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Comparison of near and medium infrared spectroscopy to predict fatty acid composition on fresh and thawed milk

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ABSTRACT

Near (NIR) and medium (MIR) infrared reflectance spectroscopy (IR) predictions of fatty acid (FA) composition, expressed as g/kg of milk or g/100 g of FA, on fresh and thawed milk were compared. Two-hundred-and-fifty bulk cow milks, collected from 70 farms in northwest Italy, were scanned by MIR in liquid form and by NIR in liquid and oven-dried forms. MIR and NIR FA (g/100 g FA) predictions on oven-dried milk were similar for the sum of even chain-saturated FA (ECSFA), odd chain-FA (OCFA), unsaturated FA (UFA), conjugated linoleic acid (CLA), n-3 FA, and C18:1cis9 to C16 ratio. The monounsaturated FA (MUFA), n-6 to n-3 ratio, polyunsaturated FA (PUFA), and n-6 FA were predicted better by NIR on oven-dried milk. The NIR showed worse predictions than MIR for almost all FA, when expressed as g/kg of milk. The NIR predictions on fresh liquid and oven-dried milk were similar, but the reliability decreased for thawed liquid milk. The high performance shown by NIR and MIR allows their use for routine milk FA composition recording.

Keywords: near infrared reflectance spectroscopy (NIR), medium infrared reflectance spectroscopy (MIR), milk fatty acids.

1. Introduction

Dairy products are an important constituent of human diet in Western Europe, with a consumption of about 92.9 kg/capita per year, and a contribution of about 30% of total animal fat consumption (Food and Agriculture Organization of the United Nations, 2012). An excess in consumption of SFA, which are in relatively large amount in milk, as well as of some trans- and n-6 fatty acids (FA) has been associated with negative effects on cholesterolemia, obesity, metabolic syndrome, and coronary heart diseases (Stark, Crawford & Reifen, 2008; Kratz, Baars & Guyenet, 2013). In contrast, the consumption of dairy products rich in n-3 FA reduced the risk of cardiovascular disease (Stark, et al., 2008; Dawczynsky, Martin, Wagner & Jahreis, 2010), and conjugated linoleic acid (CLA) could inhibit degenerative cellular proliferation and reduce obesity and cardiovascular diseases (Dilzer & Park, 2012).

The FA composition of milk also influences milk fat melting point, and thus cheese and butter texture (Martin, Verdier-Metz, Buchin, Hurtaud & Coulon, 2005; Coppa et al., 2011).

In this context, the demand by dairy farmers, dairy industry and consumers for information on FA composition of milk is growing. In some European countries (i.e. France, and The Netherlands), FA composition has already been introduced among the parameters that are considered to determine milk price (Borreani et al., 2013). This implies a need for rapid and cheap methods to perform milk FA analysis. However, the reference method for FA analysis is based on gas-chromatography (GC) determination. The GC analyses are generally expensive and time-consuming and require great expertise, making them unsuitable for routine milk recording (Rutten, Bovenhuis, Hettinga, van Valenberg, & van Arendonk, 2009). On the contrary, Infrared Spectroscopy (IR) techniques are rapid, cheap (cost of analysis of about 1:100 compared to GC methods) and multi-parametric, and are routinely used to determine milk fat, protein, and other parameters considered when determining milk price (Bogomolov, Dietrich, Boldrini & Kessler, 2012). The most commonly used IR method for milk analysis is medium IR (MIR), which can analyze a high number of samples daily (up to 500 samples/hour). However, MIR apparatus used for milk analyses is specifically set up to analyze liquid milk only, and is very expensive. As a consequence, low analysis costs can be only achieved when a huge number of samples are analyzed per day. On the contrary, near IR (NIR) has a lower potential of analysis per day (about 150-200 analysis per day), but the NIR apparatus is cheaper (about 1:20 compared to MIR), and is not set up to analyze a specific product. Thus NIR could be used in small laboratories, that perform a low number of milk analyses daily, and use NIR also to analyze other products (i.e. forages or cheese). Recently, prediction equations have been proposed for milk FA analysis. Soyeurt et al. (2011) obtained reliable prediction of the main FA (expressed as g/dL of milk), by calibrating MIR models on a large number of individual fresh milk samples from commercial farms. These equations, were then validated by Maurice-Van Eijndhoven, Soyeurt, Dehareng & Claus (2013) on different cow breeds. De Marchi, Penasa, Cecchinato, Mele, Secchiari & Bittante (2011)

predicted milk FA concentration (expressed as g/kg of milk) of individual milk from Brown Swiss cows reared in different farming systems by MIR. Rutten, et al. (2009) compared effectiveness of MIR in predicting the concentration of some FA according to the season in which individual fresh milk samples were collected and to the way reference data were expressed (g/dL of milk or g/100 g of FA). The prediction equations developed on FA expressed as g/dL seem to lead to better predictions, perhaps because this FA prediction by MIR is the combined effect of predicting fat content and FA composition on the same spectrum (Soyeurt et al., 2011). However, when used to determine milk price, FA are expressed as g/100 g FA (Borreani et al., 2013), because milk fat content is still included in milk price calculations. Equations for a detailed FA composition expressed as g/100 g of FA developed by Coppa et al. (2010) using NIR. These equations were based on individual thawed milk samples derived from controlled trials with experimental diets (including also lipid supplements), studied to modify milk FA profile to a great extent.

The heterogeneity among milk sample datasets (i.e. fresh or thawed samples, commercial or experimental milks, variation in cow feeding) and of units in which reference data have been expressed, together with the heterogeneity of results on the same FA in the previously cited studies, make it difficult to compare the reliability of the different IR techniques in predicting milk FA composition.

The aim of this research was to compare effectiveness of NIR and MIR in predicting FA composition on fresh and thawed milk, expressing reference data as both g/kg of milk and g/100 g of FA. All the equations were developed on the same sample dataset, composed of bulk milk from commercial farms, and designed to be representative of a great variation in production conditions.

2. Materials and Methods

2.1. Milk Sampling and Survey

Two hundred and fifty bulk cow milk samples were collected from 70 farms located in northwest Italy in 2011 and 2012. In order to explore the maximum variability in FA composition of milk from commercial farms, samples were selected to cover the largest possible variety in production conditions and animal feeding. During each sampling collection, data on production conditions were recorded by means of on-farm surveys at each sampling collection time according to Borreani et al., (2013). Surveys included questions on altitude, forage management, herd size, herd breed composition, and animal feeding during the indoor period or during pasture utilization, when present.

Bulk milk samples (about 1 L each) were collected on farm, kept at 4°C and transported to the laboratory where they were divided into seven sub-samples. One sub-sample was used for FA reference analysis by gas-chromatography, while the other six sub-samples were used for IR analyses.

2.2. FA Gas-Chromatography Analysis

In order to perform milk FA composition analysis by mean of the reference gas-chromatography, milk was centrifuged at 4°C and 3700 *g* for 15 min to separate the cream. The cream was centrifuged at 35°C and 20000 *g* for 35 minutes to separate the anhydrous fat. The FA *trans*-esterification was obtained according to Revello–Chion, Tabacco, Giaccone, Peiretti, Battelli & Borreani. (2010). The FA methyl esters (FAME) were analyzed by means of GC as described by Ferlay et al. (2010), using a 7890A GC-System, gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. The FAME were separated on a 100 m × 0.25 mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 250°C, and the detector temperature at 255°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), then increased by 10°C/min to 175°C (held for 40 min), and 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen. The C18:1*trans* isomers, non-conjugated 18:2 FA, and CLA isomers were identified as described in Borreani et al. (2013). A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (C4:0 to C10:0). The FA concentrations were measured by the reference GC method as g/100 g FA.

2.3. IR Analysis

Three of the six subsamples were scanned immediately as fresh (F) milk by NIR i) as liquid milk and ii) as oven-dried milk, according to Coppa et al., (2010), and by MIR iii) as liquid milk. The three other subsamples were frozen at -20°C, thawed (T) after 2 to 3 months and scanned by NIR iv) as liquid milk, and v) as oven-dried milk, and by MIR vi) as liquid milk. Subsample i) was scanned in its native form at 2 nm intervals from 400 to 2498 nm using a Foss NIRSystems model XDS (Foss NIRSystems, Silver Spring, MD, USA) and controlled via ISIscan software version 2.21 (Infrasoft International LLC, State College, PA, USA) by placing 1 mL of milk in a 50 mm-diameter, 0.2 sample thickness camlock cell. Each spectrum was time-averaged from 32 scans and it was compared with the 32 average-measurements of a ceramic reference. The absorbance was recorded as log (1/*R*). Subsample ii) was oven-dried at 40°C for 24 h on a glass microfiber filter (Whatman GF/A, 55 mm Ø, Cat. No. 1820 055, Whatman International Ltd, Maidstone, UK) and placed in a 50 mm-diameter ring cup according to Coppa et al. (2010). Spectra were obtained for these second subsamples using the same instrument in reflectance mode. The subsample iii) of 50 mL was analyzed using a Fourier transform mid infrared spectroscopy (MilkoScan FT6000, Foss System, Hillerød, Denmark), working within the MIR region from 1000 to 5000 cm^{-1}, and following the International Dairy Federation 141C:2000 procedure (2000). Milk samples was kept at room temperature for 20 min, then placed in a water bath at 40±2°C for 15 min, and mixed thoroughly,

before to be analyzed. Three spectra were generated for each sample by using the calibration mode of the spectrometer, then averaged to obtain one spectrum for each milk sample using ISIscan software, version 2.21. The same subsample scanned by MilkoScan FT6000 was also used to predict milk fat content, which was considered as the reference fat content value. The slope and bias of the equation used for milk fat prediction were 0.992 and 0.030, respectively, whereas the repeatability standard deviation (s_r) and standard error of prediction (SEP) were 0.004 and 0.03, respectively.

Once thawed, subsamples iv), v) and vi) (1 mL, 0.5 mL, and 50 mL, respectively) were agitated at 2500 rpm for 1 min, using a vortex mixer, then scanned by NIR in liquid and oven-dried forms, and by MIR in liquid form. The same methods previously described for fresh milk were applied to thawed milk.

2.4. Calibration and Statistics

The main chemical groups of FA and rations of interest for human nutrition and for cheesemaking technology were selected to compare the calibration performance of the different IR methods. The sum of even chain-saturated FA (ECSFA) included the straight and even chain-SFA from C4:0 to C24:0; the sum of odd chain saturated FA (OCFA) included the strait and odd chain-SFA from C5:0 to C23:0; the sum of branched chain FA (BCFA) included the branched chain SFA (iso and anteiso configurations) from C13:0 to C18:0. The total SFA included the sum of ECSFA, OCFA, and BCFA; the sum of monounsaturated FA (MUFA) included C10:1cis9, C12:1cis9, C14:1trans9, C14:1cis9, C16:1trans11; C16:1cis9, C16:1cis11, C17:1cis9, C18:1trans4 to C18:1trans16, C18:1cis9 to C18:1cis16, C19:1cis10, C20:1cis9, C20:1cis11, and C24:1cis9; the sum of PUFA included C18:2cis9trans13, C18:2cis9trans12, C18:2cis9trans14, C18:2trans11cis15, C18:2n-6, C18:3n-6, C18:3n-3, CLAcis9trans11, CLAcis9cis11, CLAtrans11trans13, CLAtrans9trans11, C20:2n-6, C20:3n-6; C20:4n-6, C20:5n-3, C22:3n-3, C22:4n-6, C22:5n-3, and C22:6n-3. The sum of UFA resulted from the sum of MUFA and PUFA. The sum of C18:1cis and C18:1trans isomers included all the previously cited C18:1cis and C18:1trans isomers, respectively. The sum of trans FA included all the previously cited UFA with at least one trans double bond. Similarly, the sum of CLA, n-6 and n-3 FA grouped all the previously cited PUFA belonging to respective chemical groups. To test possible relationships between milk fat content and milk FA concentration in g/100 g FA (as obtained by the GC analyses) a Pearson's correlation analysis was performed by using the SPSS for Windows software package (version 17.0; SPSS Inc., Chicago, IL).

Calibrations were performed using WinISI II Project Manager, version 1.50 (Infrasoft International, South Atherton St. State College, PA, USA). The samples were divided into calibration (n = 200) and validation (n = 50) sets. Samples from different farms from those included in the calibration set were selected for validation set, with the aim of making the validation dataset completely independent. Calibration and validation samples were chosen in order to maintain the same proportion of different feeding types in both datasets.

In order to find the best performance of all the applied IR methods, several mathematical treatments were tested to compute the prediction models. Regressions were calculated with both partial least square (PLS) and modified partial least square (MPLS) (Shenk & Westerhaus, 1995). Five different correction procedures were applied to the raw data for both regression types: no correction, standard normal variate (SNV), detrend (D), standard normal variate and detrend (SNVD), and multiple scatter correction (MSC). Three different mathematical treatments were performed for each scatter correction: no mathematical treatment (0,0,1,1, where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in the first smoothing, and the fourth is the number of data points in the second smoothing), first-order gap derivation (1,4,4,1), and second-order gap derivation (2,10,10,1). In order to improve calibration equations and decrease possible repeatability error due to instrumental derives, temperature or sample preparation-related variations and variations in pathlength, a repeatability file was included for each IR calibration during the development of equations, as described by Soyeurt et al., (2011) and Westerhaus (1990). The main advantage to including the repeatability file in a calibration is to develop an equation that gives the same predicted value across all conditions represented in the scans (Westerhaus, 1990). Regressions were developed for FA reference data expressed both as g/100 g FA and as g/kg of milk for each mathematical treatment. The FA concentration expressed as g/kg of milk were calculated using the milk fat content predicted by MIR, and considering the FA available for esterification on glycerol in average as 97% of the total milk lipid (Chilliard, Ferlay, Mansbridge & Doreau, 2000). A 12 latent variable calculation was set for each regression calculation, critical values (Student's T) for removing potential calibration outliers were T = 2.5, two elimination passes were allowed, and full cross-validation (6 cross validation groups) was used. On completion of calibration, the model was applied to the validation set. The statistics used to develop and evaluate the calibration models included standard error of cross-validation (SECV), coefficient of determination for cross-validation (R^2CV), coefficient of determination in external validation (R^2V), SEP, the slope and the bias of validation set, and the ratio of standard deviation of reference data to the SECV (RPD). The RPD statistics provide a basis for standardizing the SEP (Williams & Sobering, 1993). The RPD should be as high as possible. To facilitate the comparison of the performance of different IR methods, predictions were classified poor, approximate, promising and reliable for an R^2 (both R^2CV and R^2V) value lower than 0.66, between 0.67 and 0.81, between 0.82 and 0.90 and higher than 0.91, respectively (Coppa et al., 2010). A principal component analysis (PCA) was also performed on NIR liquid milk, NIR oven-dried milk and on MIR spectra, on both fresh and thawed milk, to identify the wavelengths showing having the highest loadings.

3. Results and Discussion

3.1. Milk Samples and FA Reference Dataset

Production conditions of milk samples are given in Table 1. The bulk milk dataset was composed of milk from more than 7700 cows, from 5 main breeds (Italian Holstein, Italian Red Pied, Piemontese, Valdostana Red Pied, and Barà-Pustertaler), but cows of 8 other breeds (Jersey, Brown Swiss, Montbéliarde, Alpine Grey, Valdostana Castana, Abondance, and Tarantaise) and several crossbreeds (including Belgian Blue crossbreeds) were also present in the herds. The average daily milk yield per cow ranged from very low values (less than 5 kg/cow × day) that are typical of dual-purpose local breeds to the high values (up to 40 kg/cow × day) of high-yielding, genetically-selected breeds. Herd size varied from small herds diffused in mountain areas to large herds that are typical of intensive dairy farming systems (Coppa et al., 2013).

Cow feeding also varied to a great extent (Table 1) and included full fresh herbage or hay diets, corn silage- or grass or legume silage-based diets and diets in which concentrates represented more than 50% of the DM. Production conditions in northwest Italy are highly inhomogeneous (especially in terms of feeding systems), due to the proximity of the Alps, whose pastures are grazed by dairy herds, and to the presence of the fertile Po Plain, where the high yield per hectare of corn silage allows energy density of cow diet to be increased and dairy farming systems to be strongly intensified (Borreani et al., 2013). This heterogeneity of territory is well described by the very large altitude range in our dataset: from 150 to 2500 m a.s.l.. Such a wide variation in production conditions is difficult to find in any other European country, as can be seen by the narrower range of variation in production conditions reported in literature (i.e. Ferlay, Agabriel, Sibra, Journal, Martin & Chilliard, 2008; Stergiadis et al. 2012). Only Coppa et al. (2013), describing a large European dataset, that also included farms from Northern Italy, have reported a similar range of variation in production conditions.

This variety of production conditions was reflected on the great variation in milk FA composition (Table 2).

The total SFA ranged from 51.64 to 72.51 g/100 g FA, whereas the UFA ranged from 26.73 to 47.49 g/100 g FA. Total trans-FA were between 2.82 and 13.03 g/100 g FA, and total CLA were between 0.39 and 2.98 g/100 g FA. Higher maximum values were observed for MUFA, total C18:1cis isomers, PUFA, and n-3 FA, and were 41.28, 33.27, 10.34, and 3.12 g/100 g FA, respectively. A similar variation in FA composition of milk in our dataset was also found when FA concentrations were expressed as g/kg of milk. Similar ranges of variation were observed by Soyeurt et al. (2011) on individual milk for almost all the studied FA, except for MUFA, PUFA, and n-3 FA, which reached higher maximum values in our dataset. The greater concentration of these FA in our dataset can be explained by the presence of milk produced by herds grazing on biodiversified highland pasture, which are known to give milk rich in MUFA, PUFA, and n-3 FA (Chilliard, Glasser, Ferlay, Bernard, Rouel & Doreau, 2007). Larger variations in milk FA composition have also been reported by Coppa et al. (2010) in a dataset that included individual milk derived from diets supplemented with different lipid sources. The range of milk fat content that has been found in the present research was narrower than that presented by Soyeurt et al., (2006) (29.2 to 46.4 *vs*. 28.8 to

75.1 g/kg of milk), but could be considered representative of the range of variation of fat content of commercial bulk milk.

3.2. Calibrations

The calibration and validation statistics obtained from NIR on liquid milk, NIR on oven- dried milk, and MIR were presented in Tables 3, 4, and 5, respectively. The differences in calibration performance among scatter correction and spectra mathematical treatment within each regression type were narrow, suggesting that the main source of spectra variability is due to milk composition. Thus we presented in tables the statistical treatment that showed the best average results in calibration results, but we reported also the best calibration performance for each individual FA, sum of FA, or FA ratio for all the calibration sets.

3.2.1. NIR on liquid milk

The statistics of models for FA prediction by NIR on liquid milk (fresh and thawed) are given in Table 3. The best average results on fresh milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with the MPLS - SNV - 2,10,10,1 models. The best average results on thawed milk were obtained with MPLS - D - 2,10,10,1, and MPLS - SNVD - 2,10,10,1 models for FA concentrations expressed in g/100 g FA and g/kg of milk, respectively. For fresh liquid milk, the predictions calculated on FA expressed as g/100 g FA were reliable for the sum of ECSFA, total SFA, and UFA (R^2 CV and R^2V > 0.91; RPD > 2.5), were promising for MUFA, PUFA total trans-FA, n-3 FA, C18:1cis9 to C16 ratio, and total CLA (R^2 CV > 0.81; RPD > 2.5), whereas poor predictions were found for OCFA and n-6 FA (R^2 CV < 0.66). For thawed liquid milk, the prediction quality of FA concentration expressed in g/100 g FA was lower than for fresh liquid milk for all the FA. Promising predictions were found for the sum of ECSFA, total SFA, PUFA, UFA, total trans-FA, and total CLA isomers (R^2CV > 0.81), while approximate or poor predictions were observed for the other FA. Our coefficient of determinations (g/100 g FA) for almost all the FA parameters were lower than those presented by Coppa et al. (2010) on thawed milk. This could be due to the original dataset structure of Coppa et al. (2010), which included milk samples produced by cows receiving lipid supplement, which are known to deeply affect milk FA composition (Chilliard et al., 2007). The prediction for milk fat content were poor on thawed milk. When FA were expressed in g/kg of milk, excellent predictions were only found on fresh milk for the sum of ECSFA, total SFA, MUFA, and UFA (R^2CV and R^2V > 0.91; RPD > 2.5), while promising predictions were found for PUFA, total C18:1cis isomers, total C18:1trans isomers, total trans-FA, n-3 FA, C18:1cis9 to C16:0 ratio, and total CLA on fresh milk, and total trans-FA and total CLA on thawed milk.

The highest loadings of the three principal component (PC) axes of the PCA on liquid milk were at wavelengths around 450-560, 880-1200, 1320-1500, 1600-2050 and 2200-2450 nm (Figure 1). The 450- 560 nm could be related to carotenoids pigment (Coppa et al., 2012). Pasture derived milk is known to have great concentration of carotenoids (Nozière Graulet, Lucas, Martin, Grolier, & Doreau, 2006), whose absorption is in the visible wavelength interval (Coppa et

al., 2012), but also great concentrations of PUFA, MUFA, C18:1cis isomers, and n-3 FA, as well as lower concentration of n-6 FA, and lower of n-6 to n-3 ratio (Chilliard et al., 2007), suggesting a correlation between carotenoids content and FA concentration in milk. The loadings at 1320-1500 and 1650-2050 nm could be related to the milk water content, being the two bands with maxima of water spectrum at 1450 and 1940 nm (Osborne & Fearn, 1988). However, the maxima at wavelengths between 1650 and 1950 nm and between 2200 and 2500 nm may be related to differences in the FA composition of milk fat. Indeed, the absorption bands in the near infrared region of the fat (C–H stretching first overtone at 1726 and 1760 nm, C–H combination bands at 2310 and 2350 nm, C–H stretching second overtone at 1212 nm) are related to the hydrocarbon bonds in the fatty acids (Osborne & Fearn, 1988). The overlap between water and fat adsorption bands could create interfering phenomena and thus limit the detection of milk fat composition (Villar, Gorritxategi, Aranzabe, Fernández, Otaduy & Fernández, 2012) and was indicated by Coppa et al (2010) as the origin of lower performance on FA prediction using NIRS on thawed milk.

3.2.2 NIR on oven-dried milk

The statistics of models for FA prediction by NIR on oven-dried milk (fresh and thawed) are given in Table 4. The presented best average results on fresh milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with the PLS - MSC - 1,4,4,1 models. The best average results on thawed milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with PLS - MSC - 2,10,10,1 models. Calibrations calculated on FA expressed as g/100 g FA were reliable for the sum of ECSFA, total SFA, MUFA, UFA, total C18:1trans isomers, total trans-FA, and total CLA ($R^2CV < 0.91$; $RPD > 2.5$), and promising for BCFA, PUFA, total C18:1cis isomers, n-3 FA, and C18:1cis9 to C16:0 ratio ($R^2CV > 0.81$), on both fresh and thawed milk. Only OCFA, n-6 FA, and milk fat content were poorly predicted ($R^2CV < 0.66$). The poor prediction of milk fat content did not appear in contradiction with the reliable and promising prediction of milk concentration of almost all FA expressed as g/100 g FA, as correlations between milk fat content and milk FA concentration, when significant, were very low (Pearson's correlation coefficients between -0.13 and 0.18; data not shown). As observed for liquid milk, coefficients of determination of our NIR equations were similar to those proposed by Coppa et al. (2010) on thawed milk for almost all FA, but slightly lower for C18:1cis isomers and total C18:1trans isomers, because their dataset included milk from cows receiving lipid supplement. When expressing FA concentration in g/kg of milk, only total trans-FA showed reliable predictions on both fresh and thawed milk. Promising predictions were found for PUFA, total C18:1cis isomers, n-3 FA, C18:1cis9 to C16:0 ratio, and total CLA on fresh milk, and for total C18:1cis isomers, C18:1cis9 to C16:0 ratio, and total CLA for thawed milk. All the other FA were approximately or poorly predicted.

The highest loadings of the three principal component (PC) axes of the PCA on oven-dried milk were at wavelengths around 450–560, 1600-1800, 1850-2150 and 2200-2400 nm, but all the near IR spectrum highly contributed to the loadings (Figure 1). The loadings at the visible wavelength interval were lower than those

observed for fresh liquid milk, probably because of carotenoids partial oxidative losses during milk drying process (Noziére et al., 2006). The other highest loadings may be related to differences in the FA composition of milk fat (Osborne & Fearn, 1988). Removing water from milk samples, made the whole NIR spectra contributing more to the PC axes of the PCA performed both on fresh and thawed milk spectra.

3.2.3. MIR

The statistics of models for the FA prediction by MIR on fresh and thawed milk are given in Table 5. The presented best average results on fresh milk for FA concentration expressed in g/100 g FA and g/kg of milk, and on thawed milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with MPLS - no corrections - 1,4,4,1 models. Calibrations calculated on FA expressed as g/100 g FA were reliable for the sum of ECSFA, total SFA, MUFA, UFA, total C18:1trans isomers, total trans-FA, C18:1cis9 to C16:0 ratio, and total CLA ($R^2CV > 0.91$, RPD > 2.5), while they were promising for BCFA, PUFA, total C18:1cis isomers, and n-3 FA ($R^2CV > 0.81$), for both fresh and thawed milk. Poor predictions were only observed for OCFA and n-6 FA. Milk fat content prediction was reliable also for thawed milk ($R^2CV = 0.95$). The prediction quality of FA concentrations, expressed as g/kg of milk, were similar to those obtained for FA concentration expressed in g/100 g FA, on both fresh and thawed milk. The prediction was only improved for OCFA that showed approximate instead of poor predictions. The determination coefficients and RPD of our MIR equations based on FA concentrations expressed as g/kg of fresh milk are higher or similar than those presented by De Marchi et al. (2011) and by Soyeurt et al. (2011) for almost all FA. On the contrary, the coefficients of determination found with MIR in predicting FA concentration expressed as g/100 g FA are significantly higher than those presented by Soyeurt et al., (2011) for all the FA parameters.

The highest loadings of the three principal component (PC) axes of the PCA were at wavelengths around 1550-1750, 2800-3020, 3020-3500, and 3500-3700 cm^{-1} (Figure 1). The loadings at 1550-1750 and 3020-3500 cm^{-1} could be related to the milk water content, being the two bands with maxima of water spectrum at 1600-1700, and 3040-3470 cm^{-1} (Jørgensen & Næs 2004). However, also the FA carbonyl group vibrate at 1745 cm^{-1} (and at 2855 and 2928 cm^{-1}) and the wavelengths between 1050 and 1600 cm^{-1} have been associated with C–H bending and C–O stretching (Lefèvre & Subirade, 2000). Furthermore, the wavelengths between about 950-1600, 1700-1800, and 2500-3000 cm^{-1} were also found by Soueurt et al., (2006), Rutten et al. (2009), Maurice-Van Eijndhoven et al., (2013) as the most informative form milk FA prediction by MIR.

3.3. NIR vs. MIR

To the authors' knowledge, the quality of milk FA prediction using NIR and MIR on the same pool of samples has never been compared before. Although, the relatively low sample number compared to other studies (Maurice-Van Eijndhoven et al., 2013; Rutten et al., 2009) makes our models not yet suitable for routine applications, the quality of prediction of almost all the FA, calibrating

with both NIR and MIR, was on average high and allowed a reliable IR method comparison. When considering FA concentration expressed as g/100 g FA, MIR predictions were similar to NIR predictions on oven dried milk for the sum of ECSFA, OCFA, total SFA, UFA, n-3 FA, C18:1cis9 to C16 ratio, and total CLA, for both fresh and thawed milk. The prediction quality using NIR on oven dried milk was higher than those of MIR on fresh and thawed milk for MUFA, n-6 to n-3 ratio, and especially for PUFA, C18:1cis, and n-6 FA, whereas the opposite trend was observed for BCFA. A possible reason for the better prediction found for NIR than MIR could be related to the contribution of visible spectrum (400-760 nm) (Figure 1), especially considering fresh milk. The important role played by the visible wavelength interval in discrimination by NIR of milk derived from cow fed different diets, which is known to determine different milk FA composition, have been shown by Coppa et al. (2012).

When FA concentrations were expressed as g/kg of milk, MIR coefficients of determination were better than those obtained by NIR on oven dried milk for almost all FA, for both fresh and thawed milk, except for total C18:1trans isomers, total trans-FA, and C18:1cis9 to C16:0 ratio, for which coefficients of determination were similar, and for n-6 to n-3 ratio, for which coefficients of determination were slightly lower. The FA concentration expressed as g/kg of milk were predicted by MIR from the same spectra on which the milk fat content was reliably predicted with a very high performance. As these values of milk fat content were used to convert FA concentrations from g/100 g FA to g/kg of milk, autocorrelation phenomena occurred among spectra, fat content, and FA concentration in g/kg of milk (Rutten et al., 2009; Soyeurt et al., 2006), which explain the better prediction performances obtained by MIR than by NIR.

3.4. Fresh vs. thawed milk

The reliability of prediction equations obtained with MIR and NIR on fresh and thawed oven-dried milk was similar for all FA parameters, but was significantly lower for thawed milk when using NIR on liquid samples, both for FA concentration expressed as g/100 g FA or as g/kg of milk (Table 3). Results are consistent with observation reported by Coppa et al. (2010). Milk fat and protein fractions separate from water fraction during freezing and thawing processes, making the liquid samples less homogeneous when thawed. This separation of phases could have increased the interfering phenomena related to water in the fat absorption peaks on NIR spectra, thus reducing the prediction reliability (Villar et al., 2012). Furthermore, the 450-560 nm loading picks of PC present in the fresh milk almost disappeared on thawed milk PC loadings (Figure 1), probably because of carotenoids oxidation processes during freezing and thawing. The low weight of the visible wavelength interval for liquid thawed milk, could also be at the origin of the lower FA prediction performance, compared to liquid fresh milk. This lack of precision was not observed for oven-dried milk, because the water fraction had been eliminated during drying, or for MIR, perhaps due to the mixing and homogenizing tools inside the MIR apparatus.

3.5. Reference data unit g/kg vs. g/100 g FA

A new finding of this study is the reliable performance of MIR when predicting FA concentration expressed as g/100 g FA. A real and important variation in milk FA profile have assured a high performance in prediction of milk FA concentrations as g/100 g FA. Thus, the structure of our reference dataset was determinant to obtain these high predicting performance. Indeed, as production conditions have been found to be the greatest factor affecting FA composition of milk (Chilliard et al., 2007; Coppa et al., 2013) and our dataset showed a great variability of production conditions, the same variability was reflected on milk FA concentrations, expressed in g/100 g FA. When this variability is scarcely explored, but milk fat content has a wide range of variation, the range of variation of FA, expressed as g/kg of milk, could seem apparently larger. Samples with a similar FA profile, but very high or very low milk fat content, result to have apparent (but no real) high or low FA. This occurs in particular for milk samples from individual cows in which milk fat content can varies considerably among individuals. These extreme values, when FA concentrations are expressed as g/kg of milk, can have a high leverage in developing prediction equations, that can results in high prediction performance. However, when FA are converted in g/100 g FA on the same pool of samples, the prediction resulted less reliable (Soyeurt et al., 2011; Rutten et al., 2009). Considering that FA concentrations in g/100 g FA of our dataset had an important variability, and that milk fat content of our samples were within a normal range for commercial bulk milk, the variability of our reference dataset and the reliability of our predictions was maintained when FA concentrations were expressed as g/kg of milk. Even though, this unit appears less interesting for application purposes. Milk FA supplementary premiums, when applied, are calculated on milk FA concentrations expressed as g/100 g FA (Borreani et al., 2013), because milk fat is still one of the parameters considered to determine milk price. Supplementary premium calculations based on FA concentration expressed in g/kg of milk would favour milk richer in fat, but may not having a more favourable FA profile. The same is true for breeding programs, for which milk fat content is still included among the selection parameters, and expressing FA concentrations as g/kg of milk would not allow comparisons to be made on real FA profiles among individuals. Furthermore, predicting milk FA concentrations as g/kg of milk, and then recalculating milk FA concentrations in g/100 g FA, through dividing by milk fat content, has been shown to give less reliable results than predicting milk FA concentrations directly as g/100 g FA (Soyeurt et al., 2011). Finally, expressing FA concentrations as g/kg of milk is also of scarce interest for dairy industry, because milk fat content is usually standardized during milk processing.

4. Conclusions

This work has highlighted the key role of the structure of the reference dataset in obtaining reliable predications of FA concentrations, both by NIR and MIR, on fresh and thawed milk, even for a relative low number of samples. The prediction performance for thawed milk were lower than for fresh milk only using NIR on liquid milk, but were maintained high using NIR on oven-dried milk and using MIR. Both IR techniques showed good prediction performance for milk FA

concentrations, both when expressed as g/100 g FA and as g/kg of milk, and expressed a great potential for routine milk recording after further implementation for calibration with larger number of samples: MIR could be used in labs that process a great number of milk samples daily, while NIR could be used in small labs that can also use this IR apparatus to analyze different products. Thus, IR methods could become an useful prediction tool that will allow milk FA composition to be widely used as parameter for milk payment (application of supplementary premiums), for farm and herd management, for cow diet formulation and for breeding programs.

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REFERENCES

Bogomolov, A., Dietrich, S., Boldrini, B., & Kessler, R. W. (2012) Quantitative determination of fat and total protein in milk based on visible light scatter. *Food Chemistry,* 134, 412-418.

Borreani, G., Coppa, M., Revello-Chion, A., Comino, L., Giaccone, D., Ferlay, A., & Tabacco E. (2013). Effect of different feeding strategies in intensive dairy farming systems on milk fatty acid profile, and implications on feeding costs in Italy. *Journal of Dairy Science, http://dx.doi.org/10.3168/jds.2013-6710*

Chilliard, Y., Ferlay, A., Mansbridge, R. M., & Doreau, M. (2000). Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales de Zootechnie*, 49, 181-205.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., & Doreau, M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology*, 109, 828-855.

Coppa, M., Ferlay, A., Chassaing, C., Agabriel, C., Glasser, F., Chilliard, Y., Borreani, G., Barcarolo, R., Baars, T., Kusche, D. Harstad, O. M., Verbič, J., Golecký, J., & Martin, B. (2013). Prediction of bulk milk fatty acid composition based on farming practices collected through on-farm surveys. *Journal of Dairy Science*, DOI: 10.3168/jds.2012-6379

Coppa, M., Ferlay, A., Leroux, C., Jestin, M., Chilliard, Y., Martin, B., & Andueza, D. (2010). Prediction of milk fatty acid composition by near infrared reflectance spectroscopy. *International Dairy Journal*, 20, 182-189.

Coppa, M., Martin, B., Agabriel, C., Chassaing, C., Sibra, C., Constant, I., Graulet, B., & Andueza, D. (2012). Authentication of cow feeding and geographic origin on milk using visible and near-infrared spectroscopy. *Journal of Dairy Science*, 95, 5544-5551.

Coppa, M., Verdier-Metz, I., Ferlay, A., Pradel, P., Didienne, R., Farruggia, A., Montel, M. C., & Martin, B. (2011). Effect of different grazing systems on upland pastures compared with hay diet on cheese sensory properties evaluated at different ripening times. *International Dairy Journal*, 21, 815-822.

Dawczynsky, C., Martin, L., Wagner, A., & Jahreis, G. (2010). *n*-3LC-PUFA-enriched dairy

products are able to reduce cardiovascular risk factors: a double-blind, cross-over study. *Clinical Nutrition*, 29, 592-599.
De Marchi, M., Penasa, M., Cecchinato, A., Mele, M., Secchiari, P., & Bittante, G. (2011). Effectiveness of mid-infrared spectroscopy to predict fatty acid composition of Brown Swiss bovine milk. *Animal*, 5, 1653-1658.
Dilzer, A., & Park, Y. (2012). Implication of Conjugated Linoleic Acid (CLA) in Human Health. *Critical Reviews in Food Science and Nutrition*, 52, 488–513.
Food and Agriculture Organization of the United Nations (FAO). 2013. Statistical dataset: http://faostat.fao.org/site/610/DesktopDefault.aspx?PageID=610#ancor
Ferlay, A., Agabriel, C., Sibra, C., Journal, C., Martin, B., & Chilliard, Y. (2008). Tanker milk variability in fatty acids according to farm feeding and husbandry practices in a French semi-mountain area. *Dairy Science and Techology*, 88: 193-215.
Ferlay, A. Martin, B., Lerch, S., Gobet, M., Pradel, P., & Chilliard, Y. (2010). Effect of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning *v.* evening milking on milk fatty acid profile in Holstein and Montbéliarde cows. *Animal,* 4, 672-640.
Jørgensen, K., & Næs, T. (2004). A design and analysis strategy for situations with uncontrolled raw material variation. *Journal of Chemometrics* 18, 45–52.
Kratz, M., Baars, T., & Guyenet, S. (2013). The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. *European Journal of Nutrition*, 52, 1-24.
International Dairy Federation (2000). Whole milk - Determination of milkfat, protein and lactose content — Guidance on the operation of mid-infrared instruments. IDF 141C:2000. International Dairy Federation, Brussels, Belgium.
Lefèvre, T., & Subirade, M. (2000). Interaction of b-lactoglobulin with phospholipids bilayers: a molecular level elucidation as revealed by infrared spectroscopy. *International Journal f Biological Macromolecules* 28, 59–67.
Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., & Coulon, J. B. (2005). How does the nature of forages and pastures diversity influence the sensory quality of dairy livestock products? *Animal Science*, 81, 205-212.

Maurice-Van Eijndhoven, M. H. T., Soyeurt, H., Dehareng, F., & Claus M. P. L. (2013). Validation of fatty acid predictions in milk using mid-infrared spectroscopy across cattle breeds. *Animal*, 7, 348-354.

Nozière, P., Graulet, B., Lucas, A., Martin, B., Grolier, P., & Doreau, M. (2006). Carotenoids for ruminants: From forages to dairy products. *Animal Feed Science and Technology* 131:418–450.

Osborne, B. G., & Fearn, T. (1988). *Near infrared spectroscopy in food analysis*. Essex, UK: Longman Scientific & Technical.

Revello-Chion, A., Tabacco, E., Giaccone, D., Peiretti, P. G., Battelli, G., & Borreani, G. (2010). Variation of fatty acid and terpene profile in mountain milk and "Toma piemontese" cheese as affected by diet composition in different seasons. *Food Chemistry*, 121, 393-399.

Rutten M. J. M., Bovenhuis, H., Hettinga, K. A., van Valenberg H. J. F., & van Arendonk, J. A. M. (2009). Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. *Journal of Dairy Science*, 92, 6202-6209.
Shenk, J.S., Westerhaus, M.O. (1995). Analysis of agriculture and food products by Near Infrared Reflectance Spectroscopy. Monograph, NIR Systems Inc., Port Matilda, PA: Pennsylvania State University and Owners of Infrasoft International,
Soyeurt, H., Dardenne, P., Dehareng, F., Lognay, G., Veselko, D., Marlier, M., Bertozzi, C., Mayeres, P., & Gengler, N. (2006). Estimating fatty acid content in cow milk using mid-infrared spectrometry. *Journal of Dairy Science*, 89, 3690-3695.
Soyeurt, A., Dehareng, F., Gengler, N., McParland, S., Wall, E., Berry, D. P., Coffey, M., & Dardenne P. (2011). Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. *Journal of Dairy Science*, 94, 1657-1667.
Stark, A. H., Crawford, M. A., & Reifen, R. (2008). Update on α-linolenic acid. *Nutrition Review,* 66, 326–332.

Stergiadis, S., Leifert, C., Seal, C. J., Eyre, M. D., Nielsen, J. H., Larsen, M. K., Slots, T., Steinshamn, H., & Butler, G. (2012). Effect of feeding intensity and milking system on nutritionally relevant milk components in dairy farming systems in the north east of England. *Journal of Agriculture and Food Chemistry*, 60, 7270-7281.
Villar, A., Gorritxategi, E., Aranzabe, E, Fernández, S., Otaduy, D., & Fernández L. A. (2012). Low-cost visible-near infrared sensor for on-line monitoring of fat and fatty acids contents during the manufacturing process of the milk. *Food Chemistry*, 135, 2756-2760.
Westerhaus, M. O. 1990. Improving repeatability of NIR calibrations across instruments. Pages 671–674 in Proc. 3rd Int. Conf. Near Infrared Spectroscopy, Brussels, Belgium. Agricultural Research Centre, Gembloux, Belgium
Williams, P.C. & Sobering, D.C. (1993). Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. *Journal of Near Infrared Spectroscopy*, 1, 25 32.

FIGURE CAPTIONS

Figure 1. Loadings for the first three principal component (PC) axes for the near infrared reflectance (NIR) spectra of fresh and thawed milk in liquid or oven-dried form and for the medium infrared reflectance(MIR) spectra of fresh and thawed milk.

TABLES

Table 1. Descriptive statistics of production condition of milk samples..

Item	Mean	Min	Max	SD[1]
Dairy cows per farm (n)	115	5	550	98
Days in milk (d)	191	100	295	28
Milk yield (kg/cow × day)	23.4	3.5	40.5	10.3
Total roughage (% of diet DM)	66	37	100	19
Total concentrates (% of diet DM)	34	0	63	19
Corn silage (% of diet DM)	22	0	63	17
Grass or legume silage (% of diet DM)	8	0	42	12
Hay (% of diet DM)	15	0	93	20
Fresh herbage (% of diet DM)	19	0	100	36
Total forages (% of diet DM)	24	0	100	19
Altitude (m a.s.l.)	546	95	2500	572

[1] SD: standard deviation

Table 2. Descriptive statistics of milk fat content and fatty acid composition, expressed as g/100 g FA and g/kg of milk, in the validation sets.

Fatty acids	g/100 g fatty acids								g/kg milk							
	Calibration set				Validation set				Calibration set				Validation set			
	Mean	Min	Max	SD[1]	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
Fat									37.44	29.20	45.60	3.18	37.22	31.50	46.40	3.21
ECSFA	60.13	47.35	68.73	4.61	59.86	49.87	65.31	3.90	22.09	15.44	38.87	3.04	21.76	17.11	28.41	2.37
OCFA	2.13	1.52	3.28	0.38	2.23	1.63	3.46	0.35	0.78	0.52	1.70	0.18	0.81	0.57	1.30	0.15
BCFA	2.02	1.37	3.91	0.63	2.07	1.34	3.45	0.57	0.75	0.46	1.94	0.27	0.76	0.47	1.51	0.24
Total SFA	64.28	51.64	72.51	4.05	64.16	56.11	69.78	3.40	23.62	17.36	41.01	3.13	23.32	18.74	30.48	2.42
MUFA	29.63	22.82	41.28	3.05	29.66	24.67	35.43	2.52	10.92	6.59	22.35	1.94	10.81	7.81	17.16	1.62
PUFA	5.17	3.60	10.34	1.20	5.23	3.73	8.22	1.14	1.91	1.01	4.53	0.56	1.90	1.28	3.60	0.48
UFA	34.80	26.73	47.49	3.93	34.89	28.95	42.74	3.31	12.82	7.61	26.87	2.40	12.72	9.16	19.29	1.97
C18:1cis	22.67	16.27	33.27	2.40	22.78	18.86	27.93	2.18	8.35	4.62	16.91	1.48	8.31	5.97	14.04	1.35
C18:1trans	3.36	1.82	7.80	1.18	3.31	1.80	6.73	1.27	1.24	0.56	3.30	0.50	1.21	0.65	2.73	0.50
Trans-FA	5.19	2.82	13.03	2.13	5.16	2.85	11.00	2.22	1.92	0.99	5.70	0.91	1.88	1.06	4.82	0.88
n-6	2.45	1.10	4.06	0.60	2.46	1.30	4.72	0.70	0.90	0.32	1.78	0.23	0.89	0.45	1.63	0.27
n-3	0.91	0.43	3.12	0.49	0.93	0.46	1.92	0.44	0.34	0.15	1.30	0.20	0.34	0.17	0.83	0.16
n-6/n-3	3.42	0.70	6.71	1.62	3.33	0.76	6.16	1.68	3.42	0.70	6.71	1.62	3.33	0.76	6.16	1.68
C18:1cis9/C16:0	0.75	0.42	1.30	0.18	0.77	0.54	1.17	0.17	0.75	0.42	1.30	0.18	0.77	0.54	1.17	0.17
Total CLA	0.93	0.39	2.98	0.62	0.95	0.39	2.76	0.62	0.35	0.16	1.36	0.26	0.35	0.15	1.21	0.24

[1] SD: standard deviation

Table 3. NIR prediction results on liquid fresh and thawed milk for fat content and fatty acid (FA) composition, expressed as g/100 g FA and g/kg of milk. The best average results on fresh milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with MPLS - SNV - 2,10,10,1 model, and on thawed milk for FA concentration expressed in g/100 g FA and g/kg of milk - presented were obtained with MPLS - D - 2,10,10,1, and MPLS - SNVD - 2,10,10,1 models, respectively.

Fatty acid (FA)	F/T[1]	g/100 g FA									g/kg milk								
		Calibration set[2]					Validation set[3] (n = 50)				Calibration set					Validation set (n = 50)			
		n	SECV	RPD	R^2CV	Best R^2CV[4]	SEP	Bias	Slope	R^2V	n	SECV	RPD	R^2CV	Best R^2CV	SEP	Bias	Slope	R^2V
Fat	F	-	-	-	-	-	-	-	-	-	193	0.95	3.73	0.93	(*t,w,z,α,δ*)	0.91	0.01	1.01	0.94
	T	-	-	-	-	-	-	-	-	-	191	2.95	1.08	0.14	0.18 (*x*)	3.67	-0.01	0.68	0.06
ECSFA	F	190	0.89	4.82	0.96	(*α,δ*)	1.12	0.00	0.95	0.92	194	0.74	3.37	0.91	(*r,x,α*)	0.60	0.11	0.96	0.94
	T	192	1.55	2.86	0.88	(*z,u,δ*)	2.34	-0.12	0.82	0.66	192	1.82	1.35	0.45	0.47 (*x*)	2.25	0.09	0.55	0.23
OCFA	F	192	0.22	1.59	0.61	0.62 (*r,x*)	0.25	0.11	0.90	0.44	191	0.09	1.88	0.72	-	0.10	0.03	0.85	0.58
	T	192	0.26	1.30	0.41	0.44 (*t,γ*)	0.31	0.12	1.03	0.33	188	0.11	1.37	0.46	0.51 (*y*)	0.13	0.04	0.86	0.33
BCFA	F	190	0.28	2.13	0.78	(*q,α,δ*)	0.30	0.08	1.05	0.69	192	0.12	1.97	0.74	0.85 (*r*)	0.12	0.02	1.12	0.74
	T	188	0.30	1.90	0.73	0.78 (*z,u,δ*)	0.31	0.09	1.04	0.72	184	0.12	1.83	0.70	0.71 (*u*)	0.16	0.03	0.89	0.56
Total SFA	F	193	0.91	4.20	0.94	0.95 (*x*)	1.07	0.19	0.96	0.91	194	0.73	3.48	0.92	(*α*)	0.62	0.14	0.95	0.94
	T	193	1.44	2.70	0.86	(*q,r*)	1.99	0.01	0.90	0.66	193	2.01	1.23	0.34	0.43 (*r,δ*)	2.37	0.26	0.51	0.15
MUFA	F	195	1.06	2.74	0.87	(*α*)	1.17	-0.14	0.97	0.79	192	0.45	3.52	0.92	0.93 (*α*)	0.47	-0.05	1.03	0.92
	T	197	1.42	2.00	0.75	0.77 (*q*)	1.87	-0.03	0.77	0.48	190	0.87	1.74	0.67	(*u*)	1.14	-0.12	0.99	0.50
PUFA	F	195	0.46	2.47	0.84	(*r,δ*)	0.53	-0.01	0.93	0.77	193	0.19	2.63	0.86	(*α*)	0.21	0.00	0.94	0.80
	T	192	0.48	2.30	0.81	(*r*)	0.62	0.05	1.13	0.70	191	0.25	1.84	0.71	0.74 (*r,x*)	0.27	-0.05	0.94	0.69
UFA	F	194	0.95	3.91	0.94	(*α*)	1.07	-0.20	0.95	0.90	193	0.45	4.48	0.95	(*α*)	0.44	-0.05	1.01	0.95
	T	193	1.39	2.67	0.86	-	1.72	0.09	1.14	0.70	191	1.04	1.83	0.70	0.72 (*u*)	1.21	-0.11	1.03	0.62
C18:1cis	F	191	1.12	2.07	0.77	(*α*)	1.45	-0.10	0.88	0.57	191	0.50	2.48	0.84	-	0.62	-0.06	0.97	0.80
	T	192	1.28	1.67	0.64	0.65 (*z*)	1.90	0.19	0.71	0.29	189	0.75	1.55	0.59	-	1.06	0.01	0.98	0.38
C18:1trans	F	191	0.50	2.18	0.79	0.80 (*α*)	0.67	-0.08	1.04	0.67	191	0.18	2.46	0.84	-	0.28	-0.04	0.93	0.66
	T	189	0.53	2.10	0.77	-	1.02	-0.03	0.81	0.36	193	0.22	1.89	0.72	0.74 (*r*)	0.30	-0.05	1.06	0.57
Trans-FA	F	192	0.76	2.45	0.84	(*x*)	1.08	-0.12	1.02	0.72	190	0.26	3.01	0.89	-	0.46	-0.07	0.93	0.70

	T	191	0.78	2.46	0.84	-	1.36	0.04	1.19	0.61	188	0.31	2.42	0.83	-	0.47	-0.09	0.98	0.68
n-6	F	198	0.38	1.54	0.58	0.59 (*r,x,γ*)	0.51	0.07	1.01	0.43	197	0.15	1.42	0.50	0.55 (*w*)	0.19	0.02	1.11	0.48
	T	193	0.37	1.53	0.57	(*q,z,u*)	0.48	0.02	1.42	0.56	192	0.16	1.24	0.36	0.44 (*γ*)	0.20	0.01	1.31	0.47
n-3	F	192	0.18	2.50	0.84	-	0.22	0.01	0.95	0.72	194	0.07	2.56	0.85	0.86 (*x*)	0.08	0.00	1.00	0.75
	T	193	0.22	1.91	0.73	0.75 (*z*)	0.23	0.03	1.16	0.73	193	0.09	2.06	0.77	(*x*)	0.09	-0.01	0.82	0.73
n-6/n-3	F	193	0.77	2.05	0.77	0.80 (*γ*)	0.97	0.04	0.90	0.66	193	0.77	2.05	0.77	0.80 (*t*)	0.95	0.02	0.92	0.68
	T	187	0.76	2.08	0.77	(*r*)	0.86	-0.17	1.11	0.74	188	0.81	1.95	0.74	0.77 (*r,x*)	1.02	-0.06	0.87	0.62
C18:1cis9/C16:0	F	191	0.06	2.49	0.84	0.85 (*α*)	0.08	0.01	1.10	0.78	191	0.06	2.49	0.84	0.85 (*α*)	0.08	0.01	1.10	0.78
	T	190	0.07	2.04	0.76	(*q,r,u,δ*)	0.11	0.03	0.93	0.56	189	0.08	2.00	0.75	0.76 (*r,u,x,δ*)	0.12	0.02	0.99	0.53
Total CLA	F	194	0.21	2.63	0.86	0.88 (*q*)	0.32	-0.01	0.91	0.71	193	0.08	2.76	0.87	0.89 (*r,x*)	0.121	-0.01	0.951	0.724
	T	189	0.19	2.94	0.89	-	0.33	0.00	0.99	0.71	189	0.07	2.91	0.88	0.89 (*δ*)	0.12	-0.01	1.09	0.75

[1] F: fresh milk; T: thawed milk

[2] n: number of samples included in the calibration set; SECV: standard error of cross validation, RPD: the ratio of standard deviation of reference data to the SECV; R^2CV: coefficient of determination in cross-validation; best R^2CV: best coefficient of determination in cross-validation, and code of statistical model with which were obtained (in brackets).

[3] SEP: standard error of prediction in external validation; R^2V coefficient of determination in external validation.

[4] q,r,t,u,w,x,z,α,γ,δ: MPLS; q,r: no spectra correction ; t,u : SNV ; w,x : D; z,α: SNVD; γ,δ: MSC; q,t,w,z,γ: 1,4,4,1; r,u,x,α,δ: 2,10,10,1.

Table 4. NIR prediction results on oven-dried fresh and thawed milk for fat content and fatty acid (FA) composition, expressed as g/100 g FA and g/kg of milk. The best average results on fresh milk for FA concentration expressed in g/100 g FA and g/kg of milk were obtained with the PLS - MSC - 1,4,4,1 models, and on thawed milk for FA concentration expressed in g/100 g FA and g/kg of milk presented were obtained with PLS - MSC - 2,10,10,1 models.

Fatty acid	F/T[1]	g/100 g FA									g/kg milk								
		Calibration set[2]					Validation set[3] (n = 50)				Calibration set					Validation set (n = 50)			
		n	SECV	RPD	R^2CV	Best R^2CV[4]	SEP	Bias	Slope	R^2V	n	SECV	RPD	R^2CV	Best R^2CV	SEP	Bias	Slope	R^2V
Fat	F	--	-	-	-	-	-	-	-	-	194	2.52	1.31	0.414	0.47 (*w*)	2.95	-0.02	1.18	0.39
	T	-	-	-	-	-	-	-	-	-	194	3.09	1.05	0.08	0.15 (*p*)	3.47	0.01	3.26	0.25
ECSFA	F	192	0.82	5.54	0.97	(*o,t,γ*)	0.95	-0.03	0.90	0.95	191	1.46	1.68	0.65	0.67 (*z*)	1.68	-0.26	0.96	0.51
	T	197	0.85	5.28	0.96	(*f,k,l,n*)	0.81	-0.14	0.94	0.96	193	1.79	1.33	0.44	0.45 (*m*)	1.94	0.14	0.83	0.35
OCFA	F	191	0.22	1.61	0.61	(*o*)	0.27	0.07	0.82	0.46	192	0.10	1.51	0.56	0.62 (*v*)	0.13	0.01	0.71	0.37
	T	196	0.26	1.37	0.47	(*c,h*)	0.27	0.11	1.04	0.53	191	0.11	1.31	0.42	0.47 (*i*)	0.14	0.05	0.97	0.28
BCFA	F	190	0.25	2.32	0.82	(*b,h*)	0.29	0.03	0.90	0.75	189	0.11	2.09	0.77	0.80 (*b,h*)	0.15	-0.01	0.79	0.62
	T	188	0.25	2.17	0.79	-	0.33	0.11	1.05	0.69	191	0.12	1.91	0.73	0.76 (*c,i*)	0.15	0.03	0.99	0.58
Total SFA	F	195	0.68	5.89	0.97	(*o,t,u,γ*)	0.89	-0.01	0.92	0.94	192	1.59	1.54	0.58	0.61 (*w,z*)	1.85	-0.24	1.01	0.43
	T	194	0.63	6.14	0.97	0.98 (*n*)	0.68	-0.10	0.96	0.96	193	1.91	1.24	0.35	0.37 (*m,γ*)	2.01	0.09	1.03	0.31
MUFA	F	196	0.77	3.86	0.93	(*o,γ*)	0.92	-0.01	0.89	0.88	191	0.79	1.85	0.71	0.72 (*t*)	1.02	-0.06	0.99	0.60
	T	196	0.67	4.48	0.95	(*n*)	0.74	-0.01	0.94	0.92	193	0.86	1.77	0.69	-	1.07	0.04	1.12	0.56
PUFA	F	195	0.37	3.16	0.90	0.92 (*c,i,o*)	0.42	0.06	0.91	0.87	192	0.20	2.41	0.83	0.86 (*n*)	0.21	-0.03	0.93	0.82
	T	193	0.33	3.23	0.90	-	0.37	0.05	1.03	0.90	192	0.23	1.98	0.75	0.79 (*c,i*)	0.25	0.01	0.95	0.71
UFA	F	195	0.66	5.89	0.97	(*o,t,u,γ*)	0.87	0.01	0.92	0.94	191	0.87	2.11	0.78	0.79 (*t*)	1.12	-0.05	0.98	0.67
	T	195	0.61	6.10	0.97	0.98 (*n*)	0.62	0.08	0.98	0.97	193	1.00	1.90	0.73	-	1.26	0.04	1.04	0.57
C18:1cis	F	188	0.76	2.82	0.88	-	1.00	0.05	0.95	0.79	193	0.67	1.68	0.65	0.67 (*t,z*)	0.73	-0.09	1.09	0.70
	T	197	0.78	3.06	0.90	-	0.88	0.08	1.00	0.84	193	0.76	1.55	0.59	0.61 (*c,i,n*)	0.99	0.08	1.13	0.45
C18:1trans	F	195	0.33	3.47	0.92	(*o*)	0.41	-0.02	1.04	0.90	193	0.14	3.00	0.89	0.90 (*o*)	0.20	-0.01	0.99	0.84
	T	192	0.29	3.78	0.93	-	0.40	0.00	1.10	0.91	191	0.14	3.17	0.90	(*c,i*)	0.16	-0.02	0.98	0.88
Trans-FA	F	193	0.45	4.37	0.95	(*o*)	0.62	0.05	1.06	0.92	192	0.22	3.62	0.92	(*o*)	0.36	-0.04	0.96	0.84
	T	194	0.45	4.15	0.94	(*n*)	0.65	0.04	1.08	0.92	195	0.25	3.37	0.91	(*c,h,i,n*)	0.29	-0.03	0.97	0.88
n-6	F	197	0.36	1.62	0.62	0.66 (*o*)	0.40	0.04	1.33	0.72	197	0.15	1.45	0.52	-	0.15	0.00	1.48	0.74

	T	194	0.34	1.62	0.62	0.65 (*n*)	0.41	0.00	1.36	0.69	195	0.15	1.40	0.49	-	0.15	0.01	1.23	0.49
n-3	F	191	0.17	2.53	0.85	0.86 (*h*)	0.14	0.01	1.03	0.90	190	0.07	2.41	0.83	0.85 (*h*)	0.07	0.00	0.90	0.84
	T	189	0.17	2.57	0.85	(*n*)	0.18	0.02	0.95	0.84	192	0.08	2.11	0.78	0.82 (*c,h,i,n*)	0.07	0.00	0.87	0.83
n-6/n-3	F	196	0.80	2.02	0.76	-	0.86	0.00	1.02	0.73	196	0.80	2.02	0.76	-	0.86	0.00	1.02	0.73
	T	196	0.77	2.09	0.77	0.82 (*n*)	0.81	-0.11	1.03	0.76	196	0.77	2.09	0.77	0.82 (*n*)	0.76	-0.05	1.02	0.78
C18:1cis9/C16:0	F	189	0.05	2.94	0.89	(*u*)	0.05	0.01	1.00	0.91	189	0.05	2.94	0.89	(*u*)	0.05	0.01	1.00	0.91
	T	195	0.06	3.10	0.90	(*n*)	0.06	0.02	1.05	0.89	195	0.06	3.10	0.90	(*n*)	0.06	0.02	1.03	0.90
Total CLA	F	191	0.17	3.60	0.92	(*b,c,i*)	0.17	0.01	0.96	0.92	191	0.07	3.20	0.90	(*b*)	0.09	0.00	0.98	0.86
	T	195	0.18	3.40	0.92	(*b,h*)	0.20	0.02	1.02	0.90	198	0.08	2.98	0.89	0.91 (*b,c,h,i*)	0.09	0.01	1.03	0.86

[1] F: fresh milk; T: thawed milk

[2] n: number of samples included in the calibration set; SECV: standard error of cross validation, RPD: the ratio of standard deviation of reference data to the SECV; R^2CV: coefficient of determination in cross-validation; best R^2CV: best coefficient of determination in cross-validation, and code of statistical model with which were obtained (in brackets).

[3] SEP: standard error of prediction in external validation; R^2V coefficient of determination in external validation.

[4] b,c,f,h,i,k,l,m,n,o: PLS; p,t,u,v,w,z,γ: MPLS; b,c,p: no spectra correction ; f,t,u : SNV; h,i,v,w: D; k,l,z: SNVD; m,n,o,γ: MSC; m,p,v: 0,0,1,1; b,h,k,n,t,w,z,γ: 1,4,4,1; c,f,i,l,o,u: 2,10,10,1.

Table 5. MIR prediction results on fresh and thawed milk for fat content and fatty acid (FA) composition, expressed as g/100 g FA and g/kg of milk. The average best results on fresh and thawed milk for FA concentration expressed in g/100 g FA and g/kg of milk, were obtained with MPLS - no correction - 1,4,4,1 models.

Fatty acid	F/T[1]	g/100 g FA									g/kg milk								
		Calibration set[2]					Validation set[3] (n = 50)				Calibration set					Validation set (n = 50)			
		n	SECV	RPD	R^2CV	Best R^2CV[4]	SEP	Bias	Slope	R^2V	n	SECV	RPD	R^2CV	Best R^2CV	SEP	Bias	Slope	R^2V
Fat	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	T	-	-	-	-	-	-	-	-	-	189	0.09	3.93	0.94	0.95 (*w*)	0.10	-0.01	0.96	0.93
ECSFA	F	192	1.10	4.00	0.96	-	1.03	-0.15	0.94	0.93	193	0.38	6.79	0.98	(*w*)	0.43	-0.05	0.94	0.97
	T	192	0.98	4.56	0.95	-	0.97	-0.27	0.98	0.94	192	0.66	4.23	0.94	(*w*)	0.66	-0.06	0.89	0.94
OCFA	F	191	0.24	1.44	0.51	0.60 (*r*)	0.25	0.05	0.76	0.37	190	0.08	2.04	0.76	0.78 (*z*)	0.09	0.02	0.84	0.59
	T	191	0.26	1.29	0.41	0.54 (*s,x*)	0.25	0.11	0.89	0.37	190	0.08	2.15	0.78	-	0.10	0.03	0.76	0.60
BCFA	F	190	0.26	2.25	0.82	-	0.32	0.10	1.14	0.70	192	0.10	2.71	0.86	0.89 (*r*)	0.12	0.03	1.11	0.74
	T	193	0.26	2.32	0.82	-	0.28	0.04	0.99	0.74	193	0.10	2.48	0.84	0.86 (*t,w*)	0.11	0.02	1.11	0.77
Total SFA	F	195	1.03	3.85	0.94	-	0.94	0.13	0.93	0.93	189	0.32	8.38	0.99	(*w*)	0.36	-0.04	0.96	0.98
	T	187	0.89	4.28	0.95	-	0.97	-0.18	0.97	0.92	189	0.68	3.70	0.93	(*p*)	0.65	-0.05	0.90	0.94
MUFA	F	195	0.92	3.24	0.91	-	1.07	-0.15	0.96	0.83	194	0.39	4.70	0.95	0.96 (*w,z*)	0.38	0.00	0.99	0.95
	T	193	0.97	2.98	0.91	-	0.99	0.08	0.94	0.85	192	0.44	3.53	0.92	(*w*)	0.53	0.04	0.98	0.90
PUFA	F	190	0.49	2.15	0.82	-	0.56	0.10	1.03	0.76	191	0.19	2.49	0.84	0.86 (*z,v*)	0.20	0.04	0.93	0.83
	T	192	0.48	2.34	0.82	-	0.45	0.05	1.02	0.82	194	0.20	2.61	0.85	-	0.17	0.02	0.91	0.85
UFA	F	195	1.04	3.70	0.93	-	0.98	-0.18	0.92	0.92	194	0.34	6.91	0.98	(*w,z*)	0.33	-0.02	0.97	0.97
	T	187	0.89	4.20	0.94	-	1.04	0.15	0.98	0.90	194	0.46	4.39	0.95	(*w*)	0.56	0.02	0.97	0.92
C18:1cis	F	197	0.96	2.33	0.82	(*z,w*)	1.14	-0.10	0.96	0.72	194	0.40	3.52	0.92	(*w,z*)	0.49	-0.01	1.04	0.87
	T	191	0.91	2.55	0.85	-	1.06	0.08	1.00	0.76	192	0.47	2.52	0.84	0.87 (*w,z*)	0.52	0.07	1.01	0.85
C18:1trans	F	192	0.34	3.40	0.91	(*w*)	0.47	-0.06	0.96	0.83	191	0.13	3.62	0.92	(*t,w,z*)	0.19	-0.02	0.98	0.82
	T	194	0.39	2.98	0.91	-	0.56	-0.12	0.98	0.77	191	0.14	3.23	0.90	-	0.19	-0.05	0.91	0.81
Trans-FA	F	191	0.62	3.28	0.91	-	0.77	-0.02	0.95	0.84	193	0.21	4.09	0.94	-	0.31	-0.01	1.00	0.85
	T	191	0.58	3.44	0.92	-	0.86	-0.14	1.01	0.81	192	0.21	3.91	0.94	-	0.30	-0.07	0.93	0.84
n-6	F	192	0.40	1.47	0.54	(*γ*)	0.58	-0.09	0.74	0.33	193	0.15	1.46	0.54	0.55 (*w*)	0.21	-0.02	0.91	0.34
	T	193	0.38	1.53	0.57	0.59 (*q,δ*)	0.50	0.01	0.98	0.39	195	0.14	1.50	0.55	-	0.21	0.01	0.93	0.36

n-3	F	192	0.18	2.35	0.82	0.84 (*p*)	0.24	0.11	1.14	0.77	193	0.07	2.45	0.83	0.84 (*w*)	0.08	0.03	0.97	0.75
	T	192	0.18	2.44	0.84	-	0.23	0.09	1.04	0.76	193	0.07	2.65	0.86	0.87 (*p*)	0.09	0.03	1.00	0.74
n-6/n-3	F	196	0.80	1.99	0.75	-	0.91	-0.23	1.12	0.71	196	0.80	1.99	0.75	-	0.91	-0.23	1.12	0.71
	T	197	0.82	1.91	0.73	0.73 (*δ*)	0.96	-0.24	1.04	0.69	197	0.86	1.85	0.71	0.73 (*γ,δ*)	0.96	-0.24	1.04	0.69
C18:1cis9/C16:0	F	190	0.05	3.27	0.91	-	0.08	0.03	1.12	0.82	190	0.05	3.38	0.91	-	0.06	0.02	1.09	0.88
	T	191	0.06	2.75	0.91	-	0.06	0.02	1.09	0.87	191	0.06	2.75	0.87	-	0.06	0.02	1.09	0.87
Total CLA	F	193	0.18	3.23	0.91	(*γ*)	0.21	0.04	1.02	0.86	192	0.07	3.30	0.91	-	0.08	0.01	1.02	0.87
	T	191	0.18	3.30	0.91	-	0.22	0.00	0.95	0.84	190	0.07	3.32	0.91	(*p*)	0.09	0.00	0.95	0.84

[1] F: fresh milk; T: thawed milk

[2] n: number of samples included in the calibration set; SECV: standard error of cross validation, RPD: the ratio of standard deviation of reference data to the SECV; R^2CV: coefficient of determination in cross-validation; best R^2CV: best coefficient of determination in cross-validation, and code of statistical model with which were obtained (in brackets).

[3] SEP: standard error of prediction in external validation; R^2V coefficient of determination in external validation.

[4] p,q,r,s,t,w,x,y,z,γ,δ: MPLS; p,q,r: no spectra correction ; s,t : SNV; w,x: D; y,z: SNVD; γ,δ: MSC; p,s,y: 0,0,1,1; q,t,w,z,γ: 1,4,4,1; r,x,δ: 2,10,10,1.