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Nondestructive detection of cheese and meat quality by portable fiber-optics near-infrared instrument

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Introduction

Regulatory bodies, food processors and consumers increasingly demand of rapid non-destructive methods for analysis of food quality and confirming authenticity. The aim of this work was to assess the feasibility of portable fiber-optics NIR instrument for pork meat and white brine cheese authentication.

Material and methods

Meat Samples

Cross-breed pigs were used in an investigation. The pH of muscle samples was measured directly at 45 minute post-mortem and according to pH values carcasses were divided into two classes – normal meat with pH₄₅ values bigger than 5.8 and pale, soft, exudative (PSE) meat with pH₄₅ values smaller than 5.8. Porcine muscle (*Longissimus thoracis et lumborum*) samples were taken 24h after slaughter from the 12-13 rib and divided into two parts. One-part of each samples were scanned immediately, another part were packed in a seal plastic bag and deep freezing at -32°C for 6h and kept at -21°C. Samples were thawed after one month storage and measured again.

Cheese samples

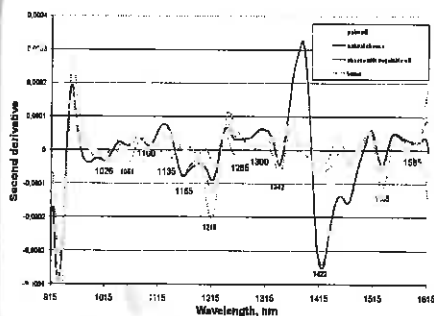
Another product were Bulgarian white brine cheese samples from cow milk – natural or cheese analogues with vegetable oil from different producers.

NIR measurements

NIRQuest 512 spectrometer (Ocean Optics, Inc.) in the region 900-1700nm using reflection fiber-optics probe. SIMCA was implemented to create models for meat and cheese types based on their NIR spectra.



Cheese samples

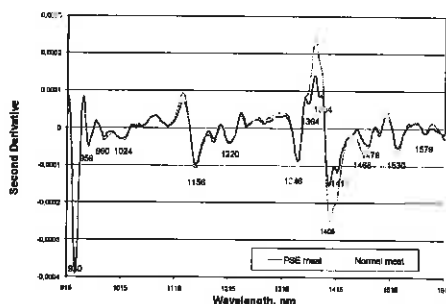


Average second derivative spectra of natural Bulgarian white brine cheese, cheese analogues with vegetable oil, palm oil and butter.

SIMCA classification of Bulgarian white brine cheese cheese analogues with vegetable oil

	Normal cheese	Cheese analogues with vegetable oil	Normal cheese	Cheese analogues with vegetable oil
Natural Cheese	56	4	23	0
Cheese analogues with vegetable oil	1	40	2	29

Results



Average second derivative spectra of normal and pale, soft, exudative (PSE) meat

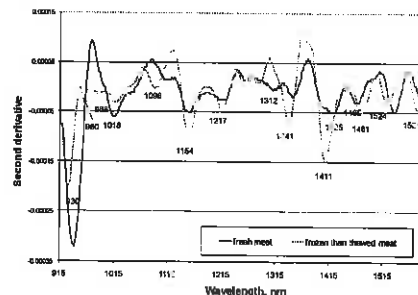
SIMCA classification of fresh and frozen than thawed meat

	Fresh meat	Normal than thawed meat	Fresh meat	Normal than thawed meat	Other classification
Fresh meat	72	0	40	1	
Frozen than thawed meat	0	66	4	32	1

Meat samples

SIMCA classification of normal and PSE meat

	Normal meat	PSE meat	Normal meat	PSE meat	Non-classified
Normal meat	35	0	21	6	0
PSE meat	0	51	4	24	1



Second derivative spectra of a meat sample measured fresh and frozen for 1 month than thawed.

Differences in absorption spectra were observed between normal and PSE meat; fresh and frozen-than-thawed meat; and natural white brine cheese and cheese analogues with vegetable oil.

Simca models based on first derivative spectral data transformation correctly classified 88.82% of samples from group of normal and PSE meat; 97.68% of samples from group of fresh and frozen-than-thawed meat and 95.45% of cheese samples.

Conclusion

The potential of NIRS for white brine cheese and meat authentication was demonstrated. Portable fiber-optics NIR instrument could be used for fast and nondestructive determination of cheese and meat quality.

Acknowledgement.

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Determination of Shell Fraction in Krill Meat by NIR

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Process



Overall

- Antarctic krill (*Euphausia superba*) represent the largest known animal biomass of ~300 mill tons, half of the total human biomass.
- High potential as a source for marine lipid and protein for feed and healthy human food.



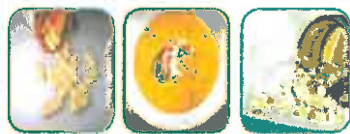
Challenge

- A limitation for use of krill is the high content of fluoride (F) content. The F is mainly located in the exoskeleton of the krill.
- The exoskeleton also contains high concentration of chitin which is associated with reduced growth and reduced digestibility of amino acids and fat in fish.



Solution

- Deshelled krill meat is produced commercially onboard vessels in Antarctica
- Strict control with amount of shell in the products must be warranted for health safety reasons.



Examples of final products

- Pharmaceutical use
- Fish feed, as a palatability enhancer
- Food flavoring and $\Omega 3$ lipids

Experimental

- 37 samples, in duplicate (calibration and test set)
- Constant portion of krill meat at ~85%
- The remaining 15% were shell, water & oil, shown in Fig. 1.
- Analyses by NIR (Fig. 2)

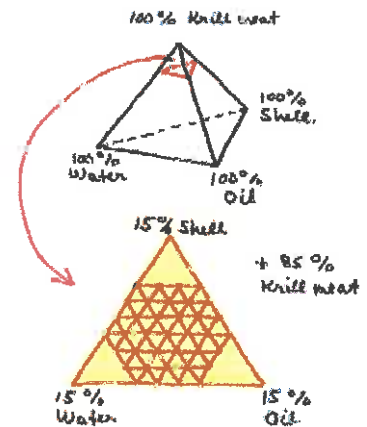


Figure 1. Experimental design. About 85 % (w/w) krill meat was held constant and 0 – 10 %, shell, water & oil was added in 7 repeatedly steps, to make the simplex mixture design.



Figure 2. The NIR instrument used (Perten DA 7200, 950 – 1650 nm).

Results

The prediction error results for the three constituents are presented in the Table. The predicted versus designed shell fraction resulted in a correlation coefficient of 0.98 (Fig. 3).

Table. Test set prediction error result for the added components shell, oil and moisture, w/w%.

Component	RMSEP *	SEP	Bias	R	#PLS
Shell	0.83	0.71	-0.45	0.98	7
Oil	0.64	0.64	-0.03	0.98	6
Moisture	0.65	0.69	0.10	0.98	2

* RMSEP = Root mean square error of prediction; SEP = standard error of performance; R = Correlation coefficient; #PLS = Number of Partial least square regression factors

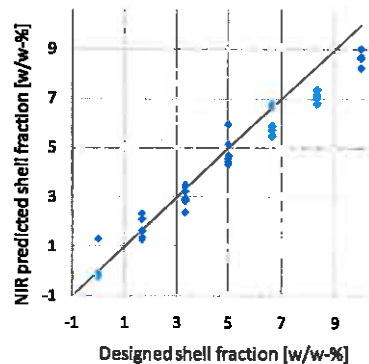


Figure 3. Predicted versus designed shell fraction in samples.

Conclusion

NIR prediction of added shell, oil and water to deshelled krill meat gave good and satisfactory results for the studied model system.

Prediction error improvements using variable selection on small calibration sets – a comparison of some recent methods

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Introduction

Two variable selection methods

- Backward Variable Selection for PLS (BVSPLS¹)
- Powered Partial Least Square (PPLS²)

have been recently proposed in order to select only the relevant variables. In this study these methods are compared to

- Full spectra PLS model
- Forward Stepwise Selection method

for data sets containing only a very limited number of calibration samples.

Materials and methods

PPLS has the ability to select variables based on X variance or X-y correlation, selectable thorough optimization of a method parameter. BVSPLS discards variables from the full spectrum model until the RMSE of a dedicated stop dataset ceases to improve. Three large NIR datasets (1100-2500 nm, 700 variables) were used for this study¹. Spectra were preprocessed with the Standard Normal Variate (SNV) method. All datasets were split with the DUPLEX algorithm³ in a calibration set (1000 samples, Table 1) and a validation set (the remaining samples). Small calibration sets from 20 to 200 samples were selected from the calibration dataset using the DUPLEX algorithm. All methods were applied to each of these small calibration sets. PLS regression was performed on all variable reduced data sets. The number of PLS factors was chosen according to a conservative Chi-square test on the cross-validated Root Mean Square Error². The resulting models were validated on the remaining test set samples. Root mean squared error of prediction (RMSEP) and the coefficient of determination (R^2) from all models were computed.

