HANDHELD NIRS SENSORS FOR ROUTINE COMPOUND FEED QUALITY CONTROL: REAL TIME ANALYSIS AND FIELD MONITORING

Sagrario Modroño, Ana Soldado, Adela Martínez-Fernández and Begoña de la Roza-Delgado*

Regional Institute for Research and Agro-Food Development (SERIDA). Department of Nutrition, Grasslands and Forages. PO Box 13, 33300 Villaviciosa, Spain

*Corresponding author: broza@serida.org

Abstract

Significant advances achieved in different sensor technologies and computer processing data have made possible to respond the needs of livestock sector, providing precise and rapid information on feed composition, being an alternative to real time quality control on compound feed the use of handheld NIRS sensors. This work aimed to evaluate two hand-held portable NIR spectrophotometers for on-site and real time analysis of nutritive parameters in raw compound feed: Phazir 1624 Polychromix Inc (PhIR) and MicroNIRTM 1700 by JDSU (MICRO). For computing data, different combinations of pre-treatments and multivariate statistical methods have been assayed to extract the valuable information of spectra data and to develop appropriate calibrations. The calibration models displayed greatest predictive capacity for Crude Protein, Crude Fiber

and Starch and the determination coefficients of cross validation were 0.90 – 0.88 for CP, 0.85-0.91 for CF, 0.89- 0.88 and 0.89-0.91 for STCH using PhIR and MICRO instruments respectively. Dry Matter showed the lowest determination coefficients of cross validation 0.67-0.73. Accuracy achieved 99-101 % for both NIRS instruments and no differences were found when applying $t_{student}$-test comparing reference and predicted data. Results obtained with both instruments were compared by using standard deviation and not significant differences were observed at the 5% level. Results so far have demonstrated the potential of these handheld NIRS instruments proposed here to estimate the individual compound feeds composition changes at farms level instantly, time avoiding the disadvantage of moving the samples to the lab.

Keywords: Near Infrared Reflectance Spectroscopy, Portable NIRS sensors, In-situ analysis, Compound feed, Chemometrics

Introduction

Nowadays one of the research topics in livestock farming is focused to measure information related to feeding/nutritional requirements, because feeding related costs are always significant part of variables costs for all types of livestock production. According to Cerosaletti and Dewing [1] the precision feed is a continuous improvement process adopted and directed by farm management to meet goals in three areas such as the improvement of nutrient efficiency, homegrown feed utilization and milk-income-over-feed cost, the optimization of purchase feed nutrient imports and crop production for the feeding system and

the reduction or minimization of nutrient overfeeding and nutrient excretion and accumulations. Therefore, automating the collection analysis and use of production related information on livestock farms will be essential for improving animal productivity [2].

An alternative for real time quality control of compound feeds is NIRS technology. The evolution on NIRS instrumentation has made considerable progress in making, available low cost miniaturized, handheld near-infrared instrument based on MEMS (micro-electro-mechanical system) [3] or LVF (linear variable filter)[4]. These type of sensors offer significant advantages in terms of size, weight, robustness, spectral range and low cost manufacturing process. They are highly resistant to mechanical stress [5] and easy to use, which represents the evolution in the analysis of the samples from taking the sample to the Lab to taking the Lab to the sample [6].

Today the role of those small portable NIRS instruments has a particular relevance [7-10]. Because of the analytical costs dominates the sampling costs, the use of these hand-held instruments increases the sub-sampling and makes analysis very quick and cheap. These NIRS analysis allow to characterize food and feed samples accurate and precisely with high representativeness of the total.

However, the development of a specific analytical methodology using NIRS technology is not a simple task. It requires proper spectra data collection and an adequate chemometric strategy. Pre-processing the spectral data is the most important step before chemometric models development to avoid the influence of non-linearities introduced by light scatter. Due to the comparable size of the

wavelengths in NIR electromagnetic radiation and particle size in feeds, NIR spectroscopy is a battle ground for undesired scatter effects (both baseline shift and non-linearities) that will influence the recorded sample spectra.

The pre-treatments have a significant impact on the predictive model performance of these portable NIRS instruments with a narrow wavelengths range. And there is always the danger of applying the wrong type or applying a too severe pre-processing that will remove some valuable information. The proper choice of pre-processing is difficult to assess prior to model validation, but in general, performing several pre-processing steps is not advisable, and as a minimum requirement, pre-processing should maintain or decrease the effective model complexity [11].

No previously-published studies have focused on the pre-treatment and development of chemometric models with spectra data coming from intact feed samples collected with handheld NIRS based on MEMS (PhIR) or LVF (MICRO) systems. Both NIRS instruments include different wavelength ranges, optics and electronics, and both technical features are the most critical items of NIRS sensors [3, 4].

Attending possibilities of using handheld NIRS sensors to optimize the real time quality control on compound feed, the objective of this research work has been to develop an analytical methodology based on the use of NIRS sensors for on-site and real time analysis of nutritive parameters in raw compound feed as an essential tool for collection nutritive feed data to improve nutrient efficiency optimizing the purchase of feed and minimizing nutrient overfeeding. This methodology could be implemented to capture feed information on farms to be

related to the welfare and productivity of animals as well as to reduce the feed cost that represents a significant item in relationship with the farm profitability. For computing data, different combinations of pre-treatments and multivariate statistical methods have been assayed to extract the valuable information of spectra data coming from intact compound feed, and to develop appropriate calibrations. The final proposed methodology could provide a great potential for on-site monitoring quality control in food and feed industry and inspection administration service as first link of production chain.

