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

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Prague, 5 November 2013

www.conffidence.eu (2008-2012)

What is the problem?

- Reemerging of the ergot presence in cereals
  - For the farmer,
    yield reduction (10%)

Ergot in 1997

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

Last yea barley producers in northeast Iowa suffered a serious outbreak of ergot, a fungal disease that can affect any sma and many grass species. The fungus is called *Claviceps purpurea*, and it produces dark scl (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

  - For the feed/food sector,
    high toxicity risk for animal and human

Rye ergot - *Claviceps purpurea* Ergot is the most frequently mentioned undesirab laboratories reported either numbers of evaluated samples, or frequencies of occurrence, or bou (Appendix 2, section 2). A frequency of occurrence of < 5 % for two samples (member state A) means 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 remark was made that ergot occurrence seems to have increased in recent years. The European

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

Jul. 20th, 2012

No Comments

Rancher warns feed buyers of ration cont ergot

The first sign of trouble, in hindsight, wa 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 Nev US State 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

Walloon Agricultural Research Centre

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

AGROINNOVA

SCIENTIFIC / TECHNICAL REPORT submitted to EFSA

CFP/EFSA/CONTAM/2008/01

Scientific information on mycotoxins and natural plant toxicants

Prepared by Battilani P.[1], Costa L.G.[2], Dossena A.[1], Gullino M.L.[3], Marchelli R.[1], Galaverna G.[1], Pietri A.[4], Dall'Asta C.[1], Giorni P.[4], Spadaro D.[3], Gualla A.[4]

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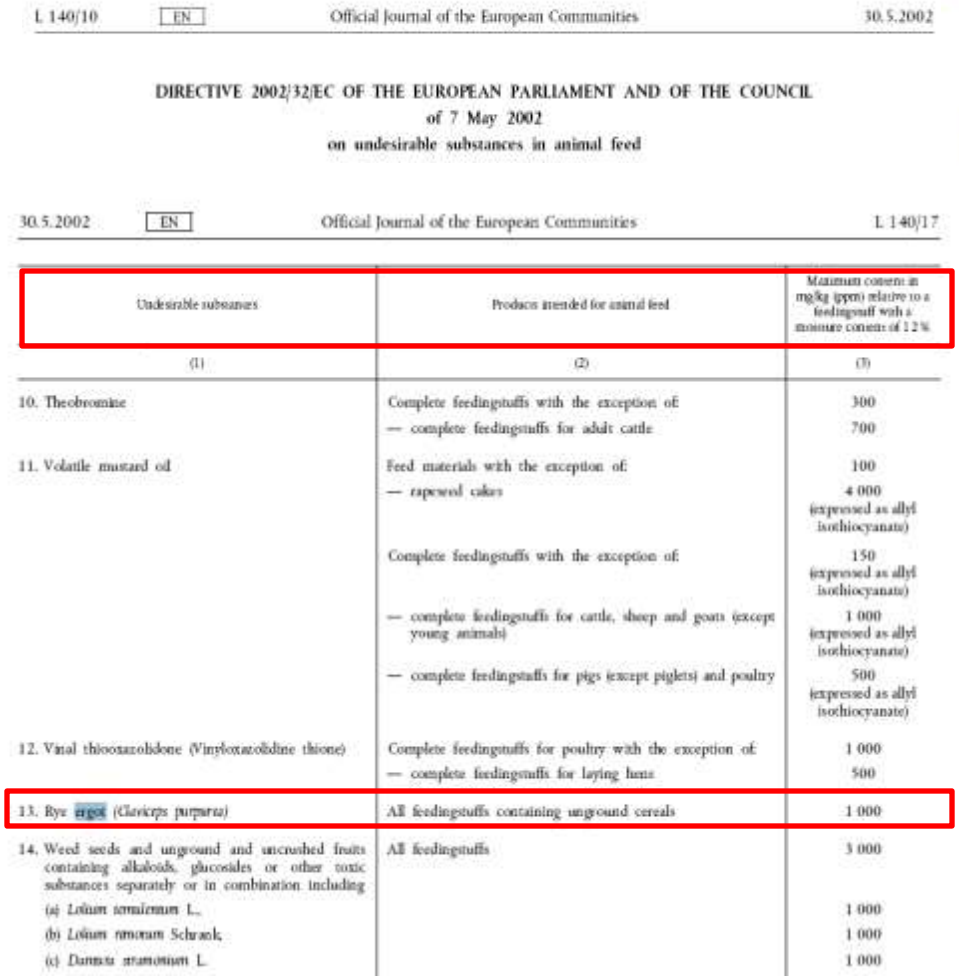
[4]Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy.

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	300
	— complete feedingstuffs for adult cattle	700
11. Volatile mustard oil	Feed materials with the exception of	100
	— rapeseed cakes	4 000 (expressed as allyl isothiocyanate)
	Complete feedingstuffs with the exception of	150 (expressed as allyl isothiocyanate)
	— complete feedingstuffs for cattle, sheep and goats (except young animals)	1 000 (expressed as allyl isothiocyanate)
	— complete feedingstuffs for pigs (except piglets) and poultry	500 (expressed as allyl isothiocyanate)
12. Vinyl thiooxazolidone (Vinyloxazolidine thione)	Complete feedingstuffs for poultry with the exception of	1 000
	— complete feedingstuffs for laying hens	500
13. Rye ergot (*Claviceps purpurea*)	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	5 000
(a) *Lolium temulentum* L.,		1 000
(b) *Lolium remotum* Schrank,		1 000
(c) *Datura stramonium* L.		1 000