Results

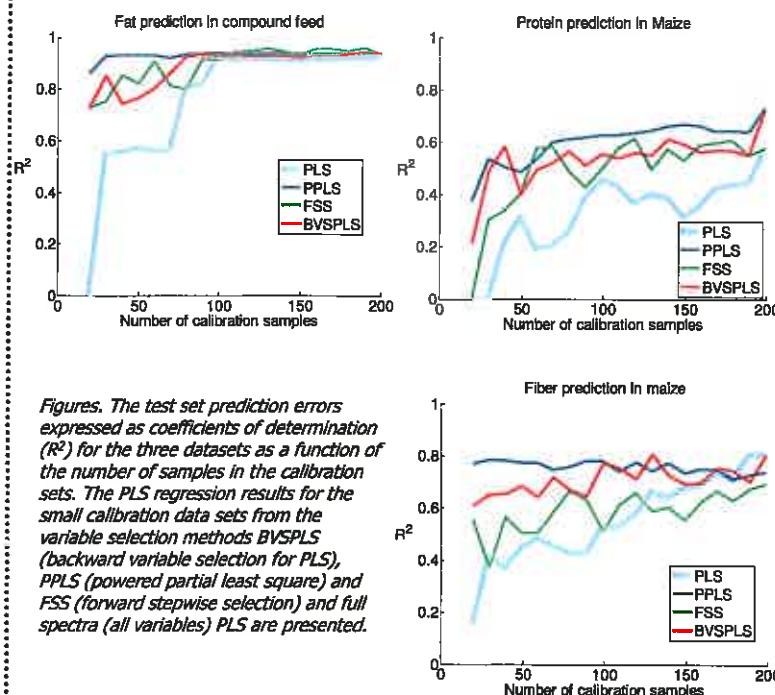
An overview of the datasets is given in the table. Typically 15 – 65 variables was selected from the 700 original variables. The model dimensionality varied from 2 – 9 PLS factors. In large calibration sets, all four methods gave similar prediction error results. Prediction errors gave satisfactory results for all three datasets. However, as the number of calibration samples decreased, especially below 50-100 samples (Figures), the PLS and FSS models gave poorer prediction error results compared to the recently reported variable selection methods, BVSPLS and PPLS. In some cases, for very small calibration sets (20-50 samples), both the methods BVSPLS and PPLS gave considerably lower prediction errors compared to the PLS and FSS models.

Results

Table. An overview of the three datasets. For further details see reference 1.

Product	Constituent	Range* [w/w-%]	STD** [w/w-%]	S _{rel} ** [w/w-%]	No. of samples in test set
Feed	Fat	0.660 – 33.9	5.07	0.20	3521
Maize	Fiber	24.3 – 67.3	6.82	0.60	1247
Maize	Protein	4.02 – 13.7	1.60	0.20	1149

* for both calibration and test sets



Figures. The test set prediction errors expressed as coefficients of determination (R^2) for the three datasets as a function of the number of samples in the calibration sets. The PLS regression results for the small calibration data sets from the variable selection methods BVSPLS (backward variable selection for PLS), PPLS (powered partial least square) and FSS (forward stepwise selection) and full spectra (all variables) PLS are presented.

Conclusions

In small datasets (20-200 samples), the recent variable selection methods BVSPLS and PPLS outperformed stepwise variables selection algorithms as well as the traditional full spectrum PLS model.

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Development of Robust Calibrations From Large Databases

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Introduction:

When using a large calibration database, selecting and retaining the most representative samples for calibration development becomes essential. Retaining too many samples may not bring any additional benefit but can introduce redundancy and incorporate unnecessary noise. The objective of this study was to build robust models for soybean protein, selecting the optimal data set from a large pool of samples from different crop years.

Materials and methods

Five Bruins NIR instruments, Puchheim, Germany, (OmegaAnalyzer G 106110 and G 106118, G 609148, Omega S 21101 and Agri Check 31002) and 9 crop seasons (2001 – 2009) were used for calibration.

The data was organized in Unscrambler 9.9 (Camo, Oslo, Norway) and the general models were made in Unscrambler MatLab 2010A, (MatWorks, Inc., Natick, MA, USA) PLS toolbox, (Eigenvector Research, Inc. Wenatchee, WA, USA) was used for the uniform distribution data models.

The validation set was a sample group from the 2010 crop.

Outliers (spectral and chemical) were removed. The models were built two ways:

1. Development of a calibration from the complete data base (all instruments and available years).

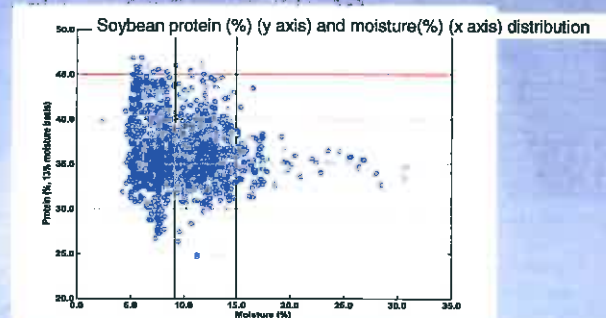
Instr/year	2001	2002	2005	2004	2006	2010	2007	2008	2009
6110	112	131	112	5	145	148	167	148	121
6118	169	163	170	163	165	5	149	147	172
21101	0	131	176	148	151	157	164	142	120
31002	0	0	0	0	0	0	165	16	115
609448	5	0	0	0	0	0	110	100	35

2. The database was split into quadrants. Protein was the main factor and moisture was considered for reality.

Moisture: Low moisture (below or equal 8.9%), medium (9.0% to equal 14.9%), and high (>15%).

Protein: elements of 3% (<29.9%, equal 30.0% to up to 34.9%, equal 35.0% and up to 39.9%, equal 40.0% to 44.9% and equal or above 45%.

Data was taken randomly from those quadrants ensuring uniform distribution, using Matlab, of both protein and moisture. Quadrants that had small amount of samples, all samples were taken.

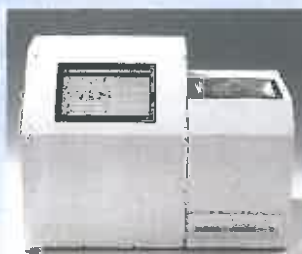


100 times of each of these combinations:

1. Randomly select 200 max per each quadrant → 1528 samples
2. Randomly select 100 max per each quadrant → 336 samples
3. Randomly select 50 max per each quadrant → 520 samples
4. Randomly select 25 max per each quadrant → 168 samples
5. Randomly select 10 max per each quadrant → 126 samples

The validation set used for all models are from the 2010 samples:

Instrument	samples
6110	76
6118	77
21101	77
31002	72



Bruins OmegaAnalyzer G



Bruins AgriCheck



Bruins Instruments
 Lindberghstraße 12
 92178 Puchheim, Germany
 Phone +49-89-800-877-0
 Email: support@bruins.de

Results

Calibration results with all instruments, all years and all samples, soybean protein, Bruins Omega

	N	Factor	R ²	RMSE(%)	SEP(%)	Bias
Calibration	4093	10	95.9%	0.71		
Validation	302	10	96.2%	0.81	0.78	0.23

Validation results of 100 models (iterations) per each sample size (N) with 10 Factors (constant)

Combination	N Calibration	Average R ²	SEP(%)				Bias(%)			
			Min	Max	Average	Std	Min	Max	Average	Std
1	1528	96.6%	0.76	0.83	0.80	0.02	-0.20	-0.31	-0.25	0.02
2	938	93.4%	0.78	0.89	0.82	0.02	-0.18	-0.35	-0.27	0.03
3	520	96.3%	0.76	0.96	0.84	0.03	-0.18	-0.40	-0.27	0.03
4	289	96.1%	0.78	1.00	0.86	0.04	-0.10	-0.43	-0.28	0.07
5	126	95.3%	0.82	1.26	0.96	0.08	0.03	-0.64	-0.30	0.14

From a large data set, an aggressive selection of samples for calibration allowed building robust models. Using about 500 samples gave results similar to models for the same instrument using over 3000 samples.

Conclusions

The SEP increases with smaller data sets; 300-500 samples appeared optimum.

APPLICATION OF NIR AND MIR SPECTROSCOPY TO ASSESS FRESHNESS IN SEA BREAM (*SPARUS AURATUS*) AND SALMON (*SALMO SALAR*) DURING ICE STORAGE

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INTRODUCTION

Fish is a food commodity extremely perishable. Modifications that take place in muscle post-mortem have a significant impact on fish quality and consumer acceptance and, as a consequence, on the aquaculture industry. Storage in ice is fairly effective and is widely used as fish preservation method. Nevertheless, a progressive deterioration in sensory and other properties still occurs, mainly due to changes in lipids and proteins.¹ Although a variety of biochemical, physical, and microbiological methods have been used to assess fish freshness, sensory evaluation is still the most satisfactory. However, this approach is cost- and time-consuming and not readily available in all situations.²

AIM

The aim of this work was to evaluate the applicability of NIR and MIR spectroscopy as rapid and inexpensive tools for the assessment of sea bream and salmon freshness during ice storage, in comparison with traditional chemical indexes.

MATERIALS AND METHODS

Aquacultured ungutted sea breams and gutted salmon, obtained 4 days after slaughter, were stored in ice as whole or as fillets, up to 21 and 17 days, respectively. At regular intervals, the muscle portion of both whole and filleted samples was minced by a Waring Blender homogeniser before chemical and spectroscopic analyses.

Chemical analyses concerned total volatile basic nitrogen content (TVB-N),³ thiobarbituric acid reactive substances (TBARS),⁴ for trimethylamine level (TMA) the AOAC 971.14 official method was used.⁵

NIR spectroscopy was performed using an FT-NIR spectrometer (MPA, Bruker Optics) with an integrating sphere (12500-3750 cm⁻¹).

MIR spectra were collected with an FT-IR spectrometer (VERTEX 70, Bruker Optics) equipped with a germanium crystal ATR cell (4000-700 cm⁻¹).

The spectral data, standardized by different pre-treatments, were processed using Principal Component Analysis (PCA), performed by using The Unscrambler software (v.9.8 Camo Software AS).

RESULTS AND DISCUSSION

Increasing values of chemical parameters were observed as a function of storage time, reflecting the progressive spoilage of fish. Similar values were reached in salmon and sea bream, both in whole and filleted samples, with the exception of TBARS. This index, used to evaluate the degree of lipid oxidation,² reached the maximum values in whole sea bream, probably due to uncutting of fish. TVB-N values reached the upper limit of acceptability (30 mg/100g)⁶ only after 17 days of storage. A TMA level of 5 mg/100g, indicating an incipient spoilage,⁷ was reached after 12 and 14 days of storage for whole and filleted samples, respectively.

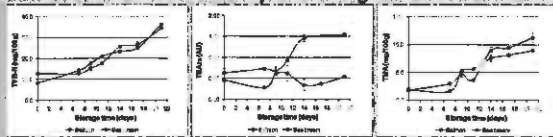


Figure 1. Evolution of freshness chemical indexes in salmon and sea bream whole samples stored in ice.

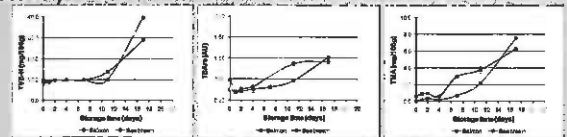


Figure 2. Evolution of freshness chemical indexes in salmon and sea bream fillets stored in ice.

PCA carried out on NIR spectra revealed a sample spectra distribution related to storage time. In particular, for sea bream a good discrimination of both whole fish and fillet samples based on storage time was observed, mainly related to molecular modifications of lipids and water. As regards salmon, PCA results were less clear, but a good distinction of the oldest samples was observed, in agreement with TBARS values, that revealed a minor lipid oxidation for whole salmon.

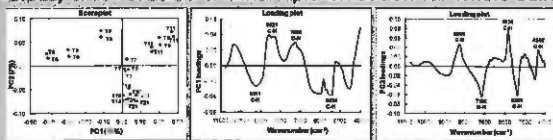


Figure 3. Results of PCA applied on NIR spectra of whole sea bream, after SNV pre-treatment.

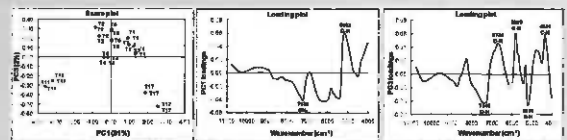


Figure 4. Results of PCA applied on NIR spectra of salmon fillets, after SNV correction.

PCA on MIR spectra gave good results for both the fish species and in particular for fillets. In this case sample discrimination was mainly related to amine groups and thus to protein degradation.

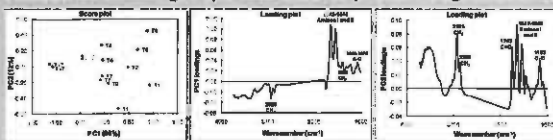


Figure 5. Results of PCA applied on MIR spectra of sea bream fillets, after SNV correction.

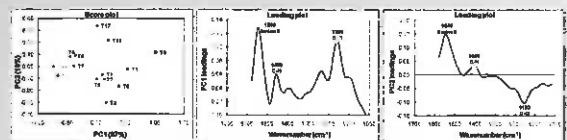


Figure 6. Results of PCA applied on MIR spectra of salmon fillets, after SNV correction.

CONCLUSION

The PCA analysis of NIR and MIR spectra of salmon and sea bream, both as whole fish and fillets, reflected the evolution of the freshness chemical parameters observed during ice storage. Sample discrimination was mainly ascribed to lipid oxidation and protein degradation. Spectroscopic techniques could thus be considered as useful tools for a rapid, easy, and low-cost evaluation of fish freshness.

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Prediction of cheese yield using Near Infrared spectroscopy (NIRS)



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INTRODUCTION



Cheese yield is considered a major factor affecting efficiency and profitability of cheese manufacturing¹. The milk selection for cheese making on the basis of its quality and adequate coagulation properties, could greatly assist the cheese makers in obtaining greater and more consistent cheese yields².

For this reason, it is necessary to elaborate a rapid method that allows for an estimation of the final cheese yield on the basis of the raw material. The use of NIR coupled with chemometrics can provide continuous information about the composition of milk during cheese making and contribute to the control of the process: thus leading to an improvement of cheese quality³.



THE OBJECTIVE OF THIS STUDY WAS TO INVESTIGATE THE APPLICABILITY OF NIR SPECTROSCOPY FOR THE DEVELOPMENT OF AN EQUATION, ABLE TO PREDICT CHEESE YIELD AT MILK COLLECTION AT THE FARM.

MATERIALS and METHODS

29 raw milk samples (collected monthly from July to October 2008) were taken from 2 tanks for creaming and were divided into several copper vats to obtain Protected Designation of Origin (PDO) Grana Padano cheese.

The samples were analysed for:

Chemical parameters:

fat and total casein percentage, caseins fractions amount (α_{S1} , β and K casein) and serum proteins (α -lactalbumin and β -lactoglobulin) concentration determined by official methods of analysis⁴;

Milk coagulation properties:

Milk coagulation time (CT), curd firmness (CF) and the aggregation rate (AG) were determined using Optigraph (Yesebaert, Frepillon, France) at fixed wavelength 11235 cm⁻¹.



Cheese yield was expressed as cheese weight (CW)/milk weight (MW);

Data analysis was carried out with: Pearson's correlation for determining the relationships among the analysed variables that can influence cheese yield (P<0.05) (SAS, 1990); Partial least squares (PLS) linear regression for the prediction of cheese yield. This regression was carried out using the Unscrambler software version 9.2 (Camo inc., Oslo, Norway).

CONCLUSIONS

These preliminary results, demonstrated the suitability of NIRS spectroscopy to predict the cheese yield, without excessive sample preparation and lengthy analyses. The suitability of NIRS for the determination of fat, protein and casein contents was already demonstrated in previous papers^{5,6,7}. The collected information could be a basis for the implementation of NIR applicability during the cheese production process in order to promote frequent controls during the whole production cycle, from milking to cheese ripening.

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RESULTS and DISCUSSION



Relationship between cheese yield and milk characteristics

> Cheese yield is more positively correlated with fat, protein and casein (R² = 0.92, P<0.001) (Table 1)⁸;

> Cheese yield is not much correlated with milk clotting ability: "coagulation time" and "curd firmness" (R² = 0.59, P<0.05 and R² = -0.24, respectively) (Table 1);

> Cheese yield is strong positively correlated with α_{S1} and β -casein (R² = 0.82, P<0.001) (Table 1). According to literature, α_{S1} casein was found to influence significantly only protein content⁷, while β -casein genotypes were found to be associated with fat percentage, fat and protein yield⁸ and curd firmness⁹.

Parameter	Fat g/L	Protein g/L	Casein g/L	Coagulation time min	Curd firmness mA	α_1 -casein g/L	β -casein g/L	K-casein g/L	Cheese yield
Fat g/L	1								
Protein g/L	0.82***	1							
Casein g/L	0.97***	0.87***	1						
Coagulation time min	0.54**	0.62**	0.56**	1					
Curd firmness mA	-0.23**	-0.25**	-0.19**	-0.30**	1				
α_1 -casein g/L	0.79***	0.97***	0.81***	0.62**	-0.22**	1			
β -casein g/L	0.83***	0.97***	0.88***	0.60**	-0.27**	0.94**	1		
K-casein g/L	0.48**	0.79***	0.51**	0.61**	-0.26**	0.81***	0.70***	1	
Cheese yield	0.95***	0.93***	0.95***	0.55**	-0.24**	0.81***	0.83***	0.56*	1

Table 1. Pearson's correlation between cheese yield and both milk quality variables and milk clotting ability. (***) P<0.001; ** P<0.01; * P<0.5; ns =not significant)



Cheese yield prediction

PLS regression was applied to the 29 analysed milk samples. The variables that most contributing to the prediction of cheese yield are: fat and casein content; total protein; milk clotting ability (CT, CF and AR).

The PLS predictive equation was the following (Figure 1):

$$\text{Cheese yield} = 9.726 + (0.581 * \text{fat}) + (0.345 * \text{casein}) + (0.017 * \text{total protein}) + (0.138 * \text{coagulation time}) + (-2.54e^{-02} * \text{curd firmness}) + (-0.153 * \text{speed of aggregation})$$

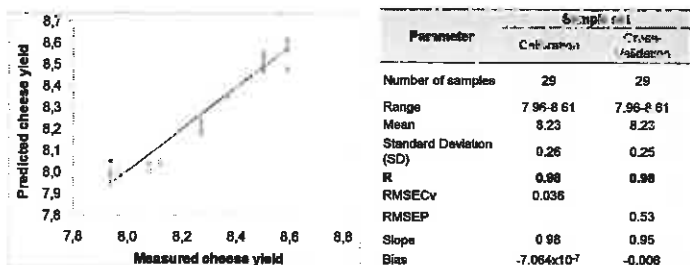


Figure 1. Linear regression between the predicted values obtained by cross-validation for the cheese yield of the 29 samples and the real cheese yield of the same samples. (Calibration: blue line; Validation: red line)

Table 1. Statistical parameters associated with calibration and cross-validation curves for cheese yield.

high R in calibration and in cross-validation (0.98) (Table 1);

low root of mean square error of cross validation (RMSECV = 0.036) (Table 1);

high ratio of performance to deviation (RPD = 4.82) = valid tool of cheese yield prediction (Table 1);

good prediction model, but with an overestimation of the real yield of an average of 1.36%.

NEAR INFRARED SPECTROSCOPY (NIRS) AS A TOOL FOR THE EVALUATION OF MILK QUALITY FOR GRANA PADANO CHEESE PRODUCTION



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INTRODUCTION

The amount of PDO Grana Padano cheese depends not only on the quantity but also on the quality of milk used. So, to maximize the cheese yield, milk must have specific cheese making characteristics: high content of casein and fat¹, an 'excellent' aptitude to coagulate².

For this reason, modern automated on-line methods, based on near infrared spectroscopy (NIRS) have been studied to evaluate the milk clotting ability³ and to provide additional information to cheese producers for increasing efficiency of cheese manufacturers⁴.

AIM

The aim of this study was to investigate the potential of NIR spectroscopy to measure the milk clotting ability in order to obtain additional information useful to predict the cheese yield at milk collection.

MATERIALS and METHODS

48 raw milk samples (collected monthly from July to October 2008) were taken from 2 tanks for creaming, and were divided into several copper vats to obtain Protected Designation of Origin (PDO) Grana Padano cheese. The samples were analyzed for:



Chemical analyses:

- > fat and total casein percentage, α_{s1} , β and k-caseins amount, α -lactalbumin and β -lactoglobulin concentration (determined by official methods of analysis⁵);



- > Milk coagulation properties: milk coagulation time (CT), curd firmness (CF), and the aggregation rate (AR) were determined using Optigraph (Ysebaert, Frepillon, France) at fixed wavelength 11235 cm^{-1} .

Cheese yield was expressed as :

cheese weight (CW)/milk weight (MW).

Chemometric analyses of milk samples were carried out with:

- ✓ Principal component analysis (PCA) of milk quality parameters and milk coagulation properties
- ✓ Partial Least Squares (PLS) linear regression for the prediction of cheese yield.

PCA and PLS regression were carried out using The Unscrambler software version 9.2 (Camo inc., Oslo Norway).

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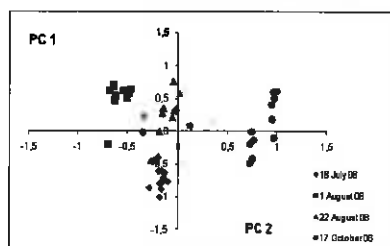
Acknowledgments

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RESULTS and DISCUSSION

Principal component analysis (PCA)

The first 3 PC_s explained 85% of the total variance (PC1= 60%; PC2=16% and PC3=9%). The component 1 (PC1) and 2 (PC2) from PCA analysis tend to segregate milk samples according to day of sampling for PC1 probably due to seasonal variations⁶ (Figure. 1).



Samples taken in October showed:

- the best chemical characteristics (high fat and casein content⁷);
- good coagulation time (parameter closely linked to the casein content)

Higher Cheese yield than that taken in summer (respectively 8.55 vs 8.18)

Figure 1. PC1-PC2 Scores-plot of the 48 analysed milk samples collected during the experimental period. (fat, total casein content, milk coagulation time, curd firmness and aggregation rate, α -lactalbumin and β -lactoglobulin = green indicators; α_{s1} , β and k-casein amount=red indicators).

The difference in concentration of whey proteins (β -lactoglobulin) that allowed the samples separation along PC2 (Figure 1), may be due to genetic polymorphism of albumin synthesis in the mammary gland, confirmed by the presence of many samples showing a β -lactoglobulin genotype of type B⁸.



This condition occurs in the formation of a stable casein network = positive influence on cheese yield!



Cheese yield prediction

To predict cheese yield, PLS regression was applied to the 48 analysed milk samples. The variables that most contributing to the prediction of cheese yield are:

- > fat and casein content;
- > milk clotting ability (CT, CF and AR);
- > α -lactalbumin and β -lactoglobulin.

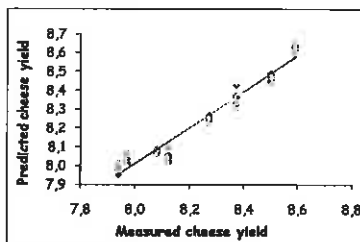


Figure 2. Linear regression relationship between predicted and measured values for cheese yield prediction (48 milk samples). Blue line = calibration curve; red line = validation curve.

Parameter	Sample set	
	Calibration	Cross-Validation
Number of samples	48	48
Range	7.95-8.64	7.96-8.65
Mean	8.23	8.23
Standard Deviation (SD)	0.22	0.22
R	0.98	0.97
RMSEC	0.04	
RMSEP		0.05
Slope	0.96	0.94
Bias	-0.001	-0.001

Table 1. Statistical parameters associated with calibration and cross-validation curves for cheese yield.

- high R in calibration (0.98) and in cross-validation (0.97) (Table1);

- high ratio of performance to deviation (RPD= 4.89) = valid means of cheese prediction (Table 1).

CONCLUSIONS

These results confirmed the close relationship between total casein content, its sub-fractions and cheese yield. On the other hand, the suitability of NIR spectroscopy was verified, combined with appropriate chemometric tools, offering a fast and accurate method for the prediction of cheese yield.

NIR AND MIR SPECTROSCOPY IN THE STUDY OF CHOLESTEROL REMOVAL ABILITY OF SOME LACTIC ACID BACTERIA

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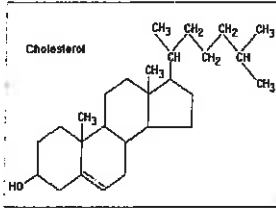
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INTRODUCTION

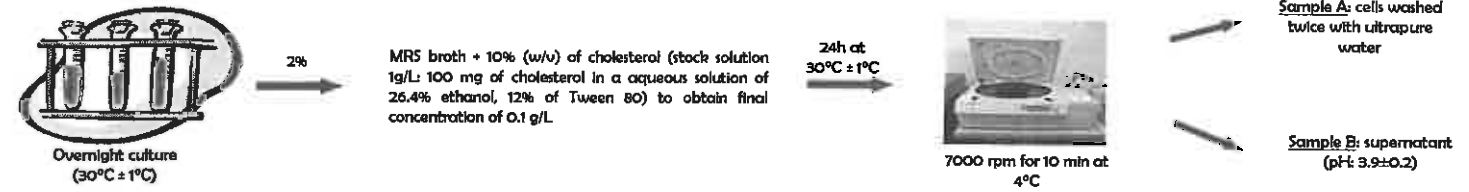


The cholesterol molecule has an important role in maintaining the integrity of cell membranes that regulate the permeability and fluidity, and is involved in many metabolic pathways such as precursor of steroids, bile acids and vitamin D. The consumers becoming more concerned about the excessive intake of fat in the diet and demand food with a lower fat content and with functional properties which can have beneficial effects on the organism. The consumption of dairy products containing probiotic bacteria has been proposed as a means to lower cholesterol in serum. Many authors have shown how these bacteria are able to decrease blood cholesterol content, proposing several *in vitro* studies about the mechanism of cholesterol-lowering action of probiotic bacteria even if the exact mechanism(s) remain unclear. Recent papers tried to show how strains isolated from dairy matrices have the ability to remove cholesterol during their growth. Methods have been proposed to investigate whether cholesterol was really assimilated by strains or whether it remains free in culture medium: cholesterol can be estimated by visible spectroscopy using *o*-phthalaldehyde reagent after extraction of unsaponified matter (cholesterol) with an organic solvent such as hexane. The application of this method requires a long time and so spectroscopic methods have been proposed using MIR and NIR techniques. The aim of this study was to verify, by the use of NIR and MIR spectroscopic techniques, whether strains with different ability to remove cholesterol can show changes in the cell wall and if it was possible to determine the amount of the cholesterol removed.

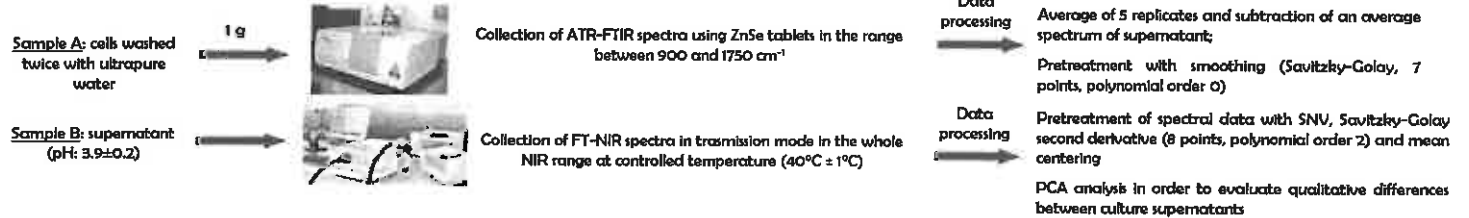
MATERIALS AND METHODS

5 Strains belonging to *Lactobacillus plantarum* species were used: Lp752, Lp885, Lp995, Lp997 and ATCC4917

a. Sample preparation



b. Spectroscopic analysis



RESULTS AND DISCUSSION

MIR SPECTROSCOPY performed on 5 strains of *L. plantarum*

Change in MIR spectra in the range from 1180 to 1300 cm⁻¹ whose entity depended on the strain characteristics

A shift of the signal centred at 1240 cm⁻¹ towards 1217 cm⁻¹ was observed.

The formation of H-bond between phosphate group and the hydroxyl group of cholesterol determined this shift.

Lp 885 shows an evident shift of spectra in the range from 1180 to 1300 cm⁻¹ → High capacity to remove cholesterol

Initial shape of bands differ for different strains → Difference in cell wall composition → Different capacity to remove cholesterol from culture broth

NIR SPECTROSCOPY performed on the supernatant of 3 strains of *L. plantarum* (Lp885, Lp995; ATCC4917)

The difference between strains in Figures 3 and 4 was observed along the PC1 (97% of total variance explained) for the samples sets Lp885 and Lp995; separation for Lp885 and Lp995 was essentially obtained along the PC2 (3% of total variance explained).

Differences in metabolism and in capacity to remove cholesterol among strains

Possibility to separate the supernatants obtained both in absence (supernatant a) and in presence (supernatant b) of cholesterol

The difference between the two sets of samples was obtained along the PC1 that was able to explain 97% of the total variance. A more detailed study is in progress to verify the feasibility of NIR spectroscopy for a rapid determination of not removed cholesterol in supernatants.

CONCLUSIONS

Preliminary results obtained supported the applicability of NIR and MIR spectroscopy to study the removal of cholesterol by some strains of LAB and suggested that the mechanism that involves cholesterol should be related to an interaction of this molecule with cell walls by hydrogen bonds. Further studies will be addressed to confirm these observations and to develop a fast method, based on NIR spectroscopy, for the determination of the cholesterol.

ACKNOWLEDGEMENT

Thanks to Dr. Flora Valeria Romeo, researcher at CRA-FLC for her precious help. A particular acknowledgment to SISNIR (the Italian Society for Near InfraRed Spectroscopy) for the financial supporting for the participation of Maria Chiara Remagni by a travel grant.

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THE USE OF NIR SPECTROSCOPY FOR MONITORING MILK-WHEY BIOTRANSFORMATION PROCESSES USING *LACTOBACILLUS PLANTARUM*



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INTRODUCTION



The disposal of whey as waste can have a high environmental impact. To overcome this problem, a better alternative can be to process whey in obtaining new added value products. Availability of high amount of lactose in whey and the presence of other essential nutrients for Lactic Acid Bacteria (LAB) growth become whey a potential raw material for the production of different bio-products through biotechnological means, such as fermentation processes^{1,2}. Among LAB, selected species of the *Lactobacillus plantarum* are present on the market as probiotic. Probiotics are defined as active microorganisms that show health benefits for the host by improving the properties of indigenous microbial community when consumed in adequate amounts. They should have technological characteristics to allow their production on large scale and their incorporation into food products without losing viability and functionality. Bioprocess optimization is often limited by the inability to measure biomass, nutrients and products concentration in a time frame that allows process adjustment. Near - InfraRed Spectroscopy (NIRS) can potentially be used to measure each of these components providing results available in short time in comparison with those obtained by official methods of analysis. Thus, this study aimed to develop a control system for monitoring fermentations using NIR techniques to correct as quickly as possible anomalies during fermentation processes originating new added products starting from milk whey recovery^{1,2}.

MATERIALS AND METHODS

a. Substrate preparation



Heat treatment with flowing stream and centrifugation
 Addition of 0.75% of yeast extract and 1% of Calcium Carbonate



b. Inoculum preparation



c. Experimental phase

Sample collection at fixed intervals



Microbial count in MRS agar and incubation for 48 hours at 30°C ± 1 °C in anaerobic conditions



HPLC determination of lactose and lactic acid using an ion exchanger column in isocratic condition and refractometer as detector



FT-NIR (FT-NIR[®] -NIRFlex N-500) spectra acquisition in transmittance mode with an optic fiber submerged directly in the whey.

Data processing using The Unscrambler 9.2 software (Camo, Inc.Oslo, Norway)

RESULTS AND DISCUSSION

a. Relationship between microbial growth and fermentation trend

Strain	OP	W	SP	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9	SP10
1	7.01	6.97	6.42	6.45	6.58	6.81	6.76	6.72	6.88	6.95			
2	7.21	7.02	7.05	6.13	6.27	6.34	6.55	6.51	6.51	6.73			
3	7.44	6.34	6.72	6.73	6.94	6.60	6.57	6.55	6.76	6.95			
4	7.44	6.42	6.67	6.80	6.80	6.95	6.76	6.82	6.74	6.95			
5	7.19	6.25	6.53	6.91	6.91	6.15	6.97	6.11	6.91	6.90			
6	7.38	6.29	6.60	6.74	6.60	6.60	6.62	6.62	6.60	6.60			

Table 1: Microbial count values expressed in log CFU/mL.

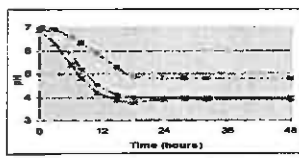


Figure 1: Example of *Lactobacillus plantarum* growth curves.

Adaptability of *Lactobacillus plantarum* on the substrate tested: within 48 hours strains reached growth values of order 9 log CFU/mL demonstrating how this species is able to multiply even in non-optimal growth conditions, thereby justifying its presence in many environmental niches, as reported in literature.

b. Lactose degradation vs. lactic acid production

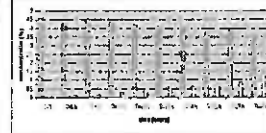


Figure 2: Lactose degradation and Lactic Acid formation.

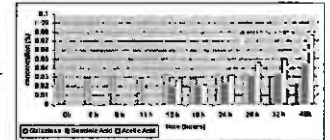


Figure 3: Examples of secondary metabolites formation during fermentation.

• Different behavior of each strains of *Lactobacillus plantarum* than others of the same species

• Not all the available lactose was metabolized to lactic acid: *L. plantarum* is an heterofermentative microorganism and therefore the degradation of this sugar leads to the formation of traces of other compounds

c. FT-NIR data analysis³⁻⁴

Parameters	Range	Sample set					
		Calibration			Validation		
		R	RMSEC (g/100g)	SEC (g/100g)	R	RMSEP (g/100g)	SEP (g/100g)
Lactose	0.24-3.56 (g/100g)	0.9985	0.0451	0.0459	0.9224	0.1802	0.173
Lactic Acid	1.093-3.49 (g/100g)	0.9893	0.0579	0.059	0.9585	0.1245	0.127
Biomass	7-9 (log CFU/mL)	0.8902	0.2531	0.2586	0.9222	0.207	0.23

Table 2: Statistical parameters for Calibration and Validation curves (Pre-processed data with smoothing Savitzky-Coley to points, polynomial order 2)

Results, obtained by applying PLS regression on the pre-processed data (smoothing Savitzky-Coley, 15 points, polynomial order 2), proved to be very satisfactory for all parameters, as shown in Table 2.

In all cases it was possible to obtain:

- High correlation coefficients and good RMSEP values in validation, using models with a low number of principal components (PCs);
- In the case of biomass produced by *L. plantarum* SEP is lower than SEC and this information demonstrated that the develop model was an adequate representation of the fermentation process.

Identification of specific absorption bands³⁻⁴

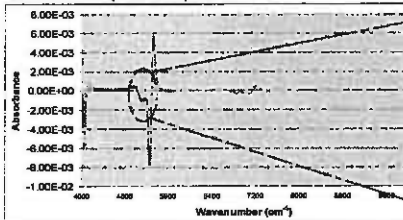


Figure 4: Examples of NIR second derivative spectra obtained from a standard whey as a function of time.

• Region between 6500-7500 cm⁻¹ appear to be super-imposable to all tested times. In this area it was possible to identify:

- 6960 and 7084 cm⁻¹ stretching and bending of C-H bond
 - 6840 cm⁻¹ stretching O-H bond
- Region around 4400 cm⁻¹ stretching O-H and C-H bonds
 • Region around 515 cm⁻¹: second overtone of C-H stretching

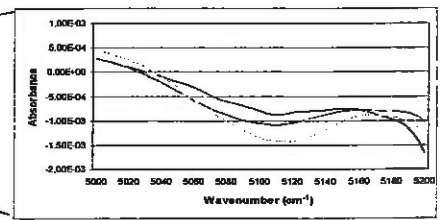


Figure 5: A move to depth profiles of the NIR second derivative spectra.

Region between 5000-5200 cm⁻¹ relationship between bands intensity and analyte concentration

Increase of the NIR bands with the incubation time

CONCLUSIONS

On the basis of the obtained statistical parameters for each studied index determined by both reference methods of analysis and FT-NIR technology, it was possible to confirm:

- The applicability of NIRS for the direct determination of lactose, lactic acid, and microbial count in milk whey.
- The use of an optic probe proved to be a viable alternative to existing methods of fermentation monitoring, particularly with respect to biomass determination.
- A complex analytical matrix, such as whey with suspended cells, at varying concentration wasn't an impediment for a rapid, reliable, and accurate determination of the studied components.

ACKNOWLEDGEMENT

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Transferability of quantitative applications between different FT-NIR Spectrometers used for Quality Control in Feed Industry



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Introduction

Feed producers organize their business in networks not just for distribution, but also for production sites, especially if it's needed to cover big areas like Russia. Quality Control Laboratories of these sites – clients – are often connected to a central R&D Laboratory master. Usually Master Lab provides protocols and guidelines to perform controls of samples, also for non-destructive techniques like Near-Infrared Spectroscopy and they should ensure that all plants are aligned. The study is about a ring-test conducted by Provimi Russia Research, Service & Consulting Dept. to check the possibility to easy transferring the same applications into a network of FT-NIR Spectrometers without additional adaptation and without using correction factors for each unit.



Materials and methods

Five FT-NIR Spectrometers NIRFlex N-500 (BUCHI Labortechnik AG, Switzerland) have been installed in Provimi Russia Quality Control Labs: 2 in 2005 (Moscow and Klin) and three in 2006 (Samara region, Rostov region and St. Petersburg region).

Since 2005, using a wavelength range from 4.000 to 10.000 cm⁻¹, with a resolution of 8 cm⁻¹, the Spectrometer in Moscow, the "Master Unit", has been used to create quantitative calibrations able to measure the chemical composition of Feed raw materials and finished products. The quantitative calibrations were created for the screening of main chemical composition of samples, with the aid of chemometric software NIRCAL (BUCHI Labortechnik AG, Switzerland) and using the technique of Evaluation Set. These calibration developed have then been transferred to the four "Client Units", as they were, without introduction of any correction factors.

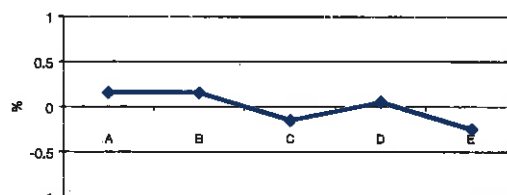
In 2009, two kilograms of 18 different samples among complete feed, concentrates and commodities like raw materials – grains, corn fish meal etc. – were grinded, mixed and split to 5 parts each. All 19 samples (400 g of each) were put in plastic bags and sealed immediately. During week 2 of 2009 these ring-test samples have been analyzed by the five labs for two parameters: moisture and protein content.

In Moscow wet chemistry analysis have been carried on same sample as well.

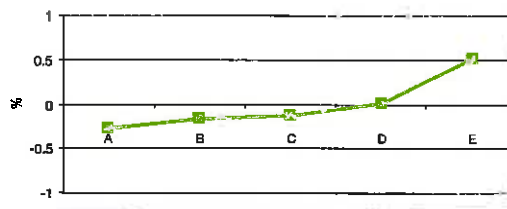
Results

The eighteen samples among finished products and raw materials sent to all the FT-NIR Spectrometer Network have been these: Wheat Grain, Wheat, Barley Grain, Barley, Corn, Sow Feed pellets 1 and 2, Sow Feed 1 and 2, Pig Starter Feed Pellet, Bran, Fish meal, Pig Concentrate, Meat Meal, Soy Bean Meal and Sunflower Meal. Below are shown, as an example the detailed results obtained with the test samples of Pig Starter Feed.

Moisture, % deviation from ring test average



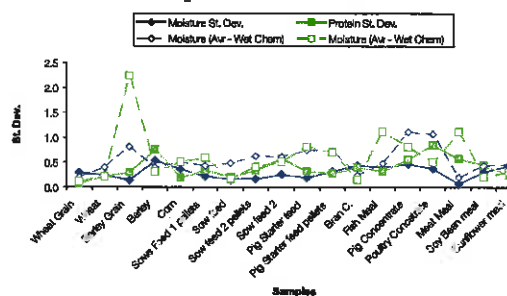
Protein, % deviation from ring test average



A = Azov Client Unit; B = Klin Client Unit; C = Volosovo Client Unit; D = Samara Client Unit; E = Moscow Master Unit

The most important result nonetheless come by the confront of Standard Deviations obtained with all different kind of 18 samples, as well as a comparison between the ring test average and wet chemistry.

Ring Test Standard Deviations



Conclusions

The statistic of results obtained shows that the five FT-NIR Spectrometers using same applications without correction factors, produce analysis reproducible within the range of specification of Provimi Russia quality control's policies.

Spectral evaluation of anti-staling enzymes

Merete Møller Engelsen¹, Jannie Krog Jensen² & Søren Balling Engelsen²

Background

This study is designed to examine the potential of using NIR spectroscopy in combination with chemometrics to predict staling in wheat bread. A fast method to predict if the breads have been added anti-staling enzymes and how stale the wheat breads are is desired in order to speed up the process of investigating the performance of new anti-staling enzymes. In the study the staling in wheat breads was measured by near infrared spectroscopy (NIR) and infrared spectroscopy (IR) and compared to texture analysis (TA) measurements.

The purpose is to find a rapid spectroscopic method that can measure changes during storage of bread. IR and NIR has previously show promising results for measuring staling and therefore the two methods was tested during storage of wheat bread with or without added anti-staling enzymes.

Experimental design

In this study 9 doughs were made that each gave 10 breads allowing for a timeframe of 10 experiments. The bread was regular white bread to which different enzymes was added, see Figure 1.

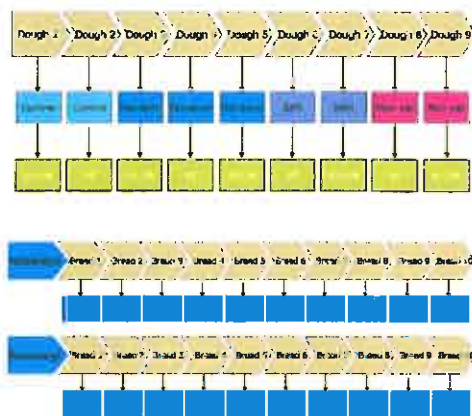


Figure 1: Design of experiment

Enzymes used

Novamyl: Novamyl® ~maltogenic α-amylase

BAN: BAN® ~bacterial α-amylase

Nov. var.: Novamyl variant Novamyl® with changed functionality

Chemometrics

Multivariate data analysis is a powerful tool in investigating and visualizing big data sets. The purpose of using chemometric is to decompose the data in order to detect and model hidden phenomena.

Two different chemometric methods were used; PCA (Principal Component Analysis) and PLS (Partial Least Squares regression) as implemented in the software Latentix (www.latentix.com).

PCA is an unsupervised method that breaks the data up into latent variables and their related scores. PLS is a supervised regression method that use latent variables and their scores to build relations to external reference variables.



Texture Analyzer (TA)

The texture analyser measurements resulted in two parameters; firmness and elasticity. Firmness is measured as the maximum force needed for 20% compression at a speed of 1 mm/sec. The elasticity is measured as the height of the bread sample at moment of release after the 5th compression (70%) at a speed of 6.5 mm/sec, compared to the original height. Bread samples for the texture analyser were cut into cubes with the dimensions 4.5 x 4.5 x 4.5 cm. All measurements were made in triplicates and the results are shown in Figure 2-4.

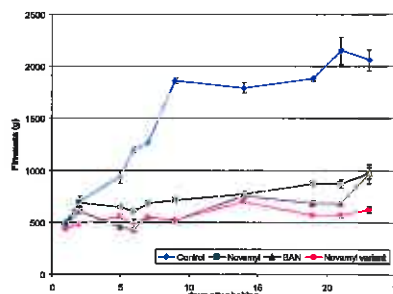


Figure 2: Firmness data during storage from Texture Analyzer measurements for the four bread types

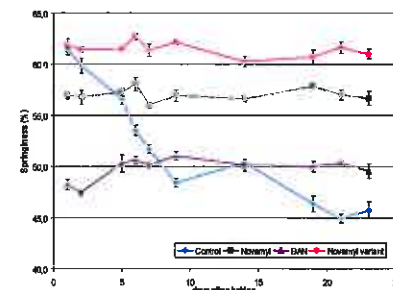


Figure 3: Elasticity data during storage from Texture Analyzer measurements

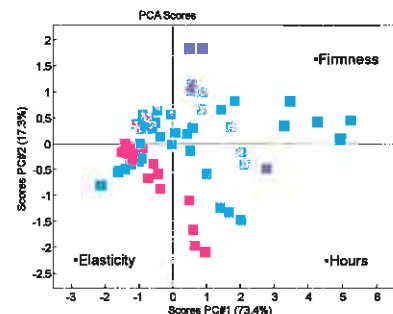


Figure 4: PCA bi-plot of texture data over time. Dough 1: control, dough 5: Novamyl®, dough 7: BAN®, dough 9: Novamyl variant.

Near InfraRed spectroscopy (NIR)

The near infrared (NIR) region of electromagnetic spectrum extends from 770 to 2500 nm. Absorption bands in this region are overtones or combinations of fundamental stretching vibrational bands. The most intense bands are usually due to CH, OH and NH vibrations. For this reason NIR has the ability to measure most of the important components in a food system simultaneously; fat, protein, moisture and carbohydrates. Very little sample preparation is required for NIR measurements, and it is a very fast method and non-destructive. The NIR spectra and the resulting PCA and PLS plot is shown in Figure 5-7.

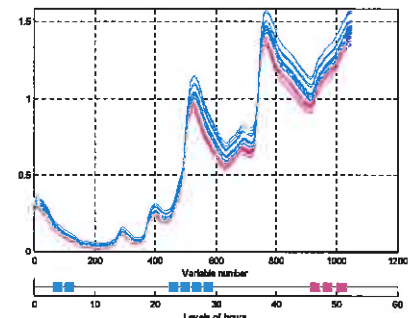


Figure 5: The raw NIR spectra of one bread colored by hours of storage. NIR spectra was recorded on a FOSS NIRSystem 6500.

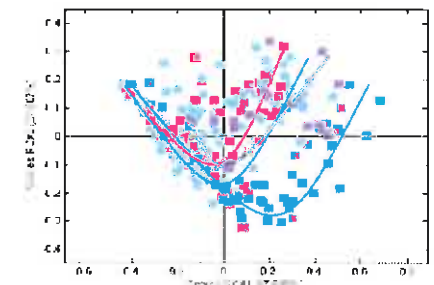


Figure 6: PCA plot of NIR colored by enzyme added. Dough 1: control, dough 5: Novamyl®, dough 7: BAN®, dough 9: Novamyl variant.

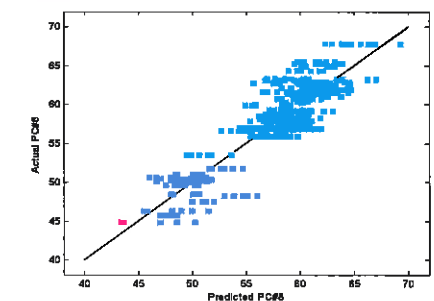


Figure 7: Predicted versus measured plot of elasticity colored by firmness from less firm to very firm

Conclusions

NIR spectroscopy proved a useful tool to monitor staling in wheat breads. NIR explained very well amylopectin being retrograded and recrystallized. The good PLS regression between the NIR spectra and the resilience data from TA showed that NIR can be used to predict the rate of staling in new samples.

Chemometrics was a useful tool to distinguish the staling characteristics between the different enzymes used. The samples that were added enzymes displayed somewhat the same anti-staling behaviour, but to different degrees, proving that the enzymes were different in anti-staling efficiency.

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Improving Mycotoxins Measurements and Analysis by Selecting NIR Instrumentation

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*Corresponding author: broza@serida.org

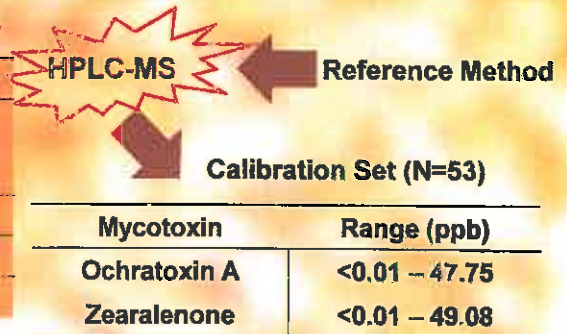
INTRODUCTION The frequent contamination of cereal grains with *Fusarium* toxins and by *Penicillium* species such as zearalenone (ZEN), and ochratoxin A (OTA) is an important issue in animal and human nutrition. They can contaminate large amounts of food and feedstuffs, making necessary their determination and incidence. The concerning showed by the researchers is due to their possible toxic effect that can cause serious health problems for animals and as well as for economical reasons.

OBJECTIVE

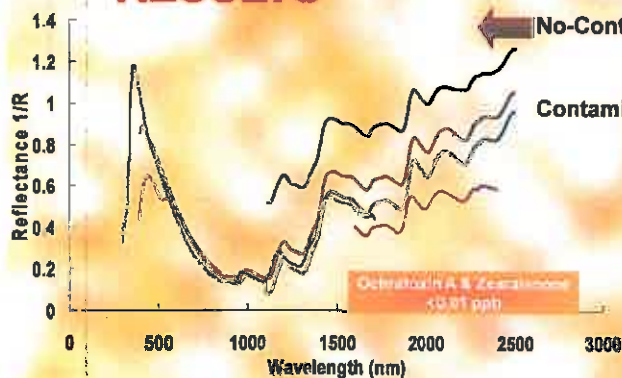
Evaluate different NIRS instruments to examine the quantitative levels of OTA and ZEN in wheat. Now, this cereal makes-up about 25-30% of the average diet.

MATERIAL & METHODS

INSTRUMENT	Sampling System	Wavelength range (nm)
Foss NIRSystems 6500	at-line transport mode & Optic fiber	400-2500
FT-NIR Spectrum One NTS	at-line	1112-2500
Zeiss Corona 45 VisNIR 7	on-line	400-1710
PHAZIR™ (Polychromix)	on-site	1600-2400



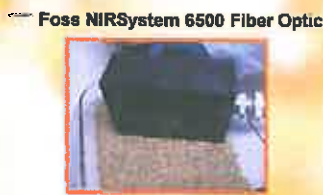
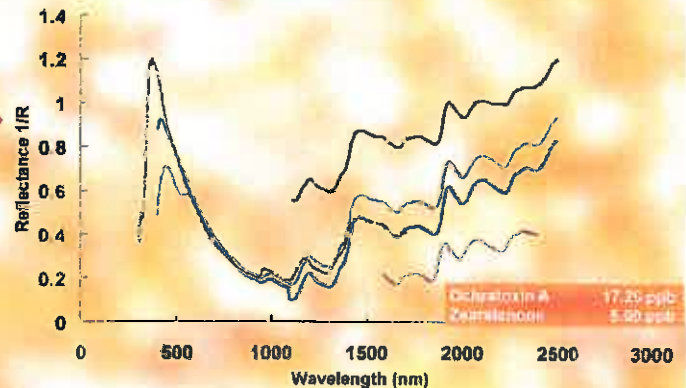
RESULTS



SPECTRA DATA

No-Contaminated Wheat

Contaminated Wheat



OCHRATOXIN A

Instrument	Math Treatment	R ²	SECV	r ²
Foss NIRSystem 6500 Transport	0,0,1,1 none	0.850	0.675	0.782
Foss NIRSystem 6500 Fiber Optic	0,0,1,1 none	0.780	0.938	0.619
FT-NIR Spectrum One	2,5,5,1 SNVD	0.978	1.095	0.631
Zeiss Corona	2,5,5,1 SNVD	0.864	1.031	0.589
Phazir (Polychromix)	1,5,5,1 SNVD	0.632	0.817	0.436

ZEARALENONE

Instrument	Math Treatment	R ²	SECV	r ²
Foss NIRSystem 6500 Transport	2,5,5,1 SNVD	0.975	0.907	0.930
Foss NIRSystem 6500 Fiber Optic	1,5,5,1 SNVD	0.985	0.844	0.931
FT-NIR Spectrum One	2,5,5,1 SNVD	0.981	0.529	0.964
Zeiss Corona	2,5,5,1 SNVD	0.985	0.935	0.905
Phazir (Polychromix)	1,5,5,1 SNVD	0.811	1.750	0.715

R²: Coefficient of determination of calibration; SECV: Standard error of cross validation; r²: Coefficient of determination of cross validation; SNVD: Standard normal variate and detrending

CONCLUSIONS

NIR Spectroscopy shows capability to detect OTA and ZEN in wheat. It makes possible to quantify these mycotoxins by *at-line* and *on-line* instrumentation to be implemented at industry level as control tool.

The results of NIR calibration have shown that scanning window size is as critical as wavelength range for quantification of the OTA and ZEN mycotoxins.



NIR SPECTROSCOPY TO EXPLORE WATER STRUCTURE MODIFICATION INDUCED BY FILTRATION PROCESSES

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INTRODUCTION and AIM

The study of very diluted solutions (solute contents below 10^{-3} M) and pure water subjected to physical treatments has recently highlighted peculiar chemical-physics properties of the water solvent which need to be rationalized in structural terms, e.g. clusters formation etc. [1-3].

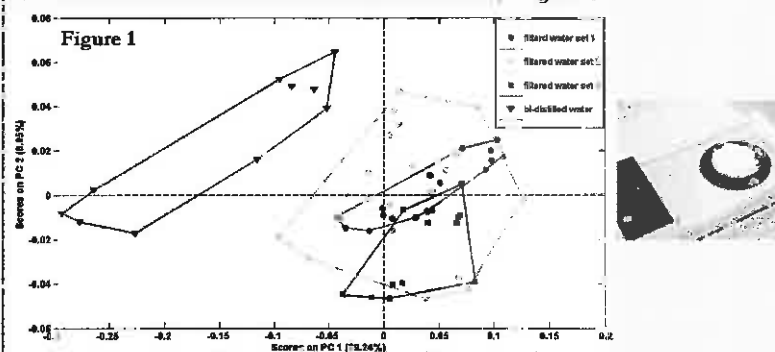
The present work was focused on the study of variations induced by filtration process of pure water by means of chemometric analysis of NIR spectra corresponding to the different conditions.

[1] V. Elia, L. Elia, M. Marchese, P. Lucantonio, E. Napoli, M. Piccoli, I. Menichelli and F. Barone, *J. Mol. Liq.* 150 15-20 (2007) [2] Shan Yin Lu, Xu Gong and David Gunn, *Environ. Technol.* 31(8), 957-967 (2009) [3] P. Giannoccolo, *Food Chemistry* 96 271-277 (2008)

PCA RESULTS

All filtered samples were grouped in a different PC1-PC2 region with respect to bi-distilled water. The two categories were discriminated mainly on PC1 (Figure 1) and the corresponding PC1 loadings (Figure 2) showed a maximum at about 6940 cm^{-1} , hence this absorption was more intense for filtered water (positive PC1 scores).

PC2 showed a loadings behaviour indicating the presence of a convolved band at about 6992 cm^{-1} which changed its position in the two categories, the interval being $6772\text{ - }7092\text{ cm}^{-1}$. This could be attributed to different hydrogen bond network.



MATERIALS and METHODS

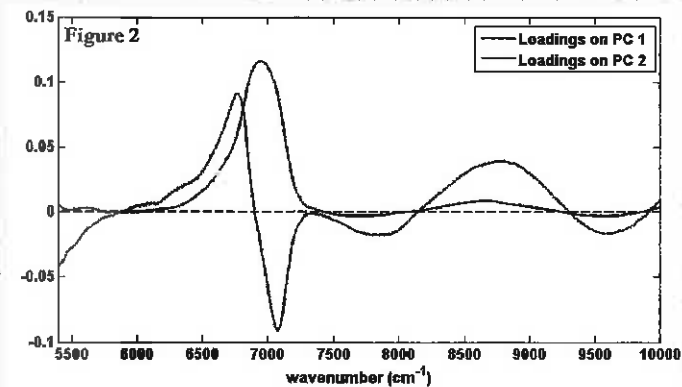
❖ **SAMPLES** 61 samples: 10 samples of MilliQ water not filtered belonging to the same stock, 38 samples divided into two sub-sets of respectively 17 (set 1) and 21 (set 2) samples filtered with the same protocol, but obtained in two different experiments, and 13 samples processed with a different protocol (set 3).

❖ **SPECTRA** NIR spectra were acquired at a constant temperature of 40°C , by a NIRFlex N500 (BUCHI Italia, Assago, Milano) in the $4000\text{ - }10000\text{ cm}^{-1}$ range, 32 scans, 8 cm^{-1} resolution. Optical path length: 1mm.

❖ **PREPROCESSING** The spectra were mean centred, the baseline was removed by using a Weighted Least Square baseline algorithm.

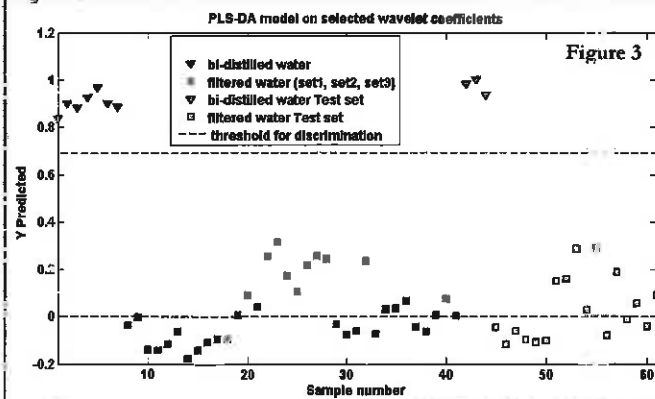
❖ **DATA ANALYSIS** Data analysis was carried out in two steps: explorative PCA, and discrimination of filtered solutions from bi-distilled water by a procedure WILMA-GA [1], which operates feature selection in the wavelet domain by using a genetic algorithm for selecting the most discriminant wavelet coefficients, according to the minimum root mean square error in cross-validation criterion obtained by PLS-DA model.

[1] Cocchi M, Duranti C, Foca G, Li Vigni M, Levari E, Ulacci A (2007) In VI Colloquio Chimico-metrologico Mediterraneo, St. Morvan, La Sainte-Baume, France, 5-7 September 2007

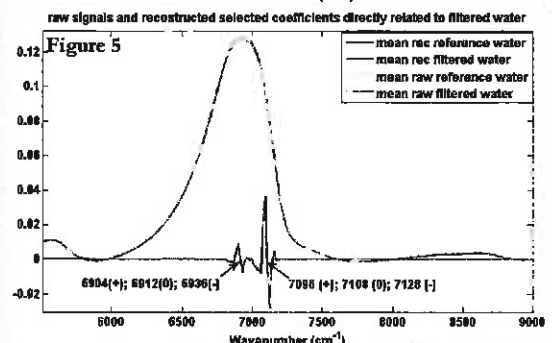
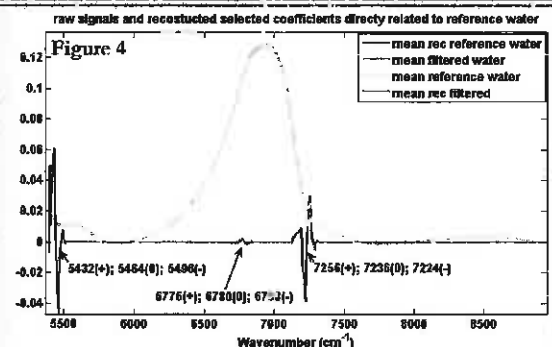


WILMA-GA / PLS-DA RESULTS

The PLS-DA model based on selected wavelet coefficients showed no classification error (training and test sets) for both categories (Figure 3). On the basis of VIP (Variables Importance in Projection) scores and regression coefficients values the most significant coefficients for discrimination were depicted. They were reconstructed in the original domain by taking separately the ones showing positive regression coefficient (Figure 4), hence directly correlated to bi-distilled water (we set the y values equal to one for this class), from those showing negative values (Figure 5), hence directly correlated with filtered water. Since the selected wavelet coefficients belonged to details (high frequency content) they looked rather different from the original signals and thus the raw signals (scaled by a factor of ten for graphical representation) were also reported in the same figures.



Considering the relevant spectral ranges for filtered water (Figure 5) $6900\text{ - }6940\text{ cm}^{-1}$ and $7096\text{ - }7128\text{ cm}^{-1}$, it was confirmed that there is a variation in the hydrogen bond networks, and thus water clusters formation, induced by the filtration processes.



FINAL REMARKS

A new insight in water structure variation induced by filtration processes was gained by near infrared spectroscopy coupled with chemometrics. Feature selection in the wavelet domain assisted data interpretation. In particular, filtration processes seemed to alter the hydrogen bonding pattern of water molecules clusters. Chemometrics pointed to discriminant spectral bands and allowed rationalization in terms of hydrogen bonds pattern variation.

ACKNOWLEDGMENTS

A particular acknowledgment goes to SISNIR (the Italian Society for Near Infrared Spectroscopy) for the financial support for the participation of Marina Cocchi and Mario Li Vigni by travel grants.



Assessment of cercospora leaf spots development on sugar beet leaves by near infrared hyperspectral imaging



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Introduction

The damage caused by cercospora leaf spot (*Cercospora beticola*) on sugar beet leads to a yield reduction. The method to assess the necrosis level on leaves consists of visual observations. The current work, carried out in collaboration with SESVANDERHAVE N.V./S.A., aims to assess by NIR hyperspectral imaging spectroscopy the development of cercospora on sugar beet leaves in the framework of a breeding program for tolerant lines. The objective of the study is to discriminate between cercospora leaf spots and health leaf as well as to quantify the disease area.



Material and methods



Figure 1: Cercospora susceptible plant at an early stage of the plant development.

For this experiment, 4 tolerant and 4 susceptible to cercospora leaf spot sugar beet plants were grown in plastic pots in a greenhouse (Figure 1). The plants were infected by spraying with cercospora beticola. Then, some leaves were analysed using a line scan NIR hyperspectral imaging system (Burgermetrics) during 6 days from the moment that the first symptoms were visible. This system includes a SWIR ImSpector N25E spectral camera from Specim Ltd, using a cooled, temperature stabilized MCT (Mercury-Cadmium-Telluride) detector, combined with a conveyor belt. All images consist of lines of 320 pixels that are acquired at 209 wavelength channels: 1100-2400 nm at 6.3 nm intervals with 32 scans by image. The acquisition is done using the software HyperPro (Burgermetrics). The samples are spread on the conveyor belt (Figure 2).



Figure 2: Image acquisition of sugar beet leaves using a line scan hyperspectral imaging system instrument.

For the necrotic area assessment, discrimination models were built using two spectral libraries corresponding to the cercospora leaf spots and the health leaves. Partial Least Squares Discriminant Analysis (PLSDA) and Support Vector Machines (SVM) were used as classification methods for the construction of these models. These models were applied to all the individual pixels in the images of the leaves in order to isolate and quantify the cercospora leaf spots. Figure 3 shows RGB pictures, SVM results using a program developed in Matlab and PLSDA results using the Hyperanalysis software (Burgermetrics) obtained by analyzing at 3 dates (6/8/2010, 9/8/2010, 12/08/2010) the same leaf from a tolerant plant (DQ100742-02) using the line scan hyperspectral imaging system. The data treatment using SVM model on each image consists of 3 steps (a to c). Figure 3a shows the image at 1000 nm. Figure 3b shows the pixels detected as health leaf or cercospora leaf spots after "background vs. leaf" equation application on the image of Figure 3a. Pixels corresponding to background (conveyor belt) are displayed in white color. Figure 3c shows the pixels detected as cercospora leaf spots after "health leaf vs. cercospora leaf spots" equation application on the not white pixels of Figure 3b. Pixels classified as cercospora leaf spots are displayed in black. The data treatment using PLSDA model shows in green the conveyor belt, in blue the health leaves and in red the cercospora leaf spots. As it can be observed, the cercospora leaf spots became detected by the discrimination models only when they are visually observed on the leaves.

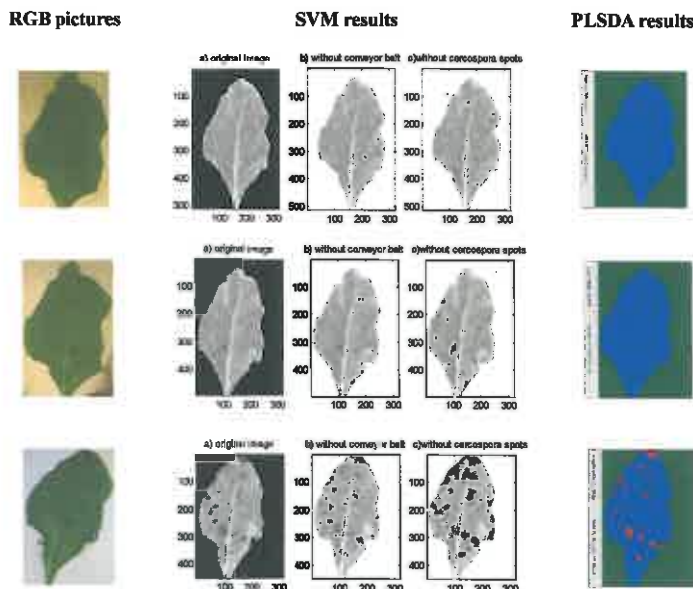


Figure 3: RGB picture, SVM and PLSDA results, showing the cercospora leaf spots observed at three dates (3 days before the first symptoms, the day of the first symptoms and 3 days after the first symptoms) on a leaf from a tolerant plant inoculated by spray with cercospora and analysed using the line scan hyperspectral imaging system.

Results

The results show clear differences between tolerant and susceptible plants (Figure 4). The disease development is slower on tolerant plants and the necrosis covers less than 20% of the leaf area. For the susceptible plants, the infection is faster and 100% of the leaf area can be infected on the same time. Similar conclusions were achieved using PLSDA or SVM models.



Figure 4: Cercospora development on leaves from tolerant (a,b) and susceptible (c,d) plants using SVM (a,c) and PLSDA (b,d) models.

Conclusion

This study has shown the potential of the NIR hyperspectral imaging to discriminate cercospora leaf spots from the health leaf and to follow the disease development. Chemometric tools and NIR hyperspectral imaging data could be used by the breeders in a sugar beet breeding program to select tolerant from susceptible plants, based on the leaf area infected with the cercospora leaf spot.

Reference

Baeten, V., Fernandez Pierna, J.A. & Dardenne, P. (2007). *Hyperspectral imaging techniques: an attractive solution for the analysis of biological and agricultural materials*. In: Techniques and Applications of Hyperspectral Image Analysis, Editors, Hans F. Grahn & Paul Geladi.



Wallonie

Walloon Agricultural Research Centre