Matching portable NIRS instruments for *in situ* monitoring indicators of milk composition

Begoña de la Roza-Delgado*[a], Ana Garrido-Varo[b], Ana Soldado[a], Amelia González Arrojo[a1], María Cuevas Valdés[a], Francisco Maroto[b] and Dolores Pérez-Marín[b].

[a]*Nutrition Research Programme. Regional Institute for Research and Agro-Food Development (SERIDA), PO Box 13, 33300 Villaviciosa. Spain*

[b] *Faculty of Agriculture and Forestry Engineering. Department of Animal Production. University of Cordoba. Campus Rabanales. Ctra. Nacional IV – Km 396. 14071 Cordoba. Spain.*

Author to whom correspondence should be addressed (telephone: +34 985890066; fax: +34 985891854; e-mail: broza@serida.org)

[1] Present Address: CETEMAS (Forest and Wood Technology Research Centre). Pumarabule, Carbayín, s/n, 33936 (Asturias) Spain.

Abstract

The real time knowledge of dairy milk composition can be used as a tool to guarantee milk quality and safety, offering additional information for dairy producers and consumers. To carry out these *in situ* analyses, methodologies based on Near Infrared (NIR) portable sensors have a great potential as an advisory tool. The main goals of the present work have been to develop a methodology using a hand-held portable NIR spectrophotometer to collect raw milk spectra, including the development of calibration models for the analysis of protein, fat and solids-non-fat (SNF) of raw milk and further to transfer the developed models to another portable unit. A total of 542 fresh milk samples were scanned over the NIR spectral range (1600–2400nm) using a hand-held MicroPhazir^{TM} (MP) NIR spectrometer and different instrumental configurations. The best results for repeatability and reproducibility calculated as root mean squared (RMS) were obtained using a 17 mm cuvette thickness. The displayed predictive ability of calibration models measured as Standard error of prediction/Standard error of cross validation were 0.96; 0.72 and 0.83 for fat, protein and SNF contents, respectively. For cloning purposes an additional MP unit (satellite) has been used. A standardization set of 10 samples enabled standardization of both instruments. After applying standardization matrix, Standard error of differences between master and satellite reached great reduction, 68% for fat, 66 % for protein and 54 % for SNF. Moreover, the demonstrated ability of sharing calibration models among several units is essential for implementation of portable instruments for in-situ analysis to provide indicators of milk composition at farm level.

Keywords: *MEMS-NIR, raw milk, in-situ NIRS analysis, standardization, calibration transfer*

Abbreviations

FNS: Foss NIRSytem 6500 monochromator

FTIR: Fourier Transform Infrared

GH: Global H

INIA: National Institute for Agricultural and Food Research

MBM: Meat and Bone Meal

MEMS: Micro-Electro-Mechanical System

MP: Microphazir ^{TM} NIRS Instrument

MPLS: Modified Partial Least Square

MP-SERIDA: Microphazir ^{TM} NIRS Instrument- Regional Institute for Research and Agro-Food Development

MP-UCO: Microphazir ^{TM} NIRS Instrument- University of Cordoba

NH: Neighbor Distance

NIRS: Near Infrared Spectroscopy

PDF: Precision Dairy Feeding

PDM: Precision Dairy Management

PLF: Precision Livestock Farming

R^2_{cv}: Coefficient of Determination in Cross-Validation

RMS(C): Root Mean Square of Differences Corrected for the Bias

SD: Standard Deviation

SECV: Standard Error of Cross-Validation

SED: Standard Error of Difference

SEP: Standard Error of Prediction

SNF: Solids-Non-Fat

SNV: Standard Normal Variate

SNVD: Standard Normal Variate plus Detrend

st1: Cloning set comprising 1 sample (the sample closest to the center of the population)

st10: Cloning set comprising 10 samples

TMR: Total Mixed Ration

UCO: University of Cordoba

1. Introduction

In the near future more and more dairy farms will uptake sophisticated Precision Livestock Farming (PLF) by sensors systems to support farm management. PLF is a combination of developing animal sensing (sensors) tools and decision-making process at the farm level. These precision systems include an instantaneous knowledge of dairy milk composition; this information can be used as a tool to guarantee milk quality and safety. It also has the potential to support animal feed suppliers, human-food retailers and other players along the supply chain to make better choices. The current challenge for PLF is the integration of the technology in the farm but not only to the pioneering farms (Halachmi, 2015). Banhazi, Babinszky, Halas & Tscharke (2012) outlined the potential role that PLF can play in ensuring that the best possible management processes are implemented on livestock farms increasing farm profitability and quality of milk products for consumers.

A new, alternative model for labour-efficient dairy production is emerging. Part of this trend in automation, robotic milking - an example of "precision dairy management" (PDM) - reduces labour requirements and minimize food safety risks (Rodenburg, 2012; Bewley, Russell, Dolecheck, Borchers, Stone & Wadsworth, 2015). However, in order to fully exploit the potential of this changing trend in dairy management, specific technologies should be considered together with the most widespread as, electronic radio frequency identification systems, robotic milking and calf- feeding systems, cameras, microphones, etc. These technologies allow control with precision as feed quality as the final product, milk, which could include under the term of Precision Dairy Feeding, (PDF). Taking into account that feed cost represents the most significant item of the total costs in milk production, and that in recent years, the volatility of the prices of cereals and flour protein, has been recurrent in world markets, it makes necessary to

use alternative rations, as far as possible, trying to introduce raw materials of low cost, and the greatest possible use of local resources and by-products, often based on a total mixed ration (TMR) that combines all ration ingredients into a single feed mix. This complicates the nutritionist roles, who must formulate rations with many raw materials, even with nutritional value and composition little known to them, maintaining quality and assessing milk safety. This situation of fragility of the dairy sector at the global level is causing, innovative nutritionists to look for alternatives such as NIRS instruments to be used as a necessary tool in PDF. There are numerous works in the NIR literature applying NIRS technology to milk analysis (reviewed by Holroyd, 2013). They have shown that it is possible to obtain high or moderate accuracy and precision in calibration models to predict the main chemical constituents. Papers dealing with the application of NIR to liquid milk can be split into several areas that involve; the determination of milk composition, authentication of cow feeding regimes and geographic origin of milk, including milk classification, calibration robustness, industrial applications and the measurement of milk microbiological content.

A high percentage of water content in samples to analyze could interfere with NIRS analyses. Water content in fresh milk is one of the major contributors to the variation in the NIR spectra due to the strong absorption bands of O-H groups in the NIR region, which can create a critical interference in quantitative analysis. Most of the research milk works are carried out using homogenized and dried samples (DESIR method) (Núñez-Sánchez et al., 2016).

The use of NIRS technology on-farm, for the analysis of forage and TMR has been demonstrated scientifically and there are some commercial solutions developed, such as a NIR Analyzer installed directly on the self-propelled mixer wagon or in the shovel of the front loader. It is able to predict dry matter for each ingredient during the loading

phase recalculating automatically the quantity to load to maintain a consistent ration (https://www.dinamicagenerale.com/Media/Default/Catalogues/PrecisionFeeding-ENG-LOW.pdf, 2016). However, research about the employment of portable NIRS sensors, susceptible to use for the on-site control of milk obtaining information on individual cow state is very limited or almost non-existent (Kawasaki et al., 2008; dos Santos, Lopo, Páscoa & Lopes, 2013). Therefore, it is urgent and important, to get scientific information about the potential of portable NIRS instruments for the analysis of raw milk, existing currently in the market.

The challenge facing this applied research is that the instruments more consolidated in the market, are not designed for this specific purpose of analyzing complex liquids such as milk. In terms of spectral characteristics and physico-chemical properties, it is necessary to show their adaptation and feasibility for the analysis of quality of raw milk. The main goals of the present work are to develop a new methodology based on use of hand-held portable NIR spectrophotometer for the analysis of fat, protein and solids-non-fat (SNF) in raw milk. Further we will evaluate the transferability of the developed methodology and calibration models to a second portable NIRS unit. Finally we will study the alternative of sharing prediction models among several units as essential tool for implementation of portable NIR instruments for in-situ analysis to provide indicators of milk composition at farm level.

2. Material and methods

2.1. NIR instruments and analysis methods

- 1) A Foss NIRSystem 6500 monochromator (FNS). This is an at-lab instrument, working in a wavelength range between 400 and 2500 nm, equipped with transport module under controlled environmental conditions (temperature 24ºC

± 1ºC, relative humidity 50% ± 10%). This instrument was used as a qualitative reference instrument to optimize sampling strategy and to evaluate the loss of spectra performance using portable instrument with small scanning window and narrow wavelength range. Spectra were collected using a liquid opaque quartz cuvette, reusable, with a 17 mm pathlength (C17) and an aluminum backside (FOSS. Ref US-ISIH-0398) for trans-reflectance measurements, combining reflectance and transmittance together into a single mode. The spectra data were recorded in reflectance mode (log 1/R) with ISI scan software (Infrasoft International Inc., Port Matilda, PA, USA). Each sample was analyzed in duplicate and each spectrum was the average of 32 scans performed on liquid milk.

- 2) MicroPHAZIR ^{TM} (MP) from Thermo Scientific, with a scanning window of 4 mm diameter (sampling area of 0.13 cm^2). All diffuse reflectance spectra were computed in a wavelength range between 1600 and 2400 nm, with a non-constant interval of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm) using a hand-held micro-electro-mechanical system (MEMS) digital transform as portable NIRS sensor. The instrumental conditions to collect raw milk spectra with this portable NIR were optimized modifying the parameters:

a) Sample presentation - two cuvettes have been assayed; the first one was C1 quartz cuvette, with a 1 mm pathlength and reusable. A liquid analysis adapter, to avoid NIR radiation losses through the quartz backside, was coupled to MP for the analysis of milk samples with this cuvette. The second one was the C17 quartz cuvette with an aluminum backside, described above (Foss NIRSystem 6500).

b) Number of scans to average for collecting one spectrum - the range evaluated was between 5, 10 and 80 scans/spectra. Five is the minimum value to be recorded using

Phazir Data Management System software (Polychromix, Inc., Wilmington, MA, USA) and 80 is the maximum value.

c) Internal reference or external reference for scanning background.

For cloning purposes two different units of MP have been used: SERIDA (MP-SERIDA; master instrument) and UCO (MP-UCO; satellite instrument) hand-held NIRS.

Nowadays there are other handhelds devices in market, however MP instruments have been selected to develop this research work because being handhelds NIRS they are easy to manage, and only these instruments were available in UCO and SERIDA labs (Modroño, Soldado, Martínez-Fernández & de la Roza-Delgado, 2017).

2.2. Samples and pretreatment

A total of 552 fresh milk samples were collected between 2014 and 2016 from individual Holstein–Friesian dairy cows of the experimental farm located in the Regional Institute for Research and Agro-Food Development (SERIDA) under different feeding experiments, and from different farms located in the North of Spain (Asturias, Spain), as suppliers from commercial milks looking at variability in their composition through the effect of supplementation, pasture biodiversity, fed different preserved forages (hay and/or silages) or changeability of TMR. Milk samples from experimental cows of SERIDA were taken from each individual animal by using the automatic sampler of Automatic milking system (DeLaval, Spain) and in farms by the farmer.

The first 50 fresh milk samples (Set 1) were employed to optimize instrumental conditions, and establish a sampling methodology for obtaining high quality milk NIR spectra using MP-SERIDA spectrophotometer. NIR analyses for this Set 1 were carried out simultaneously on portable MP-SERIDA and FNS as reference at-line instrument.

228 Set 2 comprising 492 milk samples was divided in two different groups selected with a
229 view to covering the whole range of spectral variability and product absorbance values,
230 using the SELECT algorithm included in the WinISI II version 1.50 software package
231 (Infrasoft International, Port Matilda, PA, USA):
232 Group 1 comprising 444 milk samples analyzed in hand-held MP-SERIDA. It was used
233 to develop the calibration models. NIR analyses for this Group 1 were carried out with
234 portable MP-SERIDA.
235 Group 2 comprising 48 milk samples scanned simultaneously on both hand-held
236 instruments, the master MP-SERIDA and in a second MP-UCO unit. This group was
237 divided in two different sub-groups. One sub-group comprising 10 milk samples
238 selected to obtain standardization matrixes and the other one comprising 38 milk
239 samples to validate the transference procedure.
240 As final step for practical performance, 10 milk samples coming from dairy cows of the
241 experimental farm of SERIDA were analyzed using MP-UCO device, to evaluate
242 sample by sample the calibration transfer procedure.
243 All samples were scanned without pretreatment after homogenization by hand mixing
244 for 20-30 sec. The same portion of the sample used to collect spectra in MP instruments
245 was used for reference data analysis (fat, protein and SNF). Reference analyses were
246 carried out using FTIR MilkoScan™ (Foss Electric, Hillerod, Denmark) in the
247 Professional Milk and Agro-food Laboratory of Asturias. This laboratory is accredited
248 under UNE-EN ISO/IEC 17025: 2005 (246/LE476).
249

250 *2.3. Spectral Data and Cloning Processing*
251
252 The first step when starting this research work was to export into *csv format all
253 spectral data collected from MP instruments. After that, the spectral data were adjusted

using an interpolation function to get data with a constant step of 2 nm and preserving the shape by interpolation (Fernández Pierna, Vermeulen, Lecler, Baeten, & Dardenne, 2010). This adjustment is necessary because the MP spectrometer works in the range of 1600 to 2400 nm with a non-constant step.

The WinISI software package v. 1.50 (Infrasoft 165 International, Port Matilda, PA, USA) was used to compare FNS *vs* MP spectral data and for chemometric development of MP calibration models. The equations were developed using Modified Partial Least Square (MPLS) as regression method and cross-validation to select the optimal number of factors to avoid overfitting (Shenk & Westerhaus, 1995). Chemical outliers were detected using the Student T test, to check differences between reference and predicted values; samples with a T value of over 2.5 were considered outliers (Mark & Workman, 1991).

Combined standard normal variate (SNV) plus detrend treatments were used for scatter correction (Barnes & Dhanoa, 1989). First- and second-derivative treatments were tested: 1.4.4.1; 1.8.8.1; 1.10.5.1, and 2.5.5.1, where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated (expressed in data points), the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (ISI software, 2000).

The best fitting equations, selected by statistical criteria for each parameter, on base of the lowest standard error of cross-validation (SECV), highest coefficient of determination in cross-validation (r^2_{cv}) (Williams, 2001; Pérez-Marín et al., 2008; Soldado, Fearn, Martínez-Fernández & de la Roza-Delgado, 2013) and lowest relation value between standard error of prediction (SEP, statistical parameter for testing external validation of the calibration model on 38 milk samples of group 2) and SECV (SEP/SECV) (Savenije, Geesink, van der Palen & Hemke, 2006).

279 Analytical features of NIR developed methodology was compared with reference
280 methods performance on the basis of their laboratory error and were calculated as
281 intermediate reproducibility according to ISO 5725 (ISO5725-1, 1994; ISO 5725-2,
282 1994) definitions: (i) repeatability, indicates the variability observed within a
283 laboratory, over a short time, using a single operator, item of equipment etc., and (ii)
284 intermediate reproducibility (standard deviation SD), intermediate precision relates to
285 the variation in results observed when one or more factors, such as time, equipment and
286 operator, are varied within a laboratory) on 10 different samples of Set 2 and was
287 calculated attending Eq. [1]:

288 $$R = S_R 2\sqrt{2} \quad [1]$$

289 A key factor in the cloning process is the number of samples used both when selecting a
290 procedure for standardizing NIR instruments and when selecting a cloning algorithm
291 (Zamora-Rojas et al., 2012; Pérez-Marín, Garrido-Varo ¬ Guerrero-Ginel, 2006). Since
292 cloning using numerous samples is a more complex procedure, it is advisable to
293 minimize the number of samples to be analyzed in parallel on the two instruments to
294 develop the algorithm. Two strategies using different number of samples were tested: (i)
295 10 samples comprising the cloning set (st10); and (ii) the sample closest to the center of
296 the population (st1). The cloning algorithm used for standardization process was the
297 patented algorithm by Shenk & Westerhaus (2008).

298 The statistic root mean square error (RMS) was used to select and to compare spectra
299 between subsamples in order to determine differences in repeatability and
300 reproducibility conditions (ISO5725-1 & 2, 1994).

301 This statistical parameter as the averaged root mean square of differences corrected for
302 the bias (RMS(c)) between two spectra was calculated using the CONTRAST algorithm

included in the WINISI software package, version 1.50 (Infrasoft International, Port Matilda, PA, USA), and the formula to calculate the RMS(c) is Eq. [2]:

$$RMS(c) = 10^6 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{im}-y_{ik})^2 - \frac{\left(\sum_{i=1}^{n}(y_{im}-y_{ik})\right)^2}{n}}{n-1}} \quad [2]$$

Where;

Y_{im} = log (1/R) value of m subsample at a wavelength i (λ_i).

$\overline{Y_{ik}}$ = log (1/R) value of k subsample at a wavelength i (λ_i).

n = number of wavelengths

Sample scanning modes giving spectra with the minimum value of RMS was selected for further development of calibration to predict quality parameters in milk. Besides, to evaluate the standardization process, spectra of master and host instrument were compared using the statistic RMS(c).

To evaluate the transference process of predictive NIRS models, were selected the Mahalanobis H. Values were calculated for the statistics global H (GH), i.e. the distance of a given sample from the center of the population, and neighbor (NH), i.e. the distance of that sample from its nearest neighbors (Zamora-Rojas et al., 2012) for spectral comparison, and the ratio SEPstandardized / SEPmaster and SEDstandardized / SEDmaster (SED: standard error of difference), to evaluate the transferred models.

3. Results and discussion

3.1. Sample presentation and NIRS analysis optimization

Prior to statistical assessment it was necessary to optimize sampling strategy to remove those spectra showing low quality. To attempt this work, during this optimization process all spectra were collected with FNS and MP devices. FNS analyzing with C17 cuvette was selected as reference instrument for qualitative comparison. To optimize

experimental conditions on MP-SERIDA (type of cuvettes, different number of spectra to average and the use of standard or internal reference material) was carried out the comparison between FNS and MP-SERIDA spectra shape.

The optimization results of spectra collection are shown in Fig. 1. As can be seen the strong absorption of water bands and the small scanning window of MP analyzer make it difficult to obtain spectra comparable to those obtained with the reference instrument. As it is well known, milk is a very complex matrix for NIR analysis, consisting of proteins in colloidal dispersion, fat in emulsion and minerals in solution (Marinori, Monti, Barzaghi & de la Roza-Delgado, 2013). One of the complexities facing us in the analysis of raw milk is the heterogeneity of the sample and its high water content (Schmilovivh, Shmulevich, Notea & Maltz, 2000; Tsenkova et al., 2000). It is an opaque liquid with highly light scattering effect caused by milk fat globules and casein micelles in suspension (Holroyd, 2013). Water content in raw milk is one of the major contributors to the variation in the NIR spectra, due to the strong absorption bands of O-H groups in NIR region, with a basic characteristic region at 1940 nm (Shenk, Workman & Westerhaus, 1992) that could limit the detection of analytes.

As can be seen in Fig. 1, the strong NIR absorption bands attributed to water due to the hydrogen bonds have led a high value for log (1/R) around 1940nm (water band), representing the O–H second overtone bending (Williams & Norris, 2001) and a high spectral noise at the end of scanning range when NIR analyses using MP instrument were made with 5 scans to average/sample employing both cuvettes, being much higher noise when the analysis are made with the cuvette C1 plus liquid adapter.

On the other hand, the recognition of absorption bands attributed to the other components such as fat or crude protein also was possible related with 2310 and 2180

nm, respectively, although they were very weak in comparison with the O-H bands and were more difficult to observe.

The following step was to optimize the number of scans to average for collecting one spectrum in MP instrument. To minimize spectra noise different numbers of scans were assayed 5, 10 and 80 scans/spectra. Results have shown that the spectral noise at the end of scanning range was reduced averaging 80 scans/sample and spectra were collected with high sensitivity. This value was selected for further work.

Afterwards the use of internal or external reference (material) was optimized. The use of the reference in NIR analysis is necessary to collect background, because all measurements are referred to the background. No differences were observed when analyzing milk samples using external or internal reference. For simplicity the internal reference was selected to collect spectra. This analyze mode avoids carry out and employ an external reference at farm level in order to simplify the analysis.

Table 1 shows the results of spectra repeatability and reproducibility for both cuvettes with the statistic RMS using milk samples from Set 1 to compare portable spectra (80 spectra to average and internal reference) with those recorded on FNS reference instrument. As can be seen, the best results were obtained using the C17 cuvette with an aluminum backside. Values for FNS (at-lab) were lower than MP being the ratio between at-lab and handheld device 0.5 in repeatability and 0.8 in intermediate reproducibility using C17 cuvette. Selected experimental conditions were: sampling with cuvette C17 and 80 scans/sample to average using the internal reference material.

After finishing the optimization procedure to collect spectra using the MP NIRS the samples were scanned using MP instrument to develop calibration models.

3.2. Calibration models

Calibration (Group 1) and validation (Sub-group 2) sets descriptive statistics (range, mean and standard deviation) are shown in Table 2. For each parameter, the validation set comprised samples representative of the total variance, all values lying within the range established for the calibration set. Both sets displayed, for range values, ratios calibration/validation from 0.88 to 1.28 and similar values for mean, and standard deviation (SD). As can be seen the average values of fat, protein and SNF percentage are similar to those established for milk quality payment. However, a high variability is observed in both populations, samples with high levels of fat and protein, and others with very low levels. Related with reference method error, the values were 0.114 % for fat; 0.063 for protein and 0.128 for SNF.

After assaying different derivative mathematical treatments to develop NIR calibrations (see Material and Methods section). The best results were obtained applying SNVD for scatter correction and 1,10,5,1 or 2,6,4,1 as math treatments. These pretreatments yielded the lowest SECV and highest r^2_{cv}. The external validation results were evaluated according to the minimum relation value between SEP/SECV. In base of these statistics finally were select 1,10,5,1 as math treatment for protein content and 2,6,4,1, for fat and SNF. Characteristics of the predictive models are given in Table 3.

The cross-validation statistics of calibration models displayed great predictive ability with SECV of 0.102 and r^2_{CV} of 0.961 for fat milk content. For protein content the model selected may be considered good (R^2 =0.758; r^2_{cv} = 0.676; SECV= 0.124%) whilst the model obtained for SNF would enable values for milk to be classified as high, medium or low concentration (R^2 = 0.612; SECV= 0.225%), following Williams' recommendations (2001).

The ratio SEP/SECV varied between 0.89 and 1.24. Assuming the SEP is approximately equal to SECV, this ratio is very acceptable with regard to the accuracy of the calibration. (Savenije, Geesink, van der Palen & Hemke, 2006).

Different research works using NIR laboratory instruments have established the usefulness of NIRS technology to predict milk composition and microbiological parameters (Holroyd, 2013). However, it is necessary taking into account that these evaluations were conducted using NIR instruments with wide spectral range and different possibilities of sample preparation and presentation.

In this sense, Tsenkova and co-workers (2000) evaluated the potential of NIRS to measure fat, total protein, and lactose contents of unhomogenized milk for use in dairy management, as a new tool for on-line milk analysis in the process of milking, working in the wavelength range from 400 to 2500 nm with sample thicknesses of 1 mm, 4 mm, and 10 mm based on log (1/T) data. Their found that the accuracy of fat and protein content determination of bovine milk depended strongly on the spectral regions and path lengths and the best results were obtained for the region from 1100 to 2400 nm with 1-mm sample thickness. The SECV for the model based on the first derivative spectral data transformation was 0.110 and the r^2_{cv} was 0.998 for fat content and SECV= 0.096 and r^2_{cv} = 0.848 for protein. With regard to fat content our results shown in Table 3 generally agreed with those reported by these authors by using a portable instrument with a narrow spectral range.

Related with on-line NIR analysis a publication by Masataka and co-workers (2008) provide NIR spectra of raw milk obtained in an automatic milking system (milking robot system) over a wavelength range of 600 nm to 1050 nm (transmittance). The SEP of the validation set for fat was 0.25%, this SEP value represent 200% of SEP reported here (SEP= 0.126). The value of SEP for protein obtained for these authors was 0.15%,

again the SEP value obtained in this work for this parameter is slightly lower (SEP = 0.124%).

Related with the results obtained using portable analyzer designed and developed for raw milk quality analysis during the material purchase in dairy plants (Feng et al., 2013) calibration model shows worse SEP values (0.172 and 0.201 for fat and protein content) than those obtained in this work.

3.3. Standardization process

Two standardization matrixes were developed using one milk sample (st1) or 10 samples (st10). To evaluate the success of the standardization procedure the first step was focused on the reduction of GH and NH values, in validation set (N=38) (see Table 4). These GH values were 1.497 for MP-SERIDA, 20.000 for MP-UCO before standardization and 1.550 after applying standardization matrix developed with one sample (MP-UCOst1). Related with NH the values obtained were 0.858 for MP-SERIDA, and decreasing from 15.309 to 1.043 for MP-UCO after applying standardization matrix. The GH and NH values obtained for MP-UCO before standardization, confirm the need for this process. GH and NH statistics show and excellent agreement between spectra collected in both instruments even when applying only one standardization sample and confirm that standardization successfully reduced spectral differences between both instruments for the validation-test set.

Related with the comparison between the spectra recorded in both MP evaluated instruments attending RMS(c) statistic, the best results, those with minor RMS(c), were obtained with the standardization matrix built with 10 samples. The RMS(c) values between master unit and secondary device spectra decreased from 54,590 prior to

453 standardization to 16,493 and 11,818 when applying st1 or st10 standardization
454 matrixes.
455 Fig. 2A and 2B show the mean spectra for the external validation set collected with both
456 handheld NIRS instruments before and after standardization process as raw log (1/R)
457 spectra (A) and after applying first derivative and SNVD mathematical treatments to the
458 spectral data (B). In this Fig. 2 can be seen differences between the spectra before
459 standardization in the 1880–2100 nm range. These log1/R differences are related to the
460 differences between instruments that are the same model device but they are not cloned
461 instruments. Both MP units can vary in photometric response; this is due to detectors,
462 light sources and changes over in the instrumental response function (ageing of sources,
463 replacement of some parts, etc.). However, these spectra differences must disappear
464 after standardization process showing a successful result of the standardization
465 approach.
466 The last step in the calibration transference process was to validate the transferred
467 equations with the external set of samples (Sub-group 2, N=38). Results for external
468 validation on both instruments are shown in Table 5. When the equations were applied
469 to non- standardized spectra from MP-UCO, there was a loss of performance with SEP
470 values of 0.147; 0.810 and 1.663 % for fat, protein and SNF content, respectively.
471 Nevertheless, after applying st1 or st10 standardization matrices SEP from MP-UCO
472 decreased approximately 80 % for protein and 85% for SNF content. Related with milk
473 fat content the standardization process has not too much influence over the reduction of
474 SEP values. Probably, the specific NIRS bands related with fat from 2150 to 2300 nm
475 are not affected by the standardization, because the great differences between the
476 spectra recorded in MP-SERIDA *vs* MP-UCO before standardization are in the 1880–

2100 nm range, directly related with protein wavelength ranges (Osborne & Fearn, 1986).

Additionally, to check the performance of transferred models was calculated the SED, expressed as a difference between NIR analyses on MP-SERIDA and MP-UCO instruments (see Table 5). After applying standardization matrices, SED values between MP-SERIDA and MP-UCO decreased at least eight times for SNF and five times for protein compared to non-standardized results. For fat the reduction was only 1.2 times lower. These SED values were close to SEP values.

To include a practical performance, after comparing the standardization procedure between NIR instruments (MP-SERIDA and MP-UCO), 10 milk samples coming from dairy cows of the experimental farm of SERIDA were analyzed with the MP-UCO device and applying both standardization matrices. Results are detailed in Table 6. As can be seen differences between reference and predicted values decrease after standardization. However, we must remark that there are not differences between both standardization matrices. For protein and SNF there are two samples with errors lower using st1 than using st10 standardization matrices. For fat, the prediction of 4 samples is more exact when applying st1. Nevertheless, st10 always has minor sum of residual values than st1.

To the best of our knowledge this is the first time that the ability of the MicroPHAZIR^{TM} to predict the milk composition changes of individual cows has been demonstrated. Furthermore, the ability of sharing calibration data among several units is a key point with a great importance for implementation of portable instruments at farm level for *in situ* quality control of milk.

4. Conclusions

After evaluating different sampling strategies to analyze raw milk samples using the handheld instrument Microphazir™ we can conclude that to obtain satisfactory results it is necessary to average 80 scans to collect one sample spectra using 17mm sample thickness cuvette with an aluminum backside.

This study has established a promising ability of this handheld NIR instruments to estimate the individual dairy milk composition changes. Moreover, the calibration models developed showed that the accuracy and precision of the equations obtained using the handheld instrument were similar, in terms of both calibration and validation, to those of the equations obtained on lab based instruments.

The promising results for the ability of sharing calibration data (transference procedure) after applying a simple standardization algorithm for spectral adjustment minimized spectral differences between hand-held MicroPhazir analyzers even developed with only one sample have great importance for implementation of portable instruments as a tool for *in situ* monitoring indicators of milk composition.

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635 **TABLES**

636 **Table 1.** Repeatability and reproducibility root mean square (RMS) for 80 scans by

637 spectra with C1 and C17 cuvettes types

638
639

Intrument	Cuvette type	Repeatibilty RMS	Reproducibility RMS
MP-SERIDA	C1 (1-mm + adapter)	11190	45270
	C17 (aluminum 17 mm)	5309	4799
FNS	C17 (aluminum 17 mm)	2568	3823

640
641 *MP: MicroPHAZIR NIR instrument*

642

643 **Table 2.** Statistic descriptive values for milk samples in calibration and external

644 validation sets

645

	CALIBRATION (N=444)			EXTERNAL VALIDATION (N=38)		
Parameter (%)	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
Fat	2.38 – 6.36	3.67	0.575	2.71 – 4.97	3.57	0.476
Protein	2.58 – 4.00	3.18	0.262	2.47 – 3.37	2.98	0.193
SNF	7.14-9.85	8.73	0.325	7.66-9.12	8.62	0.287

646

647 *SD: standard deviation variation, SNF: solids-non-fat*

648

649 **Table 3**. Statistics for calibrations models developed in MP-SERIDA Master Unit

650

Parameter (%)	SEC	R^2	SECV	r^2_{cv}	SEP
Fat	0.089	0.971	0.102	0.961	0.126
Protein	0.120	0.758	0.139	0.676	0.124
SNF	0.185	0.612	0.225	0.476	0.221

651

652 *SNF: solids-non-fat; SEC: Standard Error of Calibration; R^2: Determination Coefficient of*
653 *Calibration; SECV: Standard Error of Cross-validation; r^2_{cv}: Determination Coefficient of*
654 *Cross-Validation; SEP: Standard Error of Prediction*

655

Table 4. GH, NH and RMS(c) values for the "cloning set" (N=38) analyzed on the MP-SERIDA and MP-UCO before and after standardization using two matrixes (st1 and st10).

Parameter	MP-SERIDA	MP-UCO before	MP-UCOst1 after	MP-UCOst10 after
Mean GH	1.497	20.000	1.550	1.839
Mean NH	0.858	15.309	1.043	1.218
RMS(C) (µlog (1/R))	12,965	54,590	16,493	11,818

st1= Sample closest to center of population; st10= 10 samples.

Table 5. Standard errors of prediction and standard errors of difference in the validation set (N = 38) for the calibrations obtained in the MP-SERIDA and MP-UCO for predicting fat, protein and SNF content in raw milk.

Parameter	SEP				SED		
	MP-SERIDA	**MP-UCO before**	**MP-UCOst1 after**	**MP-UCOst10 after**	**MP-SERIDA *vs* MP-UCO**	**MP-SERIDA *vs* MP-UCOst1**	**MP-SERIDA *vs* MP-UCOst10**
Fat	0.126	0.147	0.167	0.145	0.179	0.193	0.146
Protein	0.124	0.810	0.190	0.178	0.762	0.133	0.179
SNF	0.221	1.663	0.460	0.274	1.573	0.361	0.214

SNF: solids-non-fat

Table 6. Practical performance using calibration models before and after transference procedure, for predicting fat, protein and SNF content in raw milk (N=10).

	Fat				Protein				SNF			
Sample	**Ref.**	**MP-UCO before**	**MP-UCOst1 after**	**MP-UCOst10 after**	**Ref.**	**MP-UCO before**	**MP-UCOst1 after**	**MP-UCOst10 after**	**Ref.**	**MP-UCO before**	**MP-UCOst1 after**	**MP-UCOst10 after**
1	3.39	3.30	3.32	3.43	2.94	1.69	2.81	2.85	8.52	7.16	8.39	8.45
2	3.40	3.32	3.34	3.48	2.77	1.98	3.10	3.15	8.54	7.15	8.41	8.55
3	3.21	3.35	3.36	3.18	2.81	0.85	2.30	2.76	8.24	6.52	8.06	8.52
4	3.33	3.29	3.31	3.36	2.47	1.48	2.72	3.05	7.66	6.82	8.19	8.55
5	3.84	3.72	3.68	3.84	3.20	2.06	3.09	3.12	8.86	7.30	8.46	8.55
6	3.93	3.71	3.67	3.84	2.97	1.68	2.78	2.83	8.45	7.16	8.35	8.44
7	3.86	3.68	3.65	3.80	2.92	1.43	2.62	2.73	8.45	7.06	8.32	8.48
8	3.52	3.39	3.40	3.50	3.06	1.79	2.92	2.98	8.69	7.16	8.40	8.47
9	3.54	3.78	3.72	3.63	3.12	1.86	3.06	3.31	9.03	7.11	8.46	8.72
10	4.38	4.24	4.13	4.34	2.86	1.93	2.96	2.98	8.57	7.50	8.61	8.65

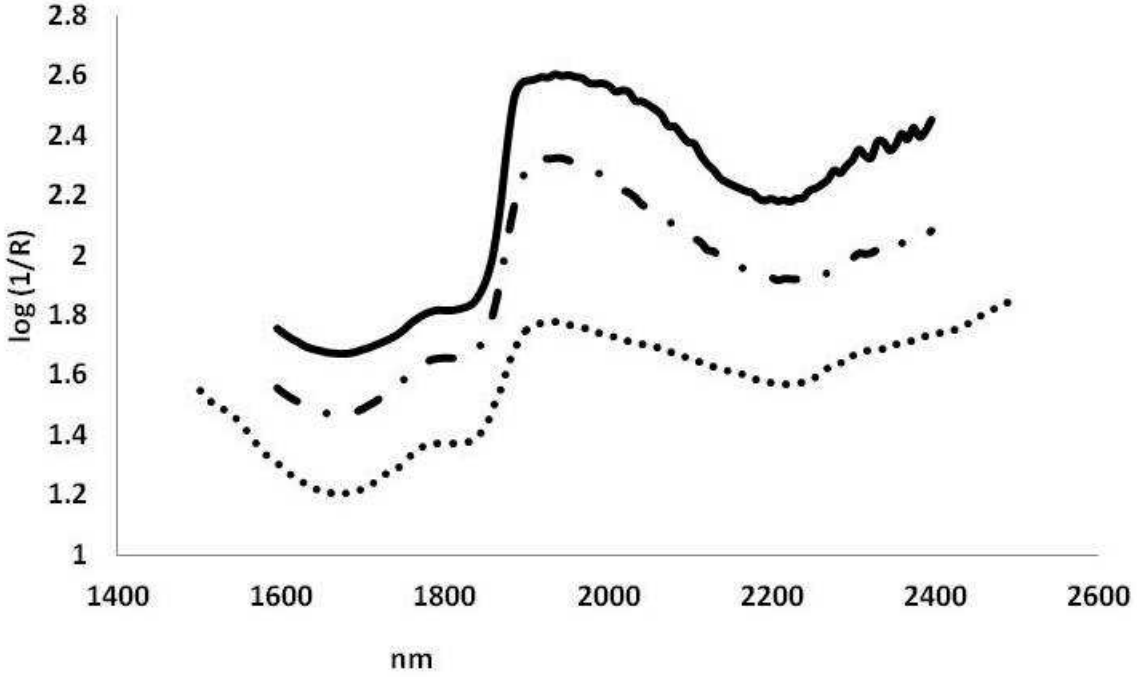
Ref. : Reference data, SNF: solids-non-fat

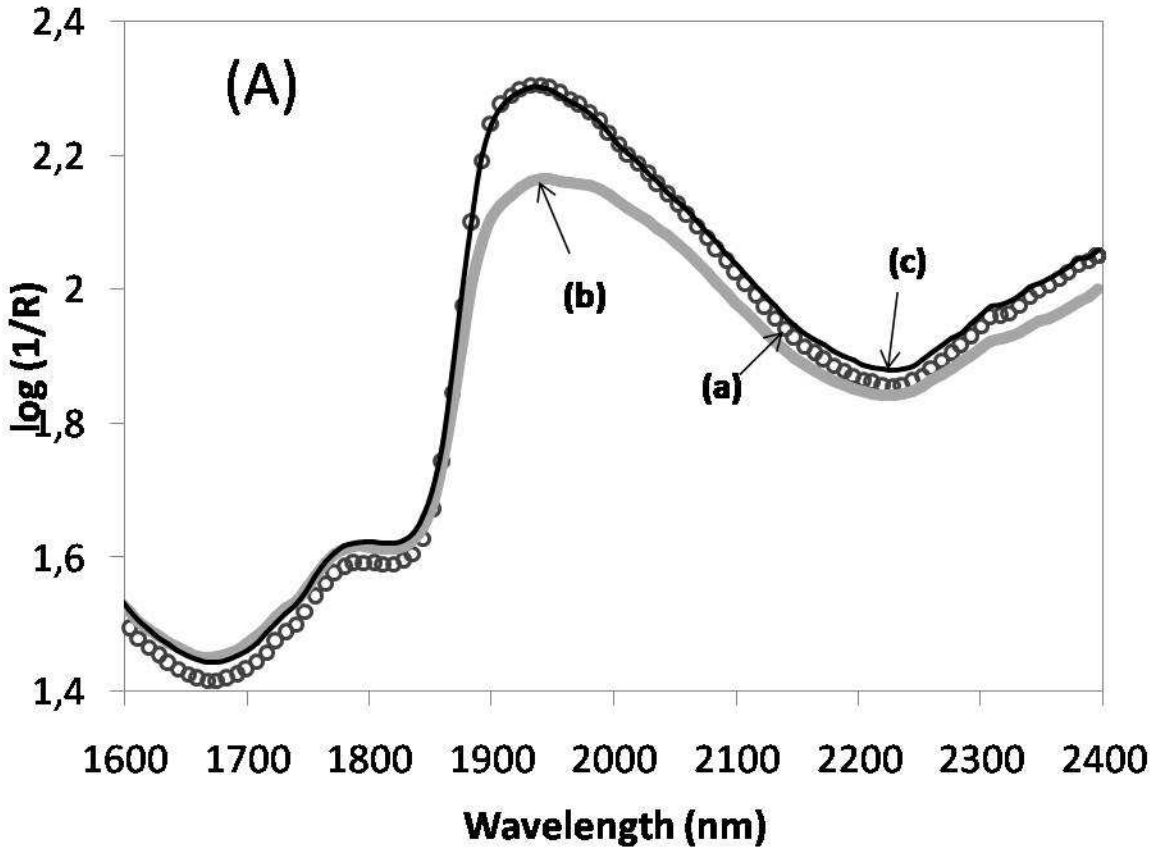
Figure captions

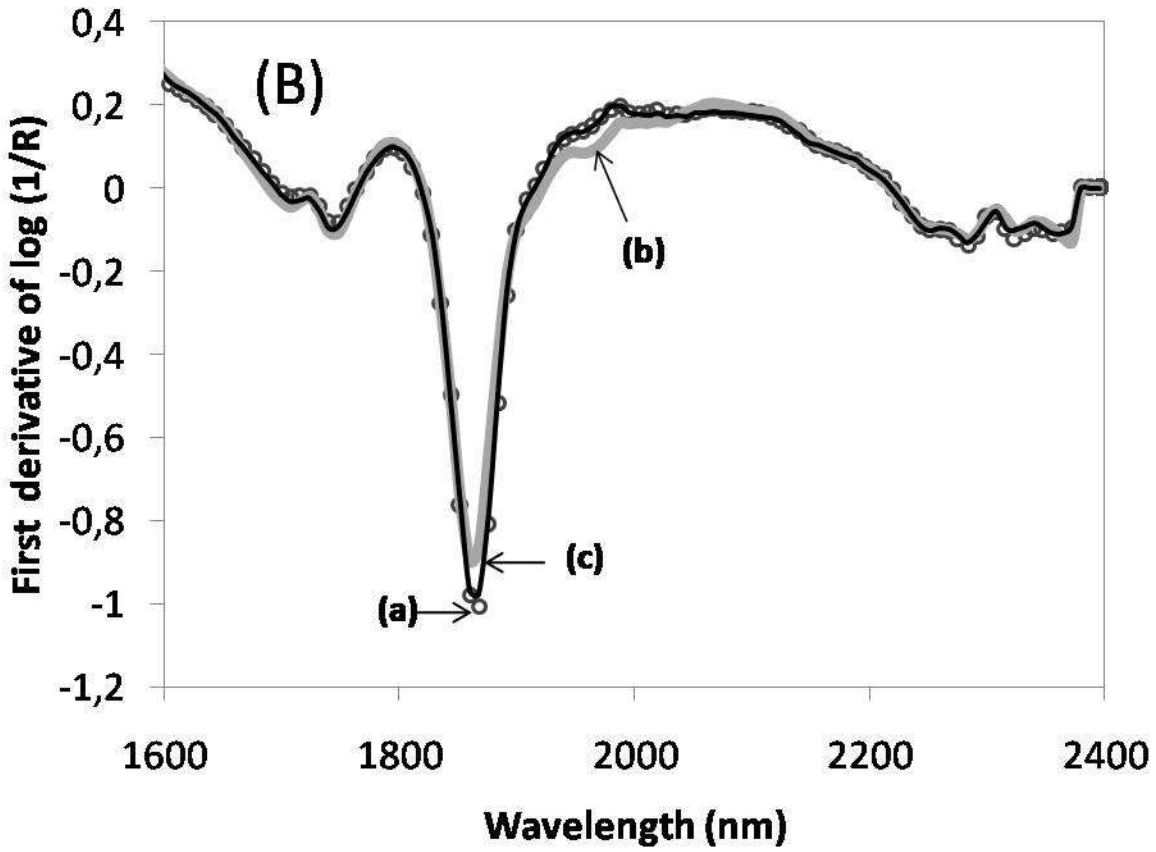
Fig. 1. Mean spectra of milk (N=25 of Set 1) analyzed averaging 5 scans/sample in MP-SERIDA and FNS instruments and different cuvettes.

A) MP-SERIDA: C1 cuvette + adapter module; B) MP-SERIDA: C17 cuvette; C) FNS: C17 cuvette

Fig.2. Mean spectra for the external validate transfer set (Set 2 (Group 2), N=38 samples and 80 scans/spectra) with both instruments. (A) Raw log (1/R) spectra with no pretreatment and (B) First derivative spectra with SNVD treatment. In both plots the line with circles (a) is the MP-SERIDA, (b) the grey solid line is the MP-UCO before standardization and (c) the thick solid black line is the MP-UCO after standardization.


2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
log (1/R)
1400
1600
1800
2000
2200
2400
2600
nm


(A)
2,4
2,2
2
1,8
1,6
1,4
log (1/R)
(a)
(b)
(c)
1600
1700
1800
1900
2000
2100
2200
2300
2400
Wavelength (nm)


(B)
0,4
0,2
0
-0,2
-0,4
-0,6
-0,8
-1
-1,2
First derivative of log (1/R)
1600
1800
2000
2200
2400
Wavelength (nm)
(a)
(b)
(c)