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

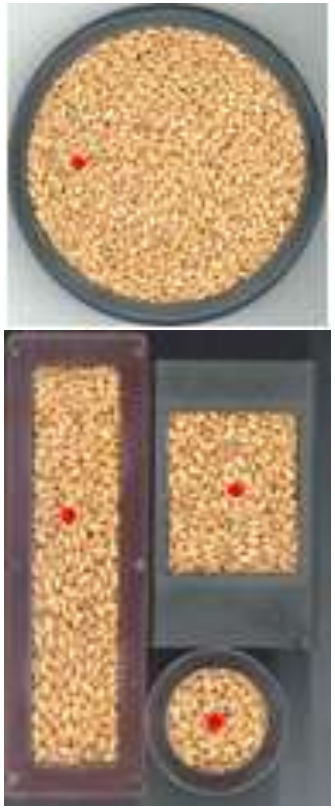
Method for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff, IAG-Method A4

International Association of Feedingstuff Analysis

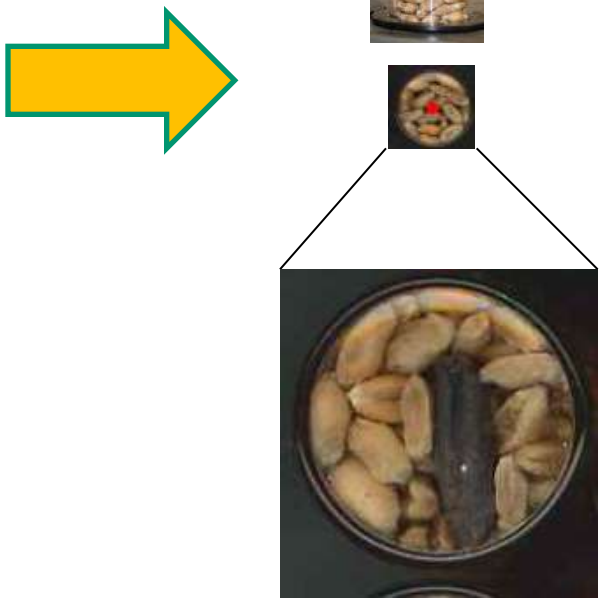
Section Feedingstuff Microscopy

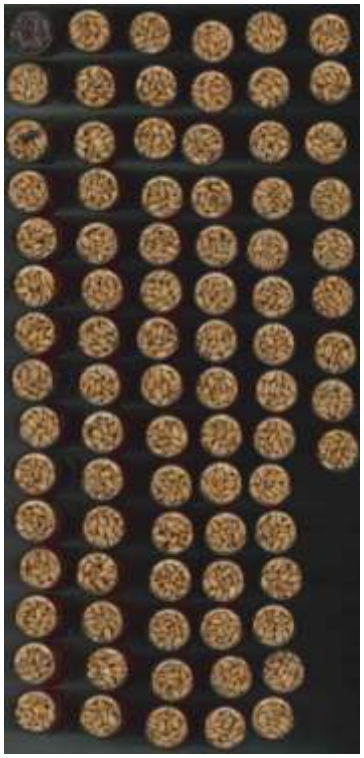
What can we do using NIRS?