Material and methods

2.1.- Samples and Reference Data

All the intact compound feeds (N=100) involved in this study were samples certified as reference material (CRM) collected from different proficiency test programs, in which SERIDA's Nutrition Laboratory has participated over an extend time period (2009-2014). Reference values were obtained from all participants as consensus results (n>20 laboratories).

This population represents a wide variability of compound feeds going from feed for dairy cows, piglets, laying hens, chicken, sheep, rabbits, horses and lambs using different ~~of~~ presentation forms (meals, crumbs, pellets and meals). From the initial data set was separated randomly a sub-set of 12 samples (validation set) for external validation. It should be stressed that selection of calibration and validation sets was only performed on the basis of spectral information [12].

The values were assigned attending the results of the proficiency test and using the following procedures: dry matter (DM) by drying in an air-forced oven at

103ºC to constant weight, crude protein (CP) by Kjeldalh method, crude fiber (CF) by Fibertec method, starch (STCH) by polarimetric methodology, fat content by solvent extraction with Soxhlet technique including an hydrochloric acid digestion prior to the extraction, according to the methods of analysis to control the composition of feed materials and compound feeds [13]. These parameters were selected because they are the main constituents to be controlled on most compound feeds [14].

2.2.- NIRS instruments and analysis

The collection of spectra data from the 100 feedstuffs raw samples was carried with handheld NIRS instruments, with two different optic and electronic characteristics. The main features of these instruments are summarized in Table 1 and detailed bellow:

Handheld micro-electro-mechanical system (MEMS) digital transform spectrometer (1.8 kg weight) from Polychromix PHAZIR^{TM} (PhIR, Phazir 1624, Polychromix Inc., Wilmington, MA, USA), works in reflectance mode in the range between 1600 and 2400 nm with a non-constant interval of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm), with a diameter window of 0.4 cm (sampling area of 0.13 cm^2). This instrument has been equipped with a special quartz protection to avoid dirt accumulation. Fifty spectra were taken for each sample at different sampling points, and each spectrum was the mean of ten scans. The final spectrum was the average of all of them. Spectra were recorded as log (1/R).

MicroNIR^{TM} 1700 spectrometer (MICRO) is a miniature near infrared spectrometer developed and manufactured by JDSU (JDSU Uniphase

Corporation). This ultracompact spectrometer is distinguished by its small size (45x42 mm diameter x height) and low weight (64 g). The input aperture dimensions are 2.5 x 3.0 mm. The scanning wavelength range goes from 910 to 1676 nm with a constant interval of 6.2 nm. It uses LVF component mounted over a diode array detector that separates incoming light into individual wavelengths. The light source is a pair of integrated vacuum tungsten lamps. MicroNIR™ has a collar to get the optimum focal point of the illumination from the spectrometer's window to the sample to be measured. A white reference measurement was obtained using a NIR reflectance standard (Spectralon™) with a 99% diffuse reflectance, while a dark reference was obtained from a fixed place in the room. Fifty spectra were taken per sample using an integration time of 1000 ms, the final spectra was the average of all spectra. The MICRO includes data collection software developed in LabVIEW™ controlled and operated by portable computer.

These different features of both NIRS sensors, are critical to collect spectra with high sensibility and low noise along the wavelength range. As detailed before, 50 different points were scanned per sample and the final spectrum was calculated averaging those 50 points. Time to scan one point was 4 s, and around 3 minutes to scan one sample. NIRS sensors are an alternative to develop sampling strategies that increase the number of observations without increasing the final analytical cost. Increasing sampling, noise is minimized, sampling variability is increased and as result the uncertainty of measurements is reduced [15].

”2.3.- Chemometric tools

The collected data were converted into a data matrix. The X and Y variables were defined as: X wavelength and Y log 1/R. Calibration development was performed in two parts; pre-treatments and mathematical treatments, and both were applied to the spectra using the Unscrambler v. 9.8 software [16].

As spectral pre-treatments, the standard normal variate (SNV) and multiplicative scatter correction (MSC) [17] procedures were assayed, together with first and second Savitzky and Golay (SG) derivatives as mathematical treatment. Both derivation techniques use smoothing to reduce the signal to noise ratio in the corrected spectra. The effect of the pre-treatment combinations and the sequence has been also studied.

After pre-treatment calibration set was centered prior to develop regression model by principal component analysis to identify and remove spectral outliers. The regression model was performed using partial least squares (PLS). To select the best equations, the statistics evaluated were: the lowest standard error of calibration (SEC) and standard error of cross validation (SECV), the highest determination coefficient of calibration (R^2), determination coefficient of cross validation (r^2) and the ratio of performance to deviation (RPD), which is the ratio between the standard deviation of the sample population and SECV [18]. The external validation was evaluated in base of the lowest standard error of prediction (SEP), the t statistic for paired samples comparing reference and NIRS methods and the confidence interval for the ratio for standard deviation of errors to compare both NIRS strategies. If the interval includes 1, the standard deviations are not significantly different at the 5% level [19].

Results and discussion

Nowadays there is no experience related to development of NIRS methodologies for analysis of raw feed spectra scanned with handheld instruments involved in

this research work. Both with narrow wavelength range and differentiated electronics and optics features (see Materials and Methods section).

These feed samples are non homogeneous raw compounds that include differences related to distribution of particles in the sample, particle size, sample density and sample morphology (shape and roughness of sample surface sometimes bigger than sensor windows). These differences from sample to sample affect to light scattering effects influencing the measured of NIR spectra and inducing baseline shifts and scaling variations (intensity variations). As consequence these altered spectra can be detrimental to subsequent quantitative analysis as inaccurate results can be obtained.

