New parameters and analytical challenges for milk recording by Fourier-Transform Mid-Infrared Spectrometry (FTMIR)

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Abstract

The increasing consumer concern over the relationship between food and human health requires to consider the analysis of new characteristics of milk composition. Due to the large number of analyzed samples, the technology used by the milk recording organizations must be fast and cheap. For these reasons, the Fourier Transform mid-infrared spectrometry (FTMIR) is largely used to quantify major milk components. The recent literature reveals that FTMIR is currently under-used in practice.

Currently, only fat and protein contents are routinely quantified by FTMIR and sometimes the concentrations of urea, lactose, and casein. Recent studies showed the potentiality of FTMIR to quantify new milk components or to predict indicators related to specific milk properties. With the condition of good analytical practices during the calibration and the use of these new equations, some of them can be implemented in milk labs. These FTMIR predictions can be executed internally in the spectrometer software or externally based on recorded spectral data.

Moreover, the FTMIR predictions can be used for additional valorisations by combining information recorded by milk recording structures and the FTMIR predictions. For instance, by using models to explain the observed variability of the studied traits, it is possible to extend the number of possible valorisations such as useful tools for herd management and breeding purposes.

Consequently, FTMIR becomes a powerful technology to quantify milk components and/or to permit a screening of the dairy cattle population based on different milk characteristics interesting for different purposes: nutritional quality (e.g. fatty acid, minerals), hygienic quality (e.g. antibiotics, somatic cells), technological quality (e.g. cheese-making), environment (e.g. methane), herd management (e.g. urea), animal health (e.g. lactoferrin, acetone), and biodiversity. The large number of FTMIR predictions will involve the development of methodologies to resume the most interesting information for the development of specific dairy products and to help farmers in their daily decisions. FTMIR still has a bright future.

Keywords: Mid-infrared, milk, FTMIR spectrometry, selection tools.

1. Introduction

The consumer is more and more conscious that the diversity, the quantity, as well as the quality of the ingested foods influence his health. This situation is reinforced by the attitude of many dieticians and nutritionists who recommend to their patients to limit drastically their consumption of dairy products due to notably the large amount of saturated fatty acids present in bovine milk fat (70% on average). It involves a truncated view in the interest of dairy products. Therefore, to promote the healthiness of dairy products, the dairy sector should take into account the detailed milk composition. Consequently, milk labs and also milk recording organizations should think about the analysis of new characteristics of milk composition showing a potential economic interest.

Traditionally, the assessment of a detailed milk composition is expensive because it requires a lot of different chemical steps and analyses such as the separation of studied constituents from the milk matrix, the use of gas chromatography or other analyses… Moreover, all of these analyses require a lot of time, skilled staff, and use often polluting products. For many years, FTMIR spectrometry has been used to quantify the major components of milk such as fat and protein contents used for the milk payment. Thanks to its fast and non-destructive advantages, this technology could be a good alternative to the traditional chemical analysis.

2. FTMIR Spectrometry

There are 3 different infrared regions (near, medium, and far infrared) with their own specificities. The analysis of milk can be realised by using near or mid-infrared. The mid-infrared has a high sensitivity to the chemical environment due to the fundamental absorptions of molecular vibrations (Belton, 1997). Mid-infrared spectrum represents the absorptions of mid-infrared ray at frequencies correlated to the vibrations of specific chemical bonds (Figure 1). Therefore, the mid-infrared spectrum reflects the global chemical composition. The near infrared gives a much more complex structural information related to the vibration behaviour of combination bonds (Cen and He, 2007). In this review, it was decided to discuss about the potentialities of FTMIR spectrometry for milk analysis because this technology is largely used by milk labs all around the world to quantify major milk components used for the milk payment or by the milk recording organizations to develop management and selection tools to help farmers in their daily decisions.

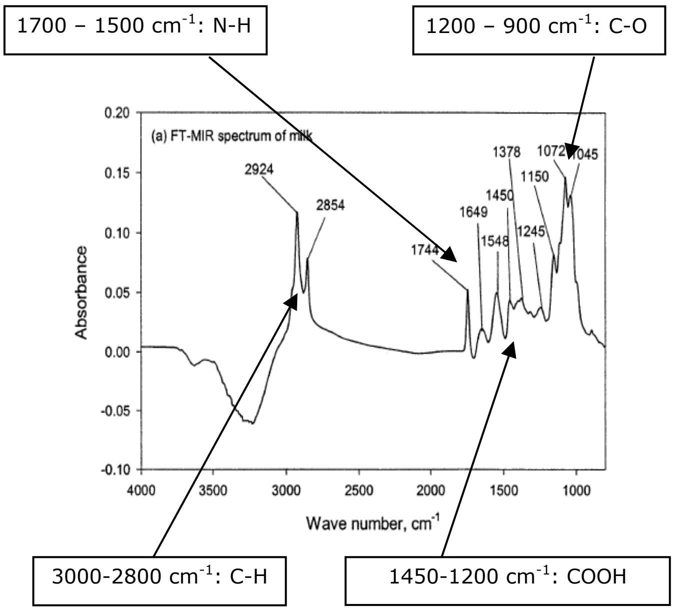

Figure 1. MIR spectrum of milk (Sivakesava and Irudayaraj, 2002).

FTMIR spectrometry is not only routinely used to quantify the contents of fat and protein in milk, but also the contents of urea, lactose, casein, and free fatty acids. Nevertheless, the recent literature reveals that FTMIR is currently under-used in practice.

3. FTMIR and milk recording

3.1 Introduction

The main objective of milk recording organizations is to develop management and selection tools useful for the dairy sector including dairy farmers and dairy industry in the current economic context. Two ways are possible to achieve this aim: first, a direct use of the FTMIR predictions of specific milk components and second, the milk recording organization can put together all available information (FTMIR predictions but also animal, lactation, and environmental information) necessary to take into account the natural variation of the considered traits in order to extend the number of potential valorisations.

3.2 Direct use of FTMIR data

The principle to obtain milk FTMIR predictions is resumed in Figure 2. The collected samples are analyzed by FTMIR spectrometry and raw data (commonly named spectral data) are generated. The number of datapoints depends on manufacturers. Finally, a specific equation is applied to the spectral data to provide the measurement of the studied trait (e.g., fat, protein…). Therefore, if you want to analyze new components in milk, you need to develop new equations.

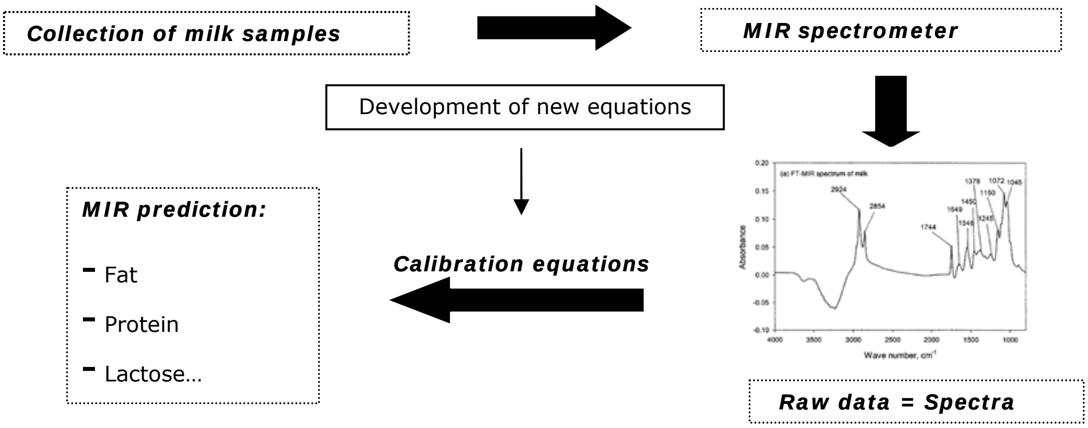

Figure 2. Principle of FTMIR prediction of milk components

Several authors have realized different research studies to extend the number of milk constituents predictable by FTMIR, which showed an interest in different fields such as the nutritional quality (e.g., fatty acid, minerals, lactoferrin), hygienic quality (e.g., antibiotics, somatic cells), technological quality (e.g., cheese-making properties of milk (e.g., casein, titrable acidity, coagulation time…), environment (e.g. urea, fatty acids, methane emissions through fatty acid predictions (Chilliard *et al.*, 2009), herd management (e.g., urea, fat, protein, lactose), animal health (e.g., fatty acids, minerals, lactoferrin, -hydroxybutyrate, acetone), and biodiversity (e.g., by studying the changes in milk composition). This review presents some examples potentially interesting for milk recording organizations.

Recently, several authors showed the potentiality of FTMIR spectrometry to quantify the fatty acid contents directly on bovine milk (Rutten *et al.*, 2009; Soyeurt *et al.*, 2006, 2008a, 2008b, and 2010). The prediction of fatty acid in milk (g/dl of milk) is more accurate if the content of considered fatty acid is high in milk. The FTMIR prediction of fatty acid in fat is less accurate because the variability of fatty acids in milk fat is lower than the one observed in milk. Table 1 describes the results obtained by Soyeurt *et al.* (2010) from a multiple breeds, multiple countries, and multiple production systems approach for major groups of fatty acids in bovine milk. RPD calculated, as the ratio of the standard deviation of reference value to the standard error of cross-validation, is a parameter assessing the robustness of a calibration equation. If this ratio for a considered equation is greater than 2, it involves a potential use of this equation for breeding and animal purposes. Therefore, all fatty acids shown in Table 1 could be used in practise to assess the nutritional quality of bovine milk fat.

Table 1. Descriptive statistics of the calibration equations for the quantification fatty acids in milk developed by Soyeurt et al. (2010).

Constituent (g/dl of milk)	N	Mean	SD	RPD	SECV
Saturated FA	496	2.40	0.80	15.7	0.0513
Monounsaturated FA	491	1.06	0.37	8.9	0.0411
Polyunsaturated FA	499	0.16	0.05	2.6	0.0204
Unsaturated FA	492	1.22	0.41	9.6	0.0428
Short chain FA	486	0.31	0.11	6.7	0.0165
Medium chain FA	496	1.78	0.60	6.5	0.0928
Long chain FA	495	1.52	0.57	6.5	0.0875

Based on Soyeurt *et al.* (2009), other traits potentially predictable by FTMIR spectrometry are the calcium, sodium, and phosphorus contents in milk as shown in Table 2. Even if this publication considered a low number of samples, the results for Ca and P were recently confirmed by using 100 additional milk samples (data not shown).

Table 2. Descriptive statistics of the calibration equations measuring minerals in milk developed by Soyeurt et al. (2009).

Mg/l of milk	N	Mean	SD	SECV	RPD
Ca 87		1333	260	95	2.74
K	61	,336	168	136	1.24
Mg	61	110	18	11	1.68
Na	87	403	107	64	1.68
P	87	1093	127	50	2.54

Interesting traits for milk recording organizations to check animal health status are ketone bodies. Hansen (1999) and Heuer *et al*. (2001) developed the first calibration equations to quantify acetone content in bovine milk. More recently, De Ross *et al*. (2007) has also developed with a relatively good success calibration equations for acetone and -hydroxybutyrate in milk (Table 3).

Table 3. Descriptive statistics of the calibration equations for ketone bodies in milk developed by De Ross et al. (2007).

mMol	N	Mean	SECV	R²c
Acetone 1063		0.146	0.184	0.72
-hydroxybutyrate 1069		0.078	0.065	0.62

The improvement of milk nutritional quality is desired. However, it is necessary to know if these changes are positively related to the technological properties of milk. In this context, several authors have developed calibration equations permitting to assess the cheese-making properties of milk through the quantification of specific traits such as titrable acidity, rennet coagulation time… Based on these results (Table 4), it appears that the cheese-making properties of milk could be assessed by relatively good FTMIR predictions of titrable acidity and rennet coagulation time.

Table 4. Descriptive statistics of the calibration equations for traits related to cheese-making properties of milk.

		N	Mean	SD	R²cv	SECV
Titrable acidity (SH°/50ml)	De Marchi *et al*., 2009	1063	3.26 0.	43 0.	66 0.	25
Rennet coagulation time (min)	De Marchi *et al*., 2009	1049	14.96 3.	84 0.	62 2.	36
	Dal Zotto *et al*., 2008	74	15.05 3.	78 0.	73 0.	80
pH	De Marchi *et al*., 2009	1064	6.69	0.12	0.59	0.07
Titrable acidity (D°)	Colinet *et al*., 2010	203	16.22 2.	01 0.	90 0.	64
Curd firmness (mm)	Dal Zotto *et al*., 2008	74	32.43	7.95	0.45	5.49

Another interesting trait is a glycoprotein present naturally in milk and entitled lactoferrin because this molecule is involved in the immune system. Soyeurt *et al*. in 2007 developed a preliminary calibration equation for the measurement this milk component. This first equation was built with 57 reference samples and the obtained RPD was equal to 2.39 with a SECV equal to 86 mg/l of milk.

3.3 Models based on FTMIR data

The milk recording organizations have an access to the animal, lactation and environmental data (pedigree, lactation stage, breed, number of lactation…). Merging these data with the FTMIR prediction permits to investigate the potential for using specific models that take into account the natural variability of these FTMIR values and therefore to extend the number of possible valorisations. To illustrate this application, two examples are presented.

Bastin *et al*. (2009) showed the possibility to model the level of milk urea in a specific herd by using a random regression test-day model. From the results given by the model, it is possible to estimate an expected value of milk urea content in a specific herd at specific test date. Based on that, it is possible to compare the expected value obtained by the model to the observed one. If the difference is too big, it can be assumed that the studied herd has a management problem (Figure 3).

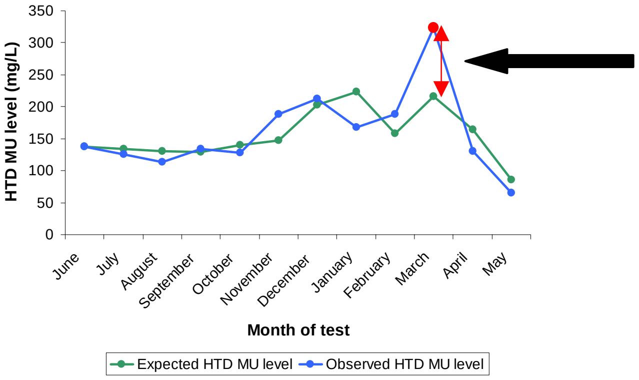

Figure 3. Evolution of observed and expected urea content (MU) in a specific herd (Bastin et al., 2009)

Another very interesting application for milk recording organizations (because these structures have individual values for cows) could be to model the contents of a specific FTMIR prediction in order to give to the farmers sufficient information to discard the less interesting cows and/or to develop an animal selection programs.

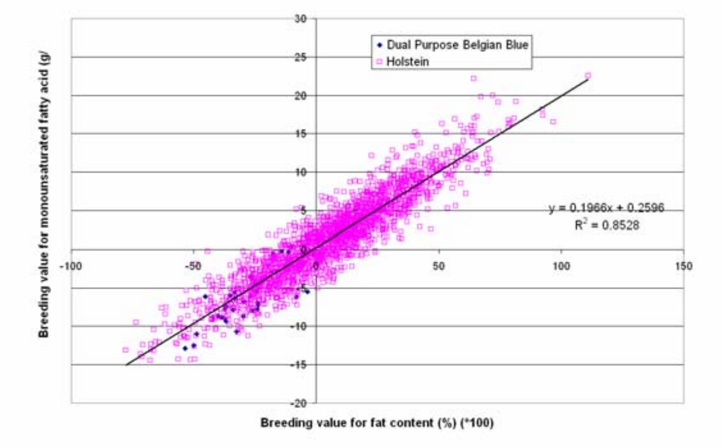

Figure 4. Relationship between the estimated breeding value for monounsaturated fatty acid in milk and the estimated breeding value for fat content.

One interest of an animal selection program is to have information for foreign bulls based on data collected from their daughters present in a country where the FTMIR analysis of a specific trait is done. For instance, through the European project RobustMilk (www. robustmilk.eu), the tools needed to implement an animal selection programs for fatty acid contents in bovine milk are developed. The contents of fatty acids in milk are heritable. The lactation heritabilities for saturated and monounsaturated FA were 44% and 22%, respectively. The first results were obtained from data collected from first parity cows. Figure 4 shows the results of the genetic evaluation for 1,993 bulls with a sufficient number of Walloon daughters with known fatty acid data. High variability of breeding values (parameters estimated to assess the individual variability of studied animals) for monounsaturated fatty acid content was observed for a considered estimated breeding value of fat content (Figure 4). Consequently, a sufficient variability of fatty acid traits exists to investigate the development of an animal selection based on the improvement of the nutritional quality of milk.

This kind of researches can be extended to all FTMIR predictions.

4. Conclusion

In conclusion, the FTMIR spectrometry is currently under-used in practice even if new traits predictable by MIR exist. A lot of work should be done by the milk recording organizations to include these new traits showing a potential economic interest in their services given to their members. Two ways will be possible to achieve this objective: a direct use of FTMIR predictions given by milk labs and/or the developments of specific models taking into account the natural variability of the studied infrared traits in order to develop specific valorisations for dairy sector including farmers, dairy companies, breeding associations…

However, this introduction of new traits in the routine milk recording will involve new challenges. The first challenge will be an analytical challenge. To avoid high bias, the FTMIR equation should be validated on the considered cow population. Indeed, breed differences or differences in the milk samples (e.g., the composition of bulk milk is less variable than the composition of milk samples collected from individual cows) used to develop the calibration equation could involve a bias. Moreover, it is currently possible to implement externally new equations thanks to the recording of spectral data. However, to use this approach successfully, it will be needed that the variability of the spectral data used for the prediction by the milk recording organization was taken into account in the calibration set used to build the used FTMIR equation. Finally, the accuracy of the FTMIR prediction should be tested regularly by the use of reference samples to correct if needed the bias and the slope of the calibration equation. Since January 2008, FTMIR fatty acid predictions is implemented in the Walloon milk lab (Battice, Belgium) and a maintenance is realized using milk samples with known contents of fatty acid (these samples are produced by Walloon Agricultural Research Centre – Valorisation of Agricultural Products Department (Gembloux, Belgium).

The second challenge will be a computational challenge. The number of studied traits by milk recording organizations will increase. Consequently, it will be necessary to study some traits simultaneously because some of them (the majority of them) will be correlated. It will be also important to know the natural variability of the studied FTMIR trait for a specific cow because the optimum of content for a studied trait can be different according to the considered aim. For instance, high lactoferrin content in milk is interesting for human health but sick cows can also produce milk samples with high content of lactoferrin. This kind of applications will require the use of multiple traits models, which need high computational cost.

In conclusion, a lot of work to do to improve the services given to the dairy farmers thanks to the extension of FTMIR possibilities.

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6. References

Bastin, C., L. Laloux, A. Gillon, F. Miglior, H. Soyeurt, H. Hammami, C. Bertozzi, and N. Gengler. 2009. Modeling milk urea of Walloon dairy cows in management perspectives. J. Dairy Sci. 92:3529-3540.

Belton, P. S. 1997. Spectroscopic approaches to the measurement of food quality. Pure Appl. Chem. 69(1):47-50.

Cen, H., and Y. He. 2007. Theory and application of near infrared spectroscopy in determination of food quality. Trends Food Sci. Technol. 18(2):72-83.

Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199-5211.

Colinet, F.G., H. Soyeurt, C. Anceau, A. Vanlierde, N. Keyen, P. Dardenne, N. Gengler, and M. Sindic. 2010. Potential etimation of titrable acidity in cow milk using mid-infrared spectrometry. Proc. 37th ICAR annual meeting, 31 May – 4 June 2010, Riga, Latvia.

Dal Zotto, R., M. De Marchi, A. Cecchinato, M. Penasa, M. Cassandro, P. Carnier, L. Gallo, and G. Bittante. 2008. Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. J. Dairy Sci. 91:4103-4112.

De Marchi, M., C.C. Fagan, C.P. O'Donnell, A. Cecchinato, R. Dal Zotto, M. Cassandro, M. Penasa and G. Bittante. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. J. Dairy Sci. 92: 423-432.

Hansen, P.W. 1999. Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. J. Dairy Sci. 82:2005-2010.

Heuer, C., H.J. Luinge, E.T.G. Lutz, Y.H. Schukken, J.H. van der Maas, H. Wilmink, and J. P.T.M. Noordhuizen. 2001. Determination of acetone in cow milk by Fourier transform infrared spectroscopy for the detection of subclinical ketosis. J. Dairy Sci. 84:575-582.

de Roos, A.P.W., H.J.C.M. van den Bijgaart, J. Horlyk, and G. De Jong. 2007. Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. J. Dairy Sci. 90:1761-1766.

Rutten, M.J.M., H. Bovenhuis, K.A. Hettinga, H.J.F. van Valenberg, and J.A.M. van Arendonk. 2009. Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. J. Dairy Sci. 92:6202-6209.

Sivakesava, S., and J. Irudayaraj. 2002. Rapid determination of tetracycline in milk by FT-MIR and FT-NIR spectroscopy. J. Dairy Sci. 85(3):487-493.

Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89(9):3690-3695.

Soyeurt, H., F.G. Colinet, V.M.-R. Arnould, P. Dardenne, C. Bertozzi, R. Renaville, D. Portetelle, and N. Gengler. 2007. Genetic variability of lactoferrin content estimated by mid-infrared spectrometry in bovine milk. J. Dairy Sci. 90(9):4443-4450.

Soyeurt, H., F. Dehareng, P. Mayeres, C. Bertozzi, and N. Gengler. 2008a. Variation of delta9-desaturase activity in dairy cattle. J. Dairy Sci. 91:3211-3224.

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008b. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. J. Dairy Sci. 91:3611-3626.

Soyeurt, H., D. Bruwier, J.-M. Romnee, N. Gengler, C. Bertozzi, D. Veselko, and P. Dardenne. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J. Dairy Sci. 92:2444-2454.

Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne. 2010. J. Dairy Sci. Submitted.