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

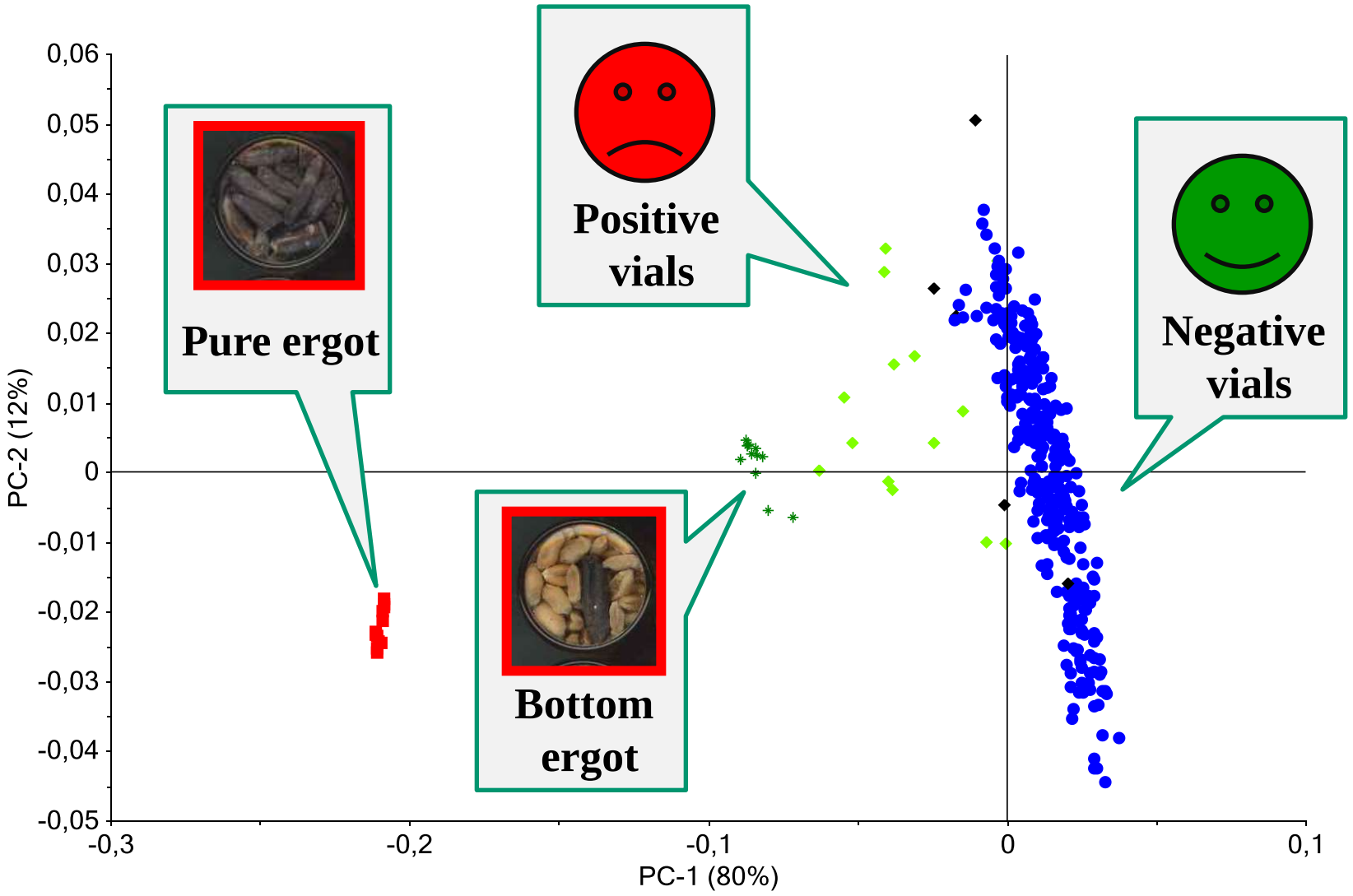
- Measurement in reflection mode

- 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%



cra-w

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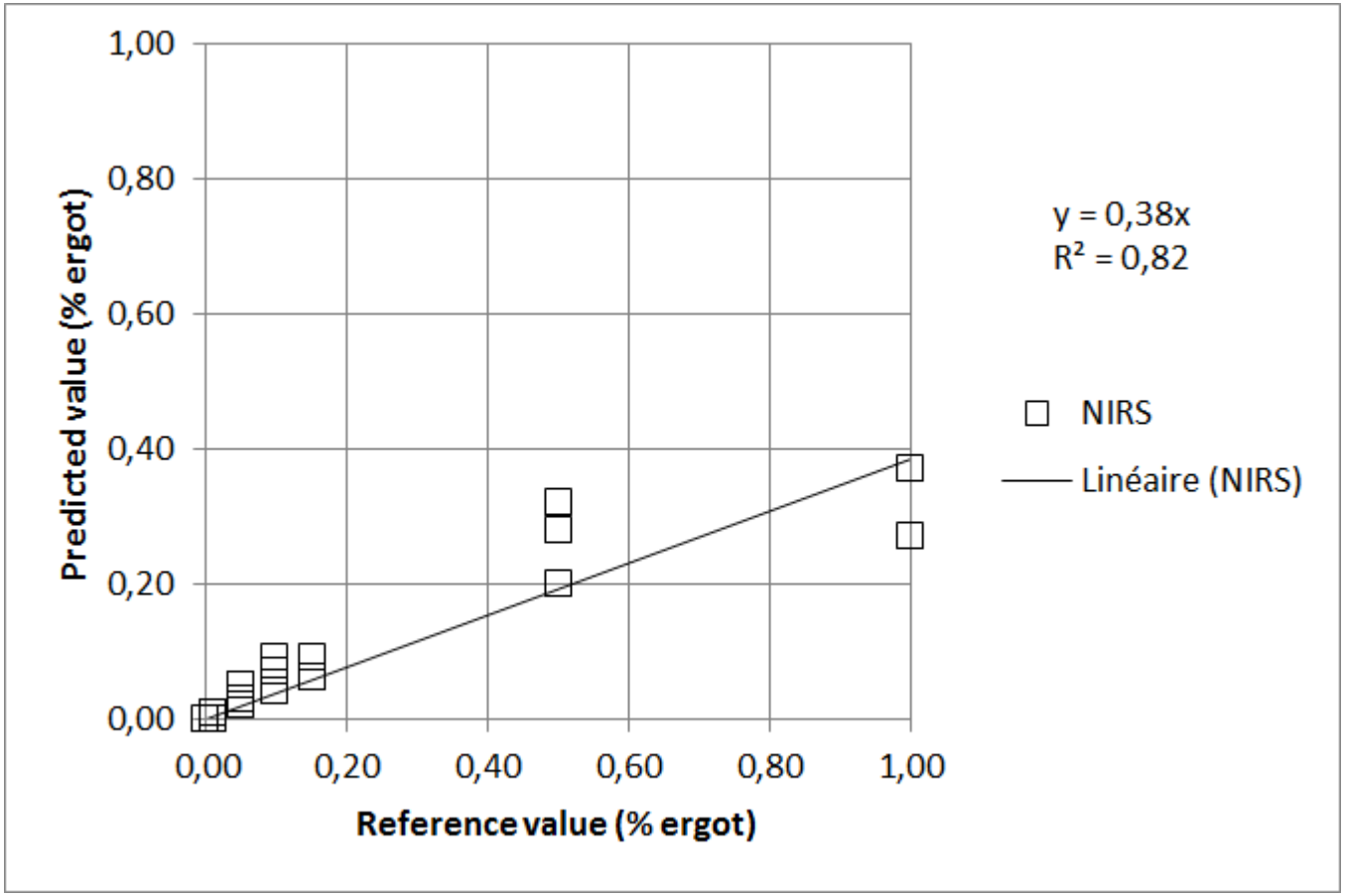
What can we do using NIRS?

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



CONFFIDENCE

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

(FP7 project n°211326)

