Using NIR spectroscopy on milk for the traceability of cows feeding

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IV Conference

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UNIVERSITÀ DEGLI STUDI DI PADOVA

MINISTERO DELLE POLITICHE AGRICOLE ALIMENTARI E FORESTALI

NIR on the GO 2010

IV Conference

Program

Thursday May 27

- **8:30 - 17:00 Registration**
- **9:15 - 9:30 Opening Remarks (delegato Ministero Politiche Agricole e Forestali)**
- **9:30 – 10:00 Invited lecture**: *On farm NIR analysis and its role in precision feeding.* David R. Mertens *(Mertens Innovation LLC)*

Plenary Session 1: *Hardware (Chairman Christian Paul).*

- *This session will have contributions related to the latest technology in NIR sensors used in On site, On line application. The Hardware session will be closed by short (10-15min) presentations of hardware suppliers.*

- **10:00-10:15** *Testing a new generation NIR diode array instruments for inline quality measurement.* P. Reyns, B. Kemps, W. Saeys, Y. Denion-Lair, J. Sentjens, M. De Winter and B.de Ketelaere, J. De Baerdemaeker
- **10:15-10:30** *Proceedings in Sample Presentation on Harvesting Machines.* L. Urbonas and H. Krüger
- **10:30-10:45** *Diode Arrays, Optical Resolution, Data Spacing and Information Content.* D. E. Honigs
- **10:45 - 11:15** coffee break - Posters
- **11:15 -12:00 Sponsor Session 1: Zeiss/ John Deere, M.U.T. AG, Polytec GmbH.**
- **12:00-12:45 Invited lecture**: *Chemometric NIR-technology to identify the quality of biological individuals as exemplified by single seed mass sorting.* L. Munk

12:45 – 13:45 Lunch

Plenary Session 2: *Software (Chairman Paolo Berzaghi).*

- *This session will focus on calibration or standardization algorithms that may beneficial for On site, On Line applications. The Software session will be closed by short (10-15min) presentations of software suppliers.*
- **13:45 -14:15 Invited lecture:** NIRS Measurement and Calibration Development for Onsite Applications: Software Requirements. Heirich Prufer & Peter Tillman

- **14:15 - 14:30** *Comparison between different standardization methods for the quality estimation of forages in portable NIR spectrometers.* P. Facco, M. Barolo and F. Benozzo
- **14:30 - 15:00 Sponsor Session 2: Sensologic GmbH, Camo**

15:00-15:30 Coffee break - Posters

Plenary Session 3: *Applications (Chairman Pierre Dardenne).*

- **15:30 - 16:00** *NIR on the Go at CRAW : 15 years of experience (1995-2010)*. G. Sinnaeve and P. Dardenne
- **16:00 – 16:30** *Portable NIR Instruments for Agriculture Applications.* J. Shenk
- **16:30 – 16:45** *On-site, on-line determination of total solids, nitrogen and phosphorus in liquid hog-manure*. P. Williams, D. Malley and E. Eising
- **16:45 – 17:00** *Nutrient based Slurry Application by Near-Infrared Spectroscopy*. C. R. Moschner
- **17:00 – 17:15** *In-Line Near-Infrared-Monitoring of the Biogas Process*. H. Andre, R. Baumgarten
- **17:15 – 17:30** *Using NIR spectroscopy on milk for the traceability of cows feeding*. M. Coppa, B. Martin, C. Agabriel, I. Constant, G. Lombardi, and D. Andueza
- **17:30 – 18:00 Round Table: NIR on the GO…where is it going?** An open discussion to make this technology a success (Christian Paul, Phil William, Pierre Dardenne, Frédéric Larsson, Paolo Berzaghi).

20:00 Gala dinner at Hotel Galileo (Padova).

Friday May 28

Plenary Session 4: *Applications (Chairman Phil Williams).*

- **09:00 – 09: 15** *Efficient Recirculation of Liquid Farm Fertiliser and Biogas Substrate using NIRS-NANOBAG® W*. Wenzl, B. Steiner, L. Haberl, W. Somitsch, S. Petrak
- **09:15 – 09:30** *Variation among days in the dry matter of silages in bunker and tower silos measured using diode-array NIRS.* J.Karlen
- **09:30 – 10:30 Sponsor Session 3: Dinamica Generale s.r.l., Haldrup GmbH, Grainit s.r.l., VDLUFA.**

10:30-11:30 coffee break – Posters

- **11:30 – 11:45** *Application of FT-NIR for determination of wood provenance*. A. Sandak, J. Sandak, M. Negri
- **11:45 – 12:00** *Precision Feeding: NIR on line & TMR consistency*. A. Barbi, A. Ghiraldi, M. Manzoli, and P. Berzaghi
- **12:00 – 12:15** *Tailored NIR Reflection Spectroscopic Methods for the Advanced Characterization of Nanomaterials*. C.W. Huck
- **12:15 – 12:30** Closing remarks.
- **12:30 – 13:30** Lunch
- Afternoon: *guided visit to Palazzo del Bo' or Cappella degli Scrovegni, only on demand.*

INVITED LECTURE

On-farm NIR analysis and its role in precision feeding.

David R. Mertens, Mertens Innovation & Research LLC, Belleville, WI 53508-9727

Introduction

Increased interest in precise feeding of dairy cows is related to three issues: (1) profit margin for milk production is narrowing and feed is the major cost of production, (2) milk production per cow has increased to the point that accurate rations are needed to meet the nutritional and health needs of high-producing cows, and (3) excretion of excess nutrients negatively impacts the environment. Off-farm analysis of feeds provides most of the information about nutrient concentrations in feed that is used to formulate dairy rations. Often the interval between analyses is too long to efficiently use feed and nourish animals. On large dairies, the amount of feed consumed each day can result in the feed being completely fed before the analytical results can be obtained for ration adjustments. Technology, such as NIR, can provide rapid estimates of nutritional value to address the dynamic feeding situation on large dairies.

Perhaps the greatest impediment to precise feeding is the daily variation in moisture or dry matter (DM) of feeds, especially fermented silages. Not only is there systematic variation in silage as a result of changes in the forage during harvest, but also there are abrupt changes due to precipitation on exposed silages surfaces. Rain can dramatically affect the ration that is mixed because water adds weight to the silage without adding nutrients. The greatest role of rapid NIR analysis on the farm may be the determination of the daily changes in feed DM so that the weights of feeds can be adjusted to obtain the same ratio of DM from each ingredient in the ration. The objective of this presentation is to: (1) describe the importance of precision feeding, (2) define the role of feed analysis in ration formulation and daily mixing of rations (3) explain the importance of DM and nutrient analysis on the farm and (4) discuss the value of on-farm NIRS analysis for precise feeding.

Materials and Methods

Generally, dairy rations are formulated using one to three forages and three to ten concentrated sources of nutrients (grains, protein feeds or mineral supplements). The nutritionist uses the nutrient concentrations of the feeds obtained from a laboratory to calculate the amounts of DM from each ingredient that is to be fed per cow per day. This is done on a dry basis because nutrients are only contained in the DM of each ingredient. However, to mix the ration on the farm, the amounts of DM from each feed ingredient must be converted into the actual weight of feed that must be mixed to make the ration. In most situations, the formulation of the ration and calculation of the amounts of feed to be mixed are based on laboratory analysis of infrequent samples. If the nutrient density of a feed ingredient changes, the ration has to be reformulated to provide the same daily nutrient needs of the cows. If the DM concentration changes, the weight of the put in the mixer has to be adjusted.

Rapid NIR analysis on the farm is dependent on the analysis of unground samples. Sample preparation requires additional time, expertise and equipment, which precludes its adoption for rapid on-farm analysis. However, forage materials in their native from are very heterogeneous, which necessitates adequate sampling protocols and scanning of large samples to obtain representative spectra. Because on-farm facilities are primitive by laboratory standards, rugged instruments are needed that can tolerate vibration and variable environments. Many fermented silages contain 60 to 70% moisture. The water peak associated with these moistures creates substantial background interference when attempting to use NIR to estimate other constituents in feed. However, DM is the most variable constituent in feeds and is the one that is needed to make adjustments in the amounts mixed on the farm.

A HarvestLab diode array sensor (Deere & Co., Moline, IL) was calibrated and used to predict DM. The HarvestLab is designed for chopper-mounted applications, but can be used as a bench-top instrument with a spinning bowl attachment (bowl is 18 cm in diameter and 9.5 cm deep). Calibrations were developed using Unscrambler v9.8 (Camo, Woodbridge, NJ) for reflectance measurements (between 950 to 1530 nm) with no mathematical treatments of the spectra. Reference samples were analyzed at the U.S. Dairy Forage Research Center, Madison, WI. Reference samples were collected by thoroughly mixing a daily sample of silage material, packing two bowls of the material and saving the spectral scan for each bowl. Two 70 g sub-samples from each bowl were dried in a forced air oven at 55 °C for 48 h and then at 105 °C for 24 h to obtain DM reference values.

The DM of silages from both bunker and tower silos were measured several times a week over a period of years to determine the typical variation in silages associated with daily sampling, systematic changes in silages during feed-out, and abrupt changes due to rain or snow events. Feeding trials were performed to compare the responses of precisely fed

dairy cows (the daily mixed ration was adjusted daily for changes in silage DM) to cows that were fed rations that were not adjusted for a single, abrupt change in forage DM.

Results and Discussion

There are many rations on a typical farm; the one the nutritionist formulates to meet the cow's needs and gives to the farmer, the one the farmer modifies and gives to the feeder, the one the feeder mixes and delivers to the cows, and the one the cow eats. Only the final diet is important, but each of the preceding rations has an impact on the diet the cow eats, in reverse order of importance. This realistic representation of the nutrition cycle of feeding on a farm highlights the importance of rapid DM determination on the farm by NIR. If the nutritionist wants 700 kg of silage DM to be fed and the silage contains 35% DM, the feeder will add 2000 kg of silage to the mixer wagon. However, if it rained on the silage the night before and now the silage contains 33% DM there is only 660 kg of silage DM in the ration. Not only have the DM proportions of the ration been changed, but also the total amount of feed DM delivered to the cows is reduced.

Changes in the nutrient concentration of the ration and in the amount of DM offered can have serious consequences to high producing dairy cows because lactation is the greatest nutrient demand of any animal production. Dairy cows typically eat 3 to 5 multiples of their maintenance feed intake, compared to 1 to 2 multiples for growth and even less for activity. The range in nutrient concentrations in dairy cow rations is very narrow for high producing cows. This occurs because competing nutritional requirements are antagonistic. On one hand, dairy cows require high densities of energy in the ration, but on the other hand they require a minimum amount of fiber, but feeds that are high in fiber are low in energy and vice versa. Thus, if a dairy ration is formulated to have minimum fiber and maximum energy density, and a decrease in DM of silages (major sources of fiber in the ration) occurs, the proportion of fiber in the ration is decreased, which can lead to digestive upsets of the cow.

This problem is magnified when we try to reduce the amounts of nutrients in the ration to reduce feed costs and environmental contamination by excretion of excess nutrients. In the past, nutritionists have "over-formulated" rations to insure that adequate nutrients were fed. This no longer acceptable, and rations are formulated much closer to the actual requirement. This practice increases the risk that the ration will be imbalanced for nutrients if the dry proportions of the ration change because the DM percentage has changed. In this new feeding situation, technology is needed that can rapidly provide DM concentrations in feeds on the farm so that the ration can be adjusted at the time of feeding. The magnitude and consistency of the water peak in the NIR spectral region makes it an excellent candidate for measuring DM in feeds quickly and reliably. However, the instrumentation must be rugged, the application simple, and the cost effective.

We have observed that DM can be predicted reliably (± 2 %-units) using unground samples with a diode array sensor. However, it appears that the calibrations may need to be evaluated and updated occasionally. The variation observed (Figure 1) in alfalfa silage indicates that the DM proportions in a ration containing 30% silage on a DM-basis would change significantly from day-to-day. Trials with lactating cows indicated that abrupt changes in the DM of silages resulted in a 2.2 kg decrease in DM intake on the day of the change and in a .9 kg decrease in milk production for two days after the change. When combined, these observations suggest that precise feeding of dairy cows can decrease losses in production due to uncontrolled changes in the ration, and that on-farm NIR technology can provide DM results reliably and quickly enough to make daily adjustments in rations.

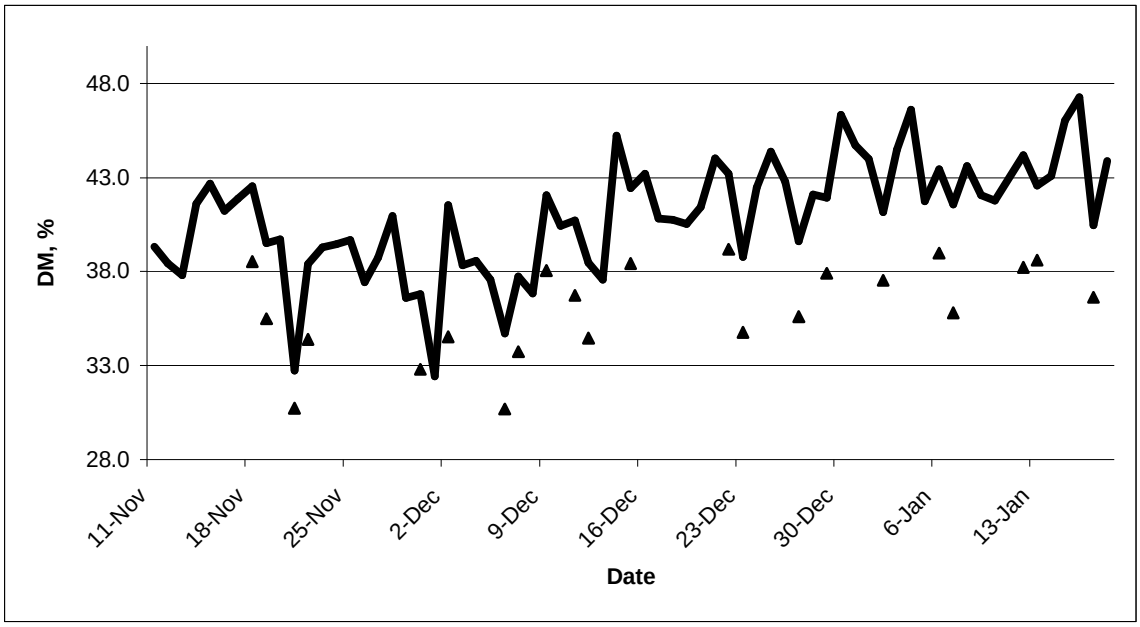

Figure 1. Variation in alfalfa silage dry matter measured by diode array NIR and its association with rain and snow events (▲) at the U.S. Dairy Forage Research Farm, Prairie du Sac, WI.

The assistance of Jacob Karlan and Paolo Berzaghi in the collection and summary of data, and of the USDA-ARS U.S. Dairy Forage Research Center, Madison, WI for feed analysis and animal trials is gratefully acknowledged.

Plenary Session 1: *Hardware*.

This session will have contributions related to the latest technology in NIR sensors used in On site, On line application. The Hardware session will be closed by short (10-15min) presentations of hardware suppliers.

Testing a new generation NIR diode array instruments for inlinequality measurement

P. Reyns[1], B. Kemps[3], W. Saeys[3], Y. Denion-Lair[2], J. Sentjens[1], M. de Winter[1], B. De Ketelaere[3], J. De Baerdemaeker[3]
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[2] Limagrain Europe, 49160 France
[3] Katholieke Universiteit Leuven, 2001 Belgium

Introduction

Since the first tests on the use of inline NIR on harvesting machines (Dardenne and Femenias, 1999), most plant breedings companies have been focusing on the Zeiss[1] corona 45 instrument. Welle et al. (2003) used a Perten[2] DA 7000 instrument in their studies. Both instruments are using the 950-1700 nm wavelength range and a resolution of about 5nm. None of these instruments approached the measurement precision achieved with laboratory NIR instruments. In their study on rapeseed quality measurement, Welle et al. (2007) got a similar precision for oil and protein like the lab, but didn't succeed to measure the glucosinolate content. Montes et al, (2007) got inferior results for oil and protein compared to the lab NIR application.

From these results, it seems the precision of the first generation diode-array instruments is inferior compared to laboratory instruments. Is it because of the lower resolution, or a lower signal-to-noise-ratio? Recently new instruments and manufacturers entered the market offering a higher resolution (2-3nm instead of 5-6) but also an extended wavelength range. In this study we tested the difference between this new generation and the Zeiss corona 45, wondering whether we could obtain the same precision as the laboratory NIR.

Materials and Methods

5 Instruments are tested in this study (Table 1). The Zeiss corona 45 NIR 1.7 bought in the year 2000, is an old generation instrument. All others are considered as the new generation, where the corona plus tested is a prototype version. Both the corona 45 and the corona 45 plus have integrated OMK optics, while the Zeiss MCS and both Polytec[3] devices are rack-based systems with fiber input (SMA connection). The external optics used with the Polytec systems is a prototype version. It consists of a collimating lens (PSS-H-217), surrounded by 3 times 5 watt halogen lamps (Figure 1). Both the PSS 1720 and PSS 2120 systems are connected to the same optics by use of a split fibre bundle.

All instruments are mounted on a test tube one after each other (**Figure 2**). The tube is divided in 4 measurement compartments, each one containing 1 device. In front of the compartment a valve is mounted to control the measurement start, and in front of the measurement window a metal plate reduces the material speed by by narrowing the tube diameter. At the start of a measurement, one sample is poured in the top of the tube. All valves are in closed position. To start the measurement for the Polytec devices in the first compartment, the first valve is opened and grain passes over the measurement window. The sample is blocked at the 2nd valve. The 1st measurement being finished, the 2nd valve is opened to start the measurement for the MCS611 device. To each spectrometer 1 industrial PC is connected. Measurements are executed with the in-house developed LINAS measurement program.

In spring 2008, spectra of 614 wheat samples and 290 spring barley samples were acquired for the different instruments by passing them through the test tube. Average sample size is about 500 gram. Samples were collected at harvest during the 2007 harvest season. Reference protein content was determined by analysis with a Foss Infratec[4] instrument. All calibrations were calculated using the Pls toolbox 5.5.1[5] under Matlab 7.5[6] . The instruments precision is compared in comparing model precision for each of them.

On the original calibration dataset a principal component analysis was performed in order to check for spectral outliers. Spectral outliers were removed manually. To make a fair comparison between devices only those samples for which good spectra were obtained for all devices were retained for the significance test. For each device, different pre-processings were tested, namely

- No treatment (No)
- Multiplicative Scatter Correction (MSC)
- Standard Normal Variate scaling (SNV)
- Detrend (Detr)

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[1] Carl Zeiss MicroImaging GmbH, 07740 Jena, Deutschland
[2] Perten Instruments AB, SE-141 05 Kungens Kurva
[3] Polytec GmbH, Polytec-Platz 1-7, D-76337 Waldbronn
[4] FOSS Tecator AB, SE-263 21 Höganäs, Sweden
[5] Eigenvector Research, Inc., 3905 West Eaglerock Drive, Wenatchee, WA 98801
[6] The MathWorks, Inc., P.O. Box 845428, Boston, MA 02284-5428

- 1st order derivative, Savitzky-Golay smoothing – 2nd order (1st d)
- 2nd order derivative, Savitzky-Golay smoothing – 2nd order (2nd d)
- SNV + 1st d
- SNV + 2nd d
- SNV + Detr

The window applied for the Savitzky-Golay smoothing was set at 22 nm (a total of 11 points, resolution 2 nm). Model validation was based on contiguous blocks (7 splits). The optimal number of latent variables was for each PLS model determined automatically using the significance test described by Haaland & Thomas (1988), which is the standard procedure in the Grams software. The resulting models for the different pre-processings were then ranked according to increasing RMSECV. In this way the best model for each device was selected and these models were sorted by increasing RMSECV. To determine whether performance difference between two devices or pre-processings is significant a paired t-test was performed with the absolute value of the prediction residuals as Y-variable. This test has been shown to be very powerful for comparing the significance of differences in model performance (Cederkvist et al., 2005).

Table 1. Overview tested devices and specifications.

manufacturer	model	optics	wavelength range	#diodes
Zeiss	corona 45 NIR 1.7	integrated OMK	940-1700	128
Zeiss	prototype corona plus 45	integrated OMK	940-1695	256
Zeiss	MCS 611 NIR 2.0 HR	external OMK 500-H NIR	1310-2010	256
Polytec	PSS 1720	prototype contact optics	840-1650	256
Polytec	PSS 2120	prototype contact optics	1090-2110	256

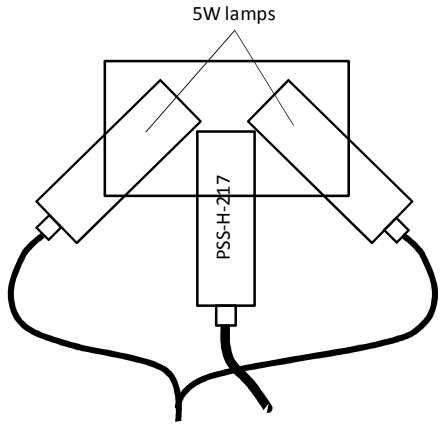

Figure 1. Schematic drawing Polytec prototype sensor head

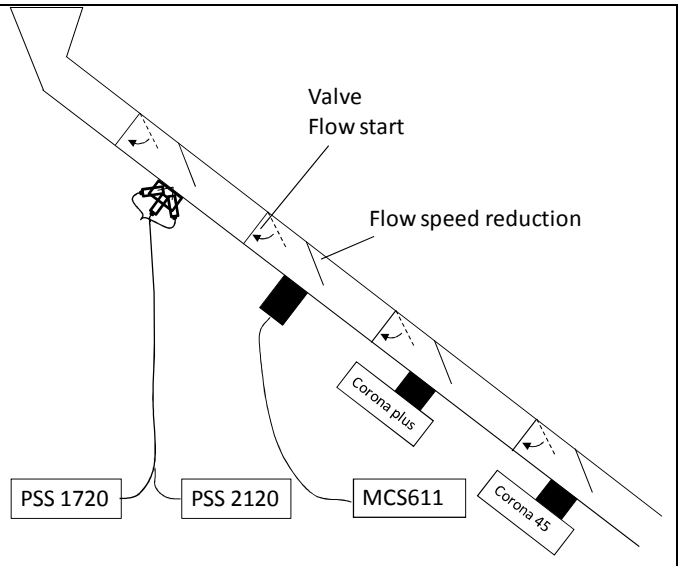

Figure 2. Schematic overview laboratory measurement setup.

Results and Discussion

In Table 2 calibration results of the wheat measurements are shown. Best results were obtained with the Corona 45 plus and Polytec PS 1720 instrument with a cross validation error of respectively 0.25 and 0.28. This difference is small, but significant according to the t-test. The difference between these 2 new generation instruments and the old Corona 45 is larger than 0.1%. This large improvement brings the precision of the new generation instruments to level of the Infratec laboratory instruments (SEP=0.23, Nils et al., 2001). The new instruments with extended wavelength range however, perform much weaker (Polytec PSS 2120 and Zeiss MCS 611). Their precision is even lower compared to the old corona 45 instruments, possibly due to a lower signal to noise ratio of the high range instruments. The instrument differences can clearly be seen in Figure 3 as well.

Table 2. Calibration results wheat samples. 1st d = first derivative; SNV= Standard Normal Variate; RMSEC=Root Mean Squared Error of Calibration; RMSECV= Root Mean Squared Error of Cross Validation.

device	Pre-processing	# LV	RMSEC	RMSECV	R^2	T-test
C 45 Plus	1^{st} d	12	0.23	0.25	0.93	1
PS1720	1^{st} d	10	0.25	0.28	0.92	1
C 45	SNV	11	0.29	0.38	0.85	1
PS2120	SNV + 1^{st} d	9	0.34	0.43	0.79	0
MCS611	SNV + 1^{st} d	12	0.34	0.52	0.72	1

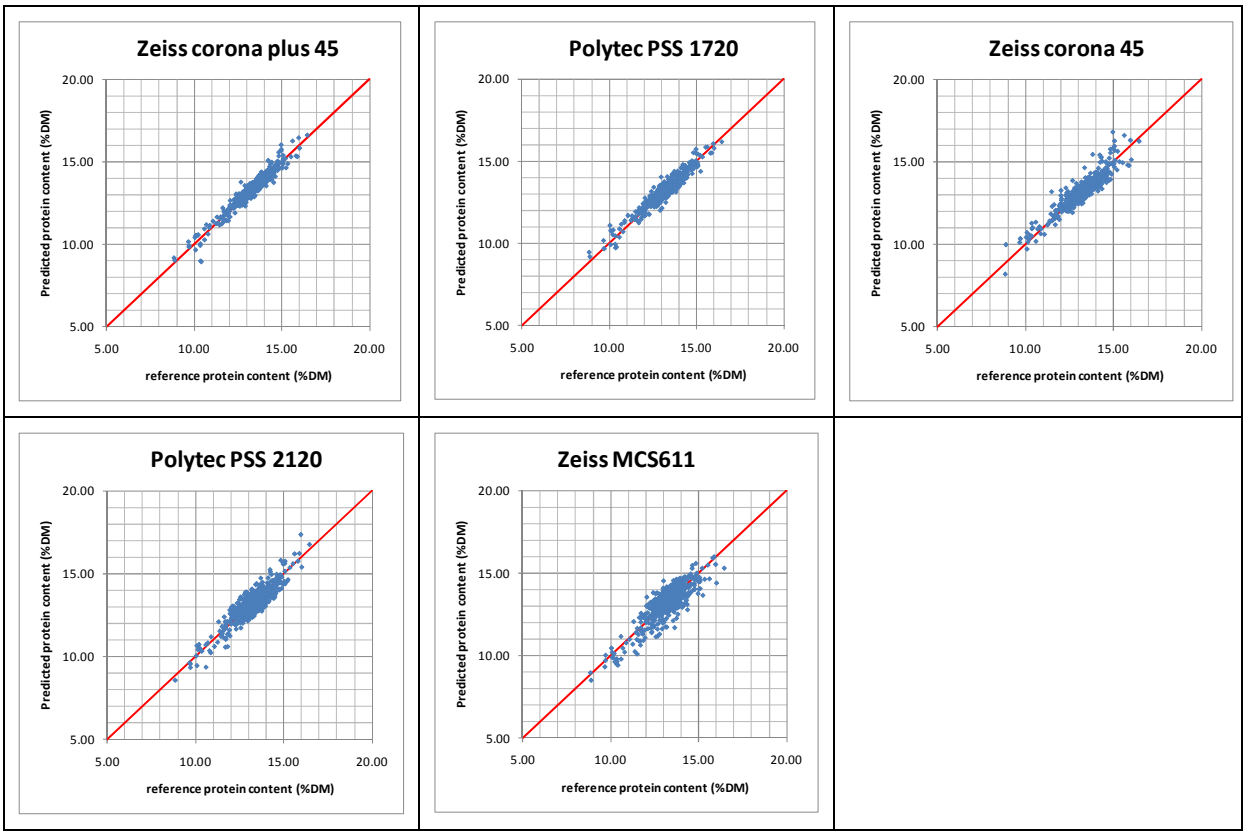

Figure 3. Cross validation scatter plots for the different instrument wheat calibrations.

Results for Barley are very similar to those of wheat. Also here the corona plus 45 instruments shows the best performance and significantly better then the PSS 1720 instrument, the difference between both is larger compared to the wheat case (RMSECV of 0.33 and 0.38 respectively). However no significant difference could be found between the PSS 1720 instrument and the old corona 45. Like in wheat, all instruments with wavelength range up to about 1700 nm (corona plus 45, PSS 1720 and corona 45) perform significantly better then the extended instruments (PSS 2120 and MCS611). Also for barley, the results for the corona plus 45 instrument are highly comparable to those of the infratec instrument (Standard Error of Prediction 0.31).

