Food Chemistry 190 (2016) 244–252

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Near Infrared Spectroscopy (NIRS) for the determination of the milk fat fatty acid profile of goats

N. Núñez-Sánchez [a,*], A.L. Martínez-Marín [a], O. Polvillo [b], V.M. Fernández-Cabanás [b,*], J. Carrizosa [c], B. Urrutia [c], J.M. Serradilla [a]

[a] Departamento de Producción Animal, Campus de Rabanales, Universidad de Córdoba, 14071 Córdoba, Spain
[b] Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, Universidad de Sevilla, Carretera Utrera, km. 1, 41013 Sevilla, Spain
[c] Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Estación Sericícola, 30150 La Alberca, Murcia, Spain

ARTICLE INFO

Article history:
Received 1 February 2015
Received in revised form 15 May 2015
Accepted 16 May 2015
Available online 19 May 2015

Keywords:
Near Infrared Spectroscopy (NIRS)
Goats
Milk fatty acids
Omega 3
Omega 6

ABSTRACT

Milk fatty acid (FA) composition is important for the goat dairy industry because of its influence on cheese properties and human health. The aim of the present work was to evaluate the feasibility of NIRS reflectance (oven-dried milk using the DESIR method) and transflectance (liquid milk) analysis to predict milk FA profile and groups of fats in milk samples from individual goats. NIRS analysis of milk samples allowed to estimate FA contents and their ratios and indexes in fat with high precision and accuracy. In general, transflectance analysis gave better or similar results than reflectance mode. Interestingly, NIRS analysis allowed direct prediction of the Atherogenicity and Thrombogenicity indexes, which are useful for the interpretation of the nutritional value of goat milk. Therefore, the calibrations obtained in the present work confirm the viability of NIRS as a fast, reliable and effective analytical method to provide nutritional information of milk samples.

1. Introduction

Milk is one of the main components of human diet, as an universal nutrient source of protein, lactose, vitamins, minerals and fats. With respect to goat's milk, France, Spain, Greece, the Netherlands, and Italy are the primary countries in the European Union that produce and consume goats dairy products, which are very important to regional and social economies (Andueza, Rouel, Chilliard, Leroux, & Ferlay, 2013).

Milk fatty acid (FA) composition is important for the goat dairy industry because of its influence on cheese yield, cheese texture and the sensory properties of dairy products (Coppa et al., 2014). Furthermore, in recent years there has been an increasing interest by consumers and researchers on the milk FA profile, due to its implications on human health (Williams, 2000). It is well known that some dietary FA are related to cardiovascular diseases (Erkkilä, de Mello, Risérus, & Laaksonen, 2008). Some of those FA present in milk fat with known negative effects on human health are medium chain saturated FA (C12:0, C14:0 and C16:0) and *trans*9- and *trans*10-C18:1 (Bauchart et al., 2007; Combe, Clouet, Chardigny, Lagarde, & Léger, 2007; Ulbricht & Southgate, 1991). However, milk fat also contains some potential beneficial FA, including short chain fatty acids (SCFA), *cis*9*trans*11-C18:2 (rumenic acid, RA), alfa-linolenic acid (ALA; 18:3 n-3) and oleic acid (*cis*9-C18:1) (Shingfield, Chilliard, Toivonen, Kairenius, & Givens, 2008). Milk FA profile can be changed and improved through the manipulation of the diet consumed by ruminants (Shingfield, Bonnet, & Scollan, 2013).

In order to consider the different effects of the various saturated and certain unsaturated FA (UFA) contents, Ulbricht and Southgate (1991) proposed two indices which might characterize the atherogenic and thrombogenic potential of the diet: the Atherogenicity Index (AI) and the Thrombogenicity Index (TI). The omega-6:omega-3 ratio (n-6/n-3) is another way to classify the milk FA, as it measures the balance between n-6 and n-3 FA, which have antagonistic physiological functions, essential for good health and development (Simopoulos, 2002).

Some European countries (e.g. France and The Netherlands) have introduced FA composition among the parameters considered to determine milk price (Coppa et al., 2014). The reference method to quantify the FA profile in milk is based on gas chromatography (GC) analysis. This method requires high expertise, is expensive and time-consuming, and, therefore, it is not feasible for the analyses of the large number of samples necessary for management

* Corresponding authors.
E-mail addresses: pa2nusan@uco.es (N. Núñez-Sánchez), victorf@us.es (V.M. Fernández-Cabanás).

http://dx.doi.org/10.1016/j.foodchem.2015.05.083
0308-8146/

purposes. Hence, it would be of great interest for both dairy farmers and dairy industry to develop a fast and reliable method to quantify FA and groups of fats in milk samples, which would allow to establish a payment system of milk according to its nutritional level, and to promote farmers to adapt their animals feeding systems accordingly (Coppa et al., 2014).

Near Infrared Spectroscopy (NIRS) has the potential for quantitative prediction of the main parameters such as protein, fat, moisture, related with the quality of the agricultural products such as grains, flours, feed, forage, oil seeds, meat and dairy products (Williams, 2001). However, water strong absorption bands in the NIR region could limit the detection of other constituents in certain foods with high water content (Thyholt & Isaksson, 1997). To solve this problem, the so called Dry-Extract System for Infra Red (DESIR) was developed (Meurens, Van Den Eynde, & Vanbelle, 1987). This method consists on oven drying a glass fiber filter previously impregnated with the liquid to test. The analytes remaining on the filter are subsequently analyzed by NIR in reflectance mode. Though, the need of sample preparation and drying of the samples prior to their analysis limits one of the main advantages of NIR technology, which is to provide instantaneous results (Thyholt & Isaksson, 1997).

NIRS technology has been reported to be a quick, consistent and economic tool to predict protein, fat, lactose and moisture contents of milk, both liquid and oven-dried, and milk products in the dairy industry for over 30 years (Holroyd, 2013). In the past years, the growing demand of consumers for knowledge about the milk quality and the compositional parameters that are related to human health has led to the development of new NIR approaches. Thereby, this technology has been successfully used for the determination of milk FA in cow milk samples, analyzed in reflectance (oven-dried milk) and transflectance mode (Coppa et al., 2010; Coppa et al., 2014). Mid Infrared (MIR) has also been used to predict FA and groups of fats in cow's milk (Coppa et al., 2014; Maurice-Van Eijndhoven, Soyeurt, Dehareng, & Calus, 2013; Soyeurt et al., 2011). Regarding goat's milk, Andueza et al. (2013) have reported the feasibility of NIR to predict FA contents and some groups of fats in oven-dried (using the DESIR method) milk from goat-feeding experiments analyzed in reflectance mode.

The objectives of this study were: (a) To evaluate the feasibility of NIRS reflectance analysis (oven-dried milk using the DESIR method) to predict milk FA contents and groups of fats in milk samples from individual goats. (b) To directly predict the AI and TI and the n-6/n-3 ratio of goat milk. (c) To compare the calibration results in two milk analysis modes, reflectance and transflectance.