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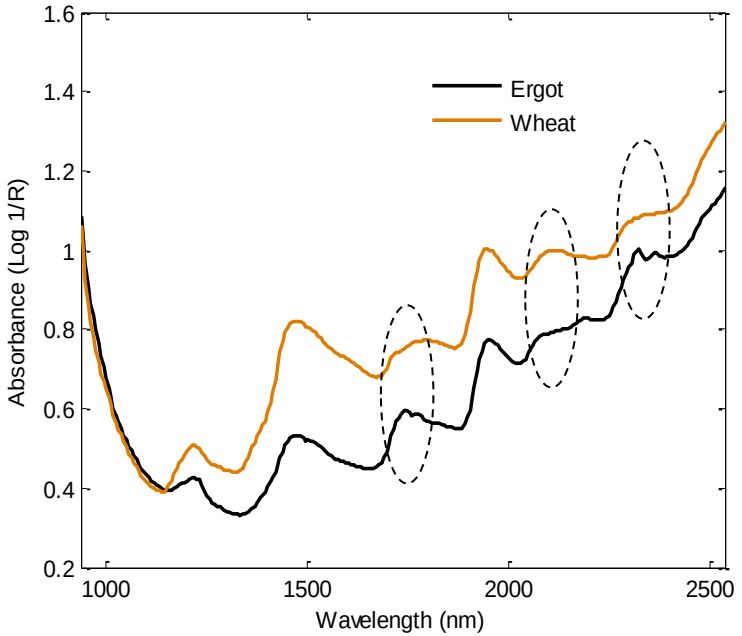
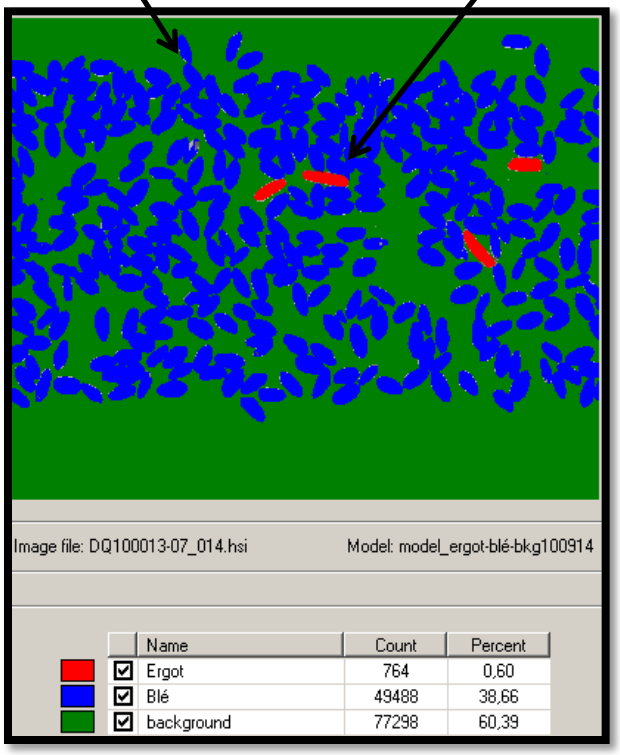
NIR line scan camera: features

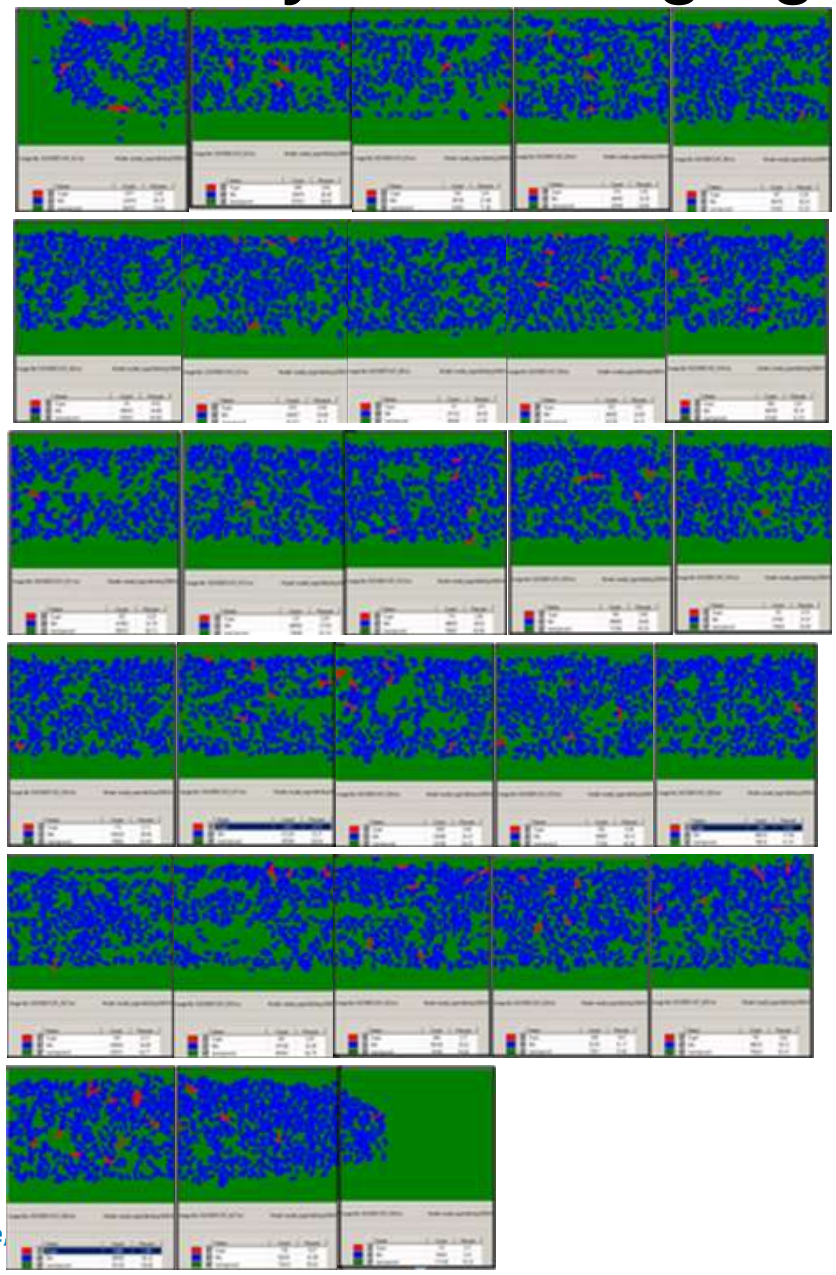
- NIR camera setup



- 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 millisec/pixel line
- Speed of the conveyor belt = 3 mm/sec
- 1 pixel = 275 µm * 275 µm = 0.075 mm2

