Near-infrared spectroscopy with fiber optic probe for determination of fatty acid profile in raw milk

NGUYEN, H.N.[(1)] *; DEHARENG, F.[(1)]; HAMMIDA, M.[(1)]; NIEMÖLLER, A.[(2)] and DARDENNE, P.[(1)]

(1) Walloon Agricultural Research Centre, Valorisation of Agricultural Products Department, 24 Chaussée de Namur, 5030 Gembloux (Belgium)
(2) Bruker Optics GmbH (NIR & Process Technology), Rudolf-Plank-Str. 27, 76275 Ettlingen (Germany)
* Corresponding author : h.nguyen@cra.wallonie.be

NIR 2011 CAPE TOWN SOUTH AFRICA
15th International Conference on Near Infrared Spectroscopy
13 - 20 MAY 2011

1. Aim

Evaluation of a Fourier Transform Near-infrared (FT-NIR) fiber optic probe system, as an alternative technique to the current methods used for quantification of milk fatty acid (FA) composition.

2. Materials and methods

Sampling and spectra recording

Broad FA variability was ensured by collecting, from August 2008 to June 2009, 583 raw milk samples derived from different farms, species and feeding regimes. Liquid milk samples were analyzed using a FT-NIR Matrix-F, a Fourier Transform Near-infrared instrument from Bruker Optics, equipped with a fiber optic probe. From the total set, 82 samples were selected for calibration using principal component analysis (PCA).

Fatty acid analysis using the GC method

The reference methods for FA composition analysis involve chemical steps such as extraction by solvents and derivation of FA before analysis by gas chromatography (GC). Detailed FA composition of the 82 selected samples was analyzed by GC according to the method of Collomb and Bühler (2000).

Calibrations and statistics

- **Predictive equations were developed using modified partial least squares (PLS1) with cross-validation.**
- **The standard normal variate (SNV) was applied to the raw data. The spectra were then transformed using a mathematical first-order gap derivation (Savitsky-Golay).**
- **The RPD statistic provides a basis for standardizing the standard error of prediction and should be as high as possible. RPD values greater than 2.5 are considered adequate for analytical purposes.**

3. Results and discussion

Table 1. Statistics models for Fat and the FA composition (variability expressed in g /dL of milk)

N=82	Min-Max	SE_C	R^2_C	SE_{CV}	R^2_{CV}	RPD
Fat	2.05-5.96	0.05	1.00	0.06	0.99	12.3
C4:0	0.07-0.18	0.01	0.61	0.02	0.48	1.4
C6:0	0.04-0.11	0.01	0.45	0.01	0.39	1.3
C8:0	0.03-0.07	0.01	0.34	0.01	0.27	1.2
C10:0	0.06-0.20	0.02	0.70	0.02	0.45	1.3
C12:0	0.05-0.27	0.02	0.74	0.03	0.50	1.4
C14:0	0.27-0.73	0.05	0.80	0.06	0.71	1.9
C14:1	0.02-0.08	0.01	0.66	0.01	0.56	1.5
C16:0	0.59-2.08	0.11	0.89	0.12	0.86	2.7
C16:1	0.03-0.16	0.02	0.72	0.02	0.64	1.7
C17:0	0.01-0.05	<0.01	0.77	<0.01	0.74	2.0
C18:0	0.16-0.71	0.07	0.66	0.07	0.62	1.6
C18:1trans	0.04-0.22	0.03	0.58	0.03	0.51	1.4
C18:1n9	0.25-1.64	0.06	0.95	0.09	0.88	2.9
C18:2n6	0.02-0.11	0.01	0.85	0.01	0.69	1.8
C18:3n3	0.01-0.04	<0.01	0.64	<0.01	0.51	1.4
CLA	0.01-0.08	<0.02	0.48	<0.02	0.37	1.3
∑Omega-3	0.01-0.06	<0.01	0.69	<0.01	0.60	1.6
∑Omega-6	0.05-0.16	<0.02	0.77	<0.02	0.65	1.7
SFA	1.57-4.16	0.11	0.97	0.14	0.94	4.2
UNSAT	0.48-2.13	0.07	0.96	0.10	0.91	3.4
MUFA	0.41-1.81	0.06	0.96	0.08	0.92	3.5
PUFA	0.07-0.27	0.02	0.79	0.03	0.69	1.8
SCFA	0.22-0.51	0.04	0.74	0.05	0.52	1.5
MCFA	1.18-3.20	0.16	0.90	0.18	0.88	2.9
LCFA	0.59-2.76	0.14	0.91	0.15	0.87	2.8