These reasons make necessary to apply and evaluate different pre-treatments, depending on the instrument, to extract all the relevant information minimizing variability unrelated to the property of interest. The success of pre-treatments lies in how effectively the mathematical treatment can separate light scattering from light reflectance in NIRS.

Taking into account these considerations, the first step in the development of this real time and on-site methodology was to analyze spectral data trying out different pre-treatments and their combinations, and assessing which pre-treatment is better by comparing subsequent model performance.

In Figure 1 are shown spectra collected with both NIRS instruments before and after combining scatter correction and mathematical derivation (SNV or MSC and 1st derivative). It can be observed the overlapping range, only from 1596 nm to 1676 nm between spectra collected in both handheld NIRS instruments. In this range, although both devices show the same trend, MICRO has a higher log (1/R) value means because more radiation has been absorbed (less reflected) by

samples. After applying mathematical pre-treatments to the spectral data could be improve their interpretation and emphasizes weak component absorbance bands. In fact, the effect of 1st derivative and SNV on mean spectrum is different depending on the application order. Those differences are not observed to the combination of MSC and first derivative.

To evaluate the influence of these pre-treatments to reduce unwanted sources of variability, to separate overlapping absorption bands and baseline variations on spectral data [17], a total of 128 chemometric models were developed as follows: 16 combinations of pre-treatments (SNV, MSC, 1st and 2nd derivative, smooth), 4 different parameters (DM, CP, CF, STCH) and two hand held NIRS instruments (PhIR and MICRO). The final calibration regression models were developed using PLS.

The mean, standard deviation, coefficient of variation and range values for chemical composition of reference samples included in the calibration (N=88) and validation (N=12) sets are given in Table 2. The wide range and the standard deviation for the parameters analysed show the diversity of compound feed involved in this research work and confirm that the structured selection using only spectral information treatment algorithms proved adequate, since the calibration and validation sets displayed similar values for descriptive statistics for all study parameters, and ranges for the validation set lay within the range recorded for the calibration set. In addition, noting that sample sets for calibration should ideally ensure uniform distribution of composition across the range of the study parameter in question [17].

No samples were removed as outliers, because all of them were satisfactory in the exploratory analysis. Tables 3 and 4 provide the calibration statistics

corresponding to the developed models using PhIR and MICRO spectra pre-processed by combining SNV and MSC before and after 1st and 2nd derivative, using PLS regression.

As can be seen, for PhIR instrument, the best statistics were obtained when applying scatter correction (MSC or SNV) after SG derivation. The calibration models displayed greatest predictive capacity for CP, CF and STCH. Attending cross validation statistics (R^2_{cv}, SECV) the best results were obtained applying 1st derivative for all the assayed models and parameters. The R^2_{cv} values could be considered between excellent and good for all nutritive parameters, CP: R^2_{cv}= 0.90, CF: R^2_{cv}= 0.85 and STCH: R^2cv= 0.89. DM showed the lowest R^2_{cv}= 0.67. Nevertheless in terms of the recommendations made by Williams [18], the predictive capacity of the models, with the exception of DM is useful because RPD ≥ 2.2 [5].

Related to MICRO instrument and summarizing results observed in Tables 3 and 4 it can be seen that the statistics confirm for first derivative the best results when scatter correction (SNV or MSC) is applied after derivation, whilst the models obtained using second derivative, with exception of CP the best statistics were showed when applying scatter correction before derivation. Cross validation statistics were excellent for all assayed parameters, with values of R^2_{cv}= 0.91 for CF and STCH, R^2_{cv}= 0.89 for CP. DM is back as the lowest correlation value (R^2_{cv} = 0.73). RPD values were higher than 2.2 for all parameter with the exception of DM. No difference related to quality of models and RPD values have been observed when comparing PhIR and MICRO.

It is interesting to compare a combination of pre-treatments in different orders, because as detailed by previous researchers [20], the order of pre-treatment

makes difference. These authors observed that for a milled sample SNV followed by 2nd derivative was not able to remove the scattering effect, while the reverse order, 2nd derivative followed by SNV worked successfully. These statements confirm our results, because with exception of CF with MICRO, the best cross validation statistics were obtained applying derivative prior scatter correction.

In our knowledge, the literature provides no data regarding the statistics values for nutritive on intact compound feed analysed using miniature devices, so the results obtained may only be compared with those reported by other authors for feed ingredients. Regarding CP calibration statistics obtained in this study compare quite well with statistics of equations reported by Biller et al. [21] using a MICRO device on some ingredients included in compound feed such as soybeans (whole grain) and distillers grains with SECV of 0.93% and 0.98%, respectively in spite of the samples were recorded in a specific sample cup and equilibrated to room temperature before spectra were collected.

To carry out a statistic comparison between cross validation results (R^2_{cv} and SECV) obtained in both instruments, highlight that calibration models constructed yielded similar precision, lightly greatest predictive capacity for MICRO instrument for all parameters than those of PhIR instrument with the exception of CP with R^2_{cv}= 0.90 vs R^2_{cv} =0.89 and SECV=1.31. vs 1.36 for PhIR and MICRO respectively.

Having got to this point, it should be mentioned that ~~our~~ heterogeneous samples require a large number of aliquots to obtain representative samples. Although the approach of taking 50 spectra is time consuming (3 minutes), it does not defeat

the purpose of rapid on-line/in-situ analyses and minimizes the sampling uncertainty [15].

For DM content, results showed the lowest R^2_{cv} (R^2_{cv}~0.7) in both instruments. DM spectra information is related to broad bands containing information about O-H interactions, the first overtone at 1450 nm and the combination band at 1930 nm [22]. MICRO wavelength range includes the band at 1450 nm (overtone) and PhIR the other one at 1930 nm (combination). Range is a critical factor to the achievement of optimal models, since often PLS-type routines cannot nullify regions containing irrelevant or null information and the inclusion of such regions can make for very poor models, perhaps the possibility of including in an instrument wavelength range covering both bands would make possible to increase these statistics. However, it is necessary to remark that these calibration statistics are in concordance with previous researchers [12] working with a single product, processed animal proteins, a feed heterogeneous ingredient but with lower particle size than compound feed. These authors obtained the following values of R^2_{cv} =0.786, and SECV=0.344, for humidity.

In addition, related with handheld PhIR instrument, previous works had evaluated the transferability from dispersive instruments (at-lab) to the handheld spectrometer, of calibration models constructed using milled feed samples analysed in at-lab monochromator employing a large set of compound feed built over the several years [23]. Although the developed methodology in this specific research work is based on transferring calibration models, a comparison of our external validation results and those obtained by this previous researcher will be carried out predicting validation set with the selected calibration models.

Assessing the predictive ability of the selected quantitative models on the external validation set (Table 5), DM accuracy achieved 99-101 % for both NIRS instruments. For CP better accuracy was observed for PhIR than for MICRO. For CF and STCH the best accuracy range was registered when analysing samples with MICRO NIRS. To confirm the performance of calibration methods developed with both instruments was calculated the confidence interval for the ratio of the true standard deviation of prediction errors [19]. Table 5 shows the lower and upper limits of a 95% confidence interval for the ratio of the true SD. As can be seen (Table 5) all the intervals include the number 1, confirming that the standard deviation are not significantly different at the 5% level ($\alpha = 0.05$ as significance level). Comparing results obtained using both instruments vs. reference data by t student statistic, we confirm that $t_{calculated} < t_{statistic}$ for all assayed parameters, no significant differences were observed.

In the context of SEP and SEL relationship, and according with our validation results for all parameters and NIRS instruments we can confirm that with exception of STCH no differences were observed between SEP/SEL ratios (SEL=1.59). Carbohydrates are a complex class of feed constituents which include starch with numerous absorption bands [22] and the determination of starch by polarimetric method had a high uncertainty associated with the results. Nevertheless, it possible to determine the levels of starch and of high molecular weight starch degradation products in compound feeds for the purpose of checking compliance with Commission Regulation EC 152/2009 [13].

A comparison between our external validation results and those obtained by Fernandez-Pierna et al. [23] on milled feed samples show that for all parameters

and instruments SEP is lower in our developed methodology using intact compound feed, with the exception STCH prediction when using PhIR instrument.

Related to the employed instrumentation, the results make necessary to remark that both handheld instruments, with different wavelength ranges, offer excellent characteristics to attempt the proposed analysis method to establish a quality control in farms in order to produce indications about the best practice management/nutrition.

Conclusions

NIRS portable technology has been successfully correlated with reference quantitative methods to predict nutritive value in compound feeds. The statistics of NIRS quantitative analysis and external validation are satisfactory and useful to attempt a compound feed control. This survey highlights the potential of handheld NIR instruments to estimate the individual compound feeds composition changes at farms level and thereby to provide advisory tools to animal production.

A comparison between NIRS instruments has confirmed the quality of spectral data using handheld MICRO and PhIR analyzers. These results, even if obtained on a limited number of reference samples, can be seen as an exciting starting point for the extension of the procedure to practical applications, even if a lot of work is still needed in this direction, in order to improve the calibration equations using a wider database of samples analyzed with reference methods, to make calibration models more stable.

It was demonstrated that in NIR spectroscopy that an inappropriate use of a pre-treatment could lead to misinterpretation of the data and inaccurate results. For instance combining derivatives (1st or 2nd) followed by SNV or MSC work better. The order of combined pre-treatments makes a difference.

Results so far have demonstrated the potential of these handheld NIRS instruments proposed here for the development of prediction models for feed analysis which is easy and faster when compared with conventional methods which require times and moving the samples to the lab.

Conflicts of Interest

All authors were Regional Institute for Research and Agrofood Development employees.

Funding

This work was supported by the Spanish Project RTA2012-00063-C02-00 from the INIA and European Regional Development Fund (ERDF).

Acknowledgements

Authors are grateful to Nutrition Laboratory Staff of SERIDA.

References

[1] P Cerosatetti, D. Dewing, Precision feed management. The Manager. PRO-DAIRY. Northeast Dairy Busines. (2008) 15-20.

[2] C. Schulze, J. Spilke, W. Lehner, Data modeling for Precision Dairy Farming within the competitive field of operational and analytical tasks, Comput. Electron. Agr. 59 (2007) 39-55. doi:10.1016/j.compag.2007.05.001

[3] E. Zamora-Rojas, D. Pérez-Marín, E. De Pedro-Sanz, J.E. Guerrero-Ginel, A. Garrido-Varo, Handheld NIRS analysis for routine meat quality control: Data base transfer from at-line instruments, Chemometr. Intell. Lab.114 (2012) 30-35. doi:10.1016/j.chemolab.2012.02.001

[4] J. Zumba, J. Rodgers, Cotton Micronaire Measurements using small portable Near-Infrared (NIR) Analyzers, Appl. Spectrosc. 70(5) (2016) 794-803. doi:10.1177/0003702816638227

[5] G. Cabassi, D. Cavalli, R. Fuccella, P.M. Gallina, Evaluation of four NIR spectrometers in the analysis of cattle slurry, Biosyst. Eng. 133 (2015) 1-13. doi:10.1016/j.biosystemseng.2015.02.011

[6] N. O´Brien, C. Hulse, D. Friedrich, F. Van Milligen, M. von Gunten, F. Pfeifer, H. Siesler, Miniature near infrared (NIR) spectrometer engine for handheld applications, Proc. SPIE 8374, 837404, 1, 2012.