Ergot detection in wheat by NIR imaging

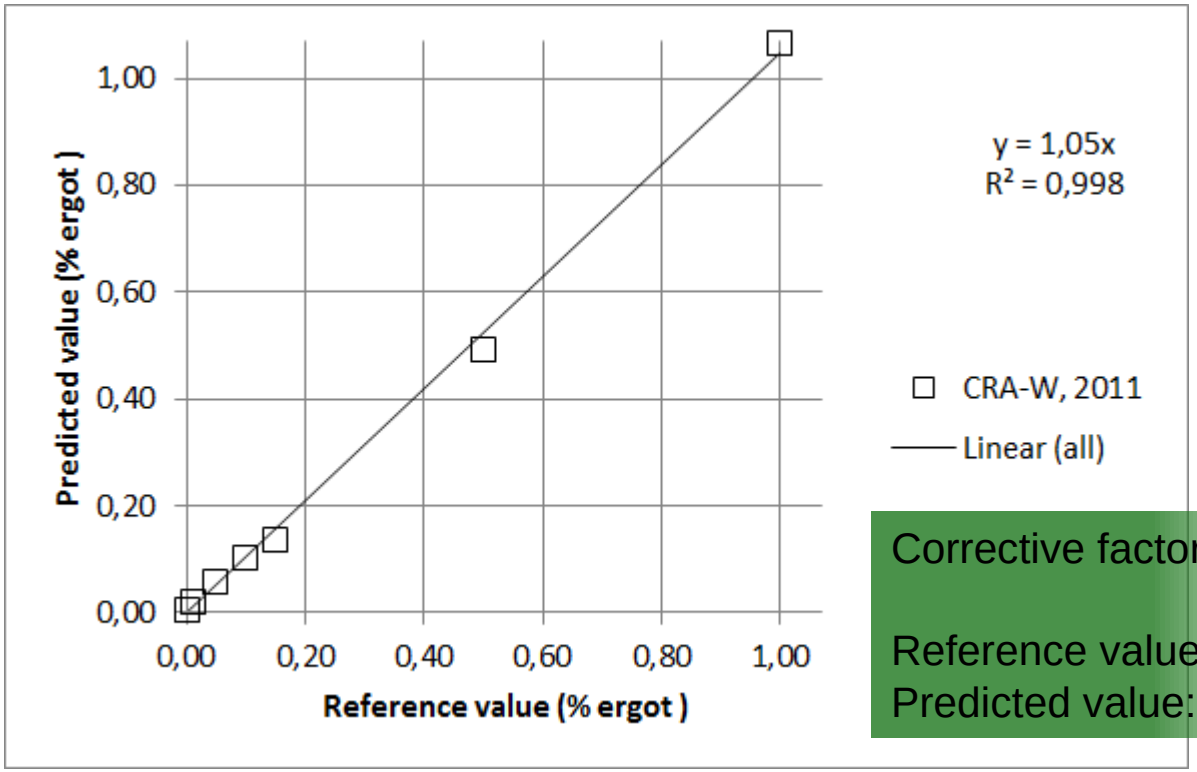




The number of pixels counted for each class of the model is also provided.

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

Peer reviewed Journal article

Food Additives and Contaminants
Vol. 29, No. 2, February 2012, 232–240

Taylor & Francis
Taylor & Francis Group

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

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(*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

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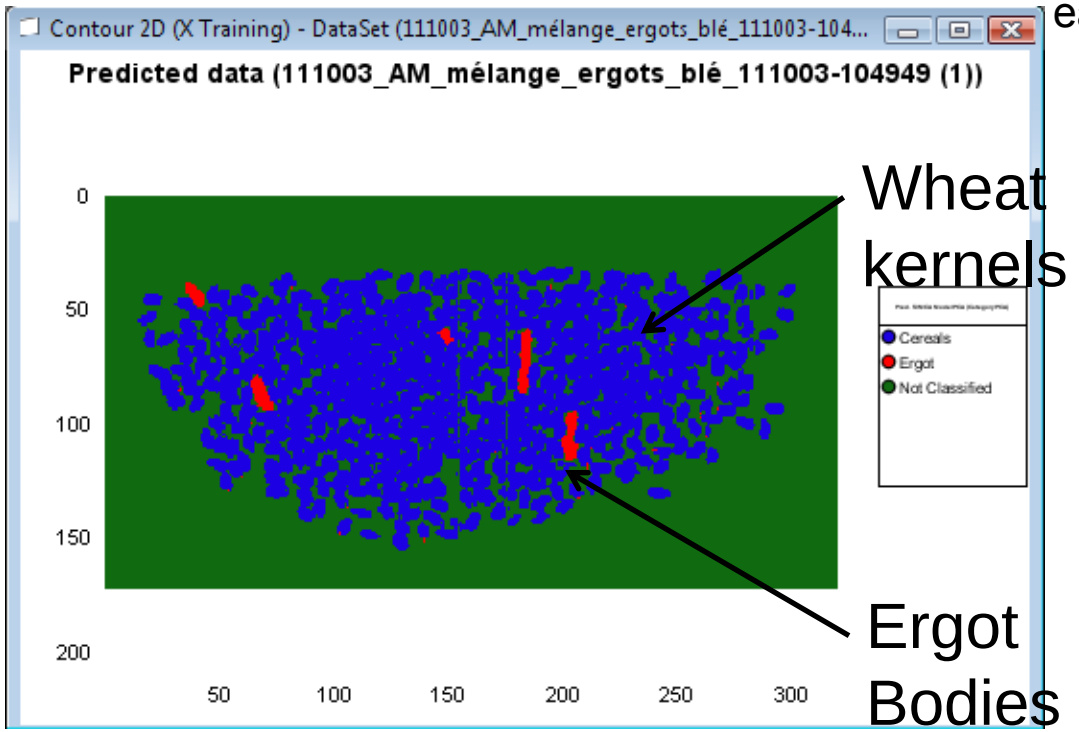
NIR line scan camera: instrument in demonstration at NUTRECO