To the best of our knowledge, this is the first attempt to develop NIR calibration equations to predict the AI and TI of milk, which would provide very useful information of the nutritional value of the samples.

2. Materials and methods

2.1. Milk samples

A total of 805 individual milk samples of Murciano Granadina goats from four commercial farms were used. The herds were under an official milk recording scheme and the diets supplied were based on alfalfa hay and commercial concentrates (Zidi et al., 2010). Milk samples were collected from a series of individual controls along three different lactation (Nov'07–Jun'08 (11 controls); Nov'08–Jun'09 (10 controls) and Feb'10–Jul'10 (7 controls), in order to obtain maximum seasonal variation. Milk samples were collected in the farm and kept at 4 °C for transportation. One sub-sample was used for the reference analysis of FAs, by gas chromatography (GC), and the other sub-sample was used for NIR analyses. Once in the laboratory, samples were added Wide Spectrum Microtablets I (Product code: 174748. Panreac Química, S.A.) for preserving the milk samples, and were frozen at −20 °C until analyses.

2.2. Fatty acid analysis

Milk fat extraction and direct methylation of FA were performed in a single-step procedure based on the method published by Sukhija and Palmquist (1988) and revised by Juárez et al. (2008) in order to minimize isomerization and epimerization in CLA. Fatty acid profile was determined by GC. Separation and quantification of the FA methyl esters (FAMEs) were carried out using a gas chromatograph Agilent 6890N Network GS System (Agilent, Santa Clara, CA), equipped with a flame-ionization detector (FID), automatic sample injector HP 7683, and fitted with a HP-88 J&W fused silica capillary column (100 m, 0.25 mm i.d., 0.2-μm film thickness, Agilent Technologies Spain, S.L., Madrid, Spain). The chromatographic conditions were described in detail by Juárez et al. (2008): initial column temperature was 100 °C, increasing at a rate of 3 °C min^{-1} up to 158 °C and then at 1.5 °C min^{-1} up to 190 °C and maintaining this temperature for 15 min, then at 2 °C min^{-1} up to 200 °C and then increasing again at 10 °C min^{-1} up to final temperature of 240 °C hold for 10 min. Temperatures of injection and detector were maintained at 300 °C and 320 °C, respectively. Hydrogen was used as carrier gas at a flow rate of 2.7 mL min^{-1}. The split ratio was 17.7:1, and 1 μL of solution was injected. Response linearity, recovery factor, precision, repeatability and reproducibility of the method were detailed by Juárez et al. (2008).

Nonanoic acid methyl ester (C9:0 ME) at 4 mg/mL was used as an internal standard. Individual FA were identified by comparing their retention times with those of an authenticated standard FA mix Supelco 37 (Sigma Chemical Co. Ltd., Poole, UK). Identification of the conjugated linoleic acid (CLA) isomers was achieved by comparing retention times with those of another authenticated standard mix (Sigma Chemical Co., Ltd.). Fatty acid content was expressed as the percentage of total methyl esters identified.

Several groups of FA were calculated from the individual FA identified: total saturated FA (Total SFA); total monounsaturated FA (Total MUFA); total polyunsaturated FA (PUFA); short chain FA (SCFA), as the sum of C4:0, C6:0, C8:0 and C10:0; medium chain FA (MCFA), as the sum of C12:0, C14:0, C16:0; saturated short and medium chain FA plus C18:0 (SFA + C18:0), as the sum of SCFA, MCFA and C18:0; monounsaturated FA of 18 atoms of carbon (MUFA-18C); *trans*-C18:1 FA (T-C18:1), as the sum of *trans*9-C18:1, *trans*10-C18:1 and *trans*11-C18:1; omega 6 FA (n-6), and omega 3 FA (n-3). Other variables calculated from GC data were the ratio n-6/n-3, the Atherogenicity Index (AI), and the Thrombogenicity Index (TI), as the content ratio of SFA/unsaturated FA, using the following formulas proposed by Ulbricht and Southgate (1991):

$$AI = (C12:0 + 4 \times C14:0 + C16:0)/(MUFA + n\text{-}6PUFA + n\text{-}3PUFA);$$

$$TI = (C14:0 + C16:0 + C18:0)/(0.5 \times MUFA + 0.5 \times n\text{-}6PUFA + 3 \times n\text{-}3PUFA + n\text{-}3/n\text{-}6).$$

2.3. Spectra collection

All spectra from milk samples were obtained on a FOSS-NIRSystems 6500 SY-I scanning monochromator (FOSS-NIRSystems, Silver Spring, MD, USA) equipped with a spinning module. Spectral absorbance values were recorded from 400 to 2498 nm, every 2 nm, as log 1/R, where R is the sample reflectance, collecting 1050 data points per sample. Each spectrum was time-averaged from 32 scans and it was compared with the 16

average-scans of a ceramic reference, before and after the sample (16–32–16, reference–sample–reference).

Two spectroscopic methods were used in this study to analyze milk samples:

2.3.1. Reflectance

Small ring cups for solid product analyses were used as filter holders. A glass microfibre filter (Millipore, AP40) per sample was impregnated with milk and oven dried at 40 °C for 24 h, as described by Núñez-Sánchez, Serradilla, Ares, and Garrido-Varo (2008). After 1 h in a desiccator, the filters were placed at the bottom of a capsule with the readable side facing the quartz window to perform NIR analysis. All the 805 milk samples obtained in this study were analyzed in reflectance mode.

2.3.2. Transflectance

A gold reflector 0.1 mm pathlength cam-lock cell for liquid product analyses was used. The capsule was filled by placing 0.35 ml of milk in the quartz window, being hermetically closed afterwards. Two capsules per sample were filled and the average spectrum was used in the data analyses. Ten samples randomly chosen from 22 controls (from control 7 till control 28, both included), were analyzed in transflectance mode, thus obtaining a set of 220 samples.

WINISI II software, version 1.50 (Infrasoft International LLC, State College, PA) was used for spectral data collection.

2.4. Data processing and calibration development

Three sample sets were used in order to accomplish the objectives of this study: (a) Set of the 805 milk samples analyzed in reflectance mode, oven-dried using the DESIR method (R). (b) Set of the 220 samples analyzed in transflectance mode as liquid samples (T). (c) Set of the 220 samples analyzed in reflectance mode using the DESIR method which were also analyzed in transflectance (RT).

The WinISI II software package was used for the chemometric management of data.