Table 3. Calibration results barley samples. 2ndd = 2nd derivative; SNV= Standard Normal Variate; RMSEC=Root Mean Squared Error of Calibration; Detr= detrending; RMSECV= Root Mean Squared Error of Cross Validation.

device	Pre-processing	# LV	RMSEC	RMSECV	R^2	T-test
C 45 Plus	2^{nd} d	11	0.25	0.33	0.85	1
PS1720	SNV + detr	12	0.34	0.38	0.78	0
C 45	Detr	16	0.31	0.39	0.8	1
PS2120	Detr	9	0.44	0.54	0.56	0
MCS611	SNV + detr	11	0.4	0.56	0.53	1

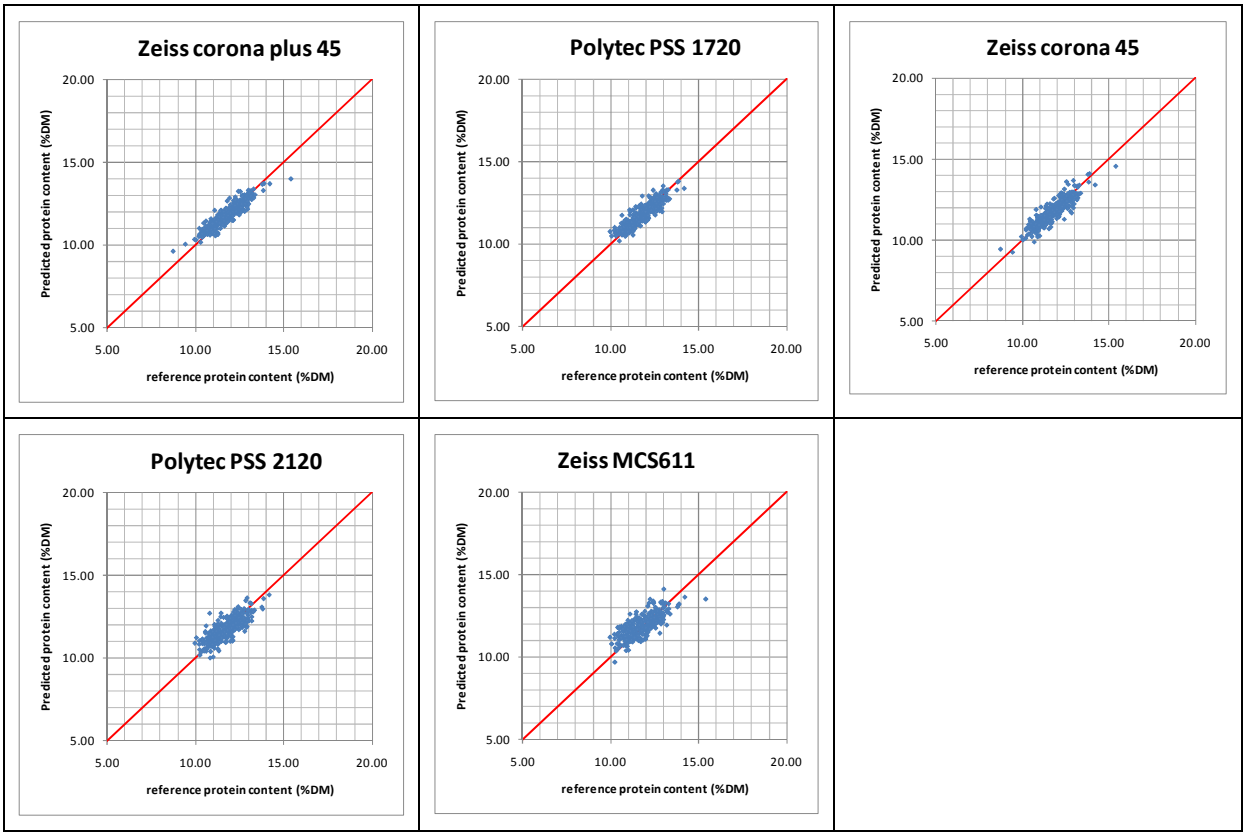

Figure 4. Cross validation scatter plots with barley results of the different instruments.

For wheat protein measurement the new generation instruments with lower wavelength range (Zeiss corona plus 45 and Polytec PSS 1720) are as accurate as conventional laboratory NIR instruments. The performance is significantly improved compared to the old generation Zeiss corona 45 (RMSECV=0.25 and 0.35 respectively). However the new generation instruments with extended wavelength range perform even worse than the old Zeiss corona 45.
The new Zeiss corona plus 45 shows improved performance for barley protein measurements as well (RMSECV=0.32 and 0.39 for new corona plus and old corona respectively). These results are comparable to those of conventional laboratory instruments. The improvement is less compared to wheat. Results of the PSS 1720 are only as good as the old instrument in barley, and also here the Polytec PSS 2120 and Zeiss MCS611 instrument perform less than the old Zeiss corona 45.

Acknowledgements
The authors acknowledge the Zeiss and Polytec companies to have borrowed their newest technologies and knowledge for this study. We thank Ko Verschuure and Bert van de Pas for the production and fine-tuning of the measurement tube. We appreciated much the time Bart Kemps and Wouter Saeys of KULeuven University freed to process all data for us.

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Proceedings in Sample Presentation on Harvesting Machines

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[Introduction] Over the past years the importance of analytical technologies has become crucial in many fields of application in order to achieve a deeper process understanding. The realization of different measurement tasks on a wide range of individual products (samples) needs highest possible system flexibility. In contrast, process analytical solutions require a high degree of reliability and an easy-to-use technology by using standard systems. Therefore two fully contradictory demands on analytical technology measurement systems have to be combined: flexibility and standardization.

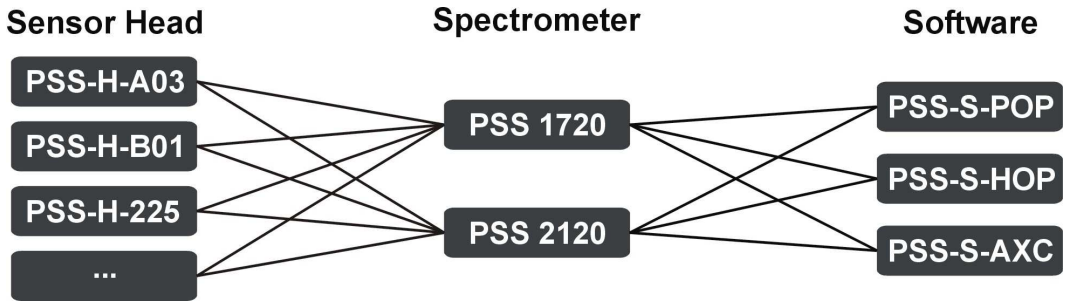

Figure 1. Modular spectroscopic system consisting of standard components being flexibly and optimally adapted to any specific application.

[Materials and Methods] A modular analytical technology system concept with fiber-coupled sensor heads offers a flexible adaptation to user-specific applications using standardized system components exclusively. Different combinations of fiber-coupled sensor heads, spectrometers for different spectral regions and dedicated software packages make almost any specific and optimized solution possible. For example, performing reflection spectroscopy requires specifically adapted sensor heads depending on sample properties or measuring distance. For the investigation of certain spectral regions, where samples show characteristic responses different spectrometers are employed. Finally, various data acquisition, processing, and control software can be chosen also.

[Results and Discussion] Modular spectroscopic systems being successfully used in many fields of application will be shown. Examples and corresponding results from three agricultural applications (see **Fig. 2**: a) funnel; b) spout; c) conveyor belt) will be discussed and presented.

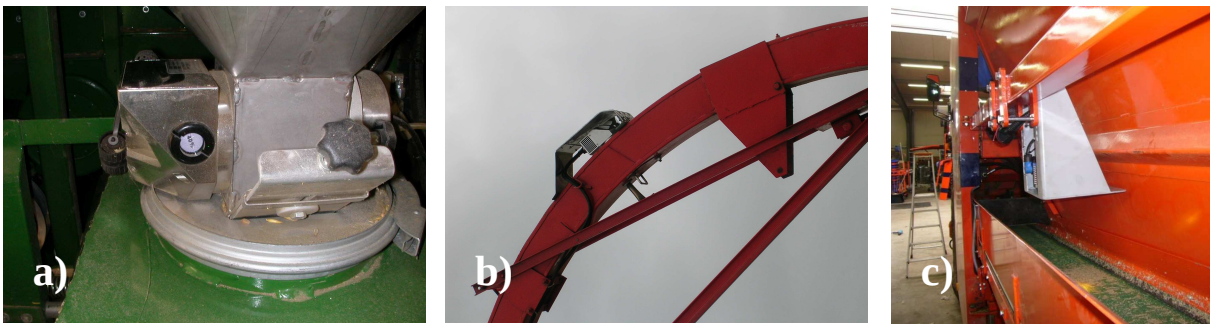

Figure 2. Integration of the sensor heads in different agricultural applications.

Diode Arrays Optical Resolution, Data Spacing and Information Content

D. Honigs,
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[Introduction] Each different NIR instrument type, whether scanning monochromator, diode array, or Michaelson (FTNIR) Interferometer uses a different number of points to represent the measured spectrum. This particular paper discusses the effect of the spectral representation on the calibration results. Simply stated, does the number of data points in the spectrum affect the result?

[Materials and Methods] Soy meal spectra of approximately 500 different soy samples were obtained on a FOSS model 5000. The spectra were measured in reflectance using packed quarter cups. The resulting spectral range was pared down to 1306 – 2390 nm (the wavelength range of the former FOSS 4500). The soy meal samples were analyzed for fat and protein. Comparison calibrations from this data set were created with WinISI(FOSS). Results for fat and protein will be displayed.

Additional spectra were obtained on a Perten Instrument model DA7200 lab unit and model DA7300 online instrument to show the wavelength axis detection limit. In one case the wavelength detection was performed on polystyrene in reflectance. To turn polystyrene into a reflecting material, the polystyrene was first powdered and then compressed to give a white surface. The other wavelength standard used was a mercury emission lamp.

[Results and Discussion] Several things are very important to understand about the way spectrometers actually record the data. The data spacing can be different from the optical resolution. For Michaelson Interferometers the data spacing is typically 2 points per resolution element unless one chooses to add points via an interpolation process. This interpolation process is commonly called zero filling. When one adds zeros to the end of the interferogram one gets additional data points in the spectrum; however, no new information is added. It is as the name implies, only zeros are added.

Scanning spectrometers frequently collect multiple data points per optical resolution element. For example, commercial units are available which collect data every 0.5, 1, 2 or 5 nm with a 12 nm optical resolution spectrometer. Like with the Michaelson interferometer, any given optical resolution requires two data points per resolution element. This comes from the Nyquist criterion (also called the Nyquist – Shannon Criterion[1]). Data collected beyond this frequency is providing some averaging effect, but does not provide additional spectral information according to the theoretical arguments put forward in Shannon Information Theory.

NIR spectroscopy provides a simple application to test the theoretical results and see if they are in line with the results observed by the traditional practices. Table 1 shows calibration data results typical for this problem. As can be seen, one can reduce the wavelength spacing by quite a bit and still get equivalent or even better results.

To some, these results seem counter-intuitive. It is commonly thought that more should always be better. But, this is not always the case. When more data points are added to a spectrum, there reaches a point where they do not add new information and actually may be a hindrance. One way this can be visualized is to rearrange the NIR spectrum by grouping every third data point. An example of this type of regrouping is shown in Figure 1. In conventional practice, when one collects repeat spectra one coadds them. One does not simply append the second spectrum to the first. However, collecting and using data points beyond the Nyquist sampling frequency in an independent manner is much like extending the first spectrum with a second rather than averaging them together.

A second way to view the importance or unimportance of the number of points in the spectrum is to recognize that most NIR calibration techniques do not regress the chemical concentrations against absorbances at wavelengths. Instead they regress those concentrations against a much smaller set of scores which have condensed a larger wavelength scale into a much smaller representation of the data. Having more data points in a spectrum is only useful to the extent that one can calculate the scores more accurately. As the results in Table 1 demonstrate, once there is enough independent wavelength data to get reasonable scores, the results do not improve by adding more. This fact is consistent with the common practice of smoothing. Most NIR calibration data, even data collected on a Michaelson interferometer, is smoothed for calibration. If it were truly important to measure the separate data points, smoothing would be a hindrance instead of an improvement.

Another common reason advanced for having more data points is that more data points will arguably match instruments together because one can supposedly measure the wavelength scale more accurately. Like the more points give better

results concept, the enhanced matching idea also conflicts with the Nyquist – Shannon theory. Once one has all of the information possible, nothing else that is done adds more information.

It turns out that for diode arrays or scanning monochromators one can measure wavelength position and wavelength accuracy far better than the typical data point spacing in the spectrum. This can be done by observing small shifts in the spectra and comparing those shifts to the first derivative of the spectrum. Using this technique, one can easily see wavelength shifts of 0.1 nm even with points spaced 5 nm apart. Similar results are seen for detecting peak positions of very narrow lines from mercury lamp emission spectra. The limit of wavelength shift that can be detected was found to be on the order of 0.02 nm in this study. This limit is observed even though the spacing is very wide by comparison. The limit of detection for peak position or wavelength shift for widely spaced data is determined by the sharpness of the wavelength transition being measured and the signal-to-noise ratio of the instrument or its ability to measure that sharp transition.

[Conclusion] The results observed with different manufacturer models and types of instruments show that NIR Spectroscopy follows the precepts of the Nyquist-Shannon theory. This theory informs designers and developers of the limits of the information content in their data.

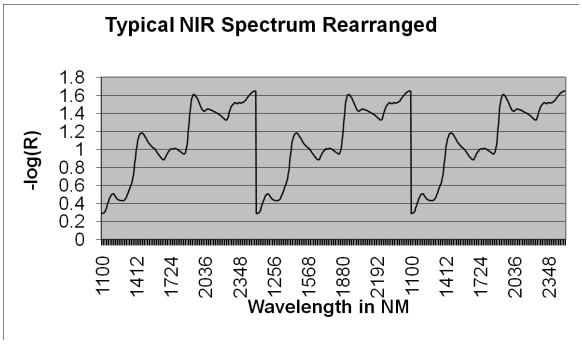

Figure 1. Soy Meal Spectrum Rearranged to Demonstrate Redundancy

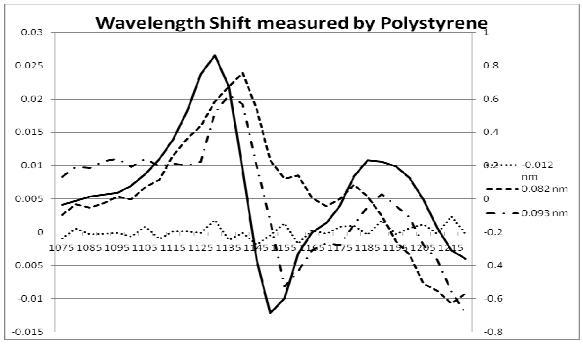

Figure 2. Smaller Wavelength Shifts Measured at 5 nm Data Spacing

Table 1. Fat in Soy Meal, (Wavelength Range 1306-2390) (541 Samples)

Wavelength Spacing in nm	2	4	6	8	16	32	64
Number of Terms in the Equation	14	14	14	14	14	13	10
SEC	0.132	0.132	0.134	0.132	0.136	0.166	0.193
R^2	0.956	0.955	0.954	0.956	0.955	0.922	0.864
SECV	0.147	0.147	0.148	0.145	0.146	0.177	0.203
F	32.82	32.72	31.4	30.35	28.08	18.11	15.01
1-VR	0.945	0.945	0.944	0.946	0.947	0.913	0.876
Number of Points in the Spectrum	543	272	181	136	68	34	17

Footnotes

1 C.E. Shannon, "Communication in the presence of noise", Proc. Institute of Radio Engineers, vol. 37, no 1, pp. 10-21, Jan. 1949 (reprinted in Proc. IEEE vol. 86, no. 2 (Feb, 1998)

INVITED LECTURE

Chemometric-NIR-technology to identify the quality of biological individuals exemplified in single seed mass sorting

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Introduction The cereal industry is based on unique individuals - seeds from wheat and barley varieties- that develop autonomously their chemical composition by growing in the field modified by the environment. In spite of that the seeds within such varieties are genetically identical there is a stunning variation in e.g. protein content within one field from 8-17 % in e.g. wheat. This variation is exploited by the BoMill TriQ single seed NIR sorter (Ref. 1) based on patterns in single seed NIR spectra indicative for chemical composition and complex food functionality such as baking quality in wheat and malting performance in barley.

Materials and Methods The BoMill AB TriQ NIR single seed sorter for wheat, durum and barley (www.bomill.com) comprises 1) The pilot plant TriQ single seed sorter for plant breeding and laboratory testing with a capacity of 1-10 kg (seeds per hour). 2) The full scale TriQ single seed sorter with a capacity of 2 tons per hour implies inspecting 20.000 *individual* kernels per second fed inside on a rotating indented cylinder (Fig. 1). The seeds in the indented pockets are inspected by the NIR sensor. The information is fed to a computer and subsequently segregated to control 3-5 separated flows by pushing seeds into fractions by compressed air. Four 2 tons units (or more) can be combined into a setup with a capacity of 8 to 20 tons per hour. In Table 1 an older model of the pilot TriQ single seed sorter (2-500 kg per hour) is exploited to sort spring wheat in three diversified seed lots that are analyzed for chemical composition and baking quality. In Table 2 a full scale 2 tons unit of the TriQ sorter is used to sort an Australian barley batch in seed fractions of different malting quality.

Results The computer in the BoMill AB TriQ single seed NIR sorter uses information from single seed NIR spectra unsupervised to span the full range of individual seed NIR fingerprints within a batch and to divide them into fractions of seeds that have a similar, more homogenous chemical composition. The result can be evaluated by single chemical analyses e.g. for protein or for more complex food functional analyses such as *baking quality* and malt performance. In Table 1 (Ref. 2) a sample of the spring wheat variety Vinjett is sorted in 3 almost equal fractions by weight (F1-F3) differentiating the original protein content (10.2 %) from 8.8 to 11.9 % as well as wet gluten content (originally 19.3 %) from 14.8 to 25.0 %. The seed sorting has an equally profound effect on dough parameters. Thus a relatively soft wheat batch can be divided into a harder fraction of improved baking quality and in a soft fraction suitable for biscuits. In the *malting industry* (Ref. 3) the homogeneity of barley quality traits are of paramount importance to optimize homogenous germination in the malting process in order to speed up wort and beer filtration by keeping ß-glucan low. At present malsters may decrease homogeneity by mixing barley batches in order to reach protein specifications (Ref. 3). In Table 2 the full scale TriQ single seed NIR sorter is used to increase seed homogeneity from the original barley batch into four sorted fractions. The original protein content of 10.8 % is spanned from 9.0 to 12.6 %. At the same time wort ß-glucan (mg/l) originally 121 mg/l is diversified from 81 to 195 mg/l. The sorting also affects malt friability (hardness) and wort filterability considerably optimizing fractions 1-3 as premium malting quality and leaving fraction 4 for sale as feed (Table 2). The disastrous effect on malt performance to reach the protein specifications 10.1% and 11.1% through mixing sorted fractions is demonstrated in Table 2. It is caused by a decrease in seed homogeneity.

Discussion The modern biometric industry is using mathematically irreducible fingerprints to identify human individuals. Now NIR sorters can be used commercially to identify the chemical fingerprints of seed individuals from batches by their unique spectral patterns in order to remove “terrorist” seeds. Karl Norris introduced the NIRS technology as a non-destructive analysis for water and protein in cereals. In Fig. 2 single wavelength absorption correlations (r) to chemical composition (x-axis) in a barley population is shown in the area 1680-1820 nm. The figure convincingly demonstrates that NIR spectra are indicative for a wide range of different chemical bonds. This property can now not only be used to predict for individual analytes such as protein, starch and ß-glucan, but also to characterize whole single seeds and barley varieties as biological individuals just as fingerprints are used for identification of human individuals in forensic technology. At the oral presentation of the paper the use of Norris statistics and chemometric pattern recognition models will be compared for analytical prediction. Chemometric models assists in over-viewing NIR fingerprints (Ref. 4 and 5) to identify by visual inspection uncompressed spectral patterns from seed batches

indicative for biological individuals and complex food functions to be used by value added sorting in plant-breeding and trade to maximize value and cereal price.

Table 1. Single seed pilot TriQ NIR sorting of spring wheat variety Vinjett (2-500 kg/h) (Ref. 2)

		Sorting fraction		
	Original	F1	F2	F3
Yield %	100	35.1	32.8	32.1
Protein %	10.2	8.8	10.0	11.9
Wet gluten %	19.3	14.8	18.6	25.0
Water abs. %	53.7	52.5	53.6	55.1
Dough:				
Development time	1.9	1.6	1.7	2.5
Stability	89	1.7	2.4	8.0
Softening	90	125	85	55

Table 2. Full scale (2 tons/h) TriQ single seed NIR sorting of Australian malting barley (Ref. 3)

		Fractions				**Blends**	
	Original	**1**	**2**	**3**	**4**	30% **4** 70% **1**	40% **4** 60% **2**
Protein %	10.8	9.0	***10.1***	*11.1*	12.6	***10.1***	*11.1*
Wort ß-glucan mg/l	121	81	***96***	*119*	195	***103***	*170*
Friability %	81	95	***88***	*83*	74	***88***	*81*
Wort filtrability ml/3 min	6.95	7.70	***6.85***	*6.85*	4.80	***5.53***	*5.60*

Conclusion: Blending the low protein fractions 1 and 2 with the high protein fraction 4 to obtain blends with identical protein content as the homogenized sorting fractions 2(10.1%) and 3(11.1%) results in marked increases in wort ß-glucan and in a corresponding much slower filterability due to decreased seed homogeneity with little effect on malt friability.

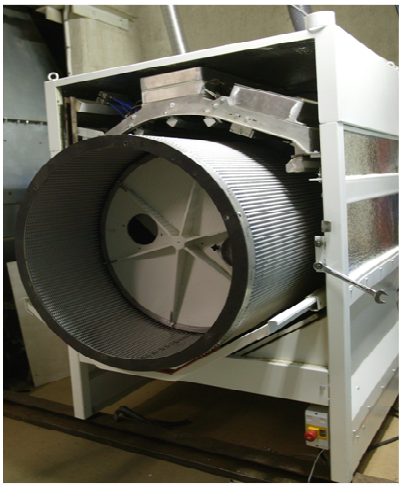

Figure 1. Industrial scale unit (2 tons/hour) BoMill AB TriQ single seed NIR sorter opened showing the sorting cylinder and the sensors (Ref.3)

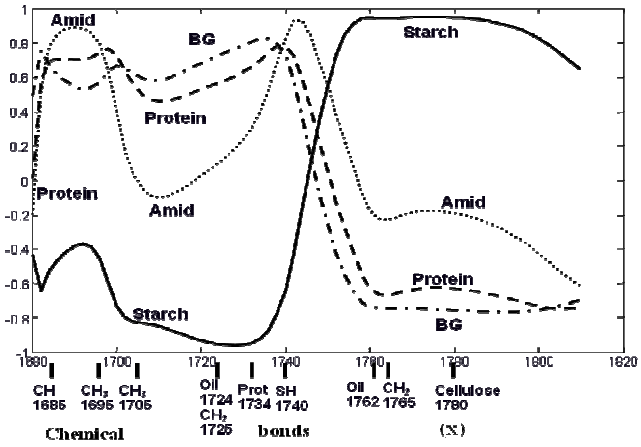

Figure 2. NIR spectra representing patterns of chemical bonds. 92 barley spectra are correlated to chemical analyses at each absorption wavelength 1680-1820 nm (Ref. 4)

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Plenary Session 2: *Software*.

This session will focus on calibration or standardization algorithms that may beneficial for On site, On Line applications. The Software session will be closed by short (10-15min) presentations of software suppliers.

INVITED LECTURE

NIRS Measurement and Calibration Development for Onsite Applications: Software Requirements

Heinrich Prüfer[1] and Peter Tillmann[2]

The application of Near Infrared Spectroscopy on site is still far from being a ubiquitous routine tool in precision farming and plant breeding experiments. Besides improvements of spectrometer modules, their mounting position and sample presentation techniques, the NIRS operation on harvest engines also leads to more challenging requirements for the data acquisition and processing software and the calibration development and maintenance tools.
Data acquisition:
1. For online applications a high data rate (up to Mbyte per sec) will be needed for full traceability. With spectra taken in a few milliseconds and several stages of data processing the demand for high performance in data processing is obvious.
2. Online spectra are usually collected without full control over the sample. The data acquisition software needs to be able to filter the incoming raw spectra before averaging and to detect outliers with appropriate chemometric outlier diagnostics.
3. The NIRS instrument is no longer a stand-alone piece of equipment: spectrometer hardware and software become integrated modules within an overall process control system. This modularity has to be reflected by the user interface concept and especially by the connectivity of the instrument software. For full control of data acquisition, processing and storage communication, more interfaces are demanded according to industry standards (e.g. different field-bus types, in Windows systems DDE and OPC).
4. For mobile applications as on harvesters a GPS interface will be needed. Plot harvesters will integrate GPS interfaces which will either be used by the instrument control software or the supervising harvester software. Calibration and other supporting software requirements:
1. With online application and long distance management of spectrometers easy and effective model updates will become more and more important. This means software for chemometric modeling which is easy to use as well as calibration management tools for safe handling and controlled calibration updates in a network.
2. To handle the large amount of spectra and other data collected in online applications there is an increasing need for storing and retrieving these data in databases. The advantages of a database design will assist in calibration development and management.
3. Due to the importance of filtering and appropriate pre-processing of spectra collected online, intelligent support tools are required which help to establish the filter parameters and to characterize the suitability of different pre-processing algorithms.
4. With the large volume of spectra and the inherent redundancy of sample information subset selection tools become indispensable to keep reference analysis costs reasonable and to avoid misleading cross-validation results.
5. In some applications with advanced real-time requirements "old-fashioned" MLR wavelength regression techniques will become important again.

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Comparison between different standardization methods for the quality estimation of forages in portable NIR spectrometers

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Introduction

Standardization of near-infrared (NIR) instrumentation is one of the most relevant issues in spectroscopy. Many sources (e.g.: electronic tolerances, differences in sampling, etc…) may determine dissimilarities between the instrument responses to the same analyzed sample. Therefore, transferring a regression equation from a reference instrument (i.e., master) to secondary devices (i.e., slaves) is of paramount importance. This issue is even more problematic when the transfer of calibration models concerns portable instrumentation. In fact, in this case the number of samples on which the estimation model has to be calibrated should be small to relieve the calibration burden of the analyst.

In this work a cross-validatory methodology is used to select a method to standardize NIR spectrometers from several state-of-the-art techniques. This methodology checks different standardization methods observing how they estimate diverse quality variables in the transfer to similar instruments, also using a reduced number of calibration samples.

The cross-validatory method is tested in the case of portable instruments with diode array technology for the estimation of quality in forages. The results show that the piecewise direct standardization is the most accurate transfer method, because it usually ensures accurate estimations of different types of forages in different slaves, and demonstrates robustness to the reduction of the number of calibration samples.

Materials and Methods

The datasets considered in this study are concerned with two different types of forages for bovine feeding: high moisture corn and grass silage. These data are split in two groups: the spectral responses of the instruments and the chemical analysis of the qualitative parameters of the forages. In particular, spectra of 4 portable NIR analyzer (one master and three slaves) are available. The spectrometers are AgriNIR™ (Dinamica generale®, Poggio Rusco – MN, Italy) equipped with diode array technology, whose InGaAs sensors measure reflectance spectra within a range of 1100-1790 nm. For every instrument, the spectra are collected in $\mathbf{X}$ ($I \times J$) matrices, where I=13 is the number of the samples analyzed in each instrument, and J = 71 is the number of measured wavelengths. The quality of the samples are characterized by Q = 6 quality variables (Table 1) measured through chemical analysis and collected in a $\mathbf{Y}$ ($I \times Q$) matrix.