NIR camera
SWIR
ImSpector
N25E
Spectra
Camera
(Specim Ltd)

Light source

Tray

Ergot detection in wheat by NIR imaging



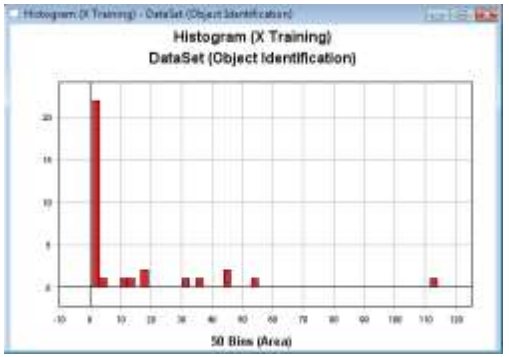
Prediction results of the SIMCA (Soft Independent Method of Class Analogy) model

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

Prediction Table (SIMCA Mo…

	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**)



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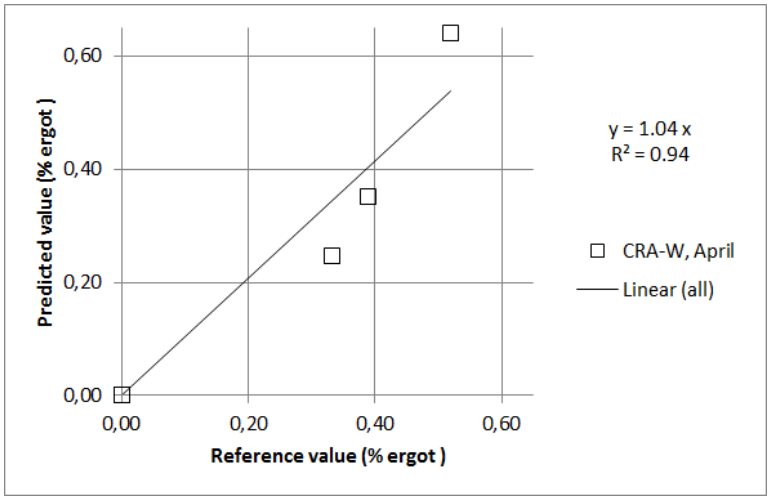
Results of ergot bodies detection

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

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

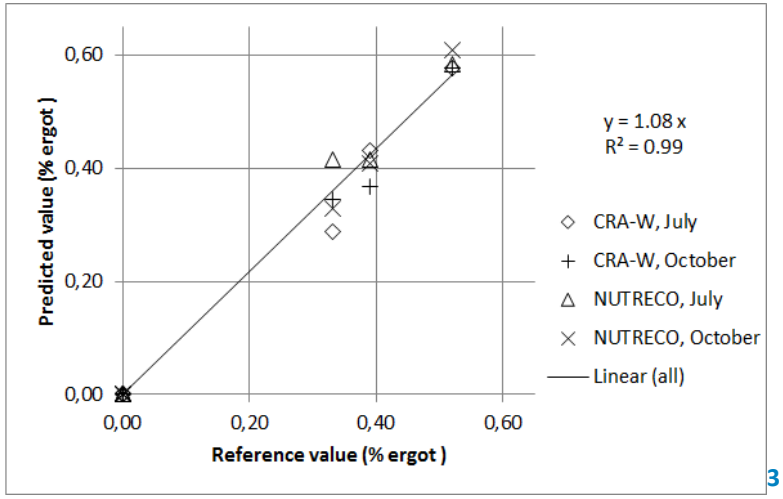
BurgerMetrics Instrument (Pilot imaging system)

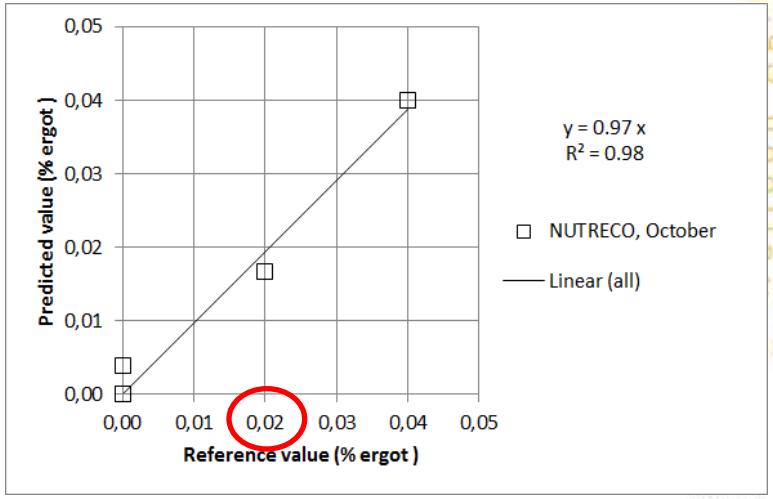


Corrective factor weight/area

Reference value: % weight
Predicted value: % area * 0,8

SisuChema Instrument (Commercial imaging system)





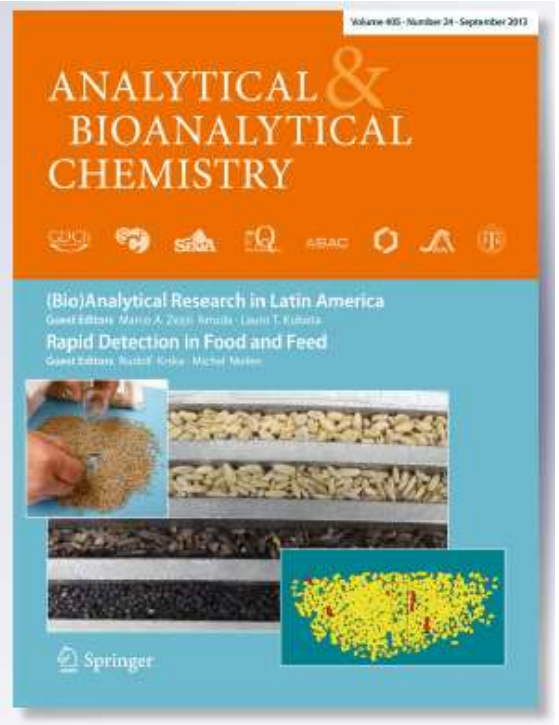
ABC special issue: CONffIDENCE outputs

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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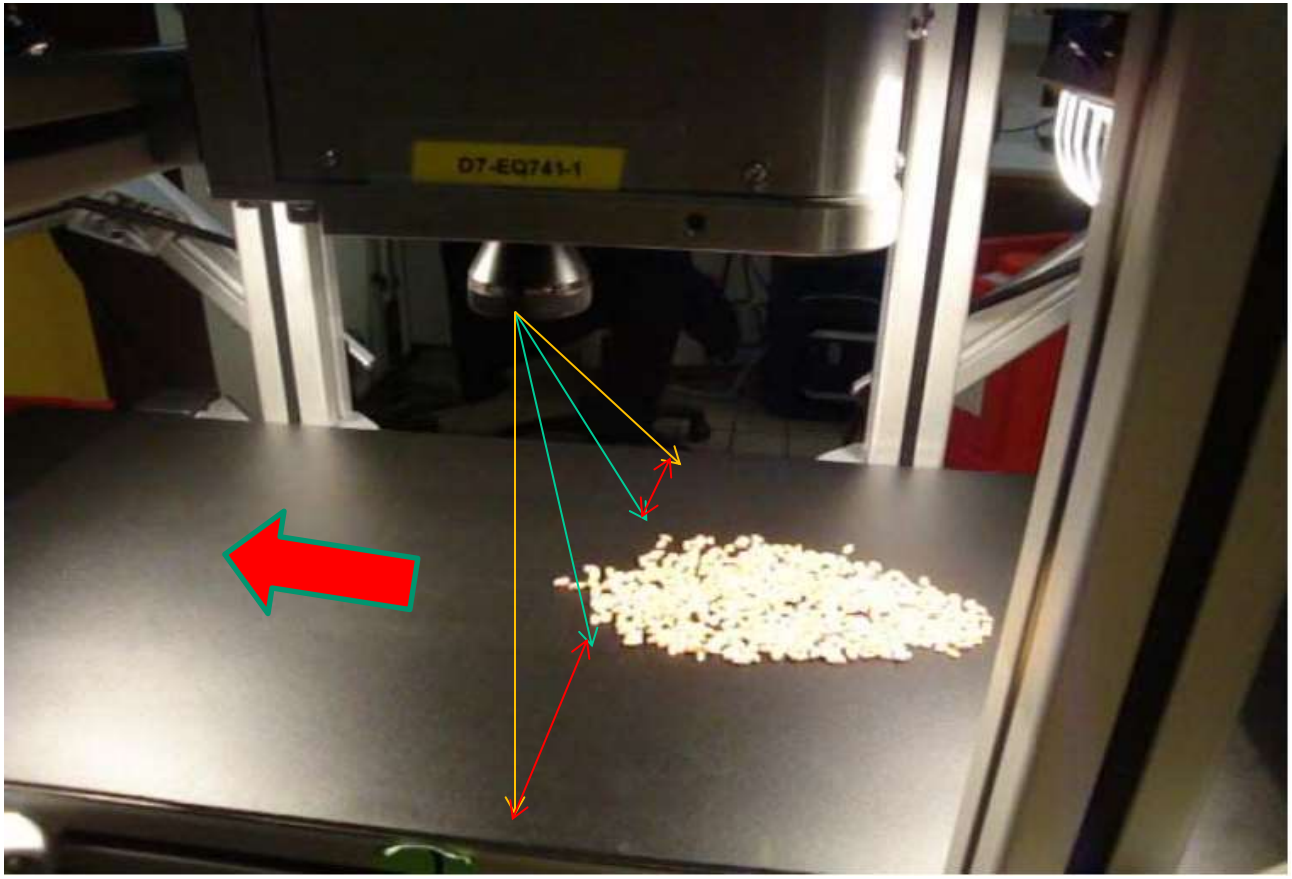
How to improve the performance? (1)

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

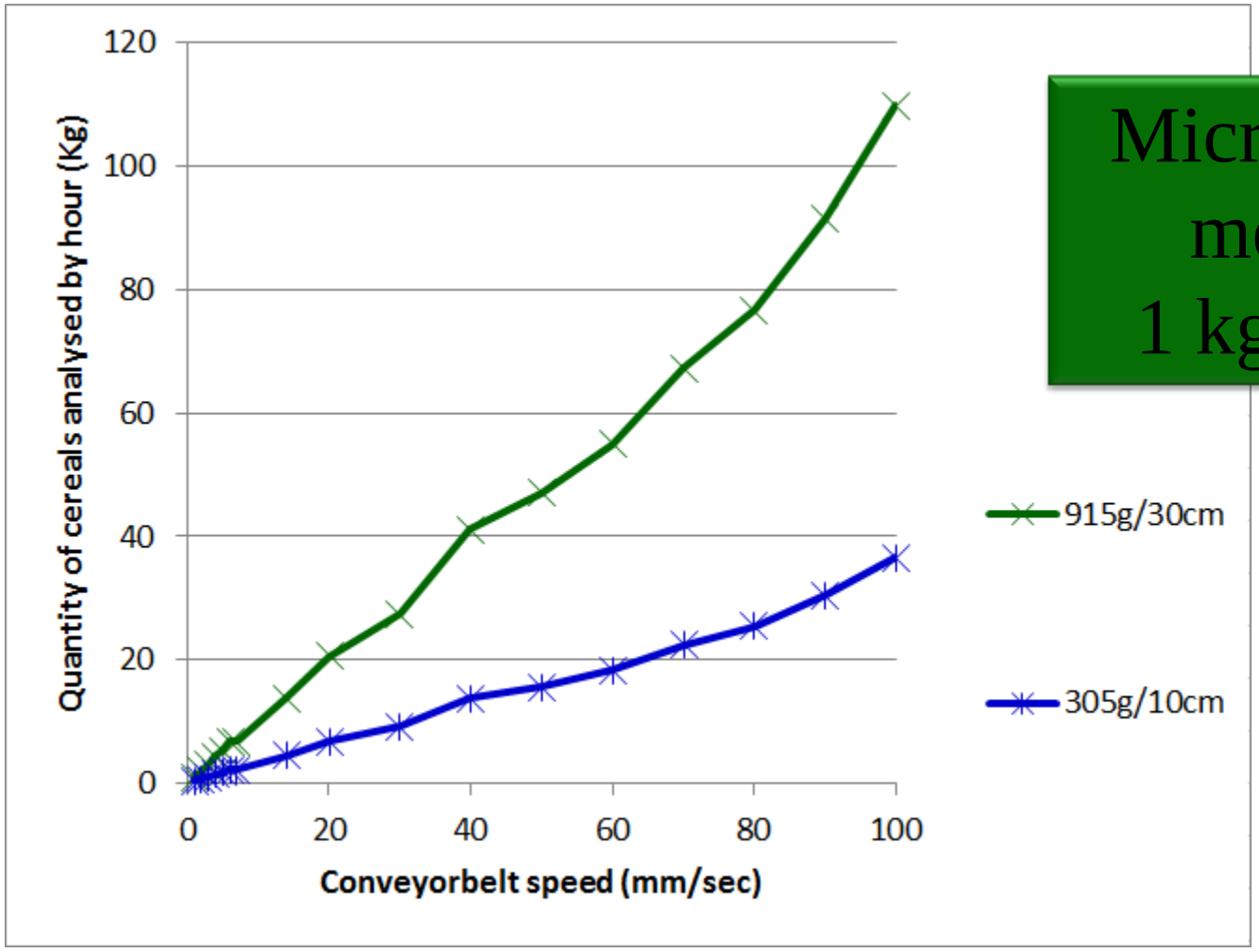
From
250 pixels by kernel
using conveyor belt
at 10 cm width
and 3 mm/sec

to

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

How to improve the performance? (3)

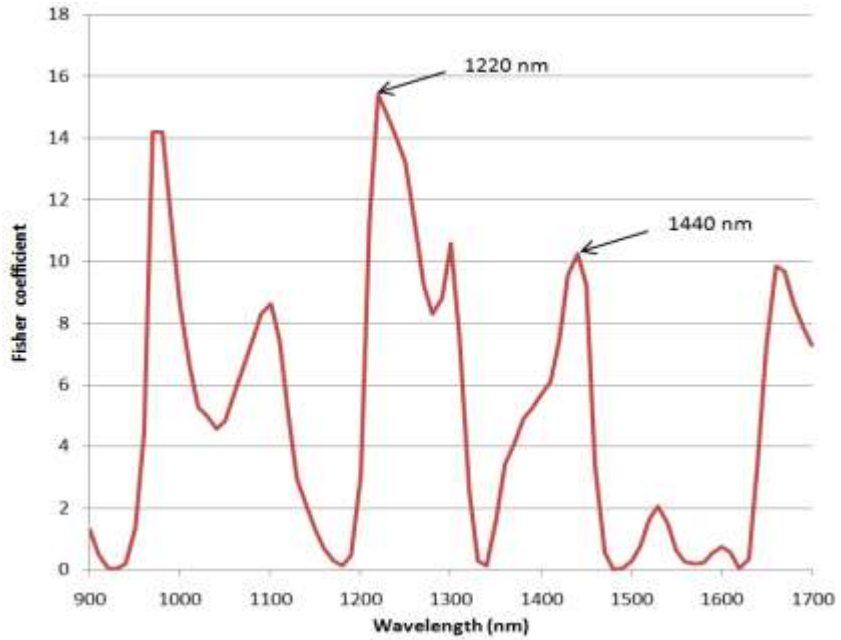
Impact on the quantity analysed by hour



Microscopic method
1 kg / hour

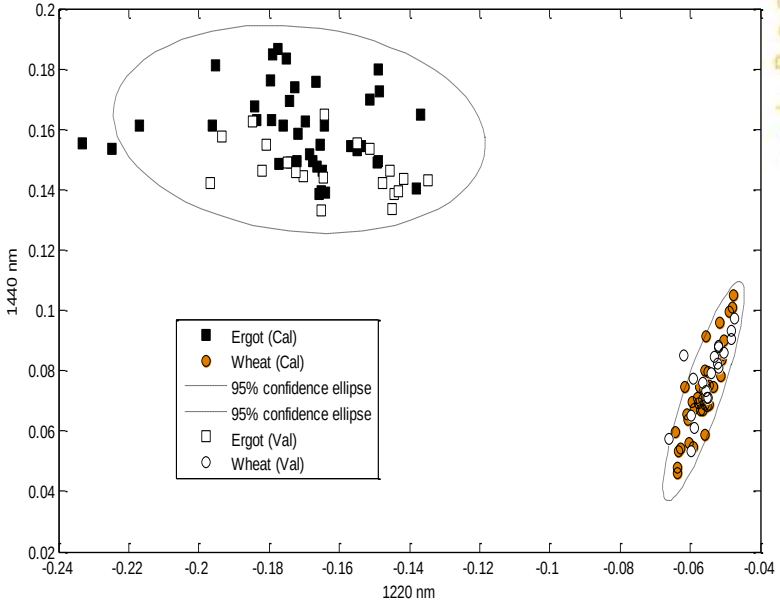
How to improve the performance? (4)

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

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

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www.con*ff*idence.eu