Prior to NIRS calibration development, the structure and spectral variability of each population were studied, and the resulting sample sets were divided into calibration and validation subsets. For that purpose, the CENTER algorithm was applied (Shenk & Westerhaus, 1991a; Shenk & Westerhaus, 1991b). This algorithm consisted of a principal component analysis (PCA) of the samples and was used to reduce the dimensionality of the data matrix and to retain the maximal amount of possible variability in the spectral data. It is common in NIRS approaches to apply pre-treatments designed to correct for scatter effects usually seen in absorbance data as well as derivatives prior to the PCA or calibrations. Hence, the initial PCA applied with the CENTER algorithm was performed by using Standard normal variate and detrending (SNV + DT) mathematical pre-treatments (Barnes, Dhanoa, & Lister, 1989) to correct scatter phenomena, together with spectral mathematical derivation treatment "1,5,5,1", 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 a running average or smoothing and the fourth is the second smoothing (Shenk, Westerhaus, & Abrams, 1989). The CENTER algorithm also calculates the GH value (Mahalanobis global distance to the center of the population) of each sample, sorts them according to it and considers as outliers those samples with GH value 3.0 or above, which are discarded (Shenk & Westerhaus, 1991a). Each resulting population of original R, T and RT sets was divided into calibration and validation sets in order to perform an external validation. For that purpose, one of each three sorted samples from the resulting set of the CENTER algorithm was selected to build up the validation set, and the rest remained as the calibration set. In this way, the samples of the validation set are consistently distributed in the population.

After calibration and validation sets were defined, milk spectra were fitted to the main FA, chemical groups, indexes and ratios of FA of interest for human nutrition (total SFA, total MUFA, total PUFA, SCFA, MCFA, SFA + C18:0, C18:0, MUFA-18C, *cis*9-C18:1, T-C18:1, *trans*11-C18:1, *trans*9-C18:1, *cis*9*cis*12-C18:2, *cis*9*trans*11-CLA, *trans*10*cis*12-CLA, C18:3n-3, n-6, n-3, n-6/n-3, AI and TI), to develop the correspondent NIR prediction equations.

Regression models were obtained using the Modified Partial Least Squares (MPLS) Regression method. With MPLS, the NIR residuals at each wavelength, obtained after each factor is calculated, are standardized (divided by the standard deviations of the residuals at a wavelength) before calculating the next factor (Shenk & Westerhaus, 1995a). Separate MPLS calibrations were performed for each parameter. Cross-validation was performed to select the optimal number of factors and to avoid overfitting (Shenk & Westerhaus, 1991b; Shenk & Westerhaus, 1995a; Shenk & Westerhaus, 1996). For cross-validation, the calibration set was partitioned in four groups; each group was then predicted using a calibration developed on the other samples; finally, validation errors were combined to obtain a standard error of cross validation (SECV).

All multivariate regression equations were developed for the spectral ranges 400–2498 nm and 1100–2498 nm. SNV + DT (Barnes et al., 1989) algorithms were used for scatter correction. Additionally, four derivative mathematical treatments were tested in the development of NIRS calibrations: "1,5,5,1"; "1,10,10,1"; "2,5,5,1" and "2,10,10,1". These treatments are routinely used in NIR calibration developments of agricultural products according to the protocol established by Shenk and Westerhaus (1995a), Shenk and Westerhaus (1995b, Shenk and Westerhaus (1996). Hence, eight regression equations per parameter were developed by combining two spectral regions and four spectral derivative math treatments. During the calibration process, T outliers, defined as samples with significant differences between their laboratory and predicted values, were identified and removed (critical value for elimination, $T = 2.5$).

The performance of the calibration models was evaluated using the standard error of calibration (SEC), the coefficient of determination between the predicted and measured parameters (R^2), the SECV, the coefficient of determination for cross validation (r^2) and the residual predictive deviation (RPD), defined as the ratio of the standard deviation of the original data (SD) to the SECV (Williams, 2001). The RPD should be as high as possible. The equations were subsequently evaluated by external validation, a procedure determining the predictive ability of an equation based on a sample set which has not been used in calibration procedures, in order to obtain the SEP value (root mean standard error of prediction). The accuracy of each model was assessed in terms of root mean standard error of prediction (SE) for each data set, that is, SEC for the calibration set, SECV for the cross-validation set, and SEP for the external validation set. Best calibrations were selected based on the higher r^2 and RPD values and lower SECV and SEP, since those parameters are routinely used to evaluate the efficiency of NIR equations.

3. Results and discussion

3.1. Fatty acid composition of milk

Mean, SD, minimal and maximal values of the FA identified by GC in the milk samples are presented in Table 1. The milk FA contents showed variability similar to that reported by Alonso, Fontecha, Lozada, Fraga, and Juárez (1999). These authors studied

the range of variation of milk FA from Murciano-Granadina goats in the Murcia region (Spain), collected from five herds during 7 consecutive months. However, the range of variation of the FA contents observed in the present work was narrower than the reported by Andueza et al. (2013), particularly in MUFA and n-3 FA, but those authors included in their study samples from trials where plant and marine oils were added to goat diets.

As expected, the selection of a number of random samples from each milk control allowed getting similar variability of milk FA in both R and T calibration sets (Table 1).

Table 1
Fatty acid contents (g/100 g fatty acid methyl esters) in milk fat of Murciano-Granadino goats quantified by gas chromatography (Set R, all the samples analyzed in reflectance mode; Set T, samples analyzed in transflectance mode).