Abbreviations - **N** : number of samples used to develop the model; SE_C : standard error of calibration; R^2_C : coefficient of determination for calibration; SE_{CV} : standard error of cross-validation; R^2_{CV} : coefficient of determination for cross-validation; **RPD** : residual predictive deviation; **SFA** : saturated fatty acids; **MUFA** : monounsaturated fatty acids; **PUFA** : polyunsaturated fatty acids; **UNSAT** : unsaturated fatty acids; **CLA** : conjugated linoleic acid; **SCFA** : short-chain FA; **MCFA** : mid-chain FA; **LCFA** : long-chain FA.

- **Our results found better prediction for milk FA in high concentrations than for the minor ones. Similarly, Coppa et al. (2010) found alike results with NIRS on milk powder.**
- **Good prediction of C16:0, C18:1n9, SFA, UNSAT, MUFA, MCFA and LCFA (RPD ≥ 2.7).**

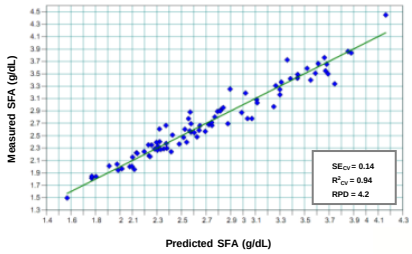

Fig. 1. Linear regression plots of measured versus predicted values of SFA

- **Failure to accurately determine Omega-3/6 fatty acids, CLA, PUFA, SCFA and most of individual FA. The quality of prediction decreases when FA are present in low to very-low concentrations (≤ 0.73 g/dL).**

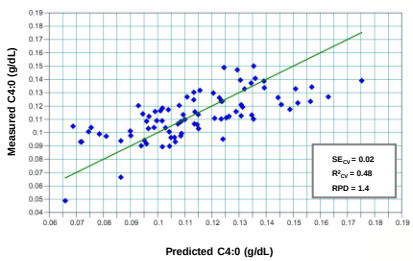

Fig. 2. Linear regression plots of measured versus predicted values of C4:0

4. Conclusion

The obtained results showed that an FT-NIR fiber optic probe system can be used to satisfactorily predict FA sums and ratios. For individual milk FA present in low concentrations, it remains valid to use prediction as an indicator for batch selection.

This study was carried out as part of the Milkinir research project supported by the Agricultural Head Office of the Walloon region - DGARNE-DGO 3 (Belgium)

Walloon Agricultural Research Centre
Valorisation of Agricultural Products Department
Agricultural Products Technologies Unit
valpro@cra.wallonie.be - www.cra.wallonie.be

Walloon Agricultural Research Centre

Near-infrared Spectroscopy with fiber optic probe for determination of fatty acid profile in raw milk

Hoang Nam Nguyen[1*], Frédéric Dehareng[1], Mohamed Hammida[1], Andreas Niemöeller[2] and Pierre Dardenne[1]

[1]Walloon Agricultural Research Centre, Valorisation of Agricultural Products Department, 24 Chaussée de Namur, 5030 Gembloux, Belgium
[2]Bruker Optics GmbH (NIR & Process Technology), Rudolf-Plank-Str. 27, 76275 Ettlingen, Germany
*Corresponding author: h.nguyen@cra.wallonie.be

Introduction

Milk is a complex beverage and contains many components such as lipids, proteins, carbohydrates and minerals in variable concentrations. The wide variety of milk fatty acids can be more or less beneficial regarding human health, especially in the context of the increasing social demand in terms of healthy food. Therefore it is now relevant to accurately estimate milk composition and quality at low cost and to identify the factors affecting milk variation. This approach is essential for obtaining high-quality dairy products.

The study presented here belongs to a broader project which aims at using NIR spectrometry (NIRS) for daily on-line measurement within the milking room (*Milkinir* research project, subsidized by the Agricultural Head Office of the Walloon region - DGARNE-DGO3, Belgium). To that purpose, it is essential to propose recording equipment which suits for multiple measurements at several places inside the same area, while preserving a sufficient spectral quality in order to predict a maximum of parameters. The use of fiber optic probes connected to a FT-NIR spectrometer is considered here. The objective of the present study is to evaluate the potential of such system as an alternative technique to the current methods used for quantification of milk fatty acids (FA) composition. The possibility of employing NIR predictive models would fasten the analytical procedure compared to the chemical steps of current reference methods which involve extraction by solvents and derivation of FA before analysis.

Materials and Methods

Samples and Near infrared spectroscopy

Broad FA variability was ensured by collecting, from August 2008 to June 2009, 583 raw cow milk samples derived from different farms, species and feeding regimes. Individual fresh milk samples were analyzed using a FT-NIR Matrix-F (834-2502 nm) from Bruker Optics, equipped with a fiber optic probe which measures samples in transflection mode (IN271P-02, Bruker Optics transflection probe for process control). NIR spectra of the milk samples were collected at 38 ± 1 °C. Each sample was measured in duplicate, and the spectral mean was used for further analysis and calibration models.

Chemometrics

From the total set, 82 samples were selected for calibration using Principal component analysis (PCA - Mahalanobis standardized distances between the closest neighbours equal or higher than 0.6). Detailed FA composition of the 82 selected milk was analyzed by gas chromatography (GC) according to the method of Collomb and Bühler (2000)[1].
The models were carried out by "OPUS 6.5", a Bruker Optics spectroscopy software package. The standard normal variate (SNV) was applied to the raw data. The spectra were then transformed using a mathematical first-order gap derivation (Savitzky-Golay). Predictive equations were developed using modified Partial Least Square regression (PLS) with cross-validation (leave one out method).

Results and Discussion

The RPD statistic provides a basis for standardizing the standard error of prediction and should be as high as possible. RPD values greater than 2.5 are considered adequate for analytical purposes (Sinnaeve *et al.*,

1994)[2]. The statistical models for Fat and the studied fatty acids are reported in **Table 1**. Our results found better prediction for milk FA in high concentrations than for the minor ones. Similarly, Coppa *et al.* (2010)[3] found alike results with NIRS on milk powder.

Table 1. Statistical models for Fat and the FA composition (variability expressed in g/dL of milk)

N=82	Min - Max	SE_C	R^2_C	SE_{CV}	R^2_{CV}	RPD
Fat	2.05 - 5.96	0.05	1.00	0.06	0.99	12.3
C4:0	0.07 - 0.18	0.01	0.61	0.02	0.48	1.4
C6:0	0.04 - 0.11	0.01	0.45	0.01	0.39	1.3
C8:0	0.03 - 0.07	0.01	0.34	0.01	0.27	1.2
C10:0	0.06 - 0.20	0.02	0.70	0.02	0.45	1.3
C12:0	0.05 - 0.27	0.02	0.74	0.03	0.50	1.4
C14:0	0.27 - 0.73	0.05	0.80	0.06	0.71	1.9
C14:1	0.02 - 0.08	0.01	0.66	0.01	0.56	1.5
C16:0	0.59 - 2.08	0.11	0.89	0.12	0.86	2.7
C16:1	0.03 - 0.16	0.02	0.72	0.02	0.64	1.7
C17:0	0.01 - 0.05	<0.01	0.77	<0.01	0.74	2.0
C18:0	0.16 - 0.71	0.07	0.66	0.07	0.62	1.6
C18:1trans	0.04 - 0.22	0.03	0.58	0.03	0.51	1.4
C18:1n9	0.25 - 1.64	0.06	0.95	0.09	0.88	2.9
C18:2n6	0.02 - 0.11	0.01	0.85	0.01	0.69	1.8
C18:3n3	0.01 - 0.04	<0.01	0.64	<0.01	0.51	1.4
CLA	0.01 - 0.08	<0.02	0.48	<0.02	0.37	1.3
∑Omega-3	0.01 - 0.06	<0.01	0.69	<0.01	0.60	1.6
∑Omega-6	0.05 - 0.16	<0.02	0.77	<0.02	0.65	1.7
SFA	1.57 - 4.16	0.11	0.97	0.14	0.94	4.2
UNSAT	0.48 - 2.13	0.07	0.96	0.10	0.91	3.4
MUFA	0.41 - 1.81	0.06	0.96	0.08	0.92	3.5
PUFA	0.07 - 0.27	0.02	0.79	0.03	0.69	1.8
SCFA	0.22 - 0.51	0.04	0.74	0.05	0.52	1.5
MCFA	1.18 - 3.20	0.16	0.90	0.18	0.88	2.9
LCFA	0.59 - 2.76	0.14	0.91	0.15	0.87	2.8

Abbreviations - **N** : number of samples used to develop the model; **SE_C** : standard error of calibration; **R^2_C** : coefficient of determination for calibration; **SE_{CV}** : standard error of cross-validation; **R^2_{CV}** : coefficient of determination for cross-validation; **RPD** : residual predictive deviation; **SFA** : saturated fatty acids; **MUFA** : monounsaturated fatty acids; **PUFA** : polyunsaturated fatty acids; **UNSAT** : unsaturated fatty acids; **CLA** : conjugated linoleic acid; **SCFA** : short-chain FA; **MCFA** : mid-chain FA; **LCFA** : long-chain FA.

Our prediction models showed good performance (RPD $\geq$ 2.7) for C16:0, C18:1n9, saturated fatty acids (SFA), unsaturated fatty acids (UNSAT), monounsaturated fatty acids (MUFA), mid-chain FA (MCFA) and long-chain FA (LCFA). But we failed to accurately determine Omega-3/6 fatty acids, conjugated linoleic acid (CLA), polyunsaturated fatty acids (PUFA), short-chain FA (SCFA) and most of individual FA. The quality of prediction decreases when FA are present in low to very-low concentrations ($\leq$ 0.73 g/dL of milk). Linear regression plots of SFA and C4:0 (measured versus predicted values, expressed in g/dL of milk) are shown in **Figs. 1** and **2** respectively.

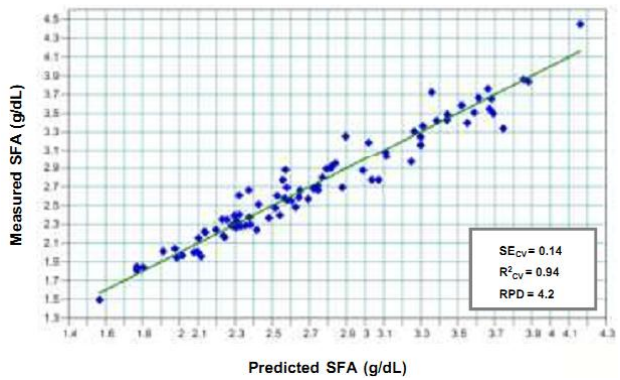

Figure 1. Linear regression plots of measured versus predicted values of SFA

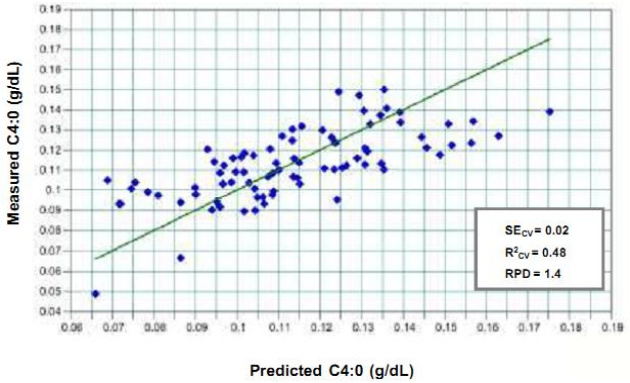

Figure 2. Linear regression plots of measured versus predicted values of C4:0

Conclusion

The statistical results showed that a FT-NIR fiber optic probe system can be used to satisfactorily predict fatty acids sums and ratios. For individual milk fatty acids present in low concentrations, it remains valid to use prediction as an indicator for batch selection.
Nevertheless, more research should be done by increasing the initial sampling size in order to try to improve the quality of prediction. Moreover, it could be useful to focus on other spectra pre-treatment procedures while simultaneously testing other regression methods in an attempt to get more accurate estimation equations of milk fatty acid profile.

Acknowledgments

The authors thank the WBA farms (Walloon Breeding Association - Ciney, Belgium) and Bruker Optics GmbH (NIR & Process Technology - Ettlingen, Germany) for the technical support and constructive discussions.
This study was carried out as part of the *Milkinir* research project supported by the Agricultural Head Office of the Walloon region - DGARNE-DGO 3 (Belgium).

References

1. M. Collomb and T. Bühler, *Travaux Chimiques en Alimentation et Hygiène* **91**, 306-332 (2000).

2. G. Sinnaeve, P. Dardenne, R. Agneessens and R. Biston, *The use of near infrared spectroscopy for the analysis of fresh grass silage*. Journal of Near Infrared Spectroscopy, **2**, 79-84 (1994).

3. M. Coppa, A. Ferlay, C. Leroux, M. Jestin, Y. Chilliard, B. Martin and D. Andueza, *Prediction of milk fatty acid composition by near infrared reflectance spectroscopy*. Inter. Dairy Jour.: Vol. **20**, Issue 3, 182-189 (2010).