Table 1. Quality variables of the forages.

Quality variable	Acronym
dry matter (%)	SS
crude protein (%)	PG
acid detergent fiber (%)	ADF
neutral detergent fiber (%)	NDF
ash (%)	CEN
crude fat (%)	EE

A cross-validatory methodology is used to test different state-of-the-art transfer techniques. The standardization strategy adopted in this study is based on the transformation of the spectra by the means of either: 1) a reproduction of the patented method PM of Shenk and Westerhaus (1985) in its original form; 2) direct standardization (DS, Wang et al., 1991); 3) piecewise direct standardization (PDS, Wang et al., 1991) with a window size of 5 wavelengths; 4) double window piecewise direct standardization (DWPDS, Wise and Gallagher, 1998) with window sizes 5 and 6 wavelengths; 5) projection to latent structures (PLS, Forina et al., 1995); 6) orthogonal signal correction (OSC, Wold et al., 1998) with one latent variable. The cross-validatory strategy evaluates different standardization methods to transfer the calibration equation between the master Agrinir™ (whose data are collected in $\mathbf{X}_m$) and three slave Agrinirs™ (whose data are collected in $\mathbf{X}_{s1}$, $\mathbf{X}_{s2}$ and $\mathbf{X}_{s3}$), for the prediction of all the quality variables $\mathbf{Y}$. The proposed technique goes

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through three fundamental steps: *i*) analysis of all the combinations of **X** data in 10 calibration samples and 3 validation samples; *ii*) calibration of a model on 10 samples for a wide series of the combinations, uniformly distributed between the entire set of permutations of the available data; *iii*) evaluation of the performance of the estimation model on the 3 validation samples. The variability of the results is inspected also for reduced numbers of calibration spectra (8, 6 and 4) selected with the criterion of the highest leverage. The standardization is operated on the row spectra and is performed prior to pretreating data and to calibrating the estimation model.
After standardization, the spectra are pretreated through standard normal variate, and then filtered both by a mean filter within a window of 4 wavelengths, and by a first derivative filter within the same window. After that a double stage principal component analysis (PCA) removes possible outliers. Finally, projection on latent structures PLS is utilized as estimation model.

Results and Discussion
Results are presented both in terms of average root mean square error of prediction (ARMSEP) and in terms of average mean relative percentage error (AMRE). These performance indicators are averaged throughout the validation dataset at every step of the cross-validatory procedure and then averaged also throughout all the steps of the procedure. Also other directions of the error variability are inspected in this study, namely: variability between different numbers of calibration spectra, variability between different slaves, variability between different quality variables, and variability between different forages. When one of these directions of variability is analyzed, the other ones are averaged to give more general results.

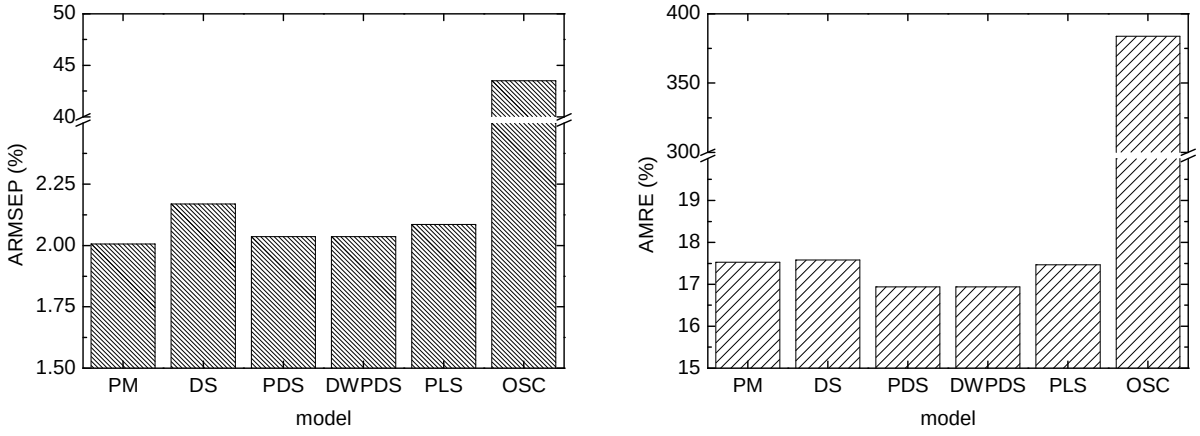

Figure 1. General results of the cross-validatory strategy for the selection of the most appropriate standardization approach in the model transfer of portable NIR spectrometers for the analysis of forages in terms of: (a) ARMSEP; (b) AMRE.

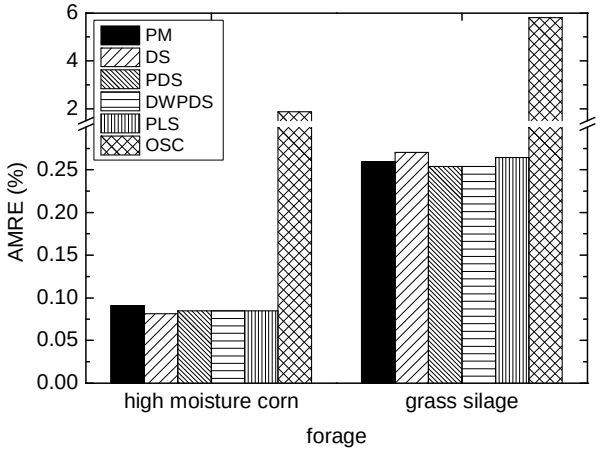

Figure 2. Evaluation of different types of standardization models applied to two different forages (high moisture corn and grass silage).

The transfer strategy which guarantees the lowest ARMSEP and the lowest AMRE is found averaging all the errors throughout the directions of forages, quality variables, number of calibration samples and slaves. Figure 1 shows that OSC is inadequate in this case, probably because the calibration dataset is not redundant and no components are present in the predictor variables **X** that are orthogonal to **Y**. The most accurate methods highlighted from both ARMSEP and AMRE are PM, PDS and DWPDS, slightly outperforming the other ones.

Quality estimation is much more difficult in the case of the grass silage (Figure 2), which is the case that determines a advantage of PDS an DWPDS. Furthermore, examining the effect of different transfer models on the quality variables (Figure 3), it is noticeable that SS and EE are estimated with great accuracy, while PG and ADF are the most difficult to be estimated. Figure 3 also highlights that the best performance are obtained with PDS and DWPDS for the prediction of almost all the quality variables.

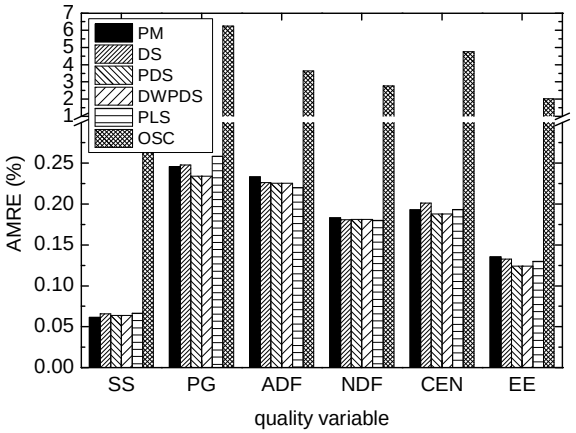

Figure 3. Accuracy of the estimation of the forages quality parameters with different standardization models.

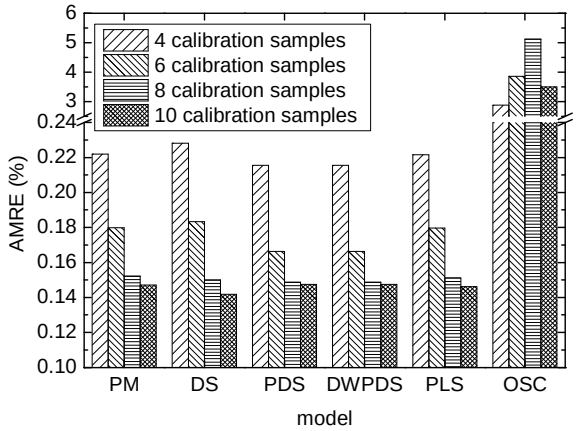

Figure 4. Effect of the number of samples used for the calibration of different models on the estimation accuracy.

Finally, Figure 4 shows that PDS and DWPDS are the most robust to the reduction of the calibration dataset, especially when the dimension of the calibration dataset is 6 or 8 samples, whereas the performance is degraded when too few calibration samples are included into the estimation equation.
In summary, PDS is recommended as the most appropriate transfer method, because of its piecewise structure. Although it does not outperform dramatically the other methods, it is suggested because of its good estimation accuracy, its greater robustness to the reduction of the calibration dataset and the simplicity of its algorithm (which determines its advantage on the DWPDS).

Acknowledgements
The authors would like to gratefully acknowledge the Ministero delle Politiche Agricole e Forestali for the financial support of the project "M.A.R.I.P.A.", within which this work was carried out, and the University of Padova for the financial support granted under Project # CPDR088921–2008 on "Innovative techniques for multivariate and multiscale monitoring of quality in the industrial production of high added-value products".

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Plenary Session 3: *Applications*.

INVITED LECTURE

NIR on the go at the CRAW 15 years of experiments (1995 – 2010)

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[Introduction]
This paper is a review of the research activities of the Walloon Agronomic Research Centre of Gembloux (CRA-W) in the field on-line NIR or MIR measurements, reaction monitoring and embedded NIR applications. It covers a wide range of applications:

- on line or in the field measurements of fruits, dairy products, tissue…
- reaction monitoring (hydrolysis, fermentation, biométhanisation)
- embedded NIR (on the harvester measurements of cereals, oil seeds and forages)

[Materials and Methods]
For on-line applications, or for the monitoring of reactions, several NIR or MIR instruments were tested according the specific applications among them: Anadis MIR (1100-3000 cm^{-1}), Delta Lactoscope MIR (2500-25000 nm), Foss NIRSystems 6500 or XDS (400-2500 nm), Phazir (940-1800 nm; 1600-2400 nm). Forr these applications continuous flow cells or fiber optics were tested and compared. Most of the embedded NIR applications were achieved with a Zeiss Corona instrument (950-1700 nm). Most of the time the spectra were acquired with the program linked to the instruments. The data treatment was performed with the Foss WinISI package.

[Results and Discussion]
Lots of applications were performed in the field of on-line measurements, monitoring of reactions or sing NIR embedded technology (on the harvester measurements).
Figure 1 shows the results obtained for the monitoring of a biomethanisation process using an at line measurement with a Foss XDS spectrometer and predicting volatile fatty acids (VFA). With a VFA concentration of less than 50 meq/l the digestion reaction is doing well (green light). VFA values between 50 and 120 meq/l lead to a warning (yellow light). When values exceed 120 meq/l actions should be taken to avoid blocking the reactor (red light).
Figure 2 shows the on-line monitoring of the cake as rapeseed is mechanically crushed. The prediction of the residual fat content in the cake allows the fine adjustment of the crushing machine as well as the seed pre-treatment.

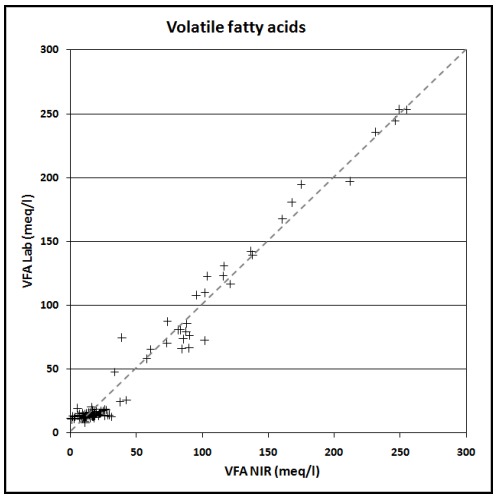

Figure 1. Calibration developed for the at line determination of the volatile fatty acids and the monitoring of a biomethanisation process (n= 94, SECv = 11.4, SD/SECv =5.5)

Figure 2. Head of a NIR Zeiss corona scanning the cake obtained during the crushing of rapeseed..

INVITED LECTURE

Portable NIR Instruments for Agriculture Applications

John S. Shenk

Introduction

There are many places a portable NIR instrument would be useful to analyze agriculture products. The most obvious short list of places would include on-farm livestock feeding operations, whole grain analysis to forecast field harvesting quality, and liquid manure field applications of nurients. With the current status of technology development in diode arrays, miniature computers, and software, portable systems may be ready to fill these needs in the agriculture marketplace.

To be successful with a portable instrument, many important factors must be considered. First of all the instrumentation needs to be customized for specific applications. These instruments will not be multipurpose as we now have with laboratory bench type instruments. The short list of factors that must be taken into account the hardware component costs, instrument accuracy, sample viewing methods, light sources, and battery life. Software will be key because product database development at the lowest possible cost is a requirement as well as networking multiple instruments across the application.

Unity Scientific has begun development of the portable instrument concept using their background and experience gained in related applications over many years. At the center of the development is the software, UniStar. This development was important to precede the entrance into this application area. What followed was the linking together of the hardware components from many different sources to meet the application requirements. Unlike bench top instruments, portable systems used in the field must be carefully designed for the application, yet low cost.

Software

The software serves three purposes. First, it must be simple with easy to use options and display for the operator, second instruments must predict alike, and third low-cost product database will need to be developed with the most efficient calibration techniques. In most portable applications, sampling will be handled by the operator. In a bench system, the sample is collected from a number of locations, dried and ground, with a carefully chosen subsample presented to the instrument. The portable software must be able to solve the sampling problem.

The second software requirement involves instrument standardization or minimization files or both. These procedures need to be imbedded in the routine operation software. The third requirement is for the user to be able to automatically select samples during routine operation to expand the database. This activity of choosing samples for expansion is critical to minimize the cost of developing the product database. Portable systems do not have the luxury of large numbers of samples with expensive reference values to build the product database. All of these software features are essential to make the analysis values accurate for the user.

The third software feature relates to product or calibration database development. Some will insist that only laboratory reference values be used. This is fine if you can afford it. A second method often rejected by many is to use the predicted values from a well calibrated bench type instrument. This will be covered in more detail under the section of application.

Hardware

The selection of the right hardware is very important. A diode array with no moving parts is an excellent choice. Selecting the scanning range is the next decision**.** The cost of the diode array is the single most expensive item in the portable system. The cost goes up as you go from 400 to 2500 nm. If the application is reflectance, you can try the lowest cost silicon detector region 400 to 1100 nm, but unless the measurement is to be made in the color region, accuracy may be a problem.

The second possible region is 900 to 1700 nm with a cooled InGaAs detector. But now the instrument becomes expensive. The cost of the instrument alone over a silicon detector instrument can be as much as S10,000 USD. This cost escalation can make a portable system impractical for the application. The other parts of the hardware, (light source, battery, electronics, instrument and carrying case) must be carefully chosen to optimize the final configuration.

Application

Our investigation into this area of portable instrumentation became focused when Renaissance Nutrition, a Pennsylvania based dairy nutrition company requested our help. They wanted to know what was the feasibility for on-farm analysis with a portable NIR instrument. After consideration of the hardware/cost and accuracy possibilities, we decided to try two instrument configurations: an Ocean Optics USB4000 (OO) scanning 600 to 1200 nm and a BaySpec x100 (BS) with cooling scanning from 900 to 1700 nm. The application could only be accomplished in reflectance

We planned a three phase development approach. We would obtain samples from a reputable forage testing laboratory for reference values for three products: hay, corn silage, and haylage. The samples would be from farmers, dried and ground, and predicted values would be obtained from the samples using a high quality NIR instrument with accurate databases. As was said earlier, everything possible had to be considered to cut the cost of development. Since the samples used for the calibration came from farmers, using the predicted values as reference values for the portable instruments was a major cost cutting feature. In addition the reference laboratory then becomes the center of the analytical hub for a portable instrument network.

Over a period of 8 weeks we scanned 100 samples undried/unground samples of each product. Four subsample positions were scanned for each sample by the two instruments. The calibrations developed for the BS instrument for all three forage products were more accurate than the OO instrument. These results were not a surprise, but we had hoped the lower cost OO instrument might have adequate accuracy.

Having selected the instrument, the second phase began. That was evaluating the calibration performance with farmer samples taken from local Pennsylvania Renaissance Nutrition customers. This phase will be continued on into the summer but our first comparisons are very encouraging.

The software will continue to play and important role. It will be used to select samples with constituents needed to expand the calibration database. It will be used to standardize additional portable instruments for the dairy/forage industry, and the minimization file will be used to minimize any remaining differences between instruments as well as control analysis variation due to temperature variation during the day.

Summary

We are working on the development of a low-cost portable instrument that will be accurate enough for formulating rations for dairy cows from on-farm analysis. This effort was made possible by combining the right software, hardware, and experience along with a high quality reference lab and the funding and support of Renaissance Nutrition.

On-site, on-line determination of total solids, nitrogen and phosphorus *in liquid hog-manure*

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Abstract

Hog (suino, Schwein, cochon, varken) production in southern Manitoba, Canada, is a major industry, involving about 10 million animals. It is located in an important grain-growing area, where the principal crops are canola, barley and wheat. The hogs are all raised in barns. Hogs produce about three times the volume of fæces of other domestic animals. The manure is washed into lagoons, which hold up to 5 million litres of liquid manure. From time to time the lagoons must be emptied by pumping. The grain farmers require nitrogen and phosphorus fertilizer for their growing crops. When the hog-producers have their lagoons pumped out, the manure is distributed on the grain farms, over a radius of up to 5 Km from the hog farms. The manure is cultivated directly into the soil. The manure represents a cheap source of both N and P to the grain farmers. The liquid manure varies widely in total solids (TS), ranging from as low as 0.4 % to over 11% TS. Consequently the nitrogen and phosphorus contents also vary, and together with the total solids, increase abruptly toward the end of a pump-out. The grain farmers need to supplement the manure with commercial fertilizer, and need to know the composition of the manure that has been pumped onto their land. The present method of estimating this is to take periodical samples during the pumping operation, and test them on small hand-held instruments, such as the Agros Nova-meter. This paper describes the application of NIR spectroscopy, using a flow-through cell interfaced with a diode-array-driven NIR instrument. The NIRS method provides a system for continuous monitoring of manure composition during the entire pump-out. This makes it possible for the manure applicators to give the grain farmers accurate advice on the total amounts of N and P that have been applied to their fields, and the amounts of commercial fertilizer that they will need to purchase to supplement the manure application. With the current focus on Precision Agriculture, as fertilizer prices continue to increase, the greatly-increased accuracy provided by on-line NIRS testing results in very significant financial benefits to the grain farmers.

Nutrient based Slurry Application by Near-Infrared Spectroscopy

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Abstract:
The objective of the project "Nutrient based Slurry Application" was to test a system to quantify nutrients concentration on line during slurry application. To protect the NIRS spectrometer against rough conditions during slurry application a specially adapted protective housing was developed. During measuring near infrared spectra, slurry reference samples were taken out of a sampling valve from the slurry tanker. The NIRS calibrations for different nutrients (DM, Ntotal, NH4-N, P and K) are all calculated by PLS regressions and validated by test set. The whole NIRS system was successfully adapted to the rough conditions on a commercial 18 m³ slurry tanker. The developed NIRS measuring system showed a reproducible and stable performance resulting from a steady state optical signal during slurry application. In general a distinct correlation between the NIRS spectra-based calculated nutrient concentrations and the concentrations detected by the chemical analysis of the reference samples was found. However, the accuracy of these calculated "on line NIRS calibrations" is not always satisfactory. Therefore, it would be best to improve these calibrations based on even larger data set of reference samples in order to span the complete variation in the population of manure samples and further check the suitability and quality of origin specific NIRS calibrations.

Introduction
The objective of the project "Nutrient based Slurry Application" was to develop and test a system to quantify nutrient concentrations during slurry application. Such a system is an essential necessity, if slurry is supposed to be applied in an efficient, economically and environmentally way; due to the composition of slurry shows a very distinct variation (table 1) depending on the slurry origin, feeding ration of the animals, degree of slurry (post) processing, homogenisation before application, etc.. Therefore a rapid on line analysis-technique for determining liquid slurry nutrients, such as a Near-infrared spectroscopy sensor (NIRS), is strongly needed.

Table 1: Review on variation of slurry composition

Slurry Composition/Nutrients		Cattle slurry [1-3]	Pig slurry [4-6]	Fermented slurry [7]
DM	[%]	1.4 - 38.6	1.8 - 17.5	2.9 - 10.2
Ntotal	[kg/m³]	0.9 - 9.5	0.6 - 14.6	2.4 - 9.1
NH4-N	[kg/m³]	0.2 - 4.7	0.3 - 10.9	1.5 - 6.8
P	[kg/m³]	0.2 - 8.6	0.1 - 11.0	0.7 - 6.0
K	[kg/m³]	0.3 - 8.4	1.13 - 11.3	2.0 - 8.8

Since other researchers - general under laboratory conditions - have used different sample presentation modes for the spectroscopic analysis of slurry and in addition tested the effect of the transflectance or reflectance sample presentation modes on the performance of the spectroscopic manure composition measurements, the development and test of a NIR spectrometer under practical conditions on a commercial available 18 m³ slurry tanker (including the "on the go" sensor calibration) has been investigated in the current research work.

Materials and Methods

In this project a commercial available 18 m³ slurry tanker was equipped with a self constructed NIR sensor located in a horizontal slurry transport pipe, so that dynamic measurements during the filling, mixing and applying of the slurry were possible (figure 1). To protect the NIRS spectrometer against rough conditions during slurry application a specially adapted protective housing was developed. The used NIR-spectrometer (Polytec PSS 1720) is equipped with an InGaS diode-array detector in a wavelength range from 850-1650 nm with a fibre optic cable light input. To reference the spectrometer the dark spectrum was collected automatically, while the white reference disc had to be referenced manual at the beginning of each measuring period. Due to a special self made software adaptation, the integration time of the spectrometer could be set at different values, to optimise the spectrometer performance depending on the "working conditions".

During the measurement of the near infrared spectra (5 subscans per sample), slurry reference samples were taken out of a sampling valve from the tanker. The composition of the taken slurry reference samples were determined by wet chemical analysis.

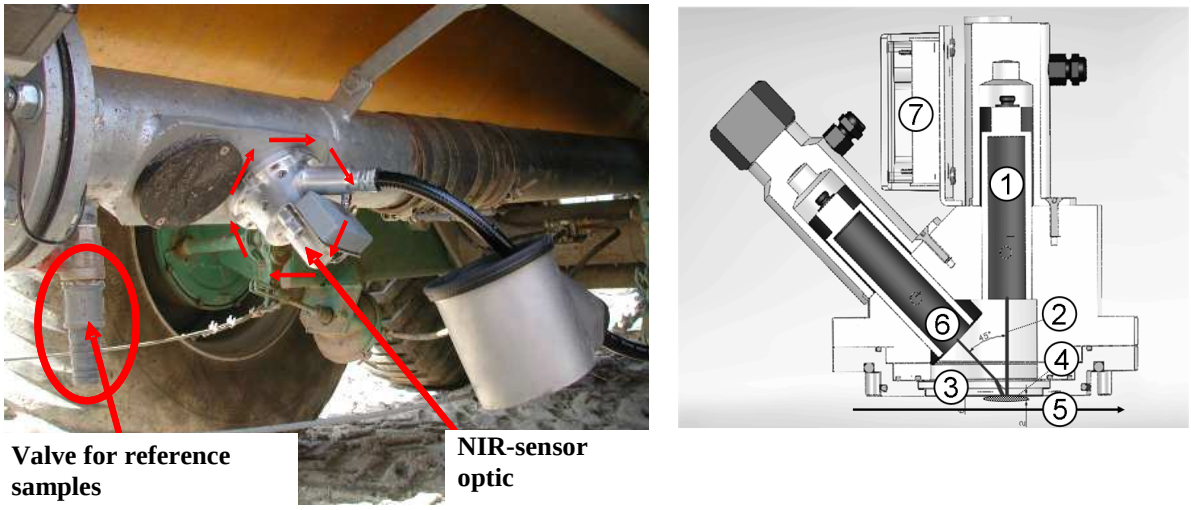


Figure 1: Location of valve to take references samples and the NIR-sensor optic at the horizontal slurry transport line (left). Measuring head: 1. light source, 2. path of light, 3. sapphire window, 4. measurement spot, 5. moving sample, 6. light uptake, 7. power unit (right)

The calibration development was performed with the chemometric-software SL Calibration Wizard v0.9.9 (SensoLogic GmbH, Norderstedt, Germany). To detect outlier (H, T, D, S)[8] in the database a preliminary calibration using the partial least square (PLS) algorithm and 1st derivative (segment 3, gab 1) of the absorbance spectra was used. Samples were removed from data set if they were detected as outlier at least in two calibration processes for the different constituents (DM, Ntotal, NH4-N, P and K). Subsequently, the sample set was split into a calibration (n = 125) and test set (n=38) by the use of Gauss Jordan algorithm (using above mentioned data pretreatment) to select significant spectra for calibration. The whole data set contains 28 samples pig slurry and 135 samples mixed slurry (mainly pig and cattle). The configuration of the calibration and test sets is given in table 2. The calibration was developed using PLS algorithm and 1st derivative (segment 3, gab 1) of the absorbance spectra without new outlier elimination. The optimum number of PLS factors was determined by full cross-validation. The developed calibrations were validated with the test set.

[8] For explanation please see SL Calibration Workshop handbook or the similar definitions at [8]: Næs et al., 2002: A User-Friendly Guide to Multivariate Calibration and Classification

Table 2: Formation of the calibration and test sets for the slurry constituents DM, Ntotal, NH4-N, P and K

	DM [%]		Ntotal [kg/m³]		NH4-N [kg/m³]		P [kg/m³]		K [kg/m³]	
	Cal	Test	Cal	Test	Cal	Test	Cal	Test	Cal	Test
Range	1.3 – 7.0	1.4 - 6.8	1.8 - 5.4	1.8 – 5.4	0.7 - 3.2	0.8 - 3.2	0.3 - 2.4	0.5 - 2.4	1.7 - 4.9	1.8 - 3.6
Mean	4.0	4.2	3.2	3.1	1.5	1.6	1.3	1.4	3.0	2.9
SD	1.2	1.3	0.8	0.8	0.5	0.8	0.4	0.6	0.5	0.4

Results and Discussion

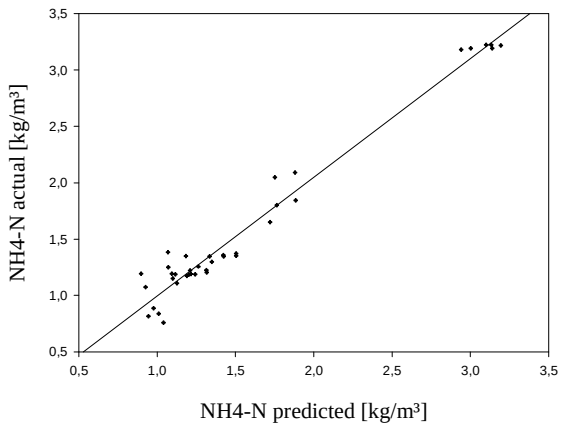
The whole NIRS measuring system (spectrometer, multiplexer, optics, fibre cable, etc.) was successfully adapted to the rough conditions on a commercial 18 m³ slurry tanker and a 12 V electrical power supply. In pre tests the ideal mounting position of the NIRS measurement optics was determined. The developed NIRS measuring system showed a reproducible and stable performance resulting from a steady state optical signal during slurry application [9]. Furthermore the system proved the general feasibility for a site specific and nutrient based slurry application, if connected to a D-GPS-system.

Table 3feature the results of the NIRS calibrations calculated for dry matter (DM), total nitrogen (Ntotal), ammonium nitrogen (NH4-N), phosphorus (P) and potassium (K). In general good correlations were achieved between the reference and predicted values.

Table 3: Statistics of calibration and validation for different slurry constituents

	Calibration			Validation			
	SEC	RMSECV	RSQcal	RMSEP	bias	RSQval	RPD
DM [%]	0.50	0.59	0.83	0.55	-0.09	0.81	2.32
Ntotal [kg/m³]	0.22	0.25	0.93	0.23	0.03	0.77	2.09
NH4-N [kg/m³]	0.13	0.15	0.93	0.14	-0.03	0.97	5.40
P [kg/m³]	0.18	0.20	0.82	0.26	0.03	0.81	2.23
K [kg/m³]	0.15	0.18	0.92	0.16	0.05	0.88	2.69

Concerning the range of the different slurry constituents (table 2) the achieved standard errors (SEC, RMSECV or RMSEP, respectively) are low. The validated methods show RPD values greater than 2; the case of the prediction of ammonium nitrogen (NH4-N) the RPD value is even greater than 5. Williams [10] characterises RPD values above 3 as an indicator for an efficient NIRS calibration for agricultural products. Moreover, the author classifies NIRS methods with an RPD value higher 5 as “good” and therefore appropriate for quality control. The validation plot for the prediction of ammonium nitrogen content as well as for dry matter content is shown in figure 2.



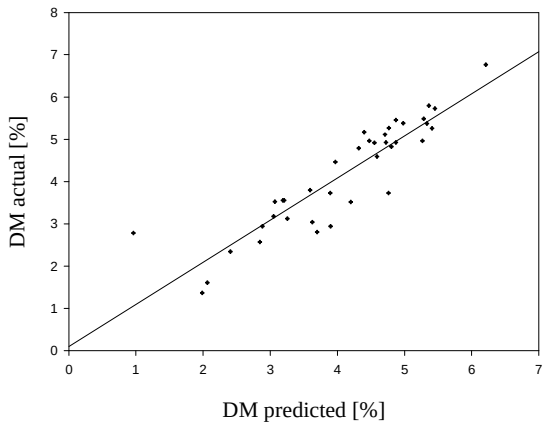


Figure 2: Validation plot for the prediction of ammonium nitrogen content, NH4-N [kg/m³], as well as for dry matter content, DM [%]

In table 4 NIRS methods with highest RPD values found in literature for the prediction of different slurry constituents are shown. Ranking and assessing the quality of the different NIRS calibrations achieved in the current research work, it has to consider that opposite to all literature the presented NIRS methods are one of the first, which are only based on an "online calibration" under practical conditions on slurry tanker. All reference samples have been taken during the online spectroscopic measurement of manure in the field, therefore these "on line calibrations" have not to be recalibrated to use them on the go directly on the slurry tanker. However, the accuracy of these on line calibrations could improve furthermore. Therefore, it would be best to build calibrations based on even larger data set in order to span the complete variation in the population of manure samples.

Table 4: Review of highest RPD values found in literature for the prediction of different slurry constituents

	RPD	Standard Error	RSQ	Range	Origin	n	Author
DM [%]	6.1	0.42* (2)	0.97	0.85 - 13*	Pig + Cattle	255	[11]
Ntotal [kg/m³]	3.38*	0.29* (1)	0.95	5.9 - 11.7*	Pig	143	[12]
NH4-N [kg/m³]	5.76	0.073 (1)	0.97	1.01 - 2.67	Pig	64	[13]
P [kg/m³]	8.46	0.055 (1)	0.99	0.043 - 1.43	Pig	64	[13]
K [kg/m³]	2.78	0.061* (1)	0.87	1.01 - 1.6*	Pig	64	[13]

* calculated; (1) SEP; (2) RMSECV

Acknowledgements
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In-Line Near-Infrared-Monitoring of the Biogas Process

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Introduction
Biogas offers attractive opportunities to utilize biomass for meeting renewable energy needs. Typical agricultural biogas feedstocks originating from organic waste, animal manure and energy crops are highly heterogeneous by nature. The fermentation process is exceedingly complex and not in depth understood in its various steps by the complex consortia of microorganisms involved. Due to the lack of appropriate monitoring and/or managing by poor controlling, the biogas process can slow down, be severely disturbed or even break down completely.
Near infrared spectroscopy (NIRS) is a desirable analytical technology for monitoring and process control in the biogas process. This work reports some field experiences and results of a new NIR sensor system (TENferm) in this sector.

NIRS-System
TENferm: TENferm is a NIR process measurement system for qualitative online analysis of organic materials in agricultural processes and facilities. It comprises hardware, software and full application services, including calibration development and on-site predictor control.
The hardware is a two component system consisting of up to four sensor heads and a central unit containing the spectrometer (TRISTAN OEM NIR-HR-U, 900 – 1700 nm, 512 pixel), an optical multiplexer (Leoni 1x4), a CPU with data interface and touch-monitor (Fig. 1). The sensor heads are connected by hybrid cables which transfer the optical measuring signal to the central unit and provide the sensor heads with energy (24 V) and control commands. The sensor heads are equipped with NIR light sources (5 W), the necessary optics to record the diffuse reflection on a sapphire window of 8 mm diameter. Automated self referencing with an internal white standard allows to eliminate environmentally induced variations. The measuring heads can be installed with 2" screw threads in conveyor pipes (Fig. 2) or lines (Fig. 3), so that continuous and representative measurement in the process is secured. Results of the in-line measurement are passed to the process control system of the plant, where the data is available for monitoring and process control.
TENferm is connected via VPN to a centralized monitoring location at the m-u-t headquarter, where the proper function and the performance of the system is monitored remotely and action is taken if necessary. Spectral as well as local sampling data are automatically synchronized with the central calibration database at the m-u-t headquarter, where comprehensive data sets containing all TENferm-systems built up the basis for calibrations as well as for software and model updates.

Figure 1. TENferm central unit with the touch-monitor displaying actual results on a biogas plant

Figure 2. TENferm measuring head mounted in a pipe (top), sampling valve (below)

Figure 3. TENferm measuring head installed in a silage auger (top), sampling plug (below)

Biogas Plant: The presented case-study is implemented on a commercial full-scale biogas plant with mesophilic operation in a 1.5000 m³ fermenter. Feedstock consists of 65 % liquid cattle manure and 35 % maize silage, premixed prior to feeding into the fermenter. Except for the silage feeding line, all substrates are pumped via a central main line - manure tank > pre-mixing tank > fermenter > effluent tank. Thus, with only two measurement points, one at the silage auger (MP-SA) and the second after the central pump (MP-CP), it is possible to completely monitor substrates in the entire biogas process.

In the first approach it was aimed to monitor characteristics and changes in organic dry matter (ODM), from raw feedstock over fermenter up to the effluent. An exemplary subset of results is presented here.

Measurement setup and data collection: Reflection spectra are beeing recorded continuously as the mean of 10 sub-scans in time-multiplex with a switch intervall of 10 sec at MP-SA and MP-CP. Since different types of substrates are flowing in a rather short sequence across the measuring line, spectral classification of the actual substrate is necessary prior to substrate specific application of specific predictors for the assigned parameters. Reference samples for calibration and validation were collected in the presented time series every two to threee weeks or as needed. Correct assignment of spectra to reference data of the samples was automatically secured by recording time-stamps at the beginning and at the end of each sampling procedure with a barcode scanner. All spectral, measurement and reference data is stored in the on site TENferm database until synchronisation with the m-u-t central database, which normally is performed once a day.
Representativity of sampling was achieved by taking incremental subsamples over a sampling time of 1 – 3 min at MP-SA and 5 -10 min at MP-CP. About 1 l of the blended subsamples was frosted right after sampling and stored at -18°C until chemical analysis in a commercial laboratory.

Calibration:
The methods in TENferm for classification and calibration are using support vector machine (SVM) algorithms based on LIBSVM and common pre- and post-processing methods. SVMs have been introduced recently in chemometrics and have proven to be powerful in NIR spectra classification tasks (SVC), as well as for regression and multivariate calibration (SVR). The advantages of SVM-based methods are that nonlinear regression can be performed easily as an extension to linear regression. In order to model non-linear processes, the Gaussian radial basis function (RBF) kernel was chosen here. The tuning of γ and σ parameters was performed using cross validation

Results and Discussion
ODM measurement on the biogas plant required two calibration models. One for maize silage (ODM MP-SA) and and one for the suspensive biogas substrates (ODM MP-CP), that were liquid manure, fermenter and premixed feedstock (Table 1). For ODM MP-SA 18 maize silage samples of the specific biogas plant were taken and supplemented with 228 silage samples from the m-u-t global database to increase range an variety. The data set for the MP-CP ODM model contains 48 samples of the specific biogas plant, evenly distributed across liquid manure, fermenter and premixed feedstock and supplemented with 209 spectrally matching samples from the m-u-t database.
Some pretesting on the selected calibration datasets showed advantages for the combination standard normal variate (SNV) as preprocessing and application of the RBF kernel. The tuning of γ and σ with leave-one-out cross validation was optimized towards decreasing the RMSEC to the piont, where no further improvement of the RPD coud be achieved, which was at 3.39 and 3.45 for ODM MP-SA and ODM MP-CP, respectively.

Table 1. SVM calibration statistics for organic dry matter in maize silage and suspensive biogas substrates

	maize_silage (ODM MP-SA)	**suspensive_biogas_substrate (ODM MP-CP)**
n	246	257
Range ODM [%]	16.1 – 45.7	1.9 – 18.5
γ	0.015	0.001
σ	11.2	300
RMSEC	0.7	0.5
RMSEP	1.3	0.8
RPD	3.39	3.45

A further challenge for in-line measurement on the pipe (MP-CP) was to securely discriminate the substrates when different types are beeing pumped in sequence. Even if at the measurement point one common prediction model can be applied, it still is necessary to assign the measured values to the specific substrate. Since individual pumping intervalls are rather short, information from the pump unit (valve switching) is not sufficiently precise with the presence of the new substrate in front of the measuring head . Pipeline length dependent time-lags and mixing processes in the pipeline lead to inaccuracies in substrate assignment. Therefore, based on the calibration data-set, a site specific spectral classification with SVC has been installed (Fig. 4).
The long-term stability and performance of the TENferm mesurement system is shown in Fig. 5. The correlation of the reference samples for validation are in good accordance with the general ODM course. Especially the frequent internal referencing with the white-standard prevents drifiting due to changes in the measurement equipment, e.g. ageing of the lamp, or environmental changes.

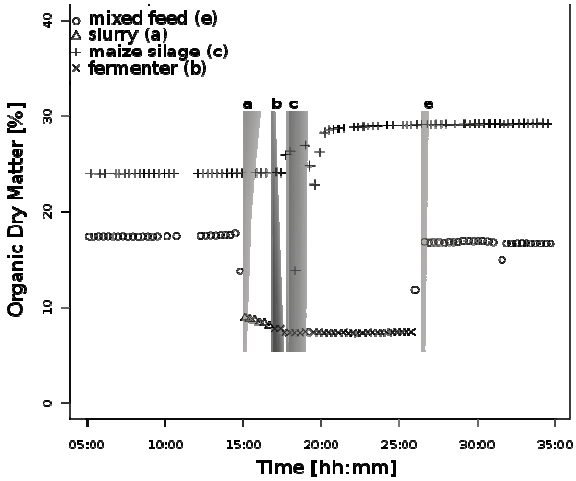

Figure 4. Typical conveying sequence on a biogas plant while feeding,, with in-line substrate classification and ODM prediction at to measuring points MP-SA (+) line and MP-CP (o)

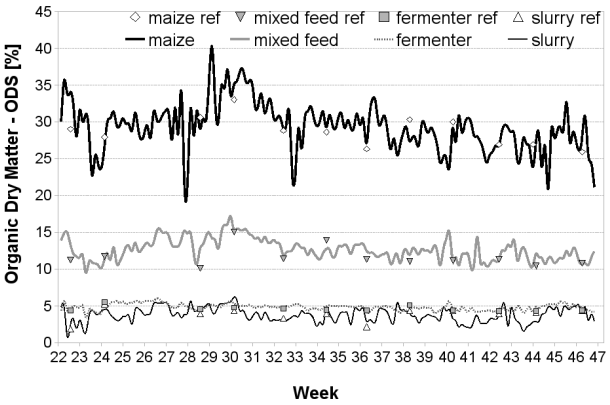

Figure 5. Long-term NIRS in-line measurement of ODM for maize silage, mixed feed, fermenter and slurry on a commercial biogas plant (values interpolated on daily average as curves, reference values as depicted by symbols, monitoring intervall 25 weeks)

A basic conclusion of the results presented here is that NIRS is well suited for process monitoring in biogas plants. Future important topics are calibration development for further parameters, improvement of a robust calibration databases, calibration transfer and automation of process control especially considering feeding strategies.

Using NIR spectroscopy on milk for the traceability of cows feeding

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Introduction

Consumer's demand of information about the way cattle are fed is recently growing, in relation to its effect on milk nutritional quality and taste. Consumer's appreciation for dairy products derived from traditional and environment sustainable production systems, such as the grass-based ones (mainly mountain or alpine extensive system) is also relevant. In fact, cow feeding affects the quality of derived milk for human nutrition, especially fatty acids (FA) profile and micronutrient contents. As compared to maize silage, grass-fed cows produce milk with lower level of saturated FA (SFA). Grass-fed cow milk is also richer in polyunsaturated FA (PUFA). Herbage in cow diet improves also β-carotene, A and E vitamins milk content. Botanical composition and agronomical practices, both changing with environmental conditions, such as altitude, have an important effect on herbage characteristics, influencing milk FA profile and terpenoids content. NIRS, allowing the prediction of milk gross composition and FA profile, could be also a useful tool to provide information about animal diet and milk origin. This work aims at discriminating the milk produced feeding cows with maize silage diet *vs.* pasture diet. Among pasture-based milk we also tested the possibility to distinguish their origin in discriminating between lowland, mountain and alpine vegetation grazed by cows.

Materials and Methods

Bulk milk samples (455) were collected from 172 farms in France and northwest Italy, during 2007 and 2008. Farms were selected to cover a wide variety of milk production conditions. During sampling, data about cows feeding, concerning forage quantity, type and conservation technique were surveyed (Table 1). As far as the diet was concerned, we selected milk samples produced in winter deriving from maize silage-fed herds (% of maize estimated > 70% of forage dry matter (DM)) and milk samples deriving from pasture-fed cows (100% of forage DM). With regard to vegetation, samples were assigned to one of the following classes of origin: lowland grassland, mountain pasture, alpine pasture (Table 1). Generally vegetation diversity increased with altitude.

Table 1. Characteristics of the two datasets (mean values, minimum and maximum values in brackets).

Dataset characteristics	Maize silage	Pasture	Lowland grassland	Mountain pasture	Alpine pasture
number of samples	37	124	69	82	78
Altitude (m a.s.l.)	253 (1 - 850)	1439 (2 - 2500)	112 (2 - 400)	859 (420 - 1100)	1986 (1200 - 2500)
Animal feeding					
Preserved forages (%)	18 (0 - 34)	0	7 (0 - 30)	9 (0 - 31)	1 (0 - 19)
Maize silage (%)*	82 (70 - 100)	0	2 (0 - 26)	1 (0 - 20)	0,0
Grass (%)*	0	100	90 (70 - 100)	90 (70 - 100)	99 (81 - 100)

*: % of preserved forages total dry matter

Milk samples were stored at -18°C until analysis. After 2 h at room temperature, 0.5 mL of milk were placed on a glass microfibre filter (Whatman GF/A, 55 mm, Cat. No. 1820 055 (Whatman International Ltd, Maidstone, UK)), and oven-dried at 40 °C for 24 h. Then each filter was placed into a 50 mm-diameter ring cup and scanned at 2 nm intervals from 400 to 2498 nm using a Foss NIRSystems model 6500 NIR scanning spectrometer (Foss NIRSystems, Silver Spring, MD, USA). NIR spectrometer was controlled via ISIscan software version 2.21 (Infrasoft International LLC, State College, PA, USA). Each reflectance spectrum was time-averaged from 32 scans and it was compared with the 32 average-measurements of a ceramic reference. Discriminant analysis was performed using the PLS-DA technique, and models were tested using a cross-validation procedure. The segment between 400 and 2500 nm was used. The standard normal variate and detrend (SNVD) scatter correction procedures were applied to the raw data. The spectra were then transformed using a mathematical first-order gap derivation

(1,4,4,1). Winisi II version 1.60 software (Infrasoft International,South Atherton St. State College, PA 16801, USA) was used.

Results and Discussion

Model classification performances are reported in Table 2 and 3. The correct classification rate observed for maize silage *vs*. pasture model exceeded 99%, with 160 samples correctly classified. Differences in milk FA composition between maize silage and pasture-fed cows' milk had been already studied; maize silage-milk had higher values of SFA and lower values of PUFA (Engel *et al*., 2007) than pasture-milk. According to the ability of NIRS to predict these FA (Coppa *et al*., 2010) they could have highly contributed to our discrimination. Moreover herbage is also rich in β-carotene resulting in more yellow milk than maize silage (Nozière *et al*., 2006). Lucas *et al*. (2008) could reliably predict β-carotene and yellowness of cheeses, which may suggest that these two parameters could contribute to milk discrimination by NIRS.

The vegetation type model correctly classified 139 milk samples out of 229, with a 39.9% error rate. Differences in milk composition among the different vegetation types reduced as compared to the ones between pasture and maize silage were probably at the origin of the low reliability of such a prediction. In fact, differences in FA profile according to vegetation altitude are mainly related to α-linolenic acid, which has higher values in mountain and in alpine pastures in particular (Engel *et al*., 2007). This FA was not satisfactory predicted by NIRS (Coppa *et al.*, 2010). Dairy product colour is also less influenced by botanical composition than by sward phenology, which depends on the grazing management, but only slightly on vegetation type.

In conclusion, NIRS could discriminate between diets (maize silage and pasture milk), as shown by the low classification error, but did not allow the prediction of the pasture type grazed by cows.

Table 2. NIRS classification of milk samples according to cows feeding expressed as number of samples classified in each category and in percent (in brackets).

Animal feeding	Predicted group membership	
	Maize silage	**Pasture**
Maize silage	37 (100.0%)	1 (0.8%)
Pasture	0 (0.0%)	123 (99.2%)

Table 3. NIRS classification of milk samples according to grassland type expressed as number of samples classified in each class and as percent (in brackets).

Vegetation	Predicted group membership		
	Lowland grassland	**Mountain pasture**	**Alpine pasture**
Lowland grassland	44 (63.8%)	22 (26.8%)	5 (7.4%)
Mountain pasture	15 (21.7%)	33 (40.2%)	11 (14.1%)
Alpine pasture	10 (14.5%)	27 (32.9%)	62 (79.5%)

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Plenary Session 4: *Applications* (continue).

Efficient Recirculation of Liquid Farm Fertiliser and Biogas Substrate using NIRS-NANOBAG®

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Introduction The proper recirculation of about three-fourths of the nitrogen being utilized in animal husbandry but not being used in the product gains more and more importance. Liquid manure represents a valuable substance as agricultural product on the one hand. On the other hand liquid manure can cause a lot of problems for environment, as is generally known. For an appropriate utilization of the recyclable materials nitrogen, phosphor and potash, which become more expensive all the time, and avoidance of exceeding stress for environment in terms of good agricultural practice (GAP) proper storage and processing of liquid manure as well as proper output and distribution on the agricultural sites are necessary. A more accurate determination of the nutrients´ contents in the liquid manure (N, ammonia, P2O5, K2O, ODM) by means of the NIRS-NANOBAG®-procedure can therefore bring an improvement of the fertilization and especially of the nitrogen-balance and it can also enforce a reduction of stress for the environment concerning water and air.

Materials and Methods

Using a beaker, the homogenised liquid manure is simply added to a sample bag which contains a carrier substance (Image 1). The special mixture of minerals, based on activated clinoptilolite zeolite, holds onto the fluid and absorbs all of the nutrients. So that it was possible to store or send the substance in an earth-moist condition within airtight packaging in a hygienic and unproblematic way. At the laboratory the sample, consisting of the mass of 50.0 g of carrier material and approx. 25 ml of liquid manure, is then weighed and dried for 3 hours at 55 ° C. The dried matter, ash content, nitrogen, phosphorous, calcium and magnesium are immediately measured in the NIR spectrometers FOSS XDS and/or UNITY SPECTRASTAR 2400. Kalibrations were performed with UNSCRAMBLER®, SENSOLOGIC® and VISION® software.

Image 1.: NANOBAG® containing activated clinoptilolite

Results and Discussion We faced two challenges: Firstly, to find a suitable mineral carrier material and optimise its grain size and secondly, to create calibration models for the near infrared spectroscopy. The chosen carrier was a carefully selected and activated mineral, clinoptilolite zeolite, which has a nanoporous crystal structure (diameter of pores: 0.4 nm, inner volume per cm^3: 0.47 cm^3) and is particularly well suited for the absorption of the aqueous phase and the volatile ammonia in the alkaline region .This is a decisive factor, as three quarters of all liquid manure has an alkaline pH value.

The calculation models created for different liquid manure samples, either separately or together, show the following correlation values and standard deviations for the projection with regards to the connection between both measurement techniques in Table 2:

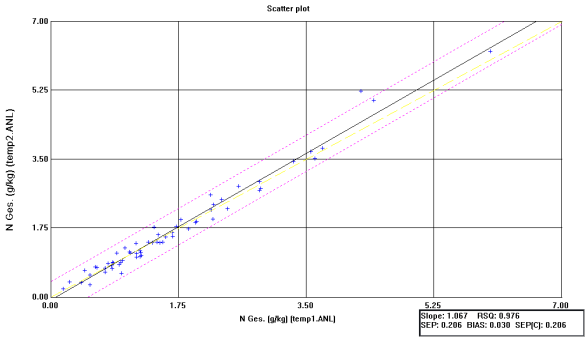

Figure 1. Multiple Regression for total Nitrogen of liquid manure

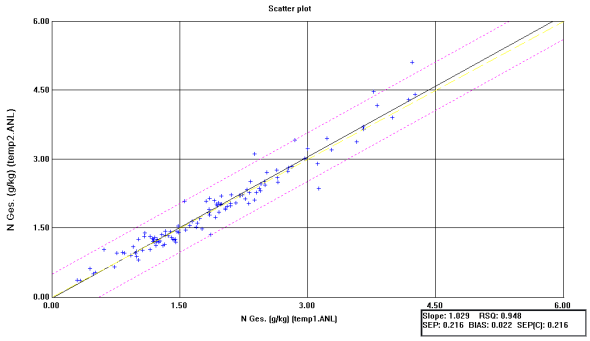

Figure 2. Multiple Regression for total Nitrogen of biogas slurries

In addition to the total nitrogen values according to FOSS-KJELDAHL decomposition, those readings obtained with the help of an alkaline distillation were also used. In the case of this alkaline decomposition, the liquid manure amides (mainly carbamide) are decomposed, but the particle-bound nitrogen is not compromised. The results showed that a differentiation between carbamide and ammonium using this method is quite possible. The subtraction of total nitrogen and ammonium therefore gives the total level of organically bonded nitrogen, while the deviations between the alkaline distillation and the colorimetrically determined ammonium indicates the amount of carbamide.

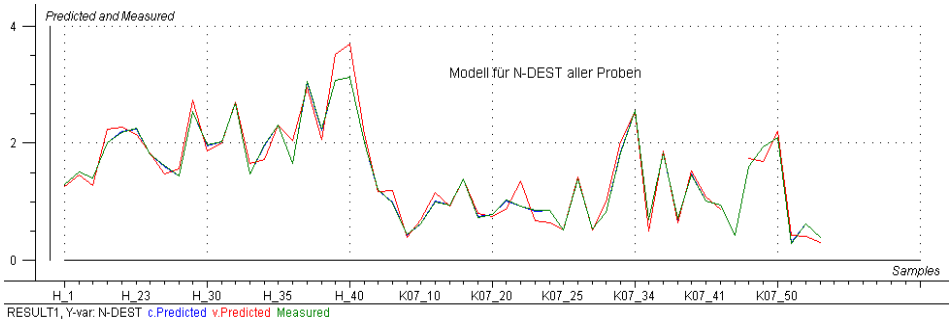

Figure 3. Cross-validated calibration model for cattle and hog liquid manure for the content of nitrogen following alkaline distillation in FOSS KJELTEC and spectroscopy in FOSS XDS

Mean value g/kg FM	N-total	NH4-NESSLER	NH4-KJELTEC	N-nitrogen *	N-organic remainder*
Cattle liquid manure	2.47	1.11	1.58	0.47	0.89
Hog liquid manure	3.88	2.59	3.39	0.84	0.49

Table 1. Comparison of the N fractions, * N-nitrogen and N-organic remainder, calculated as a difference

Parameter	Liquid manure	R^2	SEP
Dry mass	**all samples**	**0,863**	**3,45**
	Biogas liquid slurry	**0,810**	**3,81**
	Cattle liquid manure	**0,787**	**3,58**
	Hog liquid manure	**0,843**	**2,47**
Total nitrogen	**all samples**	**0,918**	**0,16**
	Biogas liquid slurry	**0,941**	**0,13**
	Cattle liquid manure	**0,910**	**0,11**
	Hog liquid manure	**0,929**	**0,15**
Ammonia nitrogen	**all samples**	**0,888**	**0,09**
	Biogas liquid slurry	**0,952**	**0,06**
	Cattle liquid manure	**0,782**	**0,07**
	Hog liquid manure	**0,883**	**0,09**

Table 2. Regression coefficients of dry mass and organic contents

Raw	**all samples**	**0,767**	**0,96**

ash	**Biogas liquid slurry**	**0,682**	**1,08**
	Cattle liquid manure	**0,501**	**1,18**
	Hog liquid manure	**0,819**	**0,80**
Phosphate	Hog liquid manure	0,990	0,143
	Cattle liquid manure	0,926	0,080
Potassium	Hog liquid manure	0,941	0,195
	Cattle liquid manure	0,883	0,555
Calcium	Hog liquid manure	0, 877	0,365
	Cattle liquid manure	0, 894	0,172
Magnesium	Hog liquid manure	0,972	0,147
	Cattle liquid manure	0,967	0,057

Table 3. Regression coefficients of raw ash and anorganic contents

Conclusion The more precise determination of the liquid manure nutrients using the NIRS-NANOBAG® process can contribute to an improvement in fertilisation and in particular the balancing of nitrogen and a reduction of environmental contamination of water and air. The method can also be used for process control in biogas production and in the targeted treatment of liquid manure.

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Variation among days in the dry matter of silages in bunker and tower silos measured using diode-array NIRS.

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Introduction

Precise feeding of dairy cows requires that the same proportions of nutrients and feed dry matter are fed each day. Rations for dairy cows are formulated on a dry basis; however, the amount of forage mixed in the ration daily depends on forage DM concentration, which is used to calculate the as-fed amounts to be added to the ration mixer. Research suggests that abrupt changes in silage DM by precipitation events can result in 1 to 2 kg of lost milk production per cow per event. Variation in silage DM has multiple sources: random sampling variation, systematic changes in the silage during harvesting, and abrupt changes due to external precipitation events. Tower silos are more prone to systematic changes because each harvested load is stratified on top of the previous load. Bunker silos are fed out across filling stratifications, but they often have an exposed face that can be contaminated by water from rain or snow.

Each source of DM variation in silage has different impacts on the rations and on the animals being fed. Random variation is simply noise around the true mean DM of the ration. Because the cow eats the true DM concentration of the silage, these random variations have no impact on the cow and would create random fluctuations in the ration. However, systematic or abrupt changes in DM reflect the true concentration of the silage on a specific day and should be taken into account when mixing rations. The objectives of this research were to use diode array near infrared reflectance spectroscopy (NIRS) to measure among-day variation in the DM concentration of silages to: (1) identify sources of variation in DM concentrations of silages, (2) determine the long-term accuracy of moisture or DM calibrations using NIRS, and (3) to determine the magnitude of the various sources of variation in silage DM in different types of silos.

Materials and Methods

Over 770 samples were collected from 25 silos between August 2008 and December 2009 at the U.S. Dairy Forage Research Center (USDFRC) farm at Prairie du Sac WI. Weather data was obtained from Wisconsin State Climatology Office for the Prairie du Sac, WI area. The farm crew was instructed to collect a handful of sample from 8 to 10 places in the silage that was defaced from bunker silos before feeding or 8 to 10 times during unloading of tower silos. These samples were mixed in a 15-50 L bucket and two sub samples were scanned to evaluate within-sample analytical variation. During parts of the experiment, the crew collected two separate buckets of samples to evaluate within-day sampling variation. These results will not be presented and only the average of all samples and analyses within a day were used to evaluate variation among days in this report. Some samples were collected during short-term animal trials and only the data from 8 silos with the most observations are presented.

A HarvestLab diode array sensor (Deere & Co., Moline, IL) was calibrated and used to predict DM. The HarvestLab is designed for chopper-mounted applications, but can be used as a bench-top instrument with a spinning bowl attachment (bowl is 18 cm in diameter and 9.5 cm deep). Calibrations were developed using Unscrambler v9.8 (Camo, Woodbridge, NJ). Calibrations were developed for reflectance measurements (between 950 to 1530 nm) with no mathematical treatments of the spectra. Reference samples were analyzed at the USDFRC laboratory in Madison, WI. Reference samples were collected by thoroughly mixing a daily sample of silage material, packing two bowls of the material and saving the spectral scan for each bowl. Two 70 g sub-samples from each bowl were transferred to tared aluminum pans and were dried in a forced air oven at 55 °C. After 24 hours, one sample was removed from the oven, equilibrated to ambient laboratory temperature and humidity, weighed, and then ground for further analysis not related to this experiment. The second sample was dried for an additional 24 hours, after which the sample was weighed without equilibration, and then placed in a 105 °C oven for an additional 24 hours. The 24 and 48 hour 55 °C DM values were regressed against their respective 105 °C values to detect suspect data. Using these regressions, the one direct measurement and two predicted values for 105 °C DM were averaged to obtain reference DM results with improved precision.

Results were statistically analyzed to obtain univariate means and standard deviations for DM among days within a silo. Local regression analysis (LOESS) was used to account for systematic trends and to estimate residual differences. When applicable, precipitation data was modeled against residual differences.

Results and Discussion

Dry matter was routinely recorded during experiments conducted at the USDFRC and these results were used to update and evaluate the DM calibration. The DM calibration was updated twice, resulting in a total of three calibration

equations (Table 1). All calibrations had acceptable standard errors of prediction. The initial calibration was based on DM determined for both fresh and silage samples. The first update improved performance by eliminating fresh material spectra and adding silage spectra to make the equation more specific for silages. The second update added both fresh and silage materials to increase the DM range of the calibration set.

Table 4 – Calibration Statistics for DM equations used with a HarvestLab sensor (Deere & Co., Moline, IL)

Calibration	N	No. of PCA	R^2	SEC	SEP
Initial	375	8	.929	1.75	1.86
Update 1	396	9	.927	1.64	1.70
Update 2	548	8	.923	1.88	1.92

After the last calibration update, 66 corn and alfalfa silages were analyzed for DM by the reference method (DM range from 25 to 65%) and used as an independent data set to evaluate the accuracy of the calibration equations (Table 2). The initial and update-2 calibrations had a significant negative bias and under predicted DM at an increasing magnitude starting at about 25% DM. Update-1 calibration tended to over predict at <36.5% DM and under predict at an increasing magnitude above 36.5% DM.

Table 5 – Evaluating the accuracy of DM equations using a set of 66 reference values collected at the end of the experiment that were not used for any calibration.

Calibration	SEP	Bias	Intercept	Slope	R^2
Initial	2.41	-1.22	2.71	0.89	.88
Update 1	2.49	-0.09	4.74	0.87	.90
Update 2	2.43	-1.32	2.73	0.89	.88

Univariate statistics for the eight silages indicate that the among-day standard deviation (SD) of DM ranged from ±1.5 to 5.0 %-units (Table 3). When systematic variation was removed by LOESS analysis, the residual variation was reduced to 38% of the univariate SD for tower silos, but was reduced to only 57% of the SD for bunker silages. This suggests that much of the variation in DM in tower silos is related to stratification during filling that is detected and removed by LOESS analysis leaving a residual variation that is due to sampling. Systematic variation detected by LOESS analysis is a smaller proportion of total variation in bunker silos. In bunker silos, the LOESS residual differences were negatively related ($P < .00001$) to precipitation events indicating that residual variation in these silos is relate to sampling and abrupt contamination.

Table 6 – Variation in silage dry matter concentration by forage and silo type.

Forage	Silo	Sample number	Sampling period (days)	Mean	Standard deviation	LOESS Residual SE
Alfalfa	Tower	48	109	43.12	3.32	0.79
Corn	Tower	32	79	38.57	3.03	1.37
Corn	Tower	41	94	36.97	1.39	0.85
Alfalfa	Bunker	38	88	43.91	2.40	2.19
Alfalfa	Bunker	33	75	40.07	3.65	1.83
Alfalfa	Bunker	104	254	35.75	5.04	2.24
Corn	Bunker	28	65	31.09	1.77	0.70
Corn	Bunker	67	147	33.12	1.76	1.42

Application of FT-NIR for determination of wood provenance

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[Introduction] Norway spruce is an important wood species growing in large areas of forests all around Europe. It is primarily raw resource for the wood and paper industries. Several wood characteristics, such as density, width of annual rings, chemical composition, and in consequence mechanical strength, aesthetical properties or durability, among others, are related to environmental and genetic factors. FT-NIR technique has already been successfully applied for predicting chemical, physical, anatomical and mechanical properties of wood (Tsuchikawa 2007). Studies on the application of FT-NIR to differentiate the origin of wood were also earlier work undertaken by the author (Sandak et al. 2010). The research presented here is therefore an attempt to exploit the potential of near infrared spectroscopy toward wood assessment in relation to varying tree provenances.

[Materials and Methods] Wood samples investigated in this project were of Norway spruce (Picea abies L. Karst.) growing in different locations and representing borders of the spruce range in Europe. Five sites have been selected for sample collection: central Finland, southern Estonia, northern Poland, southern Poland and northern Italy. The summary of samples collection and range of Norway spruce and sample locations are presented in the Table 1. From each location 30 samples have been collected. A small block (~20mm width) was cut out from each undercut slab, assuring the opening of the radial plane. The samples were carefully conditioned to guarantee constant moisture content (~12%MC), minimizing an effect of differences in the water signal measured by the NIR.

Table 1. Summary of samples collection.

Country	Location	Geographical coordinates		Elevation (m. o. s. l.)
		latitude:	longitude:	
Finland	Lieksa	63º 22’ 0’’	30º 42’ 0’’	~140
Estonia	Tartu	58º 18’ 0’’	27º 16’ 0’’	~70
Poland north	Rynkow	53º 42’ 0’’	18º 30’ 0’’	~135
Poland south	Krzeszow	50º 43’ 59’’	16º 4’ 0’’	490-540
Italy	Juribello	46º 18’ 0’’	11º 49’ 59’’	1700-1800

All the experimental samples were measured by using Fourier transform near infrared spectrometer VECTOR 22-N produced by Bruker Optics GmBH, equipped with a standard fiber-optic probe, germanium-diode detector and the thermoplastic resin Spectralon as a reference. The spectral resolution of the spectrophotometer was 8cm^{-1} and spectral range was 4000-12000cm^{-1}. Each spectrum has been computed as an average of 32 successive measurements. Dedicated tests have been performed in order to determine a routine procedure of sample measurement, since due to the heterogeneity of wood; the scanning procedure could greatly affect the results obtained. Five separate spectra have been measured on the radial plane of each wooden sample. The measurement location has been selected randomly; however any visible abnormalities of wood surface (such as resin canal, knot or discoloration) were intentionally omitted. OPUS 6.5 and National Instruments LabView 8.6 software packages have been used for signal processing and data analyzes. Signal preprocessing included computation of the second derivative, and in some cases smoothing and vector normalization. Derivatives were calculated according to the Savitzky-Golay algorithm. For the mathematical management of the spectra and then for the evaluation of the results Principal Component Analysis (PCA), Cluster Analysis (CA), Identity Test and Partial Least Squares (PLS) techniques were applied.
Traditional “wet” chemical analyses were performed in parallel to the spectroscopic measurements. The concentration of cellulose was determined according to the Seifert procedure (by using acetylacetone-dioxane-hydrochloric acid). Holocellulose content was obtained by wood delignification by sodium chlorite with addition of acetic acid (Browning 1967). Amounts of lignin, solvent extractives and ash were determined according to Tappi standards (TAPPI).

[Results and Discussion] Average NIR spectra were computed for each location in order to envisage the differences between woods harvested in different countries, as presented in Figure 1. The shapes of the curves are very similar; however there was evidence of differences after close examination of some characteristic spectral regions. The band assignments correspond to the work of Tsuchikawa et al. (2005).
Cluster analysis was performed on spectra collected from powdered wood (Figure2). It was found that all samples were clearly separated, creating five main clusters covering Finland, Estonia, Poland North, Poland South and Italy. None of the spectra were miss-classified. Similar results were obtained by PCA. All of the wood samples were clearly separated, and fitting spheres were not overlapping. The Identity Test (Opus manual) was adopted for validation of the provenance determination algorithm. 80% of samples were used for model generation, and the remaining 20% of samples were taken for validation. In all cases the hit quality calculated was lower than the threshold; and all of the samples were positively classified. The Identity Test might be therefore applied for sorting timber in relation to its origin.

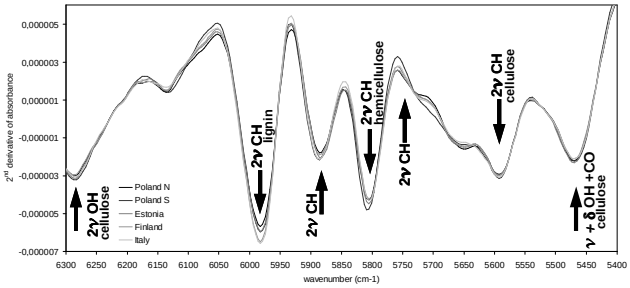

Figure 1. Second derivative of the averaged spectra for milled wood sample from different locations.

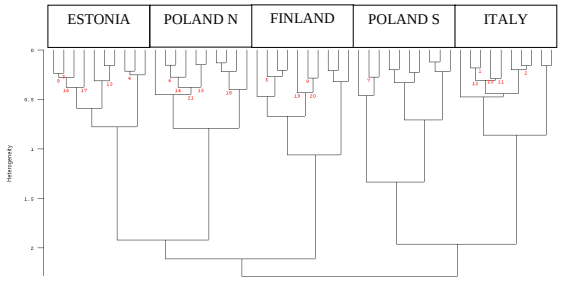

Figure 2. Cluster analysis of powdered wood fraction <0.5mm obtained form samples from different provenance. Note: Ward's algorithm, preprocessing: 1st derivative, 7500-4300cm^{-1}.

PLS models for the chemical components of wood were based on the spectra of powdered material (fraction 0.5-1 mm). Reference data were obtained during the standard chemical analysis. One averaged spectrum for each location was included in the learning group. In addition, a learning group has been enriched with twin spectra of the extracted wood powder (identical content of lignin, cellulose, holocellulose and ash, but zero extractives). Based on the above reference data separate models for each of the main chemical components of wood were developed. Models were then validated and validation parameters are shown in Table 2. Very accurate models were developed, especially for lignin and extractives content (r^2=98.7 and r^2=99.1 respectively).

Table 2. Summary of parameters characterizing PLS models of wood chemical components.

component	lignin	cellulose	holocellulose	solvent extractives	ash
range (cm-1)	7004-6557	9463-6171	9469-7440	7471-6557	9469-7440
	6175-5662	4663-4363	7004-6171	5338-4339	5338-4616
			4937-4339		
preprocessing	min/max normalization	min/max normalization	1st derivative + MSC	MSC	1st derivative + baseline correction
r2	98,67	84,98	79,99	99,13	77,24
RMSECV (%)	0,102	0,0638	0,765	0,0877	0,00711
RPD	8,86	2,58	2,24	10,8	2,1
bias	-0,00079	0,00176	-0,0384	-0,0132	0,000225
rank	5	9	5	8	7

[Conclusions] FT-NIR spectroscopy, a non-destructive, fast and low-cost technique, in combination with appropriate data managing procedures, offers an effective tool to separate groups of wooden specimens of different origin. Although trees of the same wood species, but growing in various locations, have only slight differences in chemical composition, FT-NIR spectroscopy is sensitive enough to detect such differences. The method presented in this paper has an interesting application in wide areas of forest/wood industry, for tracking wood provenances and as a technical tool for detecting logs coming from protected areas (illegal logging detection).

[Acknowledge] Part of this research has been done during a project BiQUEEN financed by Fondazione Cassa di Risparmio di Trento e Rovereto. The support was highly acknowledged.

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TAPPI standards: T 222 om-06, T 204 cm-04, T 207 cm-08, T 211 om-07, T 212 om-07
Tsuchikawa S., Yonenobu H., Siesler H.W. (2005): Near-infrared spectroscopic observation of the ageing process in archaeological wood using a deuterium exchange method. Analyst 130:379–384
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Precision Feeding: NIR on line & TMR consistency

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[Introduction]

Totally mixed rations (TMR) technique mixing all the feeds together and making it available 20-22 hours per day has the intent to obtain the best ruminal environment combining specific proportions of nutrients in the diet. But many problems are still unresolved. Differences between theoretical and prepared TMR and effectively consumed TMR by the cows were widely reported (Leonardi e Armentano, 2003)[9]. The differences, are due to errors loading feeds (wrong weight, ecc…) and change in water content that alter the amounts of dry matter of feed loaded into the wagon (Buckmaster 1998[10]; Ishler 2001[11]).

Animal producers are beginning to realize the importance of their feeding systems in terms of costs; thousands of dollars can be lost annually through:

- Ingredients inventory shrinkage;
- Inaccurate weighing of components in the ration
- Lack of knowledge of the actual Dry Matter and nutrients of the feed that are being fed.

Due to the current heavy crisis, it is a real basic requirement for all the farmers to optimize the diets, providing a more consistent TMR, controlling the accuracy of the mixer operator, measuring on line the actual Dry Matter Content of feedstuffs and monitoring the inventory of ingredients. This paper will focus on a new integrated system developed specifically to help farmers to feed animals in line with precision animal nutrition milestones. The dg precisionFEEDING™ is a kit constituted by a Feeding management software package (DTM™ 2009), scale indicator (Top Scale Indicator) and a near-infrared reflectance (NIR) analyzer.

[Materials and Methods] The near-infrared reflectance analyzer is based on a spectrophotometer working in the region 900–1800 nm, with a pixels dispersion of 8 nm and a wide temperature range [-30 °C; +50 °C] thanks to a powerful temperature compensation mechanism. Samples of fresh forages are read directly in the front loader bucket without any human intervention thanks to a mobile multi-windows scanner able to scan an extended surface of the sample. The Top Scale Indicator is connected on to the mixer wagon and it is the real control unit for the loading process: it does control the sequence as well as the target weight for each ingredient. The scanner unit is connected wireless with the Top Scale Indicator, so it is informed by Top Scale on the current ingredient in the loading phase and does transfer predictions in real time: predictions are available in about 20 seconds.

Top Scale, adjusts the target weight on the base of the NIR predictions evaluated in real time during the loading phase. In case of massive ingredients, analysis are performed on each single bucket and the weight adjustment is evaluated accordingly. All predictions are saved on the Top Scale indicator and transferred on the Feeding management software (DTM™ 2009) by memory card or wireless.

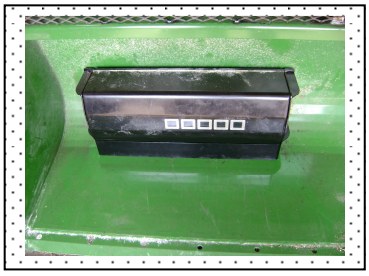

Figure 1. Scanner Unit inside the bucket of the Front

Figure 2. Front loader and Mixer wagon during a

[9] Leonardi C. and Armentano L.E. , (2003) – *Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows.* J. Dairy Sci. 86 (2): 557-564

[10] Buckmaester D.R. (1998) – *TMR Mixer Management.* Dairy Feeling System: Managements, Components, and Nutrients, Camp Hill, Pensylvenia, Natural Resource, Agricolture and Engineering Service (Nraes)

[11] Ishler V. (2001) – *The case for taking weekly silage dry matters.* Hoards Dairyman (May, 10): 341

Loader. phase of TMR preparation.

[Results and Discussion]
Here below some data about the quality of calibration curves developed till now for the NIR analyszer:

Ingredient: CORNSILAGE

Constituent	samples	Mean	SD	Est. Min	Est. Max	SEC	1-VR	SECV	1-VR	CV	RPD
SS	360	34,2	4,1	22,0	46,4	1,3	0,89	1,35	0,9	3,9	3,0
STARCH	360	11,0	2,3	4,0	18,0	1,2	0,75	1,18	0,7	10,7	2,0
NDF	360	14,70	1,46	10,32	19,08	0,70	0,76	0,72	0,76	4,87	2,04

Ingredient: GRASSSILAGE

Constituent	N	Mean	SD	Est. Min	Est. Max	SEC	1-VR	SECV	1-VR	CV	RPD
DM	196	41,44	13,51	0,90	81,99	2,37	0,97	2,48	0,97	6,0	5,45
CP	196	4,31	2,12	0,00	10,69	0,80	0,85	0,82	0,85	19,0	2,59
ADF	192	14,47	4,63	0,56	28,37	1,30	0,91	1,39	0,91	9,6	3,32
NDF	194	23,44	7,30	1,55	45,32	1,98	0,92	2,11	0,92	9,0	3,47
HASH	194	4,23	1,59	0,00	9,01	0,64	0,82	0,68	0,82	16,0	2,35
EE	198	0,88	0,24	0,17	1,59	0,13	0,67	0,14	0,67	15,6	1,72

Tailored NIR Reflection Spectroscopic Methods for the Advanced Characterization of Nanomaterials

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Introduction: In general "nanomaterial" is the collective name for matter on a scale between 0.1 nm and some 100 nm. Due to its wide distributed usage, characterization of physical, chemical and morphological properties is essential. Until now, particle size is determined by electron microscopy (ELMI), light scattering or the Coulter-Counter method, surface area by nitrogen adsorption experiments (BET), pore dimension by mercury intrusion porosimetry (MIP). For chemical characterization nuclear magnetic resonance (NMR) spectroscopy and elementary analysis are applied. We substitute all these time-consuming and expensive routine methods by NIRS and determine both physical and chemical properties imultaneously.

Experimental: NIR spectra were either recorded with a scanning polarization interferometer Fouriertransform NIR spectrometer (FT-NIR) (Büchi, Flawil, Switzerland) or a Spectrum™ Spotlight 400 combined with a Spectrum™ GX IR/NIR spectrometer (Perkin Elmer, Rodgau, Germany). The FT-NIR instrument offers a resolution of 12 cm^{-1}, an absolute wavelength accuracy of ± 2 cm^{-1} and a relative reproducibility of 0.5 cm^{-1}, the imaging system a signal-to-noise ratio of > 12000:1, a spectra collection speed of 160 spectra per second at 16 cm^{-1} and a maximum number of spectra per image of > 260,000. Chemometric software NIRCal 4.21 (Büchi), Unscrambler v9.6 (CAMO, Oslo, Norway), SpectrumIMAGE-Spotlight R 1.6.0 were used for creating the principal component analysis (PCA) and partial least squares (PLS) regression models. For testing the models the collected spectra were divided into a learning-set (c-Set, 67 %) and a test-set (v-Set, 33 %) both consisting of independent samples. Measurements were carried out at room temperature (23 °C) from 4000 – 10,000 cm^{-1}.

Results: The suitability of NIRS to determine particle size and molecular weight is demonstrated by the investigation of derivatized silica, fullerenes, nano-crystalline diamond, polymer particles, early, mid and late dendrimer generation´s. For PAMAM-NH_2 dendrimers analysis guest-host interaction with porous silica surfaces were investigated (Figure 1). The loading capacity of the silica material with adsorbed PAMAM-NH_2 was evaluated by means of capillary electrophoresis (CE), particle size and molecular weight (MW) by gas-phase electrophoretic mobility molecular analysis (GEMMA) and matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF/MS). NIR spectra show a distinct band at 4932 cm^{-1} (n_{sym} (NH) + amide II) due to the adsorbed dendrimers. On this basis multivariate calibration was performed with the theoretical data and those from CE, GEMMA and MALDI-TOF/MS resulting in better results for the NIRS method. Near-infrared chemical imaging in diffuse reflection mode was implemented as a novel tool to simultaneously determine the physical and chemical parameters of a porous poly(p-methylstyrene-*co*-1,2-bis(p-vinylphenyl)ethane) (MS/BVPE) monolith with an inner diameter of only 150 µm. The amount of MS/BVPE (%, v/v), and the quantity (%) of micropores (d < 6 nm), mesopores (6 nm < d < 50 nm) and the macropores (50 nm < d < 200 nm) could be determined with one measurement. The implication of these results is that FT-NIR spectroscopy is a suitable technique for the fast screening of samples with varying physcio-chemical nano-properties. These methods are of high interest for the pharmaceutical industry, their efficiency is shown and discussed in the proposed presentation.

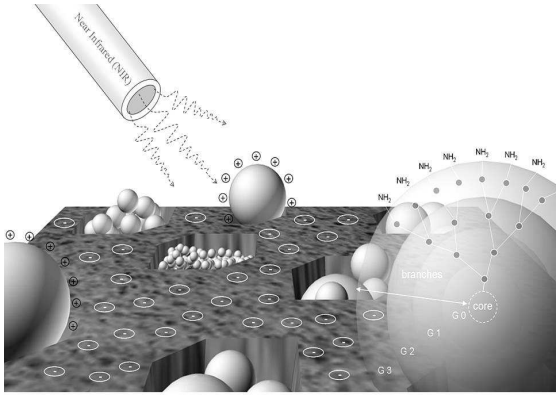

Figure 1. Guest-host interaction of PAMAM-NH_2 denrimers and porous silica

Posters session.

NIRS on-site vs. at-line: Transferability and robustness of chemometric models on fresh silages

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Introduction

Ensiling is a forage preservation method based on a spontaneous acid lactic fermentation under anaerobic conditions. However, oxygen can enter the silo through holes in the polyethylene cover or during exposure to air once the cover is open. In these situations, undesirable microorganisms can develop in the forage. The quality of the silage is currently evaluated through fermentative parameters (pH, ammonia-N, lactic acid and volatile fatty acids) and dry matter content. Due to the adverse effects of non-optimal ensiling process on animal nutrition, analytical methods are needed to estimate fermentative process at country level. Near infrared spectroscopy has been used successfully for many years in the determination of major chemical and fermentative parameters on dried and wet material by using laboratory pre-dispersive instruments. The implementation of quality control sensors at farms and country level it is possible by using diode array on-site NIR instruments, more adapted to worse conditions. However, to develop calibration models is not a simple task, it needs large data base, it is tedious, expensive and time consuming.

Although there has been a lot of progress in NIR instruments, there still exist differences that make impossible the direct transfer of calibration models from one instrument to other one. Being necessary to establish standardization models to transfer NIR calibration from laboratory to instruments more adapted for on-site analysis (i.e., diode array).

The objective of the present study has been to standardize two NIR instruments, one pre-dispersive and another one an on-site post-dispersive diode array, to transfer from one to other calibration models to predict fermentation parameters of wet silages.

Material and Methods

Instrumentation

Instrument 1: Foss NIRSystem 6500 scanning monochromator (Foss NIRSystem, Silver Spring, MD, USA) provided with a transport module in the scanning range of 400 to 2500 nm at 2 nm interval. The spectral data were recorded in reflectance mode (log 1/R) with WINISI 2 software v.1.05 (Infrasoft International Inc., Port Matilda, PA, USA). The analysis were carried out using natural cells; two different charges of each sample were scanned in duplicate, averaging the resulting spectra.

Instrument 2: On-site CORONA 45 VisNIR 1.7 (Carl Zeiss, Inc.) diode array spectrometer with a scanning range from 400 to 1680 nm with data recorded every 3 nm in reflectance mode (log 1/R).The analysis was carried out using a Petri dish to contain the wet samples. With an integration time of 100 ms, 20 scans were averaged for each measurement. All spectra were recorded using CORA software version 3.2.2 (Carl Zeiss,Inc.).

Samples

This study has been developed with three hundred and forty four (N=344) grass silage samples, collected from different farms, across North Spain. The total samples were distributed in two sets. The set one with 240 samples colleted from 2002 to 2008 (Set 1) was used for calibration development. The spectra of Set 1 were recorded using lab instrument (1). A second sample set (Set 2) containing 104 samples collected from 2009 to 2010 were scanned in both NIR instruments and served to standardizate and to validate NIRS calibrations.

The same portion of the sample used to collet spectra was used for reference data. The chemical analysis was performed using traditional analytical methodologies: Dry Matter content (DM) using dry-forced oven (60^{o}C for 24 hours), fermentative parameters were analyzed in juice extract to determine pH, ammonia-N (by destillation) and lactic acid and volatile fatty acids by HPLC.

Calibration development

NIRS calibrations were developed using SNV&D (Barnes et al., 1989) transformations to remove multiplicative interferences of scatter, MPLS and second derivative as regression model (WinISI, 2000). This model was selected taking into account the lowest standard error of cross-validation (SECV) and highest coefficient of determination in cross-validation (1-VR).

Standardization
According to Fearn (2001), who indicates that the best option in standardization procedures is to use real samples, in this work we have evaluated three different matrices developed using, one, five and ten wet silage samples and we have applied the patented algorithm by Shenk and Westerhaus (1995).

Standardization samples were selected applying the CENTER algorithm (Shenk and Weserhaus, 1991) from WinISI II in Set 2 and three outlier samples were eliminated. Afterwards, the algorithm SELECT was applied (N=101) for choosing the most representatives spectra/samples by using Mahalanobis distance among all pairs of spectra, using 1.0 as cut off value in both instruments. A total of 10 samples scanned in pre and post-dispersive instruments were selected to develop the standardization matrices. And the remaining 91 samples of Set 2 were utilized for validation.

Results and Discussion
As first step we applied the calibration model developed on instrument 1, after trim, on 91 validation spectra analyzed on instrument 2 before standardization and after applying matrices developed with one, five and ten samples. Table 1 compares the GH and NH values. The calibration developed on instrument 1 (Table 2) provide good results for validation spectra scanned in same instrument (Set 2), average GH= 1.55 and NH= 1.06, respectively; the best results, based on the lowest GH and NH values for spectra scanned in instrument 2, were obtained with the standardization matrix developed using five samples; the average GH and NH were 4.93 and 3.21 respectively.

It is clear that statistics (GH and NH) have decreased when applying standardization matrices, however these result show statistics higher than established limits (GH<3 and NH<1.2) avoiding the direct transferability of this chemometric model between these instruments.

Previous researchers (Fernández Ahumada et al. 2008) have evaluated the possibility of increase the robustness of the prediction model including spectra collected with the secondary instrument in the calibration set and recalculating a new calibration. This idea was applied to our study and 40 samples of the validation set (Set 2) were randomly selected to be included in a new calibration set containing a total of 280 samples to update the model and the remaining samples were used for external validation (N=51). The statistics show in Table 2 that the accuracy was not improved in the recalculated model. However, increasing the spectra variability updating NIR calibration model to be transferred, the external validation statistics were improved. GH and NH values became acceptable within the control limits. Calibration models to be transferred between at-line and on-site instruments must include spectra from both types of instruments. Furthermore, it is necessary to remark that this study demonstrates that calibration model already available for laboratory instrument on wet silages, the most difficult sample type due to sample presentation (wet and large fibers), to predict fermentative parameters, present in low amounts, can be transferred to on-site diode array instruments allocated in different country or farm points as real time sensors to fermentative process control.

Acknowledgements

This research work was supported by the Spanish projects RTA2006-00082-C02-01 and RTA2008-00113-C02-01from the INIA, the Asturias Regional Project PCTI-IB08-151 and PCTI-EQUIP08-15 for on-site NIRS instrument acquisition. Thanks are also given to Alfonso Carballal and Nutrition Laboratory staff for their technical assistance.

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Shenk J.S. and Westerhaus M.O., 1995. Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy. Monograph, NIRSystems.

Table 1. Global and neigborhood H Mahalanobis distances (GH and NH) values from spectra of Set 2 (N=91) collected on instrument 1 and 2 before and after application of different standardization matrices using a calibration model developed on Instrument 1.

Instrument 1		**Instrument 2**							
		No STD		**STD-1S**		**STD-5S**		**STD-10S**	
GH	**NH**	**GH**	**NH**	**GH**	**NH**	**GH**	**NH**	**GH**	**NH**
1.55	**1.06**	**79.36**	**74.38**	**48.74**	**42.64**	**4.93**	**3.21**	**30.27**	**25.22**

STD: Standardization matrix: 1S: one Sample; 5S: Five samples; 10S: Ten samples
Instrument 1: Foss NIRSystem 6500 scanning monochromator; Instrument 2: On-site CORONA 45 VisNIR 1.7

Table 2. Calibration and external validation statistics after standardization with calibration model developed on Instrument 1 and update with spectra from Instrument 2.

	CALIBRATION				**EXTERNAL VALIDATION (N=51)**		
	RSQ	**SEC**	**1-VR**	**SECV**	**SEP**	**GH**	**NH**
Calibration model (N=240)							
pH	0.87	0.220	0.82	0.257	0.776	5.29	3.35
DM (%)	0.99	0.856	0.98	1.077	7.17	5.29	3.35
N-NH_3 (mg/100ml)	0.91	19.76	0.86	25.8	165.5	4.96	3.12
Lactic acid (g/100 ml)	0.89	0.384	0.85	0.448	2.79	4.76	3.01
Acetic acid (g/100 ml)	0.74	0.168	0.63	0.203	1.00	4.76	3.01
Butiric acid (g/100 ml)	0.85	0.189	0.80	0.221	1.63	4.79	3.04
Update calibration model (N=240+40)							
pH	0.74	0.298	0.66	0.338	0.403	0.96	0.30
DM (%)	0.97	1.56	0.96	1.74	3.80	0.96	0.30
N-NH_3 (mg/100ml)	0.84	23.9	0.78	28.5	33.9	0.75	0.23
Lactic acid (g/100 ml)	0.80	0.461	0.75	0.508	0.893	0.71	0.19
Acetic acid (g/100 ml)	0.58	0.210	0.50	0.231	0.359	0.71	0.19
Butiric acid (g/100 ml)	0.75	0.253	0.68	0.287	0.458	0.72	0.20

Instrument 1: Foss NIRSystem 6500 scanning monochromator; Instrument 2: On-site CORONA 45 VisNIR 1.7
RSQ and 1-VR: Determination coefficients for calibration and cross validation; SEC; SECV; SEP: Standard errors of calibration cross validation and prediction respectively. Global and neigborhood H Mahalanobis distances (GH and NH).

Drip loss determination in pork chops with NIR

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Introduction Drip loss (DL) is an important meat quality trait that is finally perceived by consumers as juiciness, appearance and color of meat. Therefore it is of special interest for breeding schemes, meat processing industry and retailers. Limited results have been obtained in literature for the prediction of DL by NIR [1], with ratios of prediction (RPD) lower than 1.45, independently of the type of meat (beef or pork), the sample preparation (homogenized, minced or intact meat slices) or NIR using transmission or diffuse reflectance spectroscopy. The aim of this study is to evaluate the ability of NIR to predict drip loss in intact pork-chops.

Materials and Methods From 94 carcasses of castrated and female Swiss Large White pigs 25 mm thick pork loin samples were collected 1 and 24 h postmortem (pm). They were kept at 4°C until NIR and drip loss analysis. Drip loss was determined after 48 h using the bag method. The average drip loss percentage amounted to 4.34 ± 1.81% with minimum and maximum values of 0.94 and 11.35% respectively. The samples were also analyzed with diffuse reflectance NIR spectroscopy (FT, NIR 500, Büchi, Switzerland) at 1, 24 and 48 h pm, at room temperature. Intact slices were placed on a special flat accessory, comprising a sampling window of 3.5 cm in diameter. Spectra were recorded at 4 different spots on each slice, for each maturation time. Spectra were recorded scanning from 4000 to 10000 cm^{-1}, at 8 cm^{-1} intervals. A total of 32 scans were averaged for each spectrum. Calibration models were established with ~2/3 of the samples, evenly distributed over the available DL range, the other ~1/3 of samples was used as an independent set for validation. The NIRCal software, provided by Büchi with the instrument was used to establish predictive models. 1h pm: PLS model with multiplicative scatter correction full (mf) and 1st derivative BCAP 4 points, 5 PCs; 24h pm: PLS model with 1st derivative BCAP 4 points, 5PCs; 48h pm: PLS model with mf and 1st derivative BCAP 4 points, 8PCs; 48h pm (first spot per sample): PLS model with mf, 13 PCs.

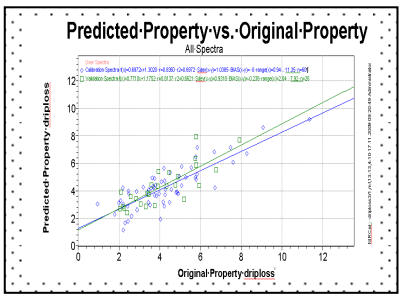

Figure 1. Predicted vs. original drip loss of 94 intact LD pork slices, 48 h after slaughter.

Results and Discussion Comparable to literature results were found for drip loss in intact pork slices when an average of 4 scanning spots per sample were used, table 1. However, the prediction quality could be considerably improved by using only the first scanning spot per sample. Thus, a slope of 0.8 together with an R of 0.81 (Table 1) were found for the validation set of samples analyzed 48h pm (first spot only, Figure 1). Furthermore, in this case a SEP of 0.93 enabled the value of 1.95 for RPD. Some authors mentioned the lack of homogeneity of intact meat slices as a determining factor for the poor quality observed of NIR predictive models for meat quality parameters. Prieto et al. [1] mentioned the possible scattering action of muscle fibers or myofibrils in intact meat slices acting as optical fibers. Hence they stated the improvement of NIR predictions by homogenizing the meat samples. However, homogenization of meat samples will severely disrupt the structure of the muscle, thus altering the water holding capacity of meat and thereby affect the drip loss. Furthermore, the ability of NIR to predict the chemical composition as well as the meat quality traits in intact samples is of great importance to meat industry and research. To broaden the DL range could help improving NIR predictions, however this is not easy to achieve with natural samples. Finally, NIR being a secondary procedure is directly dependent on the reference method used for calibration. In this respect, the reference method for DL determination needs to be strongly standardized in order to reduce the method uncertainty and to be able to compare results between different laboratories.

These results show the great potential of NIR to predict drip loss in intact pork slices which could be profitable to selection studies as well as to the meat industry.

Table 1. NIR model characteristics for the prediction of drip loss in intact pork-meat slices.

NIR DL	#spots per sample	range[cm^{-1}]	R_{CAL}	R_{VAL}	$Slope_{VAL}$	Bias	SEC	SEP
1 h postmortem	4	4000-9000	0.67	0.54	0.4	-0.16	1.25	1.31
24 h postmortem	4	4000-9000	0.79	0.53	0.5	-0.16	1.98	1.43
48 h postmortem	4	4000-9000	0.79	0.62	0.4	-0.11	1.02	1.13
48 h postmortem	1(first)	4000-10000	0.84	0.81	0.8	-0.24	1.04	0.93

#spots per sample: number of spots analyzed per sample, taken as sample replications.

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Quality Assessment of Small Grain Accessions by Means of Near - infrared Reflectance Spectroscopy on a Mobile At-line System

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Introduction
In plant breeding programs mobile at-line NIRS systems offer the opportunity to determine dry matter content and quality traits promptly after harvesting without the need to mount each combine harvester with a costly NIRS system. In a joint project of diverse German plant breeding companies with the Julius Kühn-Institute and the University of Hohenheim the potential of NIRS on a mobile at-lines system (figure 1) was assessed for determination of dry matter, crude protein, and starch content of wheat, barley, and triticale grains.

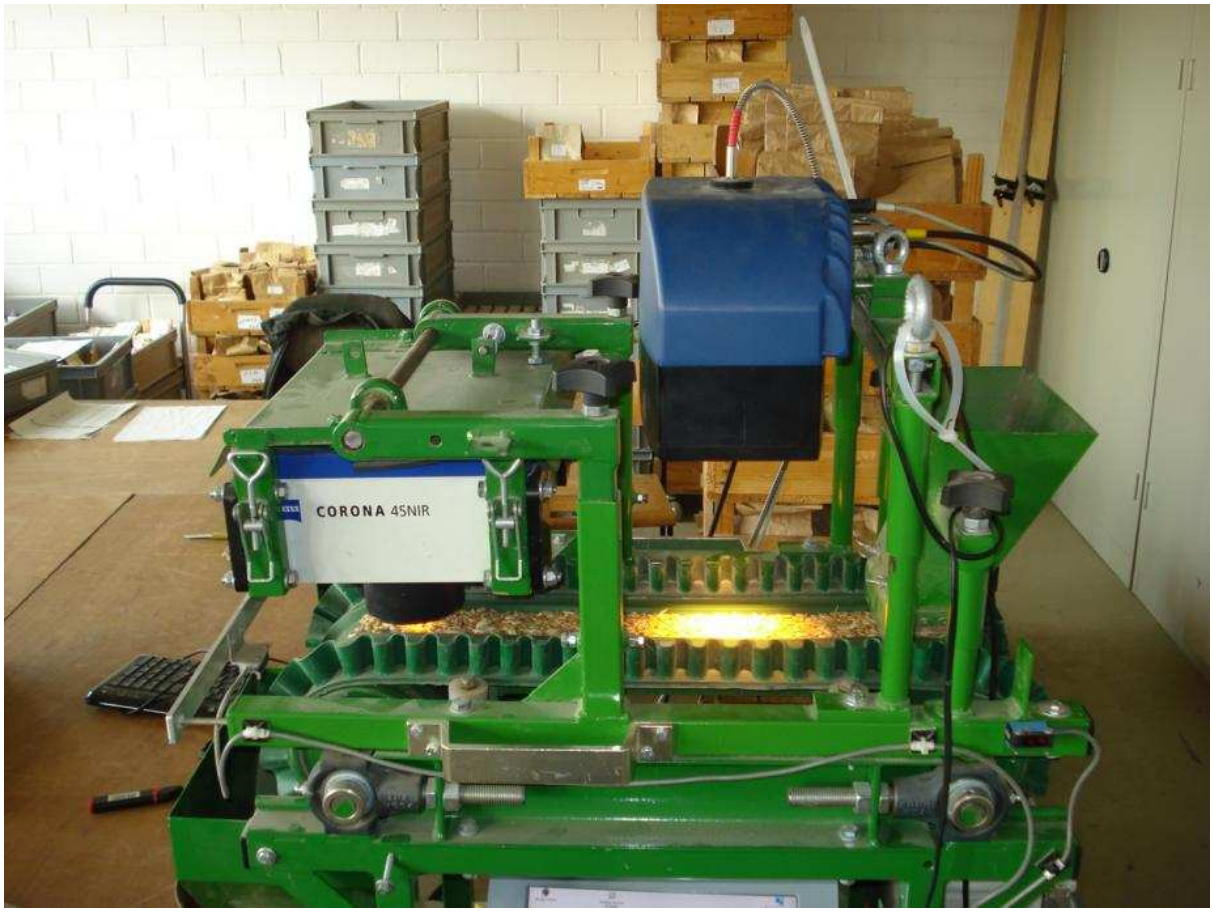

Figure 1. Combined at-line system with Zeiss Corona and Polytec NIR spectrometer.

Materials and Methods
In 2008 and 2009 samples of wheat, barley, and triticale were investigated at diverse locations from different plant breeding companies promptly after harvesting. Sample size was about 0.5 to 1.0 kg. The at-line system was equipped with a Zeiss Corona 45 near infrared diode array spectrometer (Carl Zeiss Jena GmbH, Jena, Germany), and a Polytec PSS-2120 near infrared InGaAS diode array spectrometer (Polytec GmbH, Waldbronn, Germany). NIR spectra were collected in the 960-1690 nm (Zeiss) and 1100-2100 nm (Polytec) spectral range. For the Zeiss spectra, a spectral filter was used to eliminate spectra indicating the empty conveyor belt, by removing all spectra with absorbance values higher than a threshold set for defined wavelengths. Due to different software packages this was not possible for the spectra collected with the Polytec system. Mathematical procedures on the spectral information and calibration development were performed with software package Calibration Wizard 0.8.9 (Sensologic GmbH, Norderstedt, Germany). Calibrations models were developed using partial least squares (PLS) regression and cross-validating

techniques. A total of 60 mathematical spectra pretreatments were tested for each calibration. About 10 % of all samples were randomly selected and used as validation set. Reference values were provided by the plant breeding companies mainly based on laboratory NIRS.

Results and Discussion

Both NIRS systems showed comparable results for all investigated traits (table 1). Among the traits investigated, dry matter content showed the highest potential for determination by NIRS followed by protein content. We could not develop good calibrations for starch content. Additional investigations have to show whether this is a problem of the reference values or of the NIRS technology.

The results demonstrate that the addition of different locations and breeding materials can enhance the development of a calibration method. As an example, figure 2 shows rather different values for protein content at the different environments, or breeding companies, respectively. However, the reference data originated from different breeding companies, and thus from different labs. Supplementary studies have to show whether there is an important impact of the reference labs.

Table 1: Calibration and validation statistics for the mathematical treatment with the lowest SEP value.

			Loc.	Loc.	Calibration			Validation			
Crop	Trait (% DM)	Range	2008	2009	N	R_{cal}	SEC	N	SEP	RPD	R_{val}
Zeiss-Corona											
Wheat	Protein content	9.4-14.3	4	3	1724	0.87	0.54	192	0.49	2.15	0.88
Wheat	Starch content	62.2–71.6	4	1	1533	0.87	0.76	171	0.70	2.05	0.87
Wheat	Dry matter	12.0-22.1	1	3	850	0.98	0.43	95	0.39	5.77	0.99
Barley	Protein content	8.0-15.5	4	2	449	0.96	0.35	50	0.30	3.84	0.97
Barley	Dry matter	11.8-16.2	1	2	223	0.97	0.24	25	0.19	5.24	0.98
Triticale	Protein content	9.9-17.7	5	2	890	0.98	0.28	100	0.31	4.55	0.98
Triticale	Dry matter	8.5-15.1	1	1	448	0.98	0.28	51	0.25	6.64	0.99
Triticale	Starch content	63.5-72.9	5	1	1136	0.89	0.68	127	0.73	2.05	0.87
Polytec-PSS2120											
Wheat	Protein content	9.4-14.3	4	3	1724	0.88	0.52	192	0.44	2.35	0.90
Wheat	Starch content	62.2–71.6	4	1	1533	0.87	0.77	171	0.74	1.93	0.85
Wheat	Dry matter	12.0-22.1	1	3	849	0.98	0.49	95	0.45	4.94	0.98
Barley	Protein content	8.0-15.5	4	2	350	0.96	0.38	39	0.35	3.80	0.97
Barley	Dry matter	11.8-16.2	1	2	223	0.96	0.26	25	0.19	5.21	0.98
Triticale	Protein content	9.9-17.7	5	2	892	0.98	0.29	100	0.33	4.40	0.97
Triticale	Dry matter	8.5-15.1	1	1	452	0.99	0.26	51	0.25	6.86	0.99
Triticale	Starch content	63.5-72.9	5	1	1134	0.90	0.67	127	0.72	2.09	0.88

DM = dry matter, Loc. = location, N = number; R_{cal} = correlation coefficient of calibration; R_{val} = correlation coefficient of validation, SEC = standard error of calibration, SEP = standard error of prediction, RPD = ratio of performance deviation.

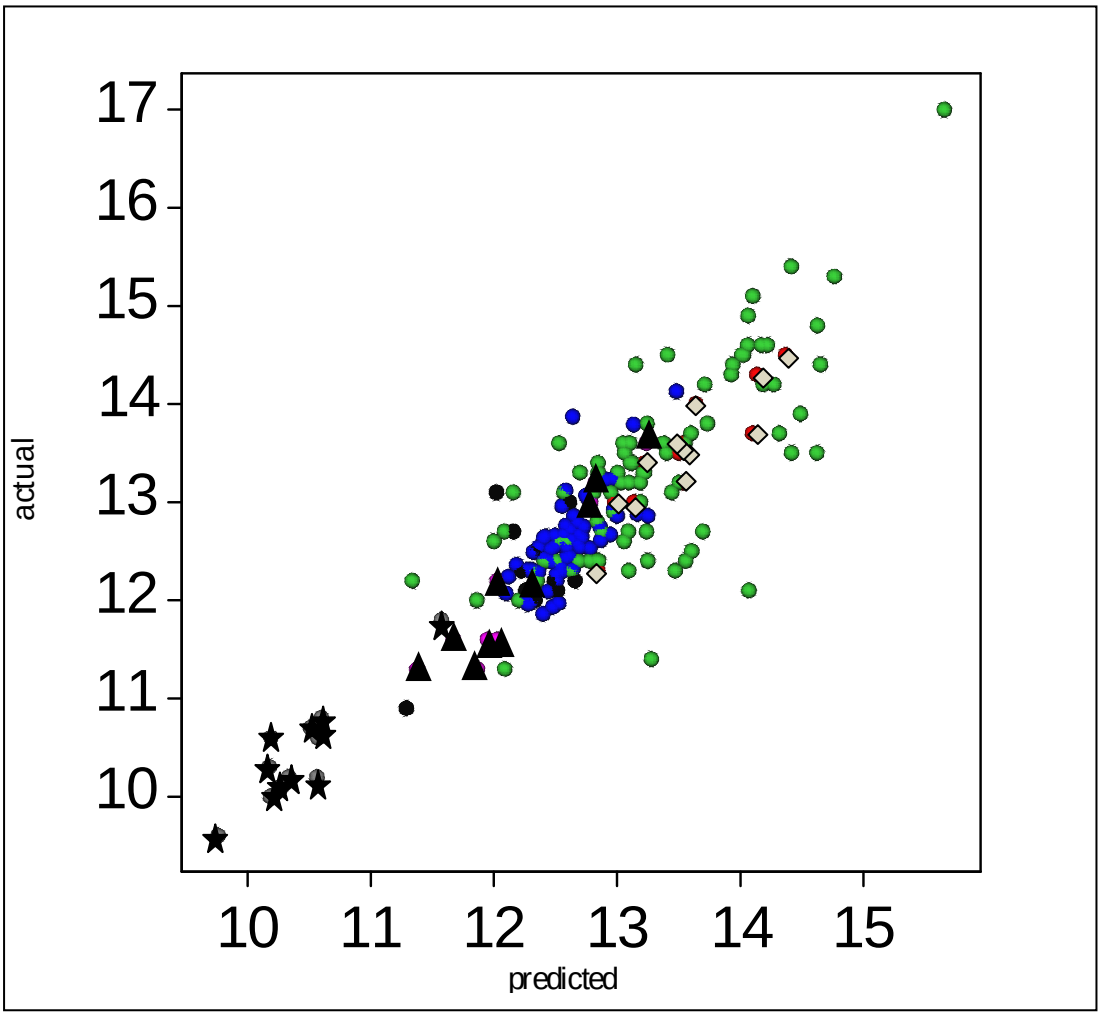

Figure 2: NIRS prediction (predicted) vs. reference values (actual) of protein content for wheat grains, investigated at six locations/breeding companies. Different symbols or colors show the different locations. NIRS spectra were collected with the Polytec spectrometer.

Acknowledgements
This research project has been carried out with the financial support of the Federal Ministry of Economics and Technology (BMWi), Germany, administered via the Arbeitsgemeinschaft industrieller Forschungsvereinigungen e.V. (AiF) and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP).

Soil Carbon Monitoring with NIR: Potentials and Challenges

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Introduction

The NIR spectroscopy technique for measuring agricultural soil properties is an investigation method that is relatively new. In the last decades NIR was mostly used to determine the quality of field crops, eg. silage maize and wheat[1,2,3]. But the soil itself plays a very important role, not only as a basis for agricultural crops production in terms of supply and retention of nutrients and moisture but also for the climate. The global carbon cycle shows that soil can store carbon and also set carbon free, whereupon the emerging carbon dioxide is one gas of the greenhouse gases[4]. NIR spectra can be used to estimate several important soil parameters such as organic carbon, nitrogen, texture and humus fractions (lignin)[5]. Global agriculture produces big amounts of the greenhouse gas. The Kyoto protocol sets binding targets for industrialized countries to reduce the greenhouse gas emissions and all greenhouse gas emission from the agricultural sector have to be reported on a annual basis to the UNFCCC. Up to now, most of the Annex I countries have not reported data for carbon stock changes in soils, so this is an insufficiently determined factor[6]. The heterogeneity of soils, the monitoring on different scales and time consuming field and laboratory measurements hamper a quantification of soil carbon stock changes on regional and national scales.

The Johann Heinrich von Thünen Institute is responsible for the German greenhouse gas inventory from the land use sectors and acts a consultant for the German federal ministry of food, agriculture and consumer protection. The development of NIR on the go techniques to monitor carbon in agricultural soils is one of our institutes´ projects. Our intention is to find out the areal distribution of carbon in soils near to the surface and down to one meter on field scale. These investigations should go fast so that various fields can be examined. The expected output will be a better understanding and estimation of carbon stocks and changes in soils in lower Saxony and Germany.

Materials and Methods

In order to investigate carbon in soils we used the visible (VIS) and near infrared (NIR) spectroscopy in a measurement range from 350 to 2200nm in absorbance mode with a resolution of 8nm[7]. Two sensors could achieve the mentioned nm-range: one for the VIS-range and one for the NIR-range. Therefore we worked with the ocean optics software for the VIS and the Hamamatsu software for the NIR. Both programs were integrated into one software, the Veris spectrometer software, so that the handling was easy and fast[8].

Spectrophotometer and software are from the Veris technologies enterprise in Kansas, USA. They produce innovative sensors that measure important soil variability within farm fields.

The very special feature within our field analysis is the on-the-go-measurement with two techniques: the shank for analyzing the upper part of the soil (spatial variation) and the probe for the vertical distribution of soil carbon down to one meter. Our spectrometer-shank is being directly mounted onto a drawbar with the tractor (Fig.1), whereupon the probe shows a direct connection to the tractor without using a front tongue (Fig.2).

Figure 1. The Veris-shank with optical unit, the subsoiler chisel, six blade coulters for measuring electric conductivity, the spectrometer and auxiliary case, two closing discs, the GPS.

Figure 2. The Veris-Probe with the foot as a robust column that connects the hydraulic arrangement with the NIR-drilling rod and window, the spectrometer and auxiliary case, the GPS.

The subsoiler of the shank penetrates the soil to a required depth (5-12 cm) and the optical sensor can be pulled throughout parallel lines with e.g. 8m distance between the lines. A chisel in the front makes a trench, through which the optical unit gets smoothened. During these measurements the NIR-window is directly in contact with the soil all the time through a self-cleaning sapphire window. Driving with 4 km/h the spectrometer took one spectra of the soil every 6 cm. The closing discs in behind close the trench to keep a relatively planar surface without significant trenches. The GPS, that is mounted upon the auxiliary case plate, gives information about the position of the shank. The advantage of this method is the rapidness of doing measurements, so that you can reach field scale measurements of soil properties without being limited in a certain number of soil samples, as you are in laboratory NIR-measurements. Furthermore the low costs analysis, the direct contact between the optical window and the soil, establishing a self-cleaning system, and also examinations that can be done after a short time of practice are remarkable characteristics of the Veris shank indicating the high potential of this instrument to apply NIR on the go techniques for mapping of spatial distributions of soil characteristics on field scale.
The probe facilitates the characterization of soil profiles by adding to the Veris-shank data the third dimension. The spectrometer collects NIR reflectance as a probe with an optical window that is pushed into the soil profile. The same spectrometers can be used for the probe as they are used in the shank system. The force to reach a depth of maximum 95 cm is being generated by the hydraulic arrangement of the tractor. As an additional parameter, the force that is needed to push the probe into the soil is recorded with a cell at the bottom of the probe. During field measurements the optical sapphire window is in direct contact with the soil, as it is for the shank. At the bottom of the probe there are also electric conductivity (EC) contacts, collecting the EC-data. Both instruments combined, the shank and the probe, lead to a three-dimensional view into the soil.
The spectrometer software from Veris provides one more special function: it can combine the information of the spectra, taken with the shank, with the GPS data in terms of filtering and clustering and calculates with a fuzzy algorithm the most representative locations of the measured field, where soil samples should be taken, covering the whole measured spectra range. The soil data is necessary for the following calibration and validation that has to be done for each measurement campaign in order to account for seasonal changes in soil moisture. Soil samples for calibration and validation of the probe results were taken via machine driven coring (8.7 cm diameter).
The above mentioned auxiliary case provides additional data along with the spectra measurements. The additional data is the soil temperature, the achieved coring depth, the driving speed of the tractor, the force needed during the drilling process, the EC-data.

Results and Discussion

Our first investigations on a 9 ha cropland site were successfully performed. Via the shank we measured 19 lines with a distance of 8m, got 4806 spectra and took 36 calibration soil samples. Via the probe we used a 24*24m grid, got 3116 spectra and took around 100 calibration soil samples from 12 locations via a 24*72m grid.
The next step will be the carbon and nitrogen analysis of the calibration samples in the lab using dry combustion technique. A calibration for the recorded NIR spectra has to be generated using also the additional measurements such as electric conductivity. New calibration techniques such as booted regression trees are going to be investigated in order to explore the full potential of the Veris instruments.
The project is in its starting phase and will aim at answering the following questions:
Temporal dynamic of soil organic carbon:

1. How is the reproducibility of soil parameters such as organic carbon with the NIR on the go instruments from Veris (precision, accuracy)?
2. Can the temporal dynamic of soil carbon stocks be estimated with NIR on the go (Minimum detectable difference in soil carbon)?
3. Can the seasonal dynamic (soil moisture) be captured in a global calibration (beyond calibration of each field campaign)?

Footnote

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Ripening monitoring of plums using NIR-instruments

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Introduction

One of the main challenges in plum crop is to harvest the fruits at their optimal stage of ripeness allowing transport, storage and market distribution while ensuring taste and flavor that meet consumer's preferences. Nowadays, optimal harvest dates are mainly based on personal/individual evaluation of skin color, fruit size and softness. Leaving these critical decisions to subjective interpretation implies that some fruits are not harvested at their optimal ripeness stage and reach consumer markets in poor condition. Since monitoring and controlling of quality parameters like firmness, total soluble solids or acidity help to determine the optimal harvest date we decided to evaluate the NIR technology as a non-destructive tool for the ripening monitoring of plums. Two approaches were tested. (a) Partial least square regression (PLS) was evaluated to quantitatively predict total soluble solids, firmness and acidity. (b) Discriminant analysis (DA) was tested as a tool to classify plum fruits according to their picking date.

Materials and Methods

Plum fruit
Plums of the varieties Fellenberg, Jojo and Tophit grown in 2009 in the experimental orchards of the Agroscope Changins-Wädenswil ACW research station (Switzerland) were used for this study. Sets of 20 fruits at four different stages of ripeness were picked for calibration models giving a total of 240 plums. On-tree measurements were performed on the same 20 fruits of the variety Tophit during the whole ripening period.

NIR Spectroscopy
Spectra were collected using two NIR-instruments (1) a portable MEMS based spectrometer (Phazir, Polychromix, USA) and (2) a diode-array Vis-NIR spectrometer (NIR Case, SACMI, Italy). The Phazir is equipped with a reflectance configuration and covers the wavelength range from 900 to 1700 nm. Fruits are illuminated by a tungsten light source and the reflected radiation is measured by a single InGaAs photodetector. Two spectra were collected from both opposite sides of each fruit along the equator. The four spectra were averaged and the mean spectrum was used for the statistical analyses. On-tree measurements were performed only with this instrument.
NIR Spectra of plums were also acquired in the laboratory using a Visible-NIR spectrometer measuring in transmission mode in the wavelength range of 600 to 1000 nm (NIR Case). Fruits were placed on the fruit holder, with the stem-calyx axis horizontal, and illuminated by 4 halogen lights. Two spectra were collected from both opposite sides of each fruit along the equator and averaged.

Quality measurements
Firmness was measured with a texture analyzer instrument (TA-xT2i, Stable micro System, plexiglas plunger, diameter 25 mm, speed 6.7 mm/sec, compression depth 2 mm). Measurements were performed on the two opposite sides corresponding to the location of NIR-measurements. After firmness measurements, each plum fruit was separately kept at -20°C before overnight thawing. Then the fruits were mashed and centrifuged (10 min. at 4000 rpm, Multifuge 3SR+, Heraeus). An electronic refractometer (Atago PR32) was used to measure total soluble solids (TSS, °Brix). Titratable acidity was determined by titration (Mettler Titrator DL67) with 0.1 M NaOH to the endpoint of pH 8.1 and expressed as g malic acid/L.

Data analyses
Calibrations were established by means of the software package 'The Unscrambler (version 9.8, CAMO, Norway). Calibration models for total soluble solids, acidity and firmness were developed using partial least square regression (PLS) with full cross validation. Several pre-treatment options were investigated including spectral smoothing, SNV, MSC and first derivative. Phazir calibrations were done on the spectral range between 930 and 1650 nm while for NIR Case models the wavelength range from 650 to 970 nm was taken into account. Calibration performance was assessed in terms of R^2 and RMSECV values. Principal component analysis (PCA) and discriminant analysis (DA) were conducted on the spectral data resulting of the on-tree measurements of the variety Tophit. Picking dates were used as grouping variable. PCA were performed in 'The Unscrambler while XLSTAT (Addinsoft, Ver. 2009.4.03) was used for DA analyses.

Results and Discussion

Fruit quality

Measurements of quality parameters using classical destructive methods helped to monitor plums ripening and to determine the optimal ripeness stage in terms of total soluble solids, acidity and firmness. Figure 1 shows the evolution of these three quality parameters for the variety Tophit during 20 days. Acidity and firmness decreased during ripening while total soluble solids continually increased. These results are in accord with observations made on other plum cultivars (Usenik *et al.*, 2008 and Crisosto *et al.*, 2004).

Calibration models

We next compared the quality parameters calibrated on Phazir and NIR Case spectra for the cultivars Fellenberg, Jojo and Tophit. Based on the values of R^2 and RMSECV the Phazir instrument shows a slightly lower performance in terms of accuracy compared with NIR Case (table 1) but it is a portable device and therefore allows fruit measurements directly on the field. For total soluble solids the calibration models yielded higher precision based on NIR Case vs. Phazir spectral data (R^2 = 0.97 and 0.88, RMSECV = 0.40 and 0.85, respectively). This might be due to the spectral range of Phazir which doesn't include the bands between 600 and 900 nm related to C-H bonds and associated with sugars. This conclusion is supported by Pérez-Marìn *et al.* (2010) who compared a Phazir instrument (1600 - 2400 nm) with a diode-array instrument (400-1700 nm) measuring firmness and soluble solid content of plums. NIR Case spectra were also better for calibrating acidity and firmness (table 1). This may be because NIR Case measures in transmission modus while Phazir is equipped with a reflectance configuration and measures a considerable smaller part of the fruit. Paz *et al.* (2008) analyzed a total of 720 plums in the region 400-1700 nm and obtained better calibration models for TSS (R^2 = 0.77) as for firmness (R^2 = 0.52). The same observation was made on apples (Gabioud *et al.*, 2007). In spite of these findings calculating the mean per batches of 10-20 fruits enhanced the performance of the calibrations (R^2 □ 0.9).

On-tree measurements

During 7 weeks, the same 20 plums of the variety Tophit were weekly measured directly on-tree using the portable Phazir spectrometer. A PCA was performed on the spectral data pre-treated with a multiple scattering correction. The score and the loadings of the first two PCs are shown in figure 2. The spectral variation along the first principal component accounted for 82% of the total variability and was mainly related to harvest dates and therefore to ripening. The second principal component explaining 9% of the total variability improved the discrimination between harvest dates. These results showed that changes during ripening can be detected using a Phazir spectrometer.

We next confirmed this by doing a discriminant analysis (DA) where fruits were classified according to their harvest date (H). Stepwise forward model selection was performed on the significant wavelengths only. Except for the first harvest date (H1), the confusion matrix of a full crossvalidation shows that all the groups were perfectly classified (table 2). Wavelengths around 900 and 1400 nm were highly correlated with the factorial scores (data not shown). Our results are consistent with findings from Pérez-Marìn *et al.* (2010) who tested discriminant models to classify plums by variety using Phazir 2400 and obtained 96.5% correctly classified samples.

Conclusions

Monitoring plum ripening by means of a portable and non-destructive technique yielded promising results. However, for single fruit analysis the prediction models for total soluble solids, acidity and firmness were not yet satisfactory. Though using sample means according to commercial practise significantly improved the quality of the predictions, reaching similar (TSS) or even better (TA, firmness) performances than obtained using a lab diode-array spectrometer working in transmission mode. Moreover, NIR-technology has been successfully used to classify plums of the variety Tophit according to the harvest date. Further studies including analyses of other cultivars, origins and years should be conducted to improve models robustness.

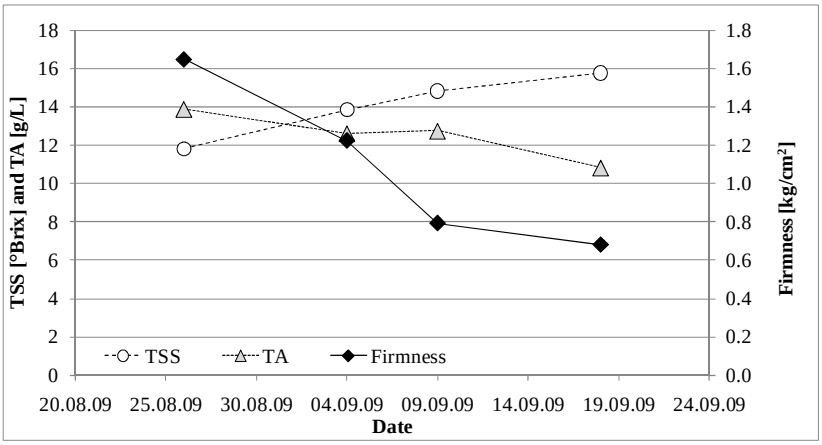

Figure 1. Ripening monitoring of plums cv. Tophit based on the evolution of total soluble solids (TSS), acidity (TA) and firmness using classical destructive methods.

Table 1. Comparison of the performance of NIR prediction models for total soluble solids (TSS), titratable acidity (TA) and firmness obtained by means of two different instruments (Phazir and NIR Case) for the cultivars Fellenberg, Jojo and Tophit. Evaluation in terms of R^2 and RMSECV after full crossvalidation. SG-1-5-2 = S. Golay 1st derivative, smoothing 5 points 2nd order.

Quality Parameter	Pre-treatment	Phazir				NIR Case		
		single fruit		batch		single fruit		
		R^2	RMSECV	R^2	RMSECV	Pretreatment	R^2	RMSECV
TSS [°Brix]	MSC	0.88	0.85	0.98	0.29	MSC	0.97	0.4
TA [g/L]	SG -1-5-2	0.58	1.46	0.92	0.57	SG -1-5-2	0.74	1.15
Firmness [kg/cm^2]	SG -1-5-2	0.75	0.31	0.96	0.11	MSC	0.8	0.28

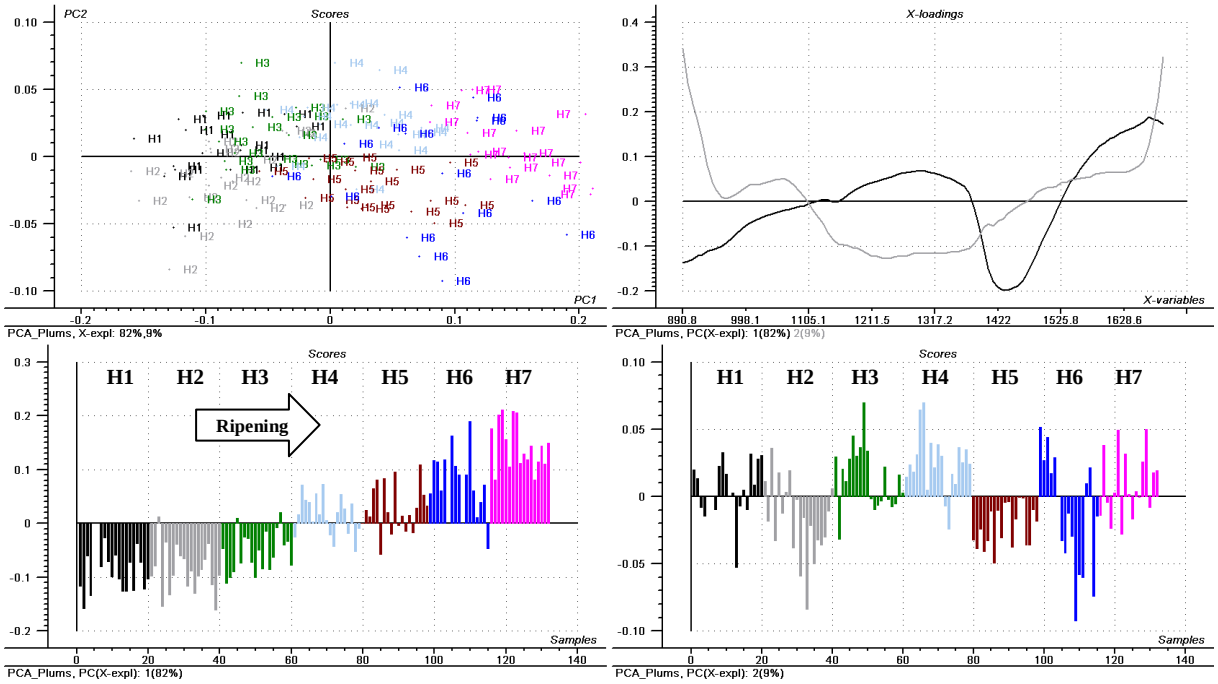

Figure 2. PCA analysis of spectral data (930 – 1650 nm) of 20 Tophit plums weekly measured on tree. H1 = 21.8.2009, H4 = 11.9.2009, H7 = 2.10.2009.

Table 2. Confusion matrix based on spectral data obtained with the portable NIR-instrument Phazir: classification of Tophit plums according to harvest date (H1 to H7).

from \ to	H1	H2	H3	H4	H5	H6	H7	Total	correct classification
H1	19	1	-	-	-	-	-	20	95%
H2	-	20	-	-	-	-	-	20	100%
H3	-	-	20	-	-	-	-	20	100%
H4	-	-	-	19	-	-	-	19	100%
H5	-	-	-	-	19	-	-	19	100%
H6	-	-	-	-	-	17	-	17	100%
H7	-	-	-	-	-	-	17	17	100%
Total	19	21	20	19	19	17	17	132	99%

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Use of NIRS for the prediction of the chemical composition of sainfoin Onobrychis viciifolia Scop.

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Introduction

Sainfoin is a forage legume adapted to cool climates, thriving well on basic and dry soils. It is much appreciated by farmers due to its high palatability, high nutritional value and non bloating properties. Sainfoin herbage contains variable amounts of condensed tannins that are implied in its nutritional characteristics and confer anthelmintic properties. Thus, their precise quantification, along with others parameters of chemical composition becomes crucial. The use of NIR technology for this characterisation is particularly relevant, especially since huge numbers of sainfoin samples are being generated by the efforts under way to select accessions from different European conditions. The aim of this work is to evaluate the suitability of NIRS for predicting the chemical composition and the content of condensed tannins of a collection of sainfoin samples

Materials and Methods

A total of 186 sainfoin samples obtained from several trials were used. Samples were oven-dried at 80° C for 48 h to determine dry matter (DM), ground through a 0.8 mm screen and then stored at environmental laboratory conditions. Approximately 5 g of ground sample were placed in a 50 mm diameter ring cup and scanned in reflectance mode at 2 nm intervals from 400 to 2498 nm using a Foss NIRSystems model 6500 scanning visible/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA) controlled by ISIscan software version 2.21 (Infrasoft International, Port Matilda, PA, USA). Each spectrum was time averaged from 32 scans.

Forage samples were analyzed for ash and crude protein (CP) according to AOAC (1990), for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to the method described by Van Soest et al., (1991). Analyses of condensed tannins were performed according to Porter et al., (1986).

Calibrations were developed using WinISI II version 1.60 (Infrasoft International, Port Matilda, PA, USA). The modified partial least squares (MPLS) regression method was used to obtain NIR equations for all the studied parameters. Spectra were subjected to standard normal variate and detrending (Barnes et al, 1989) as scatter correction, and transformed through a mathematical first order derivatisation (1,4,4,1) where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in the first smoothing, and the fourth is the number of data points in the second smoothing. Critical values for removing outliers were T=2.5, and two passes of elimination were allowed. The obtained models were evaluated by the coefficient of determination in calibration (R^2C) and cross-validation (R^2CV), the standard error of calibration and cross-validation (SEC and SECV) respectively and the residual predictive deviation (RPD) that was defined as the ratio between the SD and the SECV (Williams and Sobering, 1996).

Results and Discussion

The descriptive statistics for ash, CP, the partitioning of structural carbohydrates by the detergent systems and CT were shown in Table 1. A wide variability was observed in all studied parameters.

Table 1: Descriptive statistics for ash crude protein (CP) neutral detergent fibre (NDF) acid detergent fibre (ADF) acid detergent lignin (ADL) and condensed tannins (CT) in sainfoin samples (g/kg dry matter (DM))

	Nº	Min	Max	Mean	SD
Ash	145	65.2	159.5	108.2	17.35
CP	164	135.5	307.5	206.11	36.89
NDF	171	138.4	522.0	347.3	98.95
ADF	173	160.6	375.0	255.3	49.09
ADL	170	58.1	124.5	83.3	13.05
CT	136	0.65	4.32	2.05	0.712

Nº= number of samples; SD: standard deviation; Min= Minimum value; Max = Maximum value; SD = standard deviation

Table 2 summarizes the statistical values for the calibration equations obtained for the different determinations. R2C, R2CV and RPD values obtained for CP, NDF and ADF indicate that the prediction models have a very good precision, whereas prediction models obtained for ash and ADL are not capable of predicting these parameters adequately. Statistical results obtained for CT were also considered satisfactory, although RPD values did not reach 3, the obtained value was considered acceptable for screening purposes as well. Similar coefficients of determination values were found by Smith and Kelman (1997) for the prediction of CT concentrations in *Lotus uliginosus* Schkuhr.

Table 2. NIR calibration and cross-validation statistics for ash, crude protein (CP), neutral detergent fibre (NDF) acid detergent fibre (ADF) acid detergent lignin (ADL) and condensed tannins (CT) of sainfoin samples (g/kg DM)

	Nº	**SEC**	**R^2C**	**SECV**	**R^2CV**	**RPD**
Ash	145	10.37	0.64	11.29	0.58	1.54
CP	164	8.58	0.95	9.37	0.94	3.94
NDF	171	20.8	0.96	23.3	0.95	4.25
ADF	173	12.4	0.94	13.8	0.92	3.56
ADL	170	7.08	0.71	7.62	0.66	1.71
CT	136	0.27	0.86	0.32	0.80	2.23

Nº= number of samples; SEC= standard error of calibration; R^2C= coefficient of determination in calibration; SECV= standard error of cross-validation; R^2CV = coefficient of determination in cross-validation; RPD=residual predictive deviation;

We conclude that NIR equations developed for determination of CP, NDF, ADF and CT were adequate for the chemical characterization of sainfoin accessions. The use of NIR technology may increase dramatically the time and cost efficiency of sainfoin germplasm screening, thus facilitating the current pre-breeding programs under way.

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Title: Online Near Infrared Classification of Iberian Pigs Carcasses According to their Feeding Regime

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[Introduction] The quality, sensory characteristics and healthy properties of the Iberian pig products are highly related with the fatty acid profile of the meat and fat. The genotype, age, rearing conditions and mainly the feeding regime of the animals during the final period of growing influence in that quality. The Spanish legislation determines different commercial categories depending on genotype, production system and feeding regime. According to the feeding regime, there are three main categories: Acorn-Bellota, Recebo and Feed-Cebo which have some differences in their fatty acid composition. On-farm inspection and gas chromatography are officially used for classification purposes, however industry and consumers demand fast, accurate, objective and low cost authentication methods that informs individually of each animal. Near Infrared Spectroscopy (NIRS) has shown its viability to be a suitable at-line tool for this purpose. The aim of this work was to evaluate the performance and accuracy of a handheld NIRS instrument to classify Iberian pig carcasses according to the feeding regime of the animals during the final period of growing in an on-line application.

[Materials and Methods] One hundred and twenty five Iberian pig carcasses were used (41 of the category Acorn-Bellota, 34 of Recebo and 50 of Feed-Cebo). Reflectance spectra were taken with a handheld micro-electro-mechanical (MEMS) spectrometer (Phazir 2400, Polychromix Inc., Wilmington, MA, USA) from the subcutaneous adipose tissue located in the tail insertion area in the coxal region of the body directly from the carcasses two hours after the animals were slaughtered. The instrument measures from 1600-2400 nm. Chemometric calculations were carried out in WinISI ver 1.50 (Infrasoft International, Port Matilda, PA, USA). Principal Component Analysis (PCA) was used to visualize the structure of the data set, possible category cluster and outlier samples. The data set was divided in two: a training set (N=95) and a validation set (N=30, 10 samples per each category). These samples were selected using the SELECT algorithm by Shenk & Westerhaus included in the WinISI software. Discriminant analysis based on Partial Least Squares (PLS2) was used to develop the discrimination models. First (1,10,5,1) and second (2,5,5,1) derivative together with Standard Normal Variate (SNV) and Detrending (DT) was used as spectral pretreatments. Cross-validation with 4 groups was used to optimize the number of model factors.

[Results and Discussion] The spectra of the adipose tissues analyzed in the carcasses show a similar pattern independently of the animal category. It was observed different absorption peaks related to fat (1720-1760, 2150 and 2310-2340 nm) and water (1940 nm). The PCA score plot (10 PCs explaining the 99.09% of the variability for a second derivative) showed three different clusters, although there was slim overlapping between them (Figure 2). Table 1 shows the prediction results of the training set using PLS discriminant for a second derivative. A second derivative showed better classification results compared to a first derivative (not shown). The prediction as Acorn-Bellota category showed 96.77% of samples correctly classified, 97.5% for Feed-Cebo and 100% for Recebo. Table 2 shows the external validation of the above models. A second derivative provided larger number of samples correctly classified than a first derivative with only two samples misclassified of Feed-Cebo as Acorn-Bellota. These results indicate the viability of on-line authentication of Iberian pig carcasses at the slaughterhouse according to the feeding regime of the animals. However, models with highest number of samples are being developed. Furthermore, the handheld spectrometer needs further investigations to optimize its applicability and accuracy.

Figure 1. On-line measurement of Iberian pig carcasses

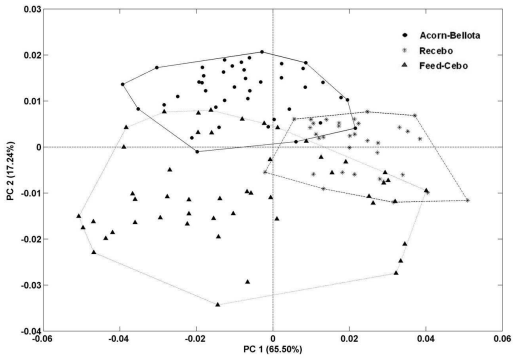

Figure 2. PCA score plot for the two first principal components (2nd derivative)

Table 1. Classification results obtained by PLS Discriminant for a second derivative

Training prediction			**Classified as**		
		Number of samples	Acorn-Bellota	Recebo	Feed-Cebo
Origin	Acorn-Bellota	31	30	0	1
	Recebo	24	0	24	0
	Feed-Cebo	40	1	0	39

Table 2. PLS discriminant validation performance for a second derivative

External validation			**Classified as**		
		Number of samples	Acorn-Bellota	Recebo	Feed-Cebo
Origin	Acorn-Bellota	10	10	0	0
	Recebo	10	0	10	0
	Feed-Cebo	10	2	0	8

Development of a graphical user interface (GUI) for on-line NIRS analysis of feed ingredients at the reception level in a feed mill plant.

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[Introduction]Real-time NIRS analysis of feed it is by far more complex that the development of an application for at-line laboratory analysis. A key issue that must be considered in the implementation of NIRS instrument for on-line analysis is the need of a simple software platform, for fast generation of analytical and statistical reports after one spectrum is read. This paper describes a graphical user interface (GUI) developed in the LabVIEW^{TM} environment to control a CORONA NIRS instrument and performs on-line analytical predictions and statistical data of feed ingredients at the reception level in a feed mill plant. Simulation and real time results will show that the developed GUI produces very fast useful results by using a friendly and practical interface. The idea of this work was to develop a flexible software tool for online prediction, capable to control different spectrometers with the same program.

[Materials and Methods] The device used to scan the samples was the CORONA 45 VISNIR (Carl Zeiss, Inc.), diode array spectrometer that took reading from 380nm to 1690nm, every 2nm. The spectral region used was 1100nm to 1690nm. Absorbance values were recorded as log(1/R), where R was reflectance. Dark and white references were taken manually by requirement of the program. LabView^{TM} Professional Ed. version 8.6 (National Instruments, Inc.) together with MCS5xx32 libraries (release 1.05) provided by Carl Zeiss Gmbh were used to develop the application. Chemometric calibrations were obtained by using SIMPLE PLS algorithm and global (GH) and neighbors (NH) distances were calculated by means of Principal Component Analysis (PCA) and Mahalanobis distances (MD) based on eigenvalues. The PLS, PCA and MD algorithms were implemented under LabView^{TM} Environment. MATLAB (version 7.0, the Mathworks, MA, USA) was employed to compare results. First and second derivative (Norris derivative), SNV, detrending and smoothing (Savitzki-Golay smoothing) were applied with different configurations. Leave-one-out was used as cross-validation method.

[Results and Discussion] A new software application presented like a user friendly and easy to use GUI for the on-line prediction of chemical composition has been developed. It was implemented to work together with a CORONA spectrometer. The selection of this spectrometer is due to his fast acquisition time, wide measuring area (window) and the availability of libraries for applications developers. These characteristics are quite interesting in case of online measurements because they allow the building of new robust applications adapted to the requirements of the problem.

MSC5xx32 libraries can run under LabView^{TM} environment. These libraries are the basis for all new applications and they manage physical and configurations parameters of the device. LabView^{TM} is a quick and powerful tool to develop new software. Its Visual programming allows a very fast development of applications and gives the possibility to control easily all the process. The developed application manages the configuration (acquisition times, wavelengths, averages, etc.), spectra collection, pretreatment, calculations, results showing, data storing and reports generation (Fig 2). The selection of different pretreatments and prediction models is easy and automatic in some cases. In this case the PLS algorithm is used for predictions. GH and NH are calculated after Principal Component Analysis (PCA).

With this kind of software there is possible to generate quickly new subroutines (VIs) based on different algorithms, like PLS, LWR, Bayesian, etc., and to incorporate these models into the program easily. Spectra collection, calibration, implementation of new algorithms and online prediction can be done in the same software. Further works are needed to implement new prediction models and spectral pretreatments.

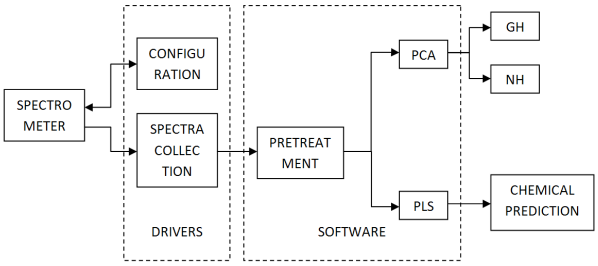

Figure 1: Diagram of the program structure.

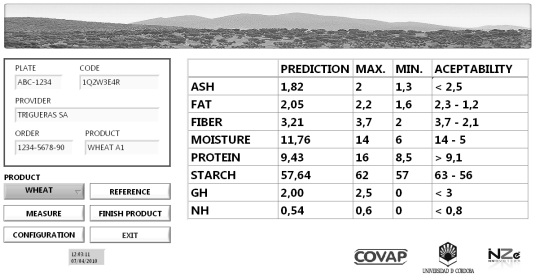

Figure 2: Graphical user in

Non-destructive non touch Visible-NIR transmittance spectroscopy for identification of Fresh and Frozen-thawed Fish

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Introduction

The online evaluation of the quality and freshness of the seafood is usually done through the employment of trained operators. This practice is costly in economic terms and requires a period of training of technicians. For certain frauds (eg. defrosted as fresh) also are not yet available objective assessment criteria, particularly for products already processed, sliced or filleted. The handling of the product reduces the shelf-life by making the fish susceptible to microbial growth. This study was carried out to evaluate the discrimination power of a portable Visible-NIR transmittance spectroscopy on fresh and thawed fish products.

Materials and Methods

Two products were studied: whole red mullet (*Mullus barbatus*) and swordfish cutlets (*Xiphias gladius*). For each product 80 fresh (F) and 80 frozen–thawed (T) samples were used as a calibration set, while another set was used to build up a validation set (F = 71; T= 71). The number (N) of samples for validation was chosen according to the formula $N = (1.96/e)2\ \theta\ (1- \theta)$ where e is the accepted error of 0.07% and θ is a presumptive sensitivity (se) and specificity (sp) value of 0.9. T samples were obtained by quickly cooling down to -80 °C and then stored at -20 °C for 6, 5 and 4 weeks respectively. Visible-NIR spectra were collected in transmittance mode between 300-1100nm using a diode array spectrometer MMS1 (Zeiss) customized in a portable device. The absorbance data have been considered between 700 and 1080 nm and developed by the MSC (Multiplicative scatter correction) algorithm using also a derivative treatment of 2^{nd} degree with a gap of 5 nm and a 5 points smoothing treatment. All calculations were performed using WinISI II (Infrasoft International, Port Matilda, USA). Discriminant analysis was performed by PLS2 (Partial Least Squares) multivariate analysis method using cross-validation. The robustness of calibration models was evaluated on the external validation data sets. For each sample (whole fish or whole cutlet) was assigned a dummy dependent variable according to cold treatment (1 for F Fresh and 2 for T Frozen-thawed), using a cut-off of 1.5 to classify samples. During validation tests a threshold between 1.65 and 1.35 was also chosen to identify the uncertain classification of samples. The positive predictive value (PPV) and negative predictive value (NPV) were calculated by using a free software WinEpiscope 2.0. PPV can be described by the following formula: n° of true positives/(n° true positives + n° of false positives) and is also called precision. The NPV consider the proportion of negative samples correctly classified.

Results and Discussion

The study of calibration data sets by principal component analysis (PCA) showed the presence of two distinct clusters (Fresh vs. frozen thawed) in both calibration groups (Figures 1-2).

The results of both validations are reported in table 1. The positive predictive values PPV (the probability that a positive test reflects the correct identification of positive samples) were very high with similar upper and lower limits. However it is necessary to highlight that this validation parameter depends on the prevalence of the studied cases (number of thawed samples; T). In this trial the prevalence of 50 % of positive case (T) was taken as assumption. In terms of Sensitivity (se) the validation on whole red mullets provided the best result (se. *Mullus barbatus* 97.2 % vs. 90.9 % *Xiphias gladius*).This suggested some lacks of Visible-NIR transmittance in the classification of processed seafood. The Specificity (sp), the proportion of negative samples (Fresh; F), is similar between validations (98.5 %) meanwhile NPV is lower in the swordfish (table 1). The performance of classification between Fresh and Thawed samples were reported in Figures 3-4. The Scatter plot reported the values of Visible-NIR transmittance prediction according to the dummy variables in the PLS analysis. The threshold evidenced an high number of uncertain samples in swordfish cutlets validation sets. This result corroborates the trouble in identification among sliced fish products. Other authors have tested the ability of Visible-NIR spectroscopy to discriminate between fresh and frozen-thawed fish demonstrating interesting performances on whole *Pagrus major* (100% of 108 fish tested) classification (1). For the fish analysis the authors used a surface interactance fiber-optic without MSC correction treatment on spectra (raw data). The model applied in the present calibration considered 80 samples for each treatment (Fresh vs. Thawed) and a training set of 142 samples. This higher number of tested samples can describe the effectiveness of the NIR in authentication of fish. In the surface interactance measurements (1) it was necessary remove the mucus and moisture on the skin, however the use of

transmittance avoids the surface contact and permit a continuously acquirement of the spectra. Those characteristics could be easily applied in industry.

These results show the feasibility of Visible-NIR non touch non destructive analysis in authentication of fresh and frozen fish products. The validation parameters suggest that Visible-NIR could be utilized as a screening method for the quality control on-line.

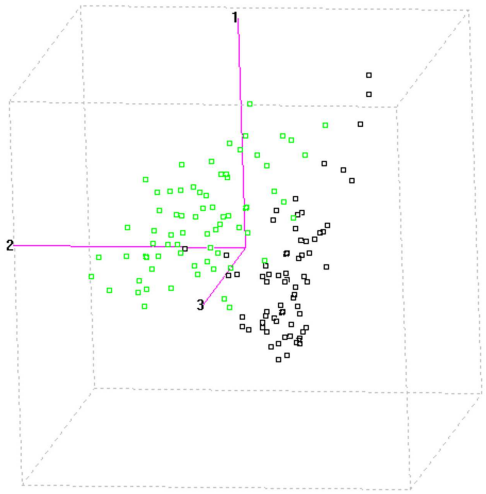

Figure 1. Score plot of PC1, PC2 and PC3 of the calibration set for Whole Red Mullet. □ Fresh ; □ Thawed

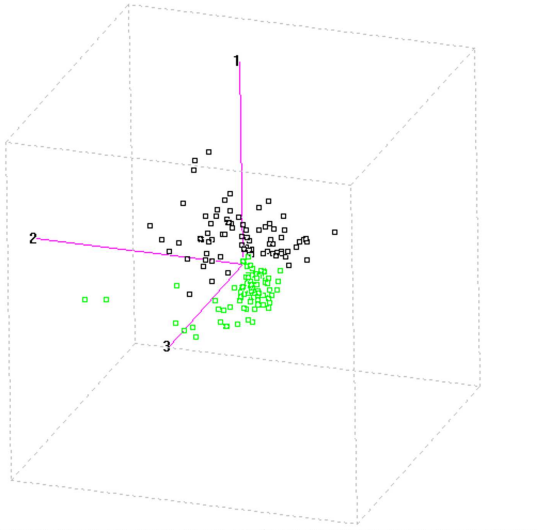

Figure 2. Score plot of PC1, PC2 and PC3 of the calibration set for Swordfish cutlets.□ Fresh ; □ Thawed

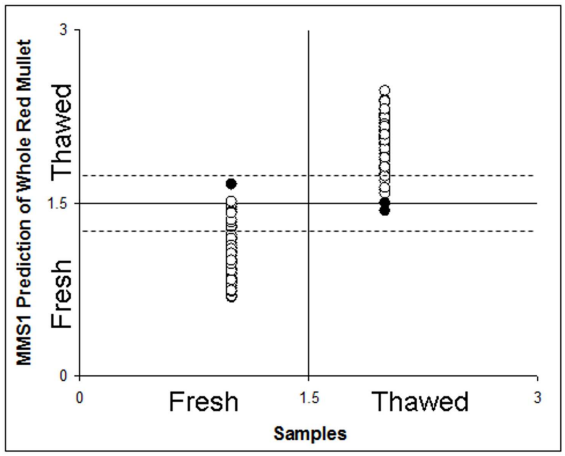

Figure 3. Scatter plot of Visible-NIR transmittance classification for Whole Red Mullet. Black spots were misclassified samples; The dotted lines evidenced a uncertain threshold between 1.65 and 1.35.

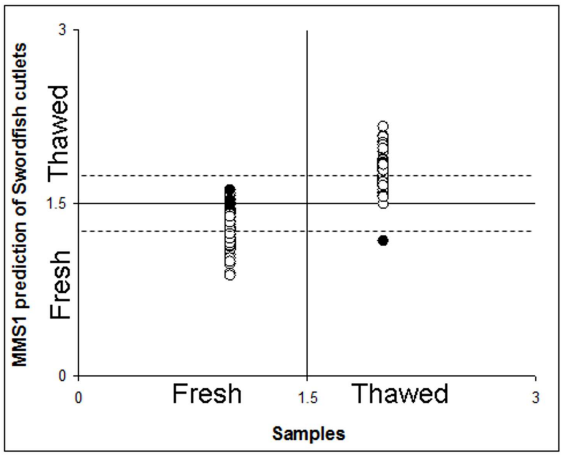

Figure 4. Scatter plot of Visible-NIR transmittance classification for Swordfish cutlets. Black spots were misclassified samples; The dotted lines evidenced a uncertain threshold between 1.65 and 1.35.

Table 1. Performances of validation (at 95% Confidence Interval)

Product	**Groups**	**Number of samples**				**Validation parameters (%)**	
		C	**F**	**U**	**T**	**PPV**	**NPV**
Whole red mullet	Fresh	70	1	9	71	98.6 (90.3-99.7)	97.2 (92.4-100.0)
	Thawed	69	2	6	71		
Swordfish cutlets	Fresh	64	7	20	71	98.6 (92.3-100.0)	90.1 (80.7-95.9)
	Thawed	70	1	14	71		

C: correct; F: False; U: Uncertain; T: total samples analyzed; PPV: Positive Predictive value; NPV Negative Predictive value. PPV and NPV were expressed as percentage with lower and upper limits.

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The potential of mobile VIS/NIR measurements in TOC prediction and organic soils delineation of the peat field in different field conditions

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Introduction

Although organic soils cover a relatively small area in Denmark they can contribute significantly to the carbon balance. More detailed information on the field scale variability of total organic carbon (TOC) is essential for improved carbon management.
The current study represents the first attempt to apply the mobile VIS/NIR system to the mapping of organic soil in Denmark with the focus on investigating it's potential as a rapid analytical method for real-time estimation of the spatial distribution of TOC. The crucial approach of this project is based on the hypothesis that VIS/NIR should be a lucid method for TOC estimation and organic soils delineation due to the organic nature of the majority bonds in this region. The possible effect of different field conditions on the spectral measurements will be investigated. As the main part of this work comparison of conventional mapping method to the new mobile VIS/NIR mapping was reported.

Materials and methods

A part of a 12 ha highly variable agricultural field in Central Jutland, Denmark was selected as the study site. In the first step 162 soil samples were collected on a 25 m grid (Figure 1, map a). Samples were dried at 80^0C over night; sieved (< 2mm) and subsamples were ground using a ball mill. Samples were analysed for TOC using a Leco induction furnace (CN-2000 instrument, LECO Corp., St. Joseph, MI). TOC values obtained from those analyses were used for a reference map generation by interpolation using kriging.

Spectral data were acquired using an on-the-go shank based VIS/NIR spectrophotometer system developed by Veris Technologies, KS, USA. The system includes two spectrometers measuring soil reflectance in the VIS/NIR regions (350-1000 and 1100-2200 nm) (Figure 2).
In order to increase the robustness of the calibration two data sets from different periods varying in field conditions were employed. First data set (3565 spectra, from 10-12m spaced transects) was obtained in very high soil moisture content (Figure 1, map b). In order to verify the accuracy of the first measurement and TOC prediction the second set of data (2144 spectra, from 25m spaced transects) in much drier field conditions was collected (Figure 1, map c).

In order to create calibrations for TOC predictions, a number of 15 representative soil samples for each data set were obtained. Samples were taken from a depth of 0-15 cm within the field. Each sample included 6 subsamples taken within 2.5 m of the sampling point.
Before regression analysis, the spectral information was compressed by calculating principal components, outliers were determined by mahalanobis distance and removed. Then clustering using of a fuzzy c- means algorithm was conducted. Within each cluster a location with the minimal spatial variability was selected. This way a map of 15 representative sample locations was proposed.

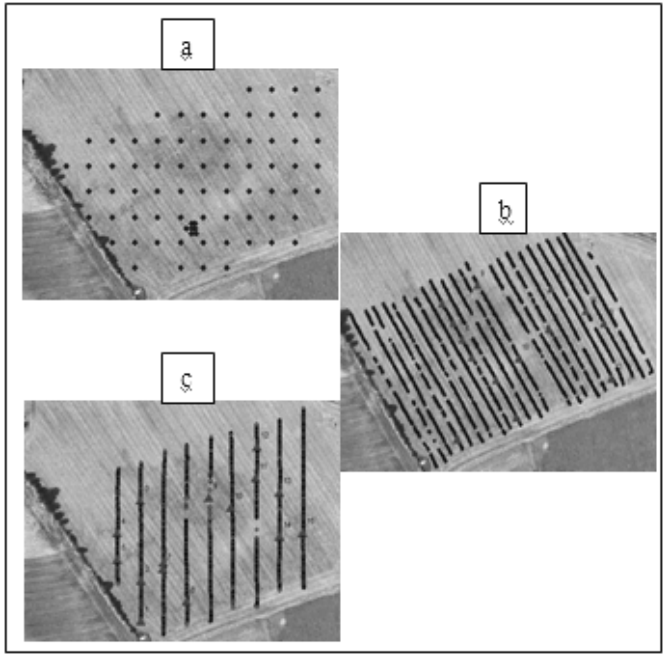

Figure 1. Sampling methods. The figure shows three different sampling methods: a)- manual sampling grid, b)- 1'st mobile VIS/NIR measurement- in wet conditions, c)- 2'nd mobile VIS/NIR measurement- in dry conditions

The collected spectra were correlated to TOC using multivariate regression techniques (Unscrambler 9.7; Camo ASA, Oslo, Norway). The calibration process involved the correlation of TOC content of the 15 samples from both data sets with their spectral data.

Three types of calibrations on both data sets were performed: using only spectra, using spectra with the auxiliary data: soil electrical conductivity EC-SH, EC- DP and soil electrical data with soil temperature. Six different spectra pre-treatments (Table 2) were conducted: (1) only spectra, (2) Savitsky-Golay smoothing over 11 wavelength points and transformation to a (3) 1'st and (4) 2'nd Savitzky and Golay derivative algorithm with a derivative interval of 21 wavelength points, (5) with or (6) without smoothing. Before auxiliary data were included they were modified by autoscaling (mean centred and scaled by 1/Sdev). The calibration equations were computed using the raw spectral data (log 1/R). Calibrations of TOC from spectral data were developed using the full cross-validation method on centred data. The best treatment was considered to be the one with the lowest RMSEP, the highest r^2 for calibration and for validation data set.

The system acquired also soil electrical conductivity for two soil depths: shallow conductivity EC-SH (0- 30 cm), deep conductivity EC-DP (0- 90 cm). During the 2'nd field campaign real-time soil temperature measurements were also collected with the use of a temperature sensor located in the shank.

Figure 2. The Veris VIS/NIR shank based system.

Results and discussion

The carbon content from the laboratory analysis of the reference samples ranged from 1.44 to 42.9 g/100g with Sdtv=13.06.
The best calibration model for the first VIS/NIR measurement using smoothed spectra with the 1'st derivative and EC-DP resulted in RMSEP=6.17, r^2=0.81, while for the second model with the additional use of temperature (RMSEP=5.39, r^2=0.86) (Table 2). The statistical differences in the calibration results of the two VIS/NIR measurements are most likely due to different calibration samples used in both models and different filed conditions.
There are two visible trends in the 1'st and the 2'nd calibration model (Figure 3). In the first calibration model samples with the lower TOC content were worse predicted than those with a higher OM content. The error of prediction of 5-6g/kg can be a problem for the areas with very low TOC resulting in negative values of predicted carbon. The second calibration model in turn shows no obvious linear correlation between TOC and VIS/NIR data within very high values of TOC (>25g/kg). From the prediction point of view the second calibration model with lower correlation in the higher TOC range seems more suitable.

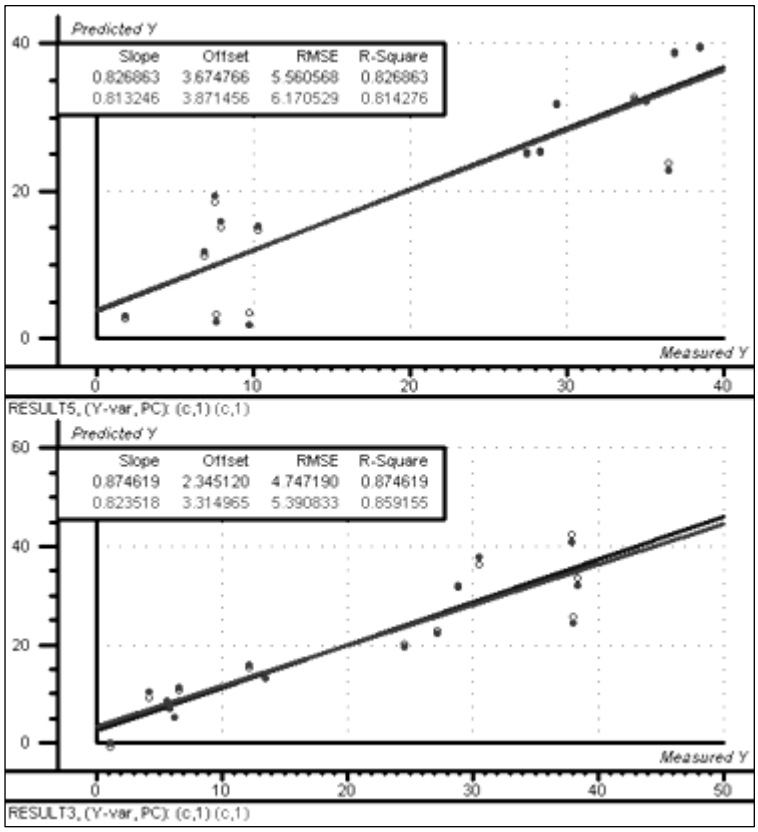

Figure 3. Results from the best calibration models; from the 1'st VIS/NIR measurement (top graph) and from the 2'nd measurement (bottom graph).

The two predicted TOC maps and a similar map based on manual sampling show the same general pattern of TOC distribution (Figure 4). The maps based on VIS/NIR measurements (maps b, c) are more detailed due to a higher amount of data points and more closely spaced measurements. The overlaid 15 calibration points from both mobile measurements correlated well with the VIS/NIR predicted TOC maps.

Table 2. Calibration/validation results of spectra, mathematical pre-treatments and accuracy of prediction for two VIS/NIR measurements.

	Spectra		**smoothed**		**1'st derv.**		**Smoothed, 1'st derv.**		**2'nd derv.**		**Smoothed, 2'nd derv.**	
	1'st	**2'nd**	**1'st**	**2'nd**	**1'st**	**2'nd**	**1'st**	**2'nd**	**1'st**	**2'nd**	**1'st**	**2'nd**
RMSEC[1]	8,02	6,59	5,39	6,60	6,79	6,47	5,32	6,47	9,17	7,89	8,7	6,76
RMSEP[1]	9,30	9,49	8,55	9,52	12,34	9,46	8,20	9,56	13,36	11,03	12,21	9,13
r^1C^1	0,64	0,76	0,84	0,76	0,74	0,77	0,84	0,77	0,53	0,65	0,57	0,74
r^1P^1	0,57	0,56	0,64	0,56	0,26	0,56	0,67	0,56	0,13	0,41	0,27	0,59
RMSEC[2]	4,13	5,02	4,01	5,04	5,56	6,07	5,56	6,07	5,56	6,07	5,56	6,07
RMSEP[2]	6,61	6,92	6,40	6,98	6,17	7,3	6,17	7,3	6,17	7,30	6,17	7,30
r^2C^2	0,90	0,86	0,91	0,86	0,83	0,79	0,83	0,79	0,83	0,79	0,83	0,79
r^2P^2	0,79	0,77	0,80	0,76	0,81	0,74	0,81	0,74	0,81	0,74	0,81	0,74
RMSEC[3]	-	4,68	-	4,68	-	4,75	-	4,75	-	4,75	-	4,75
RMSEP[3]	-	6,41	-	6,39	-	5,39	-	5,39	-	5,39	-	5,39
r^2C^3	-	0,88	-	0,88	-	0,87	-	0,87	-	0,87	-	0,87
r^2P^3	-	0,80	-	0,80	-	0,86	-	0,86	-	0,86	-	0,86

RMSEP – is the expected prediction error
RMSE - is the calibration error
rC - the raw R-square of the model
rP- adjusted R-square for future predictions
[1]- calibration using spectra only
[2]- calibration using spectra and auxiliary data
[3]- calibration using spectra, auxiliary data and temperature
1'st- 1'st sampling campaign with the mobile VIS/NIR system
2'nd- 2'nd sampling campaign with the mobile VIS/NIR system

Additional statistics have been calculated to validate the feasibility of the mobile system in predicting TOC content (Table 3) by comparison with the reference map.

Table 3. Statistical validation of TOC predicted map vs. reference sampling grid.

	RMSEP	**SEP**	**RPD**	**RER**	**R2**	**bias**
1'st VIS/NIR measurement	6.336	6.312	1.576	6.154	0.768	0.906
2'nd VIS/NIR measurement	5.876	5.844	1.815	8.437	0.786	1.037

The results support previous hypothesis that the second measurement with the mobile system performed better presenting a lower RMSEP, SEP and higher RPD, r^2 in the general prediction of TOC. Calibration representing a better fit within the lower values of TOC delivered more accurate prediction. Most importantly, when comparing the TOC final prediction maps by the Veris it shows that the system performed well in both low (TOC content <7%) and high (TOC content >40%) areas.

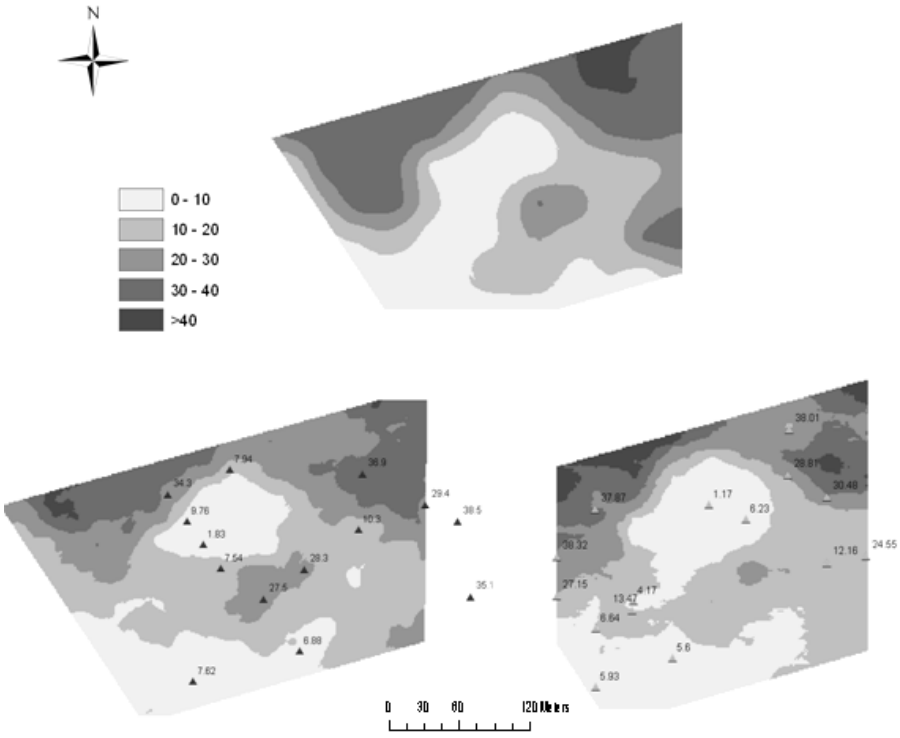

Figure 4. Maps of TOC
The figure shows three maps: a - kriging map of TOC based on manual sampling (at the top), b- co-kriging map of predicted TOC- the 1'st VIS/NIR measurement, with calibration points (left bottom), c- co-kriging map of predicted TOC- the 2'nd VIS/NIR measurement, with calibration points (right bottom).

The main differences in those two calibration models are the time of VIS/NIR measurements, varying field conditions- moisture conditions and different sampling strategy. Despite of some discrepancies between the calibration models from wet and dry field conditions they have not affected the prediction maps seriously and still correlate well with the reference map suggesting a minor effect of moisture content on spectral data.
When working on calibration models with high RMSEP it is suggested to use models with better fits within the lower values of TOC as those seem to deliver more accurate predictions.

Using of LOCAL calibration for predicting feed value of fresh forages from faeces samples

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Introduction
The prediction of forages feed value is important to estimate the ruminant performances. Near infrared reflectance spectroscopy (NIRS) has been used to predict the *in vivo* digestibility (OMD) and voluntary intake (VI) of forages using both, forages and faeces samples. Prediction models are usually built using multiple linear regression (MLR), principal component regression (PCR) or partial least square (PLS) regression techniques. Because of the large variability of fresh forage population and source, LOCAL calibration could be well adapted for their application to the prediction of forages feed value. The aim of this communication is to evaluate the potential of NIRS to predict, the feed value of fresh forages using the LOCAL algorithm on faeces samples.

Materials and Methods
A total of 1220 faeces samples of different species; rye-grass (*Lolium perenne)*, italian rye-grass (*Lolium multiflorum)*, cocksfoot (*Dactylis glomerata)*, tall fescue (*Festuca arundinacea)*, timothy (*Phleum pratense)*, soft brome (*Bromus mollis)*, lucerne (*Medicago sativa)*, red clover *(Trifolium pratense)* rye-grass + white clover (*Trifolium repens*) and permanent grassland were used. Forages samples come from digestibility and intake measurements available at INRA Clermont-Ferrand/Theix which has been largely contributed to develop the tables of nutritive value of feeds (Andrieu *et al.*, 1989).
Samples were oven-dried at 80° C for 48 h to determine dry matter (DM) and then ground through a 0.8 mm screen. They were stored at environmental laboratory conditions.
The determination of OMD and VI were determined for each forage sample on 6 sheep wethers, according to Demarquilly *et al*., (1995). From a digestibility trial a faeces sample was constituted by weighting a subsample of faeces of each animal taken daily. In each digestibility trial, forages were offered *ad libitum* to measure OMD and VI at the same time. A refusal of 10 percent of the offered quantity was allowed. Forages were offered chopped at a length of 5-7 cm twice a day, at 0800 h and 1600 h. During the experimental period, animals had free access to water and vitamin-mineral blocks.
After samples homogenization, forages were placed in a 50 mm diameter ring cup and scanned in reflectance mode at 2 nm intervals from 400 to 2500 nm using a Foss NIRSystems model 6500 scanning VIS/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA). Spectra and reference values were recorded with the NIRS3 software (Infrasoft International, South Atherton St. State College, PA 16801 USA). Each spectrum was time averaged from 32 scans. A reference scan (using the internal ceramic reference tile) was performed before and after each sample The reflectance (R) values were converted into absorbance (*A*) values using the formula A=log (1/R).
Calibrations were developed with WinISI III version 1.60 software (Infrasoft International, South Atherton St. State College, PA 16801 USA). The samples were randomly divided into calibration (n=1085) and validation (n=135) sets accordingly to the number of samples of each population samples. The LOCAL approach was used. Different models were obtained for each sample according to different options in order to find the optimised models (Shenk *et al*., 1997). The options included: number of samples used; 40-200 steps of 40, number of PLS factors used; 10-40 step 5 and number of PLS factors removed 1-10 step 1. The best setting of each determination was retained and it was then used to predict the validation set. All models were performed using NIR wavelengths (700-2500 nm) on first derivative transformation of the spectral data and a scatter correction pre-treatment; standard normal variate and detrend (SNVD) (Barnes *et al*., 1989). Validation performance for each model was assessed by the coefficient of determination of external validation (R^2V), by the standard error of prediction (SEP), by the bias and by the residual predictive deviation (RPD) which is defined as the ratio SEP to the SD of calibration set.

Results and Discussion

The samples used in this study (n=1220), all of them tested for *in vivo* OMD and VI according to the methodology described by Demarquilly *et al*., (1995) were considered as representative of the feed value of the most fresh forages found in temperate regions. The calibration and validation sets covered similar ranges for each component. Mean and standard deviation values were also similar for both sets.

Table 1: Descriptive statistics for the *in vivo* organic matter digestibility (OMD) g/g and voluntary intake (VI) g/kg $BW^{0.75}$ within calibration and validation sets.

	Calibration set (n=1085)					Validation set (n=135)			
	Min	**Max**	**Mean**	**SD**	**SEM**	**Min**	**Max**	**Mean**	**SD**
OMD	0.48	0.85	0.70	0.06	0.01	0.58	0.84	0.72	0.06
VI	22.5	115.2	68.1	11.8	5.35	38.7	101.1	70.6	10.9

SD: standard deviation; Min= Minimum value; Max = Maximun value; SEM = standard error of the method

Calibration statistics are shown in Table 2. For both determinations, OMD and VI, the best LOCAL model does not use a large number of factors for predicting the validation set. A total of 25 factors were selected with the first four not used for the prediction of the OMD and 15 factors for the prediction of VI.

Table 2: Validation statistics for prediction of organic matter digestibility (OMD) g/g and voluntary intake (VI) g/kg $BW^{0.75}$ using the LOCAL algorithm

	N	**Factors**	**SEP**	**Bias**	**R^2V**	**RPD**
OMD	130	25 (-4)	0.017	0.004	0.91	3.5
VI	130	15 (-6)	6.04	0.61	0.67	1.8

N= number of samples; Factors= Number of PLS factors, in brackets number of PLS factors excluded SEP=standard error of prediction; R^2V= coefficient of determination in validation set; RPD=residual predictive deviation

Statitstics associated to the OMD predictions show that LOCAL algorithm explains more than 90 percent of the variability. SEP and RPD values are better than those obtained by Andueza *et al*., (2010) using a similar database of forages but scanning forages samples. For VI the 67 percent of the variability was explained using the LOCAL approach. Although statistic values are better than those obtained by Andueza *et al*., (2010), the calibration model was not adequate for using in quantitative applications according to the criteria proposed by Williams and Sobering (1996).The high variability of the reference method can partially explain these results. Bias values were negligible for both determinations

We concluded that LOCAL approach is appropriate to predict the OMD values. More effort should be made to expand the variability or reduce the error for the VI determination.

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