	Set R (N = 805)*				Set T (N = 220)*			
Fatty acids	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
C4:0	0.35	0.48	0.01	3.22	0.14	0.12	0.01	0.59
C6:0	0.29	0.22	0.01	1.65	0.27	0.20	0.01	0.87
C8:0	2.04	0.94	0.12	5.44	1.76	0.78	0.12	5.44
C10:0	11.09	3.13	4.32	21.21	10.14	2.18	5.30	17.09
C11:0	0.10	0.05	0.02	0.57	0.09	0.04	0.02	0.25
C12:0	5.04	1.28	1.92	9.74	4.82	1.13	1.92	9.74
C13:0	0.08	0.04	0.03	0.29	0.08	0.04	0.03	0.28
C14:0	10.18	1.37	6.62	14.80	10.29	1.49	6.87	13.88
C14:1	0.25	0.13	0.02	0.86	0.29	0.13	0.06	0.72
C15:0	0.76	0.16	0.38	1.84	0.75	0.15	0.41	1.22
C15:1	0.13	0.08	0.01	0.49	0.10	0.07	0.02	0.34
C16:0	32.86	3.04	22.53	42.10	33.86	2.33	28.92	42.10
C16:1	0.98	0.29	0.01	2.55	0.99	0.31	0.31	2.49
C17:0	0.64	0.27	0.21	2.43	0.75	0.31	0.21	1.58
C17:1	0.21	0.08	0.03	0.68	0.23	0.07	0.04	0.68
C18:0	10.84	2.41	5.37	19.48	11.45	2.17	6.53	19.28
*trans*9-C18:1	1.92	1.56	0.13	9.08	1.42	1.38	0.19	6.64
*trans*11-C18:1	2.24	1.18	0.24	8.56	2.16	1.01	0.27	6.63
*cis*9-C18:1	15.08	3.20	3.95	27.82	15.31	3.08	5.82	26.33
C18:2n6t	0.33	0.14	0.05	1.55	0.34	0.13	0.05	1.31
*cis*9*cis*12-C18:2	2.59	0.52	0.76	4.62	2.65	0.53	1.38	4.38
*cis*9*trans*11-CLA	0.51	0.27	0.05	1.57	0.57	0.29	0.09	1.47
*trans*10*cis*12-CLA	0.10	0.09	0.01	0.65	0.07	0.06	0.01	0.30
Other CLA	0.12	0.07	0.02	0.46	0.12	0.07	0.02	0.43
C18:3n-3	0.22	0.11	0.02	0.52	0.24	0.13	0.02	0.52
C18:3n-6	0.06	0.05	0.01	0.64	0.06	0.04	0.01	0.34
C20:1	0.09	0.06	0.01	0.50	0.07	0.04	0.01	0.22
C20:2	0.08	0.08	0.01	0.47	0.10	0.09	0.01	0.47
C20:3n-6	0.11	0.08	0.01	0.55	0.10	0.07	0.01	0.39
C20:4n-6	0.14	0.11	0.01	0.87	0.17	0.08	0.01	0.53
C20:5n-3	0.04	0.02	0.01	0.15	0.04	0.02	0.01	0.15
C21:0	0.14	0.17	0.01	1.42	0.09	0.11	0.01	0.70
C22:2	0.02	0.02	0.01	0.12	0.02	0.01	0.01	0.09
C22:5n-3	0.06	0.03	0.01	0.20	0.07	0.03	0.01	0.20
C22:6n-3	0.02	0.02	0.01	0.22	0.02	0.01	0.01	0.10
Total SFA	74.65	3.48	62.59	83.99	74.81	3.51	64.68	83.99
Total MUFA	20.93	3.16	13.36	32.06	20.58	3.21	13.36	29.40
Total PUFA	4.42	0.83	2.25	6.93	4.61	0.87	2.25	6.93
Total CLA	0.73	0.29	0.22	1.96	0.76	0.30	0.27	1.79
n-6	3.20	0.61	1.21	5.25	3.29	0.63	1.62	5.09
n-3	0.38	0.16	0.12	1.30	0.43	0.17	0.16	1.28
SCFA	13.77	4.23	4.98	25.01	12.26	2.78	6.29	23.75
MCFA	48.07	3.45	37.67	60.38	49.11	3.16	41.03	60.38
SFA + C18:0	72.68	3.48	61.13	82.72	72.70	3.44	63.05	82.72
MUFA-18C	19.24	3.17	4.49	30.02	18.89	3.08	12.13	28.09
T-C18:1	4.16	2.22	0.54	12.20	3.67	1.83	0.95	10.10
n-6/n-3	9.35	3.54	2.50	27.58	8.63	3.02	3.30	20.94
AI	3.18	0.67	1.60	6.25	3.27	0.72	1.99	6.25
TI	4.11	0.66	2.60	7.20	4.26	0.71	2.81	7.20

SD: standard deviation.
Total SFA: saturated fatty acids (FA).
Total MUFA: monounsaturated FA.
Total PUFA: polyunsaturated FA.
SCFA: short chain saturated FA = C4:0 + C6:0 + C8:0 + C10:0.
MCFA: medium chain saturated FA = C12:0 + C14:0 + C16:0.
SFA + C18:0: short and medium chain saturated fatty acids + C18:0.
MUFA-18C: monounsaturated FA of 18 atoms or carbon.
T-C18:1: *trans* C18:1 fatty acids.
n-6/n-3: ratio omega 6 FA/omega 3 FA.
AI: Atherogenicity Index, calculated as AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + n-6 PUFA + n-3 PUFA) (Ulbricht & Southgate, 1991).
TI: Thrombogenicity Index, calculated as TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6) (Ulbricht & Southgate, 1991).
* Set R: all the milk samples analyzed in reflectance mode. Set T: milk samples analyzed in transflectance mode.

3.2. Visible and NIR spectral features

Fig. 1 shows visible + NIR average spectra of the goat milk samples analyzed in transflectance (liquid milk; left scale) and in reflectance (oven-dried, DESIR method; right scale) modes.

Discrepancies in the visible region were observed in both spectra. The average spectrum of transflectance mode exhibited a strong band around 460 nm, associated with the absorbance of the golden (yellow) reflector disk used in this transflectance cup. This strong band in transflectance mode was split into three bands in the reflectance spectrum at 436 nm, 596 nm and 692 nm. Probably, the drying process of the milk samples in the reflectance mode and the interaction of the samples with the glass fiber filters used in the DESIR method, together with the addition of Wide Spectrum Microtablets for preserving the milk samples which provided a violet-blue color to the milk, were responsible for these changes.

With respect to the NIR region, the average spectrum of milk analyzed in its liquid form, in transflectance mode, was similar to that of water, with two dominant bands around 1450 nm and 1940 nm, related with O–H first overtone and O–H combination band, respectively (Murray & Williams, 1987; Westad, Schmidt, & Kermit, 2008; Williams, 2001). In addition, small bands corresponding to fatty acids and fat contents appeared at 1210 nm, related with second overtone from C-H stretching vibration; 1726 nm and 1760 nm, associated with the first overtone from C–H stretching vibration of methyl ($-CH_3$), methylene ($-CH_2$), and ethenyl (–CH=CH–) groups; and around 2308 and 2348 nm, due to combination of C–H stretching and bending vibrations of methyl and methylene functional groups (Westad et al., 2008; Williams, 2001). According to Hourant, Baeten, Morales, Meurens, and Aparicio (2000), the band near 1725 nm refers to oleic acid, while 1760 nm peak is assigned to the saturated components. Šašić and Ozaki (2000) identified bands at 2316, 2340, and 2368 nm arising from combinations of CH_2 stretching and bending modes of protein side chain groups in milk NIR transmission spectra, although, upon their results, they concluded that protein content of the milk samples did not interfere with the fat bands found in this region.

The oven-drying process of the milk samples analyzed using the DESIR method caused the almost disappearance of the two large bands characteristic of moisture absorption (1450 and 1940 nm). The rest of the peaks obtained for milk samples analyzed under reflectance mode using the DESIR method appeared stronger than those for samples analyzed in transflectance mode. Additionally, new bands which were hidden under the strong water band around 1940 nm appeared after the drying process of the samples in the reflectance mode at 2060 nm, 2174 nm and 2466 nm, corresponding to protein absorption bands: the band around 2056 nm corresponds to N-H stretching vibrations of various types, the band at 2174 nm corresponds to N-H second overtone, CH stretch/C=O stretch combination band and C=O stretch/N–H amid combination band, and the band at 2468 nm corresponds to the C–N–C stretching first overtone (Berzaghi, Zotte, Jansson, & Andrighetto, 2005).

3.3. NIR calibration results

Regression models for the prediction of the main FA, chemical groups and ratios of FA of interest for human nutrition were developed in each of the three sample sets (R, T and RT) described in

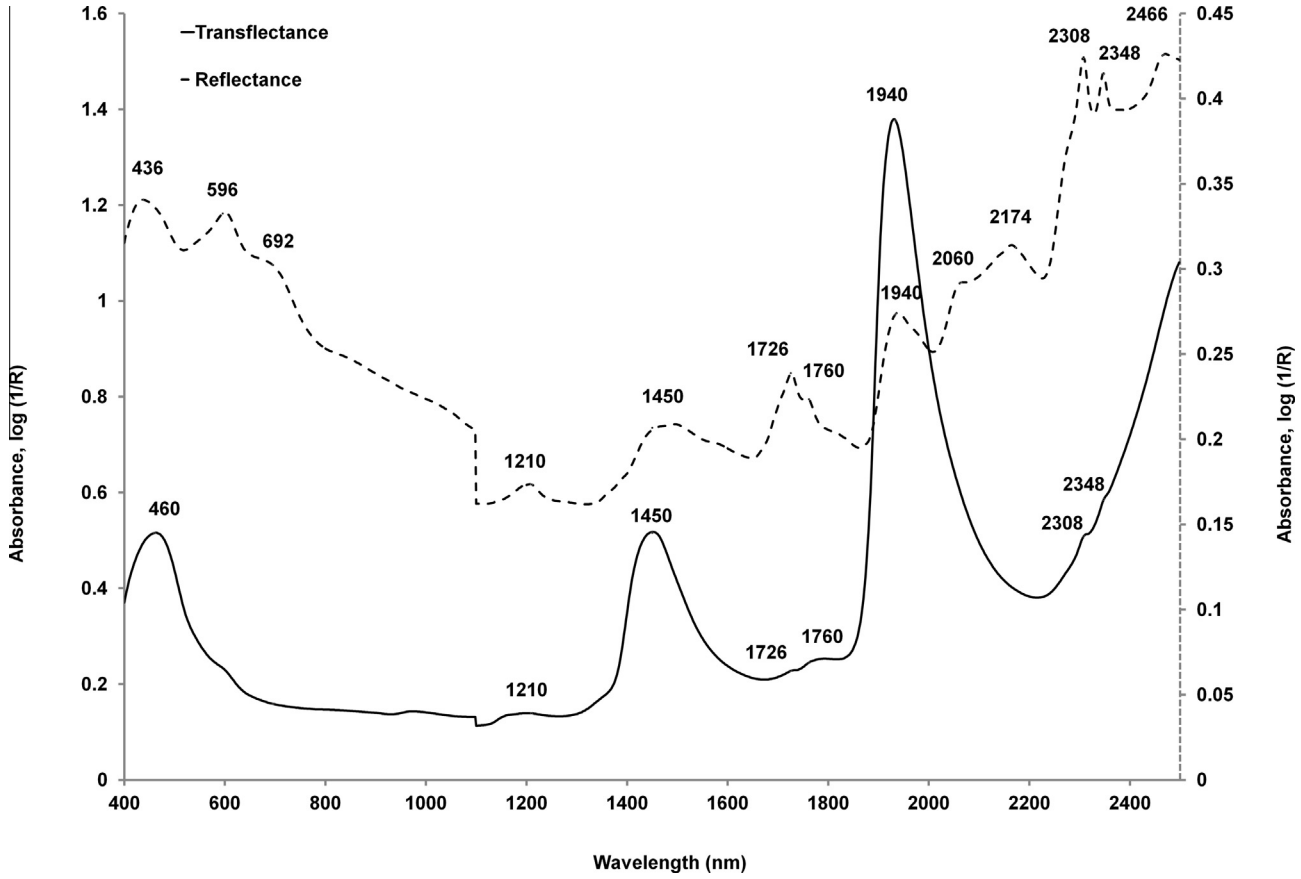

Fig. 1. Visible + NIR average spectra of the goat milk samples analyzed in transflectance (liquid milk; left scale) and in reflectance (oven-dried, DESIR method; right scale) modes. Numbers indicate absorption peaks in each spectrum.

Table 1. The calibration statistics of the selected equations obtained for each parameter, based on the higher r^2 and RPD values and lower SECV and SEP, are presented in Table 2 (Set R), Table 3 (Set T) and Table 4 (Set RT), respectively. In general, the performance of prediction models showed variations with the mathematical pre-treatment used (data not shown), and indeed, not always the selected treatment for a parameter in one of the sets was the best choice for the same parameter in any other of the sample sets. This confirms the importance of the optimization of derivatives when analyzing fats (Fernández-Cabanás, Garrido, García-Olmo, De Pedro, & Dardenne, 2007). None of the derivatives provided a clear better result over the rest, but 2,5,5,1 and 1,5,5,1 are among the most frequently selected. Nor any of the spectral regions used provided clear advantages over the other.

3.3.1. Calibration results for NIRS reflectance analysis

During the structuring process of the initial Set R, composed by 805 samples, 7 samples were removed as outliers, and the remaining population was split into calibration (533 samples) and validation (265 samples) subsets. By following this strategy, the samples in the validation set were consistently distributed in the population and had similar variability (data not shown). The statistics of models for NIR predictions of FA on oven-dried milk samples are presented in Table 2.

NIR calibration statistics of milk samples analyzed in reflectance mode (Set R) showed that the best equations were obtained for total SFA, total MUFA, SCFA, SFA + C18:0, MUFA-18C, *cis*9-C18:1, C18:3n-3, n-3, AI and TI, with r^2 higher than 0.7 and RPD close to or above 2 (Table 2).

In general, the coefficients of determination for cross validation (r^2) obtained here for almost all the FA parameters were lower than those presented by Coppa et al. (2010) and Soyeurt et al. (2011) for cow milk, and by Andueza et al. (2013) for goat milk. This fact could be explained because those authors worked with milk samples produced by animals fed lipid supplements, which are known to deeply affect milk FA composition (Chilliard et al., 2007).

However, SECV values (Table 2) were similar to those obtained by Andueza et al. (2013) for the prediction of individual FA, except for C18:3n-3, for which SECV value was lower in this study (0.05 vs 0.22). Furthermore, SECV values obtained for FA total SFA, total MUFA and total PUFA were lower than those reported by Andueza et al. (2013). As r^2 values depend on the variation range of the analytical parameter, other statistics should be used for the evaluation of the equations, such as calibration and prediction errors and RPD values, which measure the magnitude of the error in relation to the standard deviation in the calibration population.

The results obtained for the prediction of n-3 FA (Table 2) are comparable to those described by Andueza et al. (2013) in goat milk and by Coppa et al. (2014) with cow milk. The determination of milk n-3 FA in the laboratory is expensive and time consuming, but this parameter would allow to qualify the feeding system of the animals and thus would permit a differentiated milk payment system for farmers. With respect to n-6 FA, the results obtained here showed higher r^2 and similar SECV values to those reported by Andueza et al. (2013), but similar r^2 to Coppa et al. (2014) and Soyeurt et al. (2011) in cow milk. The n-6 to n-3 FA ratio (n-6/n-3) NIR predictive model has only been previously reported, for cow milk, by Coppa et al. (2014), with slightly better results than those obtained here.

Interesting results for NIR prediction of the Atherogenicity and Thrombogenicity Indexes of milk have been obtained (Table 2). To

Table 2
Calibration statistics including *T* (number of PLS terms), Mean (mean of the calibration set), SD (standard deviation of the calibration set), SECV (standard error of cross-validation), r^2 (coefficient of determination of cross validation), SEP (standard error of prediction) and RDP (ratio SD/SECV) of the selected equations obtained for the prediction of milk fat fatty acids and groups of fats of oven-dried milk samples analyzed in reflectance mode (Set R).

Fatty acid	Spectral region (nm)	Derivative pre-treatment	*T*	*N*	Mean	SD	SECV	r^2	RPD	SEP
Total SFA	1100–2500	2,5,5,1	15	492	74.61	3.40	1.53	0.80	2.22	2.31
Total MUFA	400–2500	2,5,5,1	13	506	20.86	3.03	1.47	0.76	2.06	1.99
Total PUFA	1100–2500	2,5,5,1	15	515	4.40	0.81	0.46	0.68	1.76	0.53
SCFA	1100–2500	2,5,5,1	13	504	13.87	4.14	2.06	0.75	2.01	2.30
MCFA	400–2500	2,5,5,1	9	521	48.08	3.39	2.17	0.59	1.56	2.68
SFA + C18:0	400–2500	2,5,5,1	11	506	72.81	3.31	1.82	0.70	1.82	2.38
C18:0	400–2500	1,10,10,1	10	491	10.54	1.99	1.28	0.59	1.55	1.94
MUFA-18C	400–2500	2,5,5,1	12	503	19.16	2.98	1.53	0.73	1.94	2.10
*cis*9-C18:1	400–2500	1,10,10,1	14	491	14.97	2.91	1.34	0.79	2.17	1.73
T-C18:1	1100–2500	1,5,5,1	14	497	4.02	2.08	1.24	0.65	1.68	1.70
*trans*11-C18:1	1100–2500	2,10,10,1	15	501	2.19	1.05	0.72	0.53	1.47	0.88
*trans*9-C18:1	400–2500	1,10,10,1	11	486	1.63	1.23	0.73	0.65	1.69	1.21
*cis*9*cis*12-C18:2	400–2500	1,5,5,1	12	501	2.57	0.46	0.31	0.56	1.51	0.39
*cis*9*trans*11-CLA	1100–2500	2,5,5,1	13	496	0.48	0.23	0.17	0.47	1.37	0.23
*trans*10*cis*12-CLA	400–2500	1,10,10,1	10	468	0.08	0.06	0.03	0.65	1.69	0.07
C18:3n-3	400–2500	1,10,10,1	12	494	0.23	0.11	0.05	0.75	2.00	0.06
n-6	400–2500	2,5,5,1	10	509	3.19	0.60	0.35	0.65	1.69	0.43
n-3	400–2500	2,5,5,1	10	500	0.37	0.12	0.07	0.70	1.82	0.10
n-6/n-3	400–2500	2,5,5,1	9	459	8.35	1.93	1.23	0.60	1.58	2.99
AI	400–2500	1,10,10,1	12	495	3.13	0.59	0.28	0.77	2.11	0.40
TI	400–2500	2,5,5,1	11	491	4.05	0.59	0.30	0.74	1.98	0.44

Derivative pre-treatment: 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 a running average or smoothing and the fourth is the second smoothing.
Total SFA: saturated fatty acids (FA).
Total MUFA: monounsaturated FA.
Total PUFA: polyunsaturated FA.
SCFA: short chain saturated FA = C4:0 + C6:0 + C8:0 + C10:0.
MCFA: medium chain saturated FA = C12:0 + C14:0 + C16:0.
SFA + C18:0: short and medium chain saturated fatty acids + C18:0.
MUFA-18C: monounsaturated FA of 18 atoms or carbon.
T-C18:1: *trans* C18:1 FA = *trans*9-C18:1 + *trans*10-C18:1 + *trans*11-C18:1.
n-6/n-3: ratio omega 6 FA/omega 3 FA.
AI: Atherogenicity Index, calculated as AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + n-6 PUFA + n-3 PUFA) (Ulbricht & Southgate, 1991).
TI: Thrombogenicity Index, calculated as TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6) (Ulbricht & Southgate, 1991).

Table 3
Calibration statistics including *T* (number of PLS terms), Mean (mean of the calibration set), SD (standard deviation of the calibration set), SECV (standard error of cross-validation), r^2 (coefficient of determination of cross validation), SEP (standard error of prediction) and RDP (ratio SD/SECV) of the selected equations obtained for the prediction of milk fat fatty acids and groups of fats of liquid milk samples analyzed in transflectance mode (Set T).

Fatty acid	Spectral region (nm)	Derivative pre-treatment	*T*	*N*	Mean	SD	SECV	r^2	RPD	SEP
Total SFA	400–2500	2,5,5,1	9	134	74.33	3.27	1.61	0.76	2.03	2.26
Total MUFA	1100–2500	2,5,5,1	8	130	20.93	2.99	1.61	0.71	1.86	1.96
Total PUFA	1100–2500	1,5,5,1	9	135	4.61	0.79	0.54	0.54	1.48	0.57
SCFA	400–2500	2,5,5,1	9	130	12.28	2.46	1.41	0.67	1.74	2.28
MCFA	400–2500	1,10,10,1	11	127	48.68	3.05	2.00	0.56	1.52	2.54
SFA + C18:0	400–2500	2,5,5,1	7	132	72.15	2.92	1.76	0.63	1.66	2.81
C18:0	1100–2500	2,5,5,1	6	131	11.53	1.98	1.46	0.45	1.36	1.99
MUFA-18C	400–2500	2,5,5,1	7	134	19.34	2.90	1.55	0.71	1.87	2.32
*cis*9-C18:1	1100–2500	1,5,5,1	14	127	15.39	2.62	1.41	0.71	1.87	2.37
T-C18:1	1100–2500	1,5,5,1	14	127	3.56	1.54	1.13	0.46	1.36	1.57
*trans*11-C18:1	1100–2500	1,10,10,1	10	132	2.03	0.89	0.70	0.39	1.28	1.08
*trans*9-C18:1	1100–2500	2,5,5,1	12	123	1.21	0.95	0.61	0.59	1.57	1.01
*cis*9*cis*12-C18:2	400–2500	2,5,5,1	5	135	2.65	0.48	0.35	0.47	1.38	0.4
*cis*9*trans*11-CLA	1100–2500	2,10,10,1	3	133	0.54	0.26	0.24	0.11	1.06	0.22
*trans*10*cis*12-CLA	400–2500	2,10,10,1	10	125	0.06	0.04	0.02	0.58	1.54	0.05
C18:3n-3	1100–2500	2,5,5,1	8	131	0.22	0.12	0.06	0.79	2.19	0.06
n-6	400–2500	2,5,5,1	5	134	3.30	0.59	0.39	0.57	1.54	0.48
n-3	1100–2500	1,10,10,1	12	126	0.40	0.13	0.06	0.78	2.15	0.1
n-6/n-3	400–2500	2,5,5,1	9	132	8.51	2.71	1.51	0.69	1.79	2.05
AI	400–2500	2,10,10,1	12	123	3.08	0.54	0.27	0.75	2.02	0.45
TI	400–2500	2,10,10,1	13	131	4.13	0.61	0.34	0.68	1.79	0.5

Derivative pre-treatment: 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 a running average or smoothing and the fourth is the second smoothing.
Total SFA: saturated fatty acids (FA).
Total MUFA: monounsaturated FA.
Total PUFA: polyunsaturated FA.
SCFA: short chain saturated FA = C4:0 + C6:0 + C8:0 + C10:0.
MCFA: medium chain saturated FA = C12:0 + C14:0 + C16:0.
SFA + C18:0: short and medium chain saturated fatty acids + C18:0.
MUFA-18C: monounsaturated FA of 18 atoms or carbon.
T-C18:1: *trans* C18:1 FA = *trans*9-C18:1 + *trans*10-C18:1 + *trans*11-C18:1.
n-6/n-3: ratio omega 6 FA/omega 3 FA.
AI: Atherogenicity Index, calculated as AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + n-6 PUFA + n-3 PUFA) (Ulbricht & Southgate, 1991).
TI: Thrombogenicity Index, calculated as TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6) (Ulbricht & Southgate, 1991).

the best of our knowledge, this is the first time that direct prediction of these parameters in milk has been attempted by NIR.

Models selected for the estimation of AI and TI in reflectance mode showed good calibration results: 0.77 and 0.74 for r^2 values, 2.11 and 1.98 for RPD; and 0.40 and 0.44 for SEP; respectively. Despite that spectral acquisition takes less than 5 min, analytical results cannot be obtained immediately, as this technique requires oven drying of the filters for 24 h before NIR sample scanning.

3.3.2. Calibration results for NIRS transflectance analysis

For this calibration development, a group of 220 milk samples was used (Set T). As a result of the population structuring, 10 samples were removed as outliers, and the remaining population was split into calibration (140 samples) and validation (70 samples) subsets, so the samples in the validation set were consistently distributed in the population (data not shown).

NIR calibration statistics of milk samples analyzed in transflectance mode (Table 3) showed similar statistics than those obtained with reflectance mode with the set of 805 samples (Table 2). Only total PUFA, SCFA, T-C18:1, *trans*10*cis*12-CLA and *cis*9*trans*11-CLA had slightly better results in reflectance mode, despite the fact that the number of samples used for the calibrations in reflectance mode are four fold higher than those in transflectance mode.

Transflectance analysis mode provided r^2 values of 0.75 and 0.68, RPD 2.02 and 1.79 and SEP values of 0.45 and 0.5 for AI and TI calibrations, respectively. Therefore, the predictive ability of this analysis mode with a set of 220 samples was comparable to the results obtained in reflectance mode with the set of 805 samples.

In order to evenly compare the predictive ability of the two analysis modes (reflectance and transflectance), new reflectance calibrations were developed only with the milk samples analyzed in transflectance (Table 4). Transflectance calibrations showed similar or better results than reflectance mode. Only total PUFA, and *cis*9*trans*11-CLA showed more accurate results in reflectance mode. Therefore, it seems that NIR reflectance analysis mode needs a higher number of samples to provide similar results to transflectance. This fact is remarkable for dairy industry, as oven drying operations involved in the DESIR method could delay to obtain an analytical result.

Coppa et al. (2010) achieved more accurate results in reflectance mode, compared with transflectance mode, using the same data set of cow milk samples. Also Coppa et al. (2014) compared the effectiveness of NIR in reflectance and transflectance in predicting FA composition on fresh and thawed cow milk. Similar results obtained in both modes, and only MUFA and PUFA were predicted better by NIR on oven-dried milk. Probably, the elimination of water in the drying process allowed to better correlate parameters found in a low concentration.

It is remarkable that, in this first study about the use of transflectance for the determination of the FA profile of liquid milk from goats, resulting calibrations showed similar predictive ability to those obtained in this study and in previous works in reflectance mode. In this sense, even calibration statistics for AI in transflectance were better than those obtained in reflectance with the same training set, while results for TI were similar. This fact is considered critical, as time required for reflectance analysis is higher than 24 h, while transflectance analysis can be carried out in less than 5 min.

Table 4
Calibration statistics including *T* (number of PLS terms), Mean (mean of the calibration set), SD (standard deviation of the calibration set), SECV (standard error of cross-validation), r^2 (coefficient of determination of cross validation), SEP (standard error of prediction) and RDP (ratio SD/SECV) of the selected equations obtained for the prediction of milk fat fatty acids and groups of fats of the oven-dried milk samples analyzed in reflectance mode coincident with the transflectance set (Set RT).