[7] A. Garrido-Varo, S. Vega, F. Maroto-Molina, M.J. De la Haba, D. Pérez-Marín, On-site quality control of processed land animal proteins using a portable micro-electro-mechanical-system near infrared spectrometer, J. Near Infrared Spec. 24 (2016) 47-58. doi: http://dx.doi.org/10.1255/jnirs.1192

[8] D. Pérez-Marín, P. Paz, J.E. Guerrero, A. Garrido-Varo, M.T Sánchez, Miniature handheld sensor for the on-site non-destructive assesment of post-harvest quality and refrigerated storage behavior in plums, J. .Food Eng. 99 (2010) 294-302. doi:10.1016/j.jfoodeng.2010.03.002

[9] N. Prado, V. Fernández-Ibáñez, P. González, A. Soldado, On-site NIR spectroscopy to control the shelf life of pork meat, Food Anal. Method 4(4) (2010) 582-589. doi: 10.1007/s12161-011-9208-2

[10] B. De la Roza-delgado, A. Soldado, A.F. Gomes de Faria Oliveira, A. Martínez – Fernández, A. Argamentería, Assessing the Value of a Portable Near Infrared Spectroscopy Sensor for Predicting Pork Meat Quality Traits of Asturcelta Autochthonous Swine Breed, Food Anal. Method 7 (2014) 151–156. doi: 10.1007/s12161-013-9611-y

[11] J.S. Shenk, , J.J. Workman, M.O. Westerhaus, "Application of NIR Spectroscopy to Agricultural Products". Handbook of near infrared analysis 1st ed. Burns D.A. y Ciurczak E.W. (Eds.). Practical Spectroscopy Series, vol. 13. Marcel, Dekker, USA, 1992.

[12] S. Vega, D. Pérez-Marín, J. E. Guerrero-Ginel, M. J. de la Haba, L. Cabotá, T. Burón, A. Garrido-Varo, Evaluation of a MEMS-NIR portable instrument for quality control of processed animal proteins (PAPS) at the rendering plant in NIR2013 Proceedings, Ed. by V. Bellon-Maurel, P. Williams and G. Downey, La Grande-Motte, France, p. 94, 2013.

[13] Commission Regulation EC No 152/2009 of 27 January 2009 laying doen the methods of sampling and analysis for the official control of feed, Official Journal of the European Union, L54, 2009.

[14] G.H. Lenser, R.R McEllhiney, In-plant quality commitment, in Feed Manufacturing Technology IV, Ed by R.R. McEllhiney. American feed Industry Association, Inc. Arlington, VA, USA, p.282 , 1994.

[15] T. Fearn, Sampling errors: problems and opportunities, Feed quality and safety: technology, traceability and labeling. Feed for Health: International Workshop COST Action FA0802, Gijón, Spain, 2011.

[16] Camo Software Inc., Unscramble v.8.0, 2008.

[17] R.J. Barnes, M.S. Dhanoa, S.J. Lister, Standard Normal Variate transformation and De-trending of near-infrared diffuse reflectance spectra, J. Appl. Spectrosc., 43 (1989). 772-777. doi: http://dx.doi.org/10.1366/0003702894202201.

[18] P.C. Williams, Implementation of near-infrared technology, in Near-infrared technology in the agricultural and food industries, Ed by P.C. Williams and K.H. Norris. AACC Inc, St. Paul, p. 145, 2001.

[19] T. Fearn, Comparing Standard Deviations, NIR News. 7(5) (1996) 5.

[20] J. Huang, S. Romero-Torres, M. Moshgbar, Practical Considerations in Data Pre-treatment for NIR and Raman Spectroscopy, <http://www.americanpharmaceuticalreview.com/Featured-Articles/116330-Practical-Considerations-in-Data-Pre-treatment-for-NIR-and-Raman-Spectroscopy/>, (accessed 04.20.16).

[21] C. Biller, C. Hurburgh, N. Cao, G. Rippke, Calibration of the JDSU MicroNir 1700 for agricultural product analysis, NIR News, 25, 16-18 (2014). doi: 10.1255/nirn.1471

[22] B.G. Osborne, T. Fearn, Applications of near infrared spectroscopy in food analysis" in Near Infrared Spectroscopy in Food Analysis, Longman Group UK Limited, Essex CM20 2JE, England, p. 117, 1986.

[23] J. A. Fernández Pierna, P. Vermeulen, B. Lecler, V. Baeten, P. Dardenne, Calibration Transfer from Dispersive Instruments to Handheld Spectrometers, Appl. Spectrosc. 64 (2010) 644-648. doi:10.1366/000370210791414353

[24] B. Savenije, G.H. Geesink, van der Palen, G. Hemke, Prediction of pork quality using visible/near-infrared reflectance spectroscopy, Meat Sci. 73 (2006). 181-185. doi:10.1016/j.meatsci.2005.11.006

Legend of Figure

Figure 1. Average spectra for intact compound feed measured with PhIR and MICRO instruments: a) Raw spectra, b) Spectra pre-processed by SNV and 1st Derivative, c) Spectra pre-processed by MSC and 1st Derivative. Code: SG N1 N2 N3: Savitzky Golay, Derivative order, Smooth, polynomial order.

Table 1.- Technical features of spectrophotometers: MEMS (Phazir-1624) and LFV (MicroNIR 1700).

Property	Phazir-1624	MicroNIR 1700
Detector type	Indium-Gallium-Arsenide,	128-pixel uncooled InGaAs photodiode array
Wavelength range (nm)	1600-2400	950-1650
Sampling integration time	10 spectra to average	1000 ms

Table 2. – Descriptive statistics of calibration (N=88) and external validation (N=12) sample sets.

	Set	DM %	CP %	CF %	STCH %
Range	Calibration	86.46 – 94.79	7.91 – 23.74	2.31 – 17.43	9.98 – 50.49
	Validation	87.53 – 90.19	11.17 – 18.08	3.58 – 10.15	32.48 – 44.64
Mean	Calibration	89.04	15.59	5.393	36.75
	Validation	89.05	15.18	5.619	37.64
SD	Calibration	1.260	2.955	2.876	7.154
	Validation	0.799	1.879	2.119	3.717
CV	Calibration	1.42	18.95	53.33	19.47
	Validation	0.90	12.38	37.71	9.88

DM: Dry matter; CP: Crude protein; CF: Crude fiber; STCH: Starch; SD: Standard deviation; CV: Coefficient of Variation; N: Number of samples

Table 3.- PLS calibration and cross validation statistics for predicting parameters with different pretreatment combinations and first derivative using PhIR and MICRO NIRS instruments (N=88).

		R^2c	SEC(%)	R^2cv	SECV(%)	RPD	R^2c	SEC(%)	R^2cv	SECV(%)	RPD
		SG 1 10 2+MSC					MSC+ SG 1 10 2				
PhIR	DM	0.752	0.83	0.67	0.942	1.3	0.711	0.886	0.63	0.986	1.3
	CP	0.912	1.209	0.889	1.354	2.2	0.919	1.167	0.89	1.351	2.2
	CF	0.91	1.194	0.845	1.54	1.9	0.889	1.314	0.807	1.703	1.7
	STCH	0.93	2.637	0.885	3.338	2.1	0.913	2.92	0.853	3.751	1.9
MICRO	DM	0.848	0.668	0.682	0.945	1.3	0.703	0.896	0.618	0.994	1.3
	CP	0.926	1.118	0.87	1.471	2.0	0.924	1.129	0.874	1.445	2.0
	CF	0.935	1.019	0.907	1.213	2.4	0.912	1.179	0.886	1.334	2.2
	STCH	0.932	2.589	0.885	3.356	2.1	0.935	2.539	0.894	3.22	2.2
		SG 1 10 2+SNV					SNV+SG 1 10 2				
PhIR	DM	0.753	0.829	0.674	0.935	1.3	0.723	0.871	0.614	1.003	1.3
	CP	0.923	1.138	0.896	1.315	2.2	0.919	1.163	0.886	1.373	2.2
	CF	0.911	1.187	0.838	1.575	1.8	0.867	1.431	0.8	1.736	1.7
	STCH	0.93	2.637	0.888	3.287	2.2	0.914	2.909	0.858	3.684	1.9
MICRO	DM	0.851	0.662	0.714	0.911	1.4	0.859	0.646	0.708	0.907	1.4
	CP	0.918	1.17	0.867	1.484	2.0	0.917	1.18	0.856	1.537	1.9
	CF	0.935	1.019	0.902	1.241	2.3	0.911	1.189	0.886	1.337	2.2
	STCH	0.94	2.436	0.902	3.091	2.3	0.935	2.544	0.894	3.208	2.2
		SG 1 5 2+MSC					MSC+SG 1 5 2				
PhIR	DM	0.752	0.83	0.663	0.949	1.3	0.711	0.885	0.624	0.99	1.3
	CP	0.924	1.132	0.897	1.31	2.3	0.919	1.164	0.888	1.358	2.2
	CF	0.913	1.171	0.84	1.565	1.8	0.905	1.225	0.808	1.722	1.7
	STCH	0.937	2.499	0.892	3.23	2.2	0.913	2.917	0.857	3.697	1.9
MICRO	DM	0.845	0.675	0.707	0.906	1.4	0.888	0.578	0.715	0.894	1.4
	CP	0.921	1.155	0.876	1.432	2.1	0.919	1.165	0.867	1.481	2.0
	CF	0.927	1.078	0.903	1.24	2.3	0.936	1.01	0.902	1.248	2.3
	STCH	0.933	2.571	0.889	3.283	2.2	0.937	2.491	0.903	3.085	2.3
		SG 1 5 2+SNV					SNV+SG 1 5 2				
PhIR	DM	0.753	0.829	0.644	0.968	1.3	0.723	0.87	0.645	0.968	1.3
	CP	0.924	1.1.32	0.896	1.31	2.3	0.907	1.246	0.881	1.4	2.1
	CF	0.914	1.164	0.839	1.576	1.8	0.892	1.299	0.812	1.693	1.7
	STCH	0.93	2.632	0.891	3.251	2.2	0.914	2.906	0.853	3.754	1.9
MICRO	DM	0.853	0.658	0.728	0.886	1.4	0.862	0.638	0.719	0.895	1.4
	CP	0.922	1.144	0.874	1.442	2.0	0.919	1.164	0.862	1.507	2.0
	CF	0.93	1.06	0.902	1.246	2.3	0.913	1.174	0.889	1.319	2.2
	STCH	0.943	2.373	0.913	2.927	2.4	0.936	2.52	0.901	3.109	2.3

DM: Dry matter; CP: Crude protein; CF: Crude fiber; STCH: Starch; SG N1 N2N3: Savitzky Golay, Derivative order, Smooth, polynomial order; R_C^2: determination coefficient of calibration; R^2_{CV}: determination coefficient of cross validation; MSC: multiplicative scatter correction; SNV: standard normal variate; SEC: standard error of calibration; SECV: standard error of cross validation; RPD:SD/SECV

Table 4.- PLS calibration and cross validation statistics for predicting parameters on intact compound feed with different pretreatment combinations and second derivative using PhIR and MICRO NIRS instruments (N=88).

		R^2c	SEC (%)	R^2cv	SECV(%)	RPD	R^2c	SEC(%)	R^2cv	SECV(%)	RPD
		SG 2 10 2+MSC					MSC+SG 2 10 2				
PhIR	DM	0.746	0.839	0.632	0.984	1.3	0.769	0.805	0.647	0.983	1.3
	CP	0.925	1.119	0.889	1.355	2.2	0.92	1.158	0.879	1.414	2.1
	CF	0.909	1.199	0.827	1.628	1.8	0.896	1.28	0.814	1.686	1.7
	STCH	0.935	2.534	0.866	3.59	2.0	0.922	2.777	0.825	4.068	1.8
MICRO	DM	0.854	0.655	0.701	0.906	1.4	0.717	0.878	0.648	0.962	1.3
	CP	0.933	1.064	0.884	1.395	2.1	0.926	1.114	0.883	1.391	2.1
	CF	0.927	1.08	0.903	1.239	2.3	0.929	1.064	0.904	1.233	2.3
	STCH	0.934	2.549	0.889	3.298	2.2	0.933	2.58	0.883	3.364	2.1
		SG 2 10 2+SNV					SNV+SG 2 10 2				
PhIR	DM	0.799	0.757	0.665	0.957	1.3	0.773	0.8	0.626	1.011	1.2
	CP	0.913	1.205	0.883	1.385	2.1	0.938	1.027	0.88	1.408	2.1
	CF	0.909	1.199	0.809	1.712	1.7	0.896	1.279	0.798	1.753	1.6
	STCH	0.935	2.538	0.849	3.804	1.9	0.922	2.778	0.844	3.857	1.9
MICRO	DM	0.814	0.731	0.706	0.9	1.4	0.803	0.751	0.67	0.94	1.3
	CP	0.935	1.051	0.890	1.36	2.2	0.928	1.1	0.88	1.411	2.1
	CF	0.931	1.047	0.905	1.225	2.3	0.93	1.054	0.909	1.197	2.4
	STCH	0.937	2.508	0.892	3.242	2.2	0.943	2.385	0.887	3.327	2.2
		SG 2 5 2+MSC					MSC+SG 2 5 2				
PhIR	DM	0.743	0.843	0.604	1.012	1.2	0.769	0.805	0.589	1.057	1.2
	CP	0.937	1.031	0.88	1.416	2.1	0.929	1.092	0.856	1.537	1.9
	CF	0.926	1.084	0.817	1.675	1.7	0.9	1.255	0.792	1.779	1.6
	STCH	0.895	3.185	0.823	4.065	1.8	0.91	2.949	0.818	4.154	1.7
MICRO	DM	0.851	0.661	0.696	0.91	1.4	0.886	0.583	0.709	0.903	1.4
	CP	0.911	1.216	0.844	1.596	1.9	0.94	1.014	0.873	1.454	2.0
	CF	0.924	1.102	0.88	1.374	2.1	0.938	0.998	0.906	1.217	2.4
	STCH	0.938	2.484	0.88	3.403	2.1	0.95	2.238	0.894	3.235	2.2
		SG 2 5 2+SNV					SNV+SG2552				
PhIR	DM	0.798	0.761	0.62	1.017	1.2	0.772	0.8	0.601	1.035	1.2
	CP	0.938	1.025	0.878	1.423	2.1	0.929	1.091	0.862	1.512	2.0
	CF	0.926	1.087	0.83	1.608	1.8	0.9	1.254	0.787	1.799	1.6
	STCH	0.921	2.789	0.821	4.1	1.7	0.884	3.351	0.806	4.244	1.7
MICRO	DM	0.829	0.705	0.666	0.942	1.3	0.898	0.553	0.719	0.889	1.4
	CP	0.921	1.155	0.869	1.468	2.0	0.94	1.001	0.873	1.453	2.0
	CF	0.933	1.036	0.891	1.306	2.2	0.939	0.99	0.91	1.19	2.4
	STCH	0.942	2.406	0.886	3.323	2.2	0.953	2.178	0.884	3.368	2.1

DM: Dry matter; CP: Crude protein; CF: Crude fiber; STCH: Starch; SG N1 N2N3: Savitzky Golay, Derivative order, Smooth, polynomial order; R_C^2: determination coefficient of calibration; R^2_{CV}: determination coefficient of cross validation; MSC: multiplicative scatter correction; SNV: standard normal variate; SEC: standard error of calibration; SECV: standard error of cross validation; RPD:SD/SECV

Table 5.- External validation statistics for predicting nutritive parameters on intact compound feed using PhIR and MICRO NIRS instruments (validation set, N=12).

	Math pre-treatment	SECV %	SEP %	Accuracy Range %	t student Ref vs. Handheld NIRS	$SD_{interval}$
DM_{PhIR}	SG 1 10 2 + SNV	0,935	0,522	99-101	0.51	0.510-1.334
DM_{MICRO}	SG 1 5 2 + SNV	0,886	0,664	99-101	0.28	
CP_{PhIR}	SG 1 5 2 + MSC	1,310	0,953	88-105	1.88	0.446-1.655
CP_{MICRO}	SG 2 10 2 + SNV	1.360	1,132	86-111	1.12	
CF_{PhIR}	SG 1 5 2 + MSC	1,565	1,085	56-153	1.54	0.520-1.568
CF_{MICRO}	SNV + SG 2 5 2	1,190	1,239	62-129	1.23	
$STCH_{PhIR}$	SG 1 5 2 + MSC	3,230	3,635	84-122	0.54	0.883-2.840
$STCH_{MICRO}$	SG 1 5 2 + SNV	2,627	2,361	91-110	0.59	

DM: Dry matter; CP: Crude protein; CF: Crude fiber; STCH: Starch; SG N1 N2N3: Savitzky Golay, Derivative order, Smooth, polynomial order; SECV: standard error of cross validation, SEP: Standard error of prediction, $SD_{interval}$: lower and upper limits of a 95% confidence interval for the ratio of the true standard deviations (if the interval includes 1, the standard deviations are not significantly different at the 5% level)
*$t_{0.975, 11}$=2.20

Highlights

The performance of two handheld NIR spectrometers were investigated and compared to analyze raw compound feed.

One hundred intact compound feed were characterized and used for instrument calibration.

NIRS analysis of compound feed allowed to accurately predict their nutritive composition

NIR calibrations for nutritive value, using handheld NIRS instruments, were developed for the first time on intact compound feed.

Figure

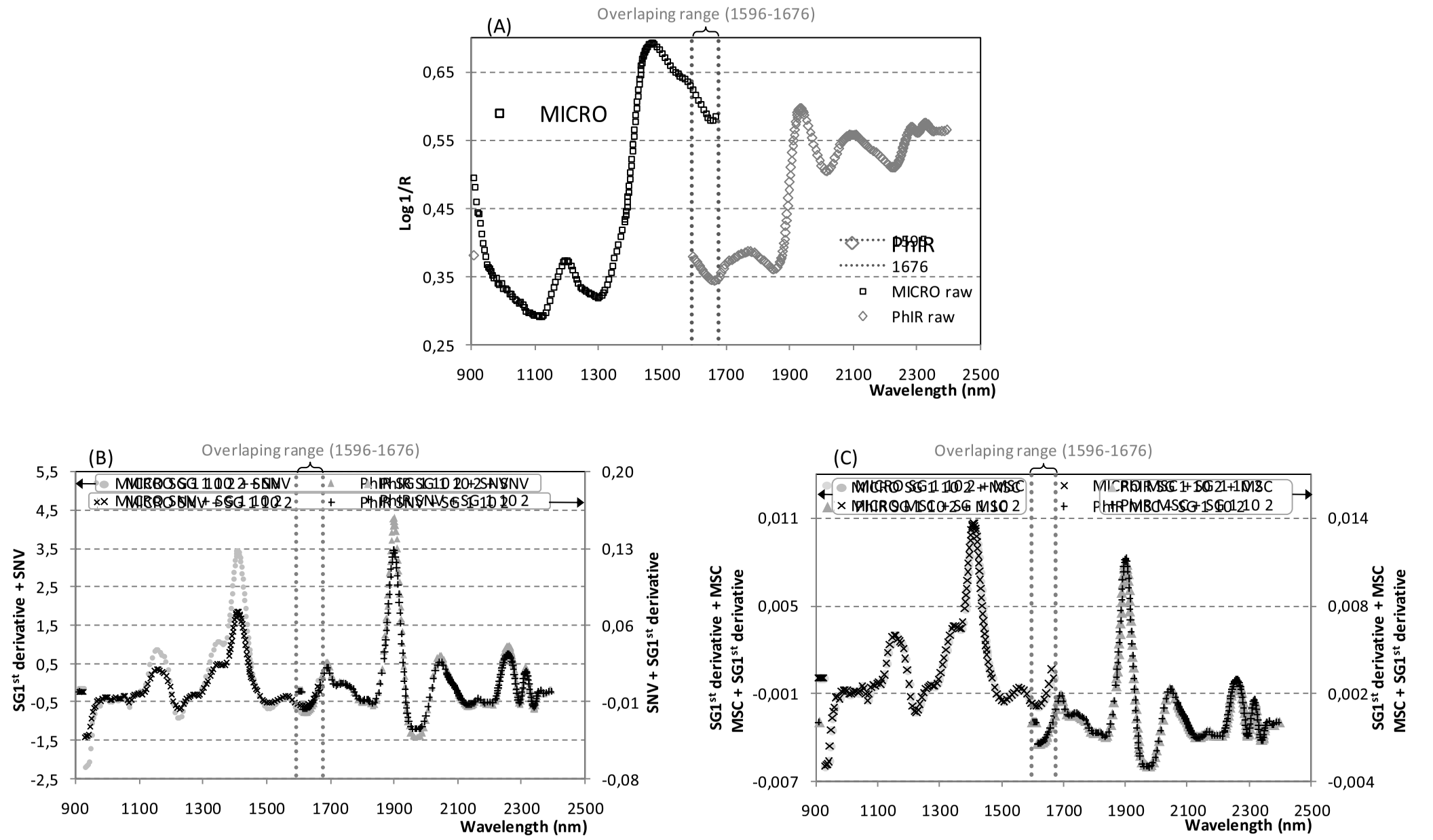

Figure 1

*Graphical Abstract (for review)

Graphical Abstract