loss and economic hardship

Rye ergot - *Claviceps purpurea*

laboratories reported either numbers of evaluated samples, or frequencies of occurrence of < 5 % for two samples (member state A) means (Appendix 2, section 2). A frequency of occurrence of < 5 % for two samples (member states IRL and ES). In effectively an occurrence of 0 %. Two member states reported no results (member states IRL and ES). In all other cases (nine labs) ergot appears to be present in low or relevant frequencies, up to 25-50 %. The European remark was made that ergot occurrence seems to have increased in recent years. The European

An outbreak of ergotism in Ethiopia in 1978 resulted in exposure to ergot alkaloids from *C. purpurea* sclerotia grain contained up to 0.75% ergot;

THREAT TO LIVESTOCK

Jul. 20th, 2012
[No Comments](#)



Rancher warns feed buyers of ration contaminated with ergot

The first sign of trouble, in hindsight, was the behaviour of the yearlings, said...
by [Barb Glen](#)
Section: [Livestock](#) | Tagged [cattle feed](#)

ERGOT IN CEREAL CROPS, GRASSES POSES THREAT TO LIVESTOCK
From: [US Fed News](#) | Date: June 21, 2007

Producers urged to test for ergot

It isn't only in wheat — ergot affects other cereals and forages
Posted Oct. 16th, 2013 by [Agri-news](#)

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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



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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



L 140/10 EN Official Journal of the European Communities 30.5.2002

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

30.5.2002 EN Official Journal of the European Communities L 140/17

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
(1)	(2)	(3)
10. Theobromine	Complete feedingstuffs with the exception of — complete feedingstuffs for adult cattle	300 700
11. Volatile mustard oil	Feed materials with the exception of — rapeseed cakes	100 4 000 (expressed as allyl isothiocyanate)
	Complete feedingstuffs with the exception of — complete feedingstuffs for cattle, sheep and goats (except young animals) — complete feedingstuffs for pigs (except piglets) and poultry	150 (expressed as allyl isothiocyanate) 1 000 (expressed as allyl isothiocyanate) 500 (expressed as allyl isothiocyanate)
12. Vinal thiooxazolidone (Vinylthioazolidine thione)	Complete feedingstuffs for poultry with the exception of — complete feedingstuffs for laying hens	1 000 500
13. Rye ergot (<i>Claviceps purpurea</i>)	All feedingstuffs containing unground cereals	1 000
14. Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including	All feedingstuffs	3 000
(a) <i>Lolium temulentum</i> L.		1 000
(b) <i>Lolium rigidum</i> Schrank		1 000
(c) <i>Danthonia stramonium</i> L.		1 000

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[European Commission, directive 2002/32/EC of the European parliament and of the council of 7 May 2002 on undesirable substances in animal feed, in Official Journal of the European communities, L140, 10-21 \(2003\).](#)





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

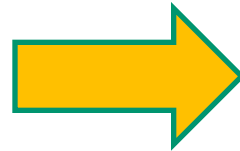
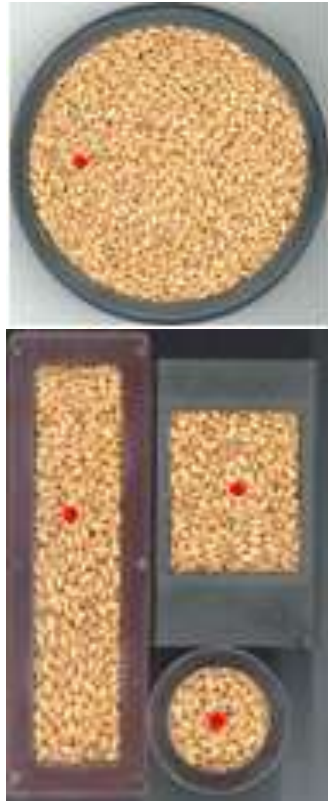


International Association of Feedstuff Analysis
Section Feedstuff Microscopy



What can we do using NIRS?

Detection based on one mean spectrum of the sample



Detection based on the analysis of several subsamples



What can we do using NIRS?



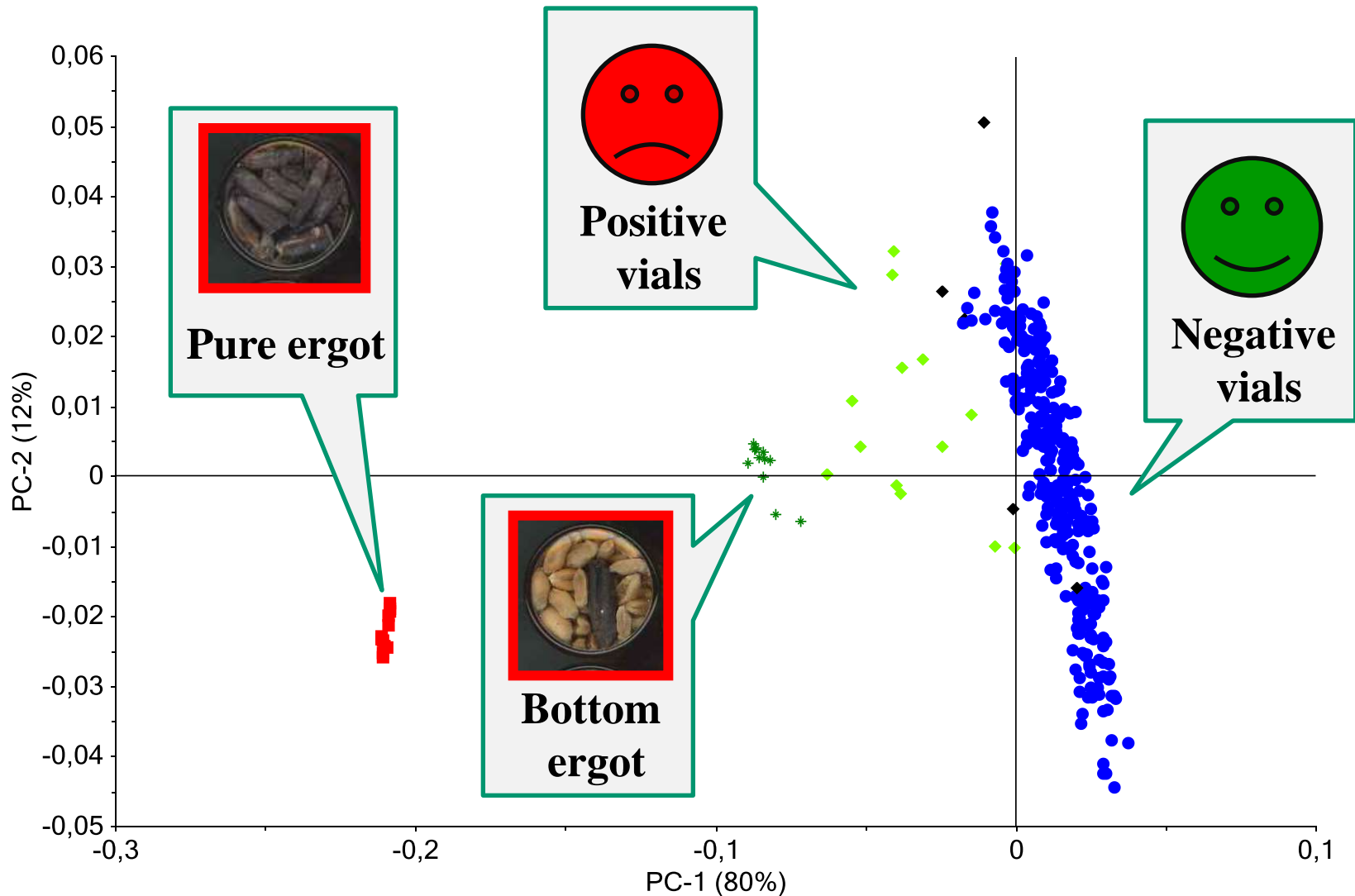
DQ Sample	Nb ergot bodies	Ergot (%)
DQ100013-01	0	0.00
DQ100013-02	1	0.01
DQ100013-03	5	0.05
DQ100013-04	10	0.10
DQ100013-05	15	0.15
DQ100013-06	50	0.50
DQ100013-07	100	1.00

- Measurement in reflection mode
- Wheel of 30 vials
- Analysis time: +/- 4 hours !!!
for 3 wheels replicated 4 times
by sample



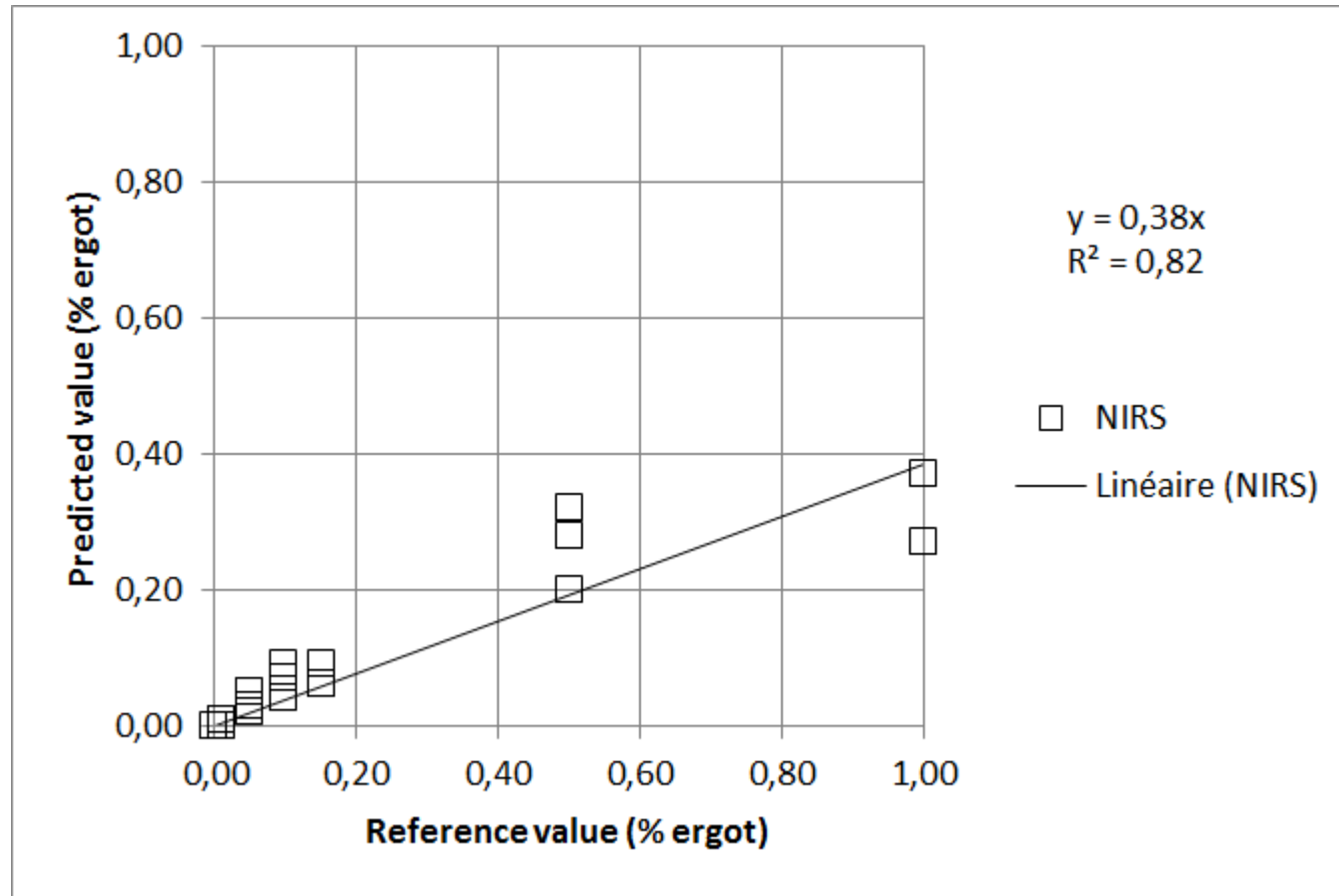
What can we do using NIRS?

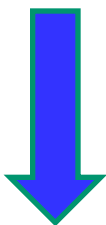
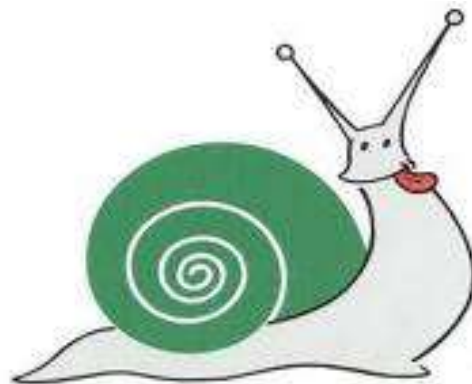
PCA on 324 spectra of one sample adulterated at 0,05%



What can we do using NIRS?

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





*Contaminants in food
and feed: Inexpensive
detection for control of
exposure*

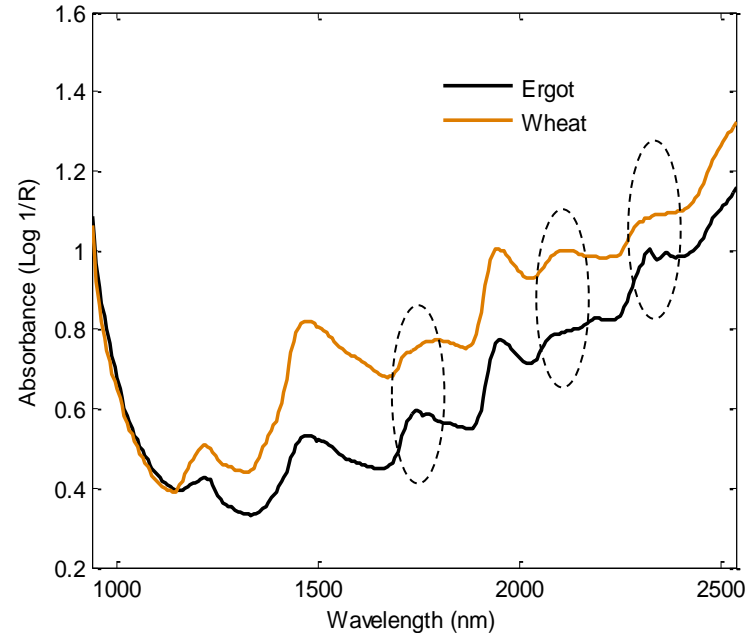
(FP7 project n°211326)



NIR line scan camera: features



- NIR camera setup



- Wavelength range: 1000-2500 nm by step of 6 nm
- 1 line = 320 pixels = 320 spectra
- field of view: 10 cm
- Analysed surface = continuous
- Time of acquisition = 50 millisecc/pixel line
- Speed of the conveyor belt = 3 mm/sec
- 1 pixel = $275 \mu\text{m} * 275 \mu\text{m} = 0.075 \text{ mm}^2$

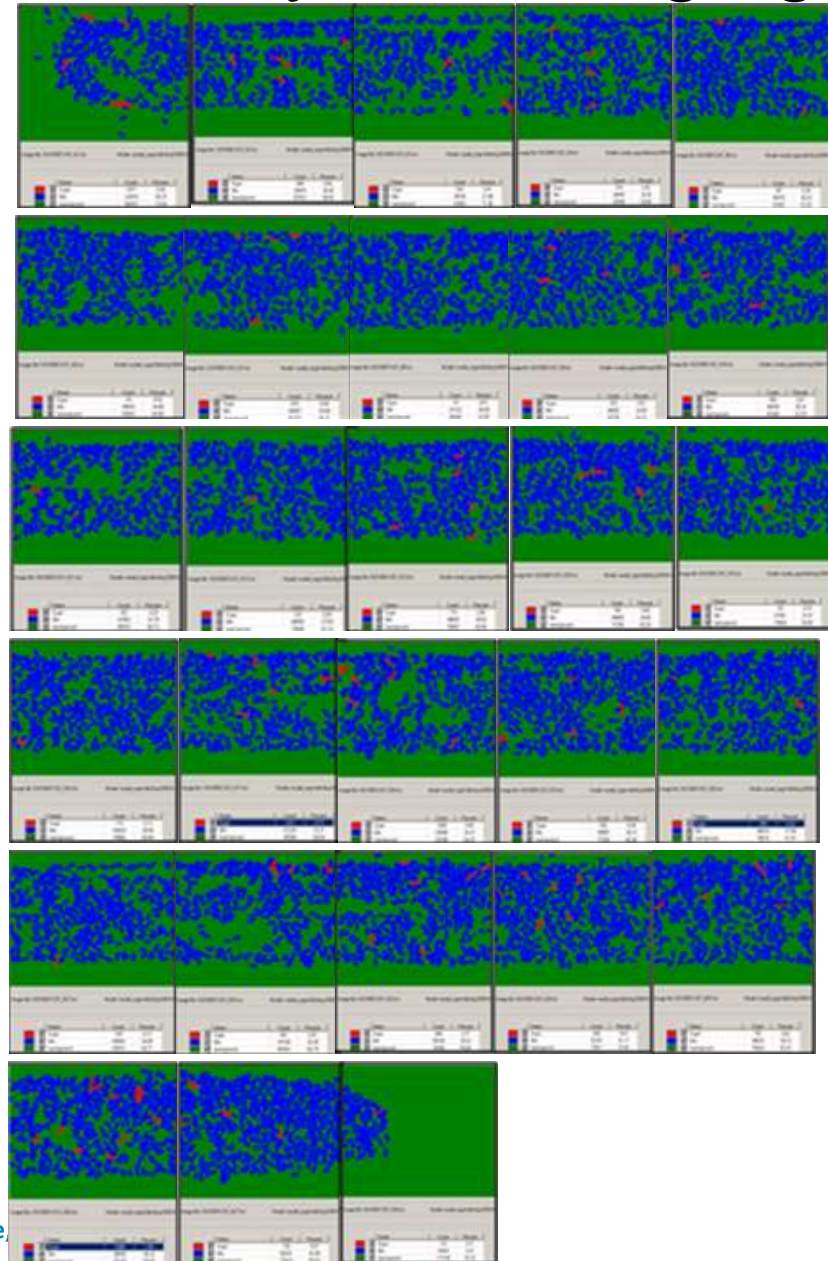
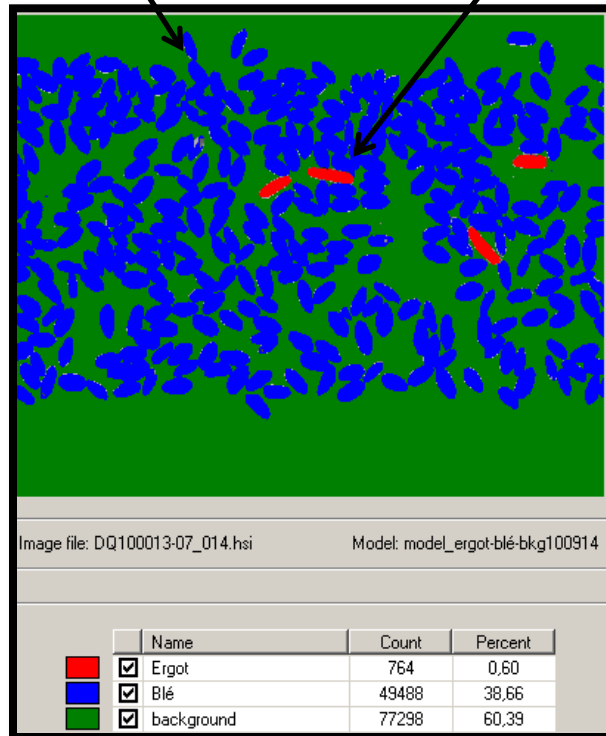




Ergot detection in wheat by NIR imaging



Wheat kernels
Ergot Bodies



The number of pixels counted for each class of the model is also provided.

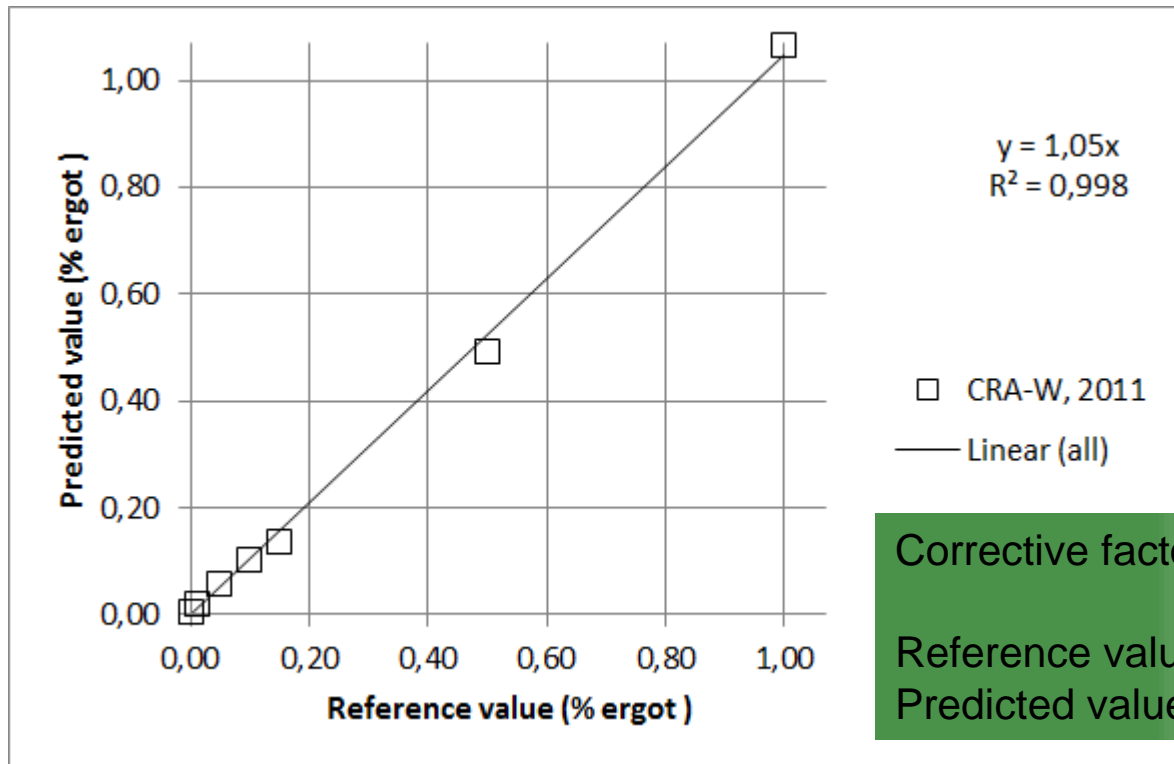




Results of ergot bodies detection



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

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Peer reviewed Journal article



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Vol. 29, No. 2, February 2012, 232–240



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

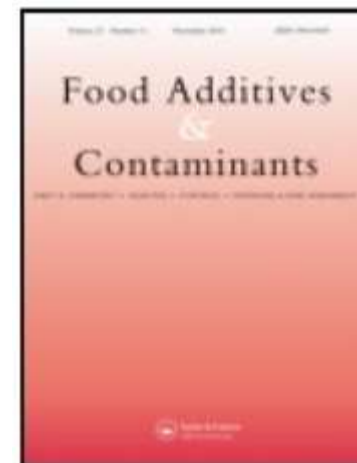
Ph. Vermeulen^{a*}, J.A. Fernández Pierna^a, H.P. van Egmond^b, P. Dardenne^a and V. Baeten^a

^a*Food and Feed Quality Unit (U15), Valorisation of Agricultural Products Department (D4), Walloon Agricultural Research Centre (CRA-W), Henseval Building, 24 Chaussée de Namur, 5030 Gembloux, Belgium;* ^b*Cluster Natural Toxins and Pesticides, RIKILT-Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, The Netherlands*

(Received 1 August 2011; final version received 25 September 2011)

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

RAFA , Prague, 5 November 2013

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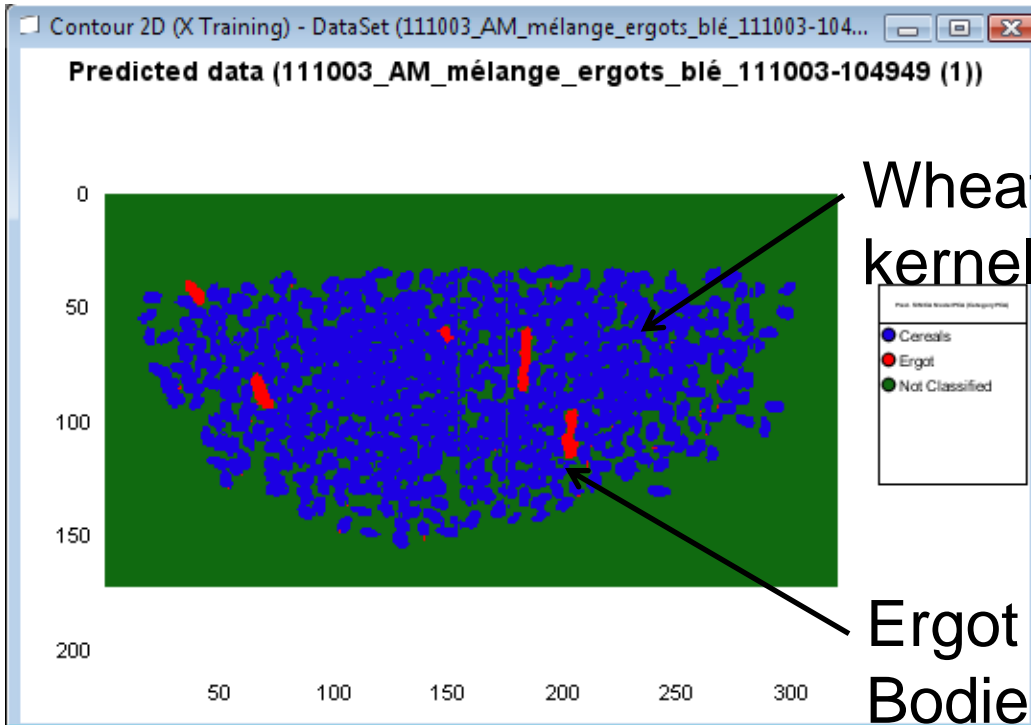


Ergot detection in wheat by NIR imaging

The number of pixels counted for each class of the model and ...

(object quantification)

ID 1	1
Predicted as:	# Predicted
1	Not Classified 36062 (65.5...)
2	Ergot 425 (0.772%)
3	Cereals 18553 (33.7...)
4	
5	Total 55040 (100%)



... 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



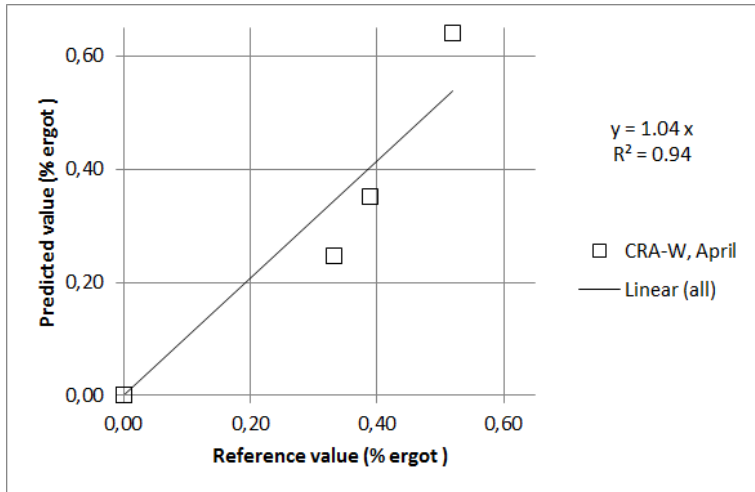
Results of ergot bodies detection



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

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

BurgerMetrics
Instrument
(Pilot
imaging system)

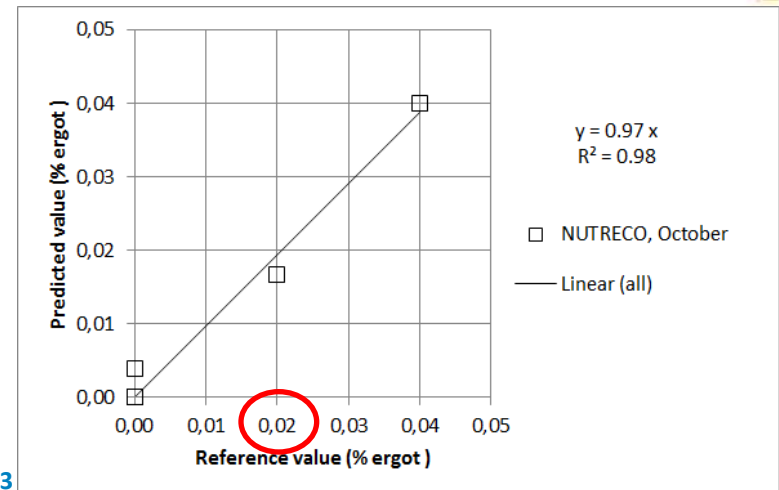
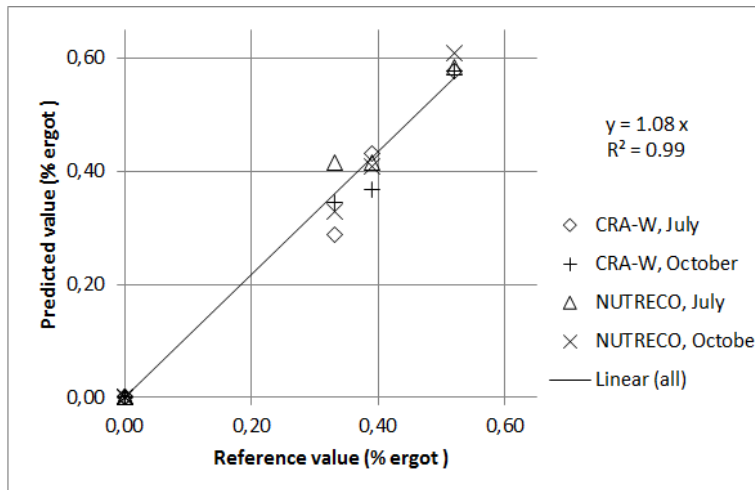


Corrective factor weight/area

Reference value: % weight

Predicted value: % area * 0,8

SisuChema
Instrument
(Commercial
imaging system)





ABC special issue: CONffIDENCE outputs



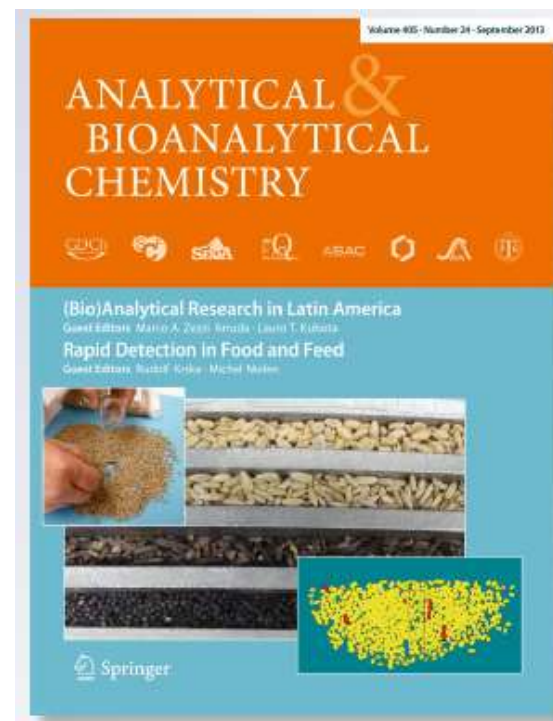
Anal Bioanal Chem (2013) 405:7765–7772

DOI 10.1007/s00216-013-6775-7

ORIGINAL PAPER

Validation and transferability study of a method based on near-infrared hyperspectral imaging for the detection and quantification of ergot bodies in cereals

Ph. Vermeulen • J. A. Fernández Pierna •
H. P. van Egmond • J. Zegers •
P. Dardenne • V. Baeten



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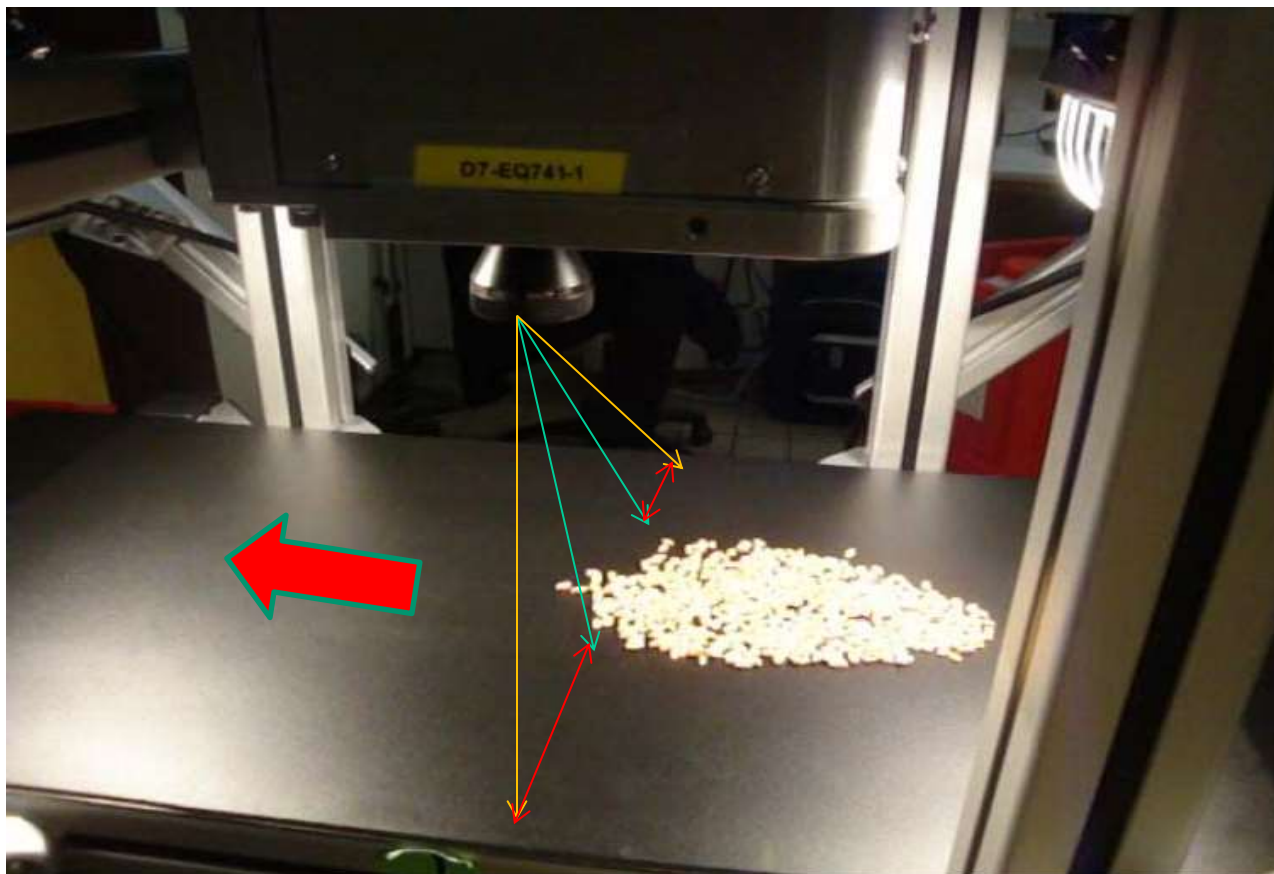
Wallonie



How to improve the performance? (1)



Increase the field of view and the move of the grains



Use the full width of the conveyor belt: 30 cm instead of 10 cm
Increase the speed of the conveyor belt: from 3 to 100 mm/sec





How to improve the performance? (2)

Impact on the acquired information



From
250 pixels by kernel
using conveyor belt
at 10 cm width
and 3 mm/sec

to

2,5 pixels by kernel
using conveyor belt
at 30cm width
and 100 mm/sec



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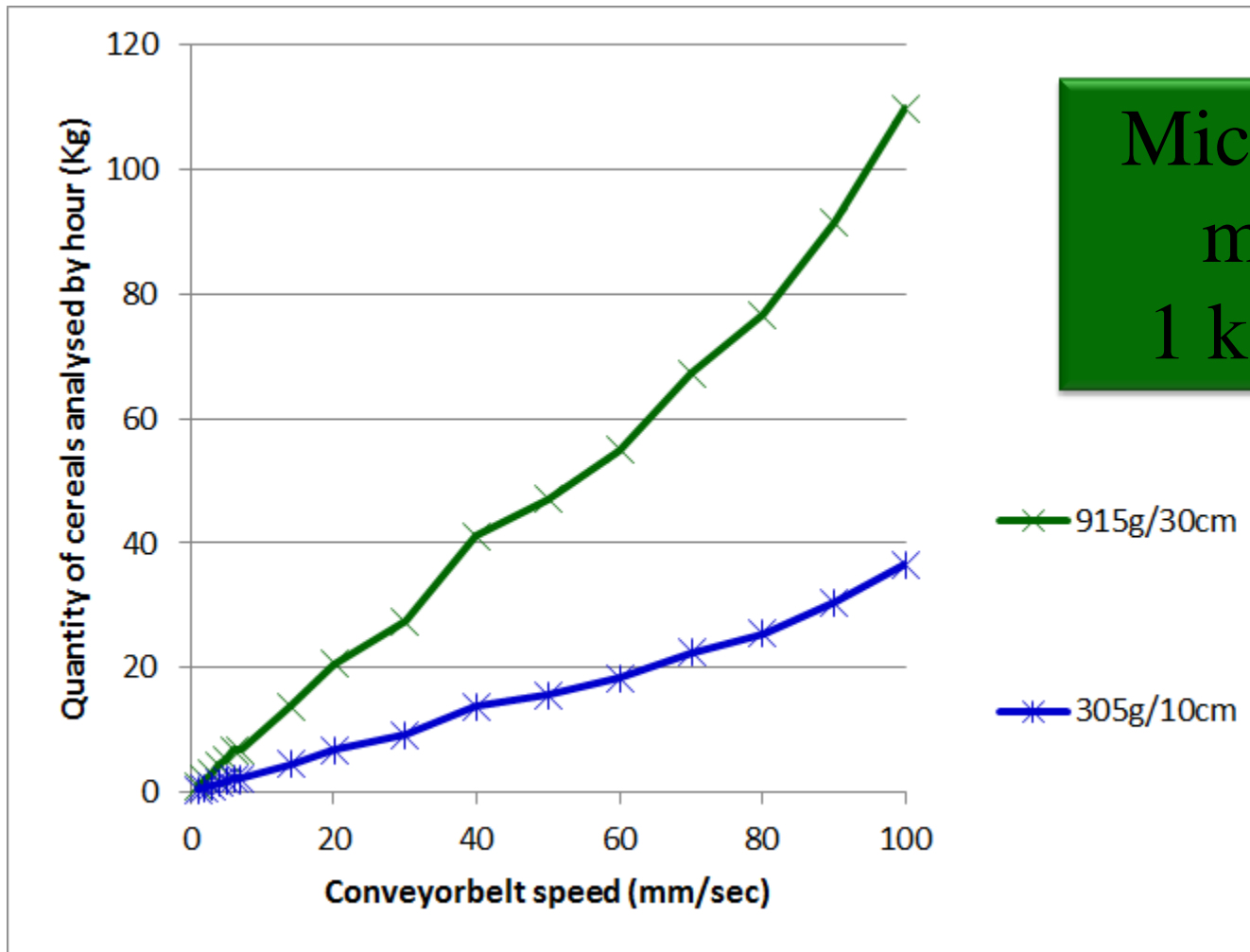




How to improve the performance? (3)



Impact on the quantity analysed by hour



**Microscopic method
1 kg / hour**

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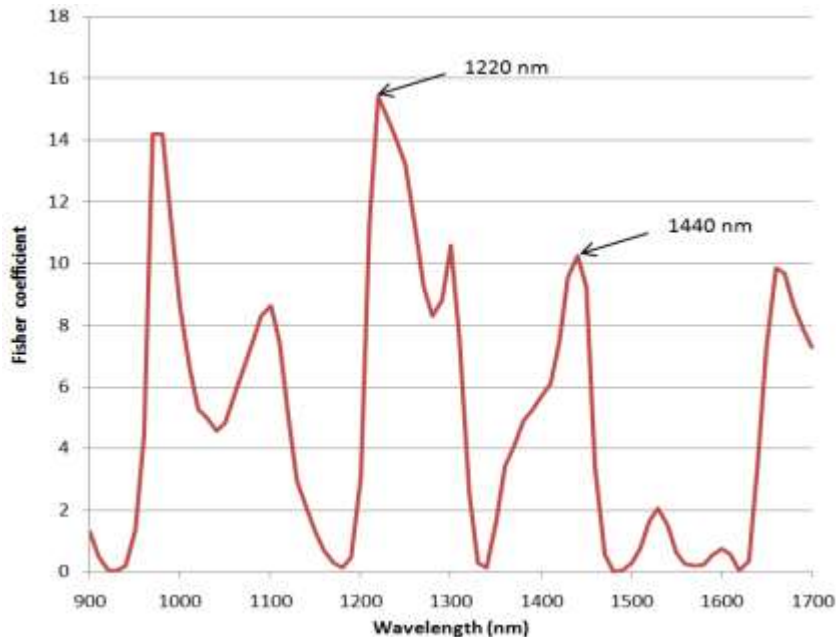




How to improve the performance? (4)

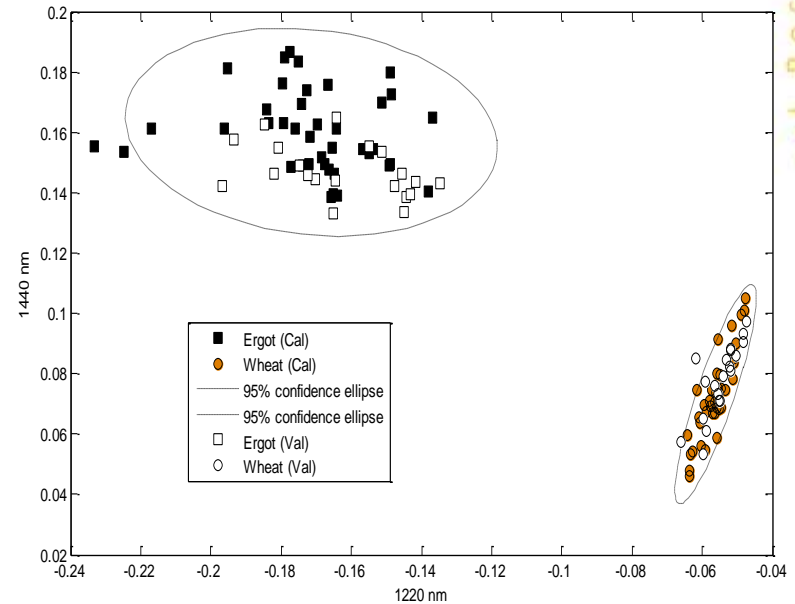


Reduce the image acquisition time: wavelength selection



The Fisher coefficient was used to select the wavelengths where the between-classes variation is higher than the within-classes variation

Discrimination Ergot/Wheat based on 2 wavelengths (1220 nm/ 1440 nm)



Vermeulen, P. , Dardenne, P. , Baeten, V. & Fernandez Pierna, J.A. (2011). *Detection of ergot bodies in cereals by near infrared spectroscopy and hyperspectral near infrared imaging*. Proceedings in: 14th International Conference on Near Infrared Spectroscopy (ICNIRS): Breaking the dawn, Bangkok - Thailand, 7-13 November 2009, 997-1002.



Other applications

- ergot detection in black oat



RAFA , Prague, 5 November 2013

Other applications

- Multicontaminants detection: ergot, datura, ...



Mixture of wheat, black oat, rape seed, ergot and datura



Benefits of the method for a feed Company



Classical microscopy	NIR hyperspectral imaging
High skilled personal	Low skilled personal
15 min / 250g	A few minute / 250 g
Reduced samples	Large samples (sampling more representative)
Dedicated to ergot	Multiple contaminants





Thank you for your attention



More information

Contact:

Ph. Vermeulen (CRA-W)
vermeulen@cra.wallonie.be

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RAFA , Prague, 5 November 2013