Fatty acid	Spectral region (nm)	Derivative pre-treatment	*T*	*N*	Mean	SD	SECV	r^2	RPD	SEP
Total SFA	400–2500	2,5,5,1	10	130	74.85	3.54	1.65	0.78	2.15	2.30
Total MUFA	400–2500	2,5,5,1	12	136	20.44	3.18	1.78	0.68	1.79	2.34
Total PUFA	400–2500	1,5,5,1	7	133	4.62	0.84	0.46	0.70	1.83	0.60
SCFA	1100–2500	2,10,10,1	8	139	12.19	2.89	2.01	0.51	1.43	2.23
MCFA	1100–2500	1,5,5,1	10	139	49.05	2.94	2.44	0.31	1.21	3.35
SFA + C18:0	400–2500	2,5,5,1	9	133	72.76	3.45	1.91	0.69	1.81	2.58
C18:0	1100–2500	2,5,5,1	5	132	11.40	1.70	1.49	0.23	1.14	2.44
MUFA-18C	1100–2500	2,10,10,1	10	133	19.02	2.99	1.80	0.63	1.66	2.47
*cis*9-C18:1	1100–2500	2,5,5,1	8	132	15.03	2.68	1.75	0.57	1.53	2.37
T-C18:1	1100–2500	2,5,5,1	10	131	3.46	1.51	1.15	0.42	1.31	1.65
*trans*11-C18:1	1100–2500	1,5,5,1	9	135	2.13	0.87	0.74	0.28	1.18	0.93
*trans*9-C18:1	400–2500	2,5,5,1	10	124	0.96	0.70	0.53	0.41	1.31	1.29
*cis*9*cis*12-C18:2	1100–2500	1,5,5,1	10	129	2.65	0.47	0.26	0.68	1.78	0.51
*cis*9*trans*11-CLA	400–2500	1,5,5,1	9	127	0.52	0.21	0.17	0.32	1.22	0.30
*trans*10*cis*12-CLA	1100–2500	1,5,5,1	13	121	0.06	0.03	0.03	0.46	1.37	0.05
C18:3n-3	400–2500	1,5,5,1	7	141	0.25	0.12	0.08	0.55	1.49	0.09
n-6	1100–2500	1,5,5,1	9	131	3.33	0.58	0.33	0.68	1.78	0.56
n-3	400–2500	1,10,10,1	13	133	0.41	0.13	0.07	0.69	1.81	0.12
n-6/n-3	400–2500	2,5,5,1	5	125	8.03	2.02	1.23	0.62	1.64	2.72
AI	400–2500	2,5,5,1	10	128	3.20	0.62	0.34	0.70	1.84	0.46
TI	1100–2500	2,5,5,1	10	130	4.25	0.70	0.35	0.74	1.98	0.49

Derivative pre-treatment: 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 a running average or smoothing and the fourth is the second smoothing.
Total SFA: saturated fatty acids (FA).
Total MUFA: monounsaturated FA.
Total PUFA: polyunsaturated FA.
SCFA: short chain saturated FA = C4:0 + C6:0 + C8:0 + C10:0.
MCFA: medium chain saturated FA = C12:0 + C14:0 + C16:0.
SFA + C18:0: short and medium chain saturated fatty acids + C18:0.
MUFA-18C: monounsaturated FA of 18 atoms or carbon.
T-C18:1: *trans* C18:1 FA = *trans*9-C18:1 + *trans*10-C18:1 + *trans*11-C18:1.
n-6/n-3: ratio omega 6 FA/omega 3 FA.
AI: Atherogenicity Index, calculated as AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + n-6 PUFA + n-3 PUFA) (Ulbricht & Southgate, 1991).
TI: Thrombogenicity Index, calculated as TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6) (Ulbricht & Southgate, 1991).

4. Conclusions

This work confirms the viability of NIRS as a fast, reliable and effective analytical method to provide nutritional information of milk samples. NIRS analysis of milk samples allowed to estimate with high precision and accuracy chemical composition, ratios and calculated indexes in both analysis modes. The best equations were obtained for total SFA, total MUFA, SCFA, SFA + C18:0, MUFA-18C, *cis*9-C18:1, C18:3n-3, n-3, AI and TI, with r^2 higher than 0.7 and RPD close to or above 2.

In general, transflectance analysis gave similar or better results than reflectance mode. Hence, in terms of accuracy and speed of analytical response, NIRS analysis of liquid milk (transflectance) is recommended instead of NIRS analysis of oven-dried milk (reflectance), as oven drying operations involved in DESIR method could delay to obtain an analytical result.

Direct prediction of the Atherogenicity and Thrombogenicity Indexes, significant for the interpretation of the nutritional value of goat milk, is possible in both NIRS analysis modes, reflectance and transflectance.

Further work would be done to improve the robustness of the equations already started in this work. Hopefully, the inclusion of samples with a wider range of MUFA and n-3 FA would help to prepare the models to precisely predict future real samples coming from animals fed diets rich in vegetable or fish oils.

Acknowledgements

The study was performed using NIR equipment and facilities belonging to SCAI (NIR/MIR Unity), University of Cordoba (Spain) and chromatography facilities from CITIUS (SGIAUS), University of Seville (Spain). This research was funded by projects AGL2007-66161-C02-01 and AGL2007-66161-C02-02. We also wish to thank Francisca Baena for her technical assistance in the NIR analyses and Francisco Moya and KAPRA S.C. for providing the experimental animals.

References

Alonso, L., Fontecha, J., Lozada, L., Fraga, M. J., & Juárez, M. (1999). Fatty acid composition of caprine milk: Major, branched-chain, and *trans* fatty acids. *Journal of Dairy Science, 82*, 878–884.
Andueza, D., Rouel, J., Chilliard, Y., Leroux, C., & Ferlay, A. (2013). Prediction of the goat milk fatty acids by near infrared reflectance spectroscopy. *European Journal of Lipid Science and Technology, 115*, 612–620.
Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. *Applied Spectroscopy, 43*, 772–777.
Bauchart, D., Roy, A., Lorenz, S., Chardigny, J. M., Ferlay, A., Gruffat, D., et al. (2007). Butters varying in *trans* 18:1 and *cis*-9, *trans*-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. *Lipids, 42*, 123–133.
Berzaghi, P. . Dalle., Zotte, A., Jansson, L. M., & Andrighetto, I. (2005). Near-Infrared Reflectance Spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. *Poultry Science, 84*, 128–136.
Coppa, M., Ferlay, A., Leroux, C., Jestin, M., Chilliard, Y., Martin, B., et al. (2010). Prediction of milk fatty acid composition by near infrared reflectance spectroscopy. *International Dairy Journal, 20*, 182–189.
Coppa, M., Revello-Chion, A., Giaccone, D., Ferlay, A., Tabacco, E., & Borreani, G. (2014). Comparison of near and medium infrared spectroscopy to predict fatty acid composition on fresh and thawed milk. *Food Chemistry, 150*, 49–57.
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.
Combe, N., Clouet, P., Chardigny, J. M., Lagarde, M., & Léger, C. L. (2007). *Trans* fatty acids, conjugated linoleic acids, and cardiovascular diseases. *European Journal of Lipid Science and Technology, 109*, 945–953.

Erkkilä, A., de Mello, V. D., Risérus, U., & Laaksonen, D. E. (2008). Dietary fatty acids and cardiovascular disease: an epidemiological approach. *Progress in Lipid Research, 47*, 172–187.

Fernández-Cabanás, V. M., Garrido, A., García-Olmo, J., De Pedro, E., & Dardenne, P. (2007). Optimisation of the spectral pre-treatments used for Iberian pig fat NIR calibrations. *Chemometrics and Intelligent Laboratory Systems, 87*, 104–112.

Holroyd, S. E. (2013). Review: The use of near infrared spectroscopy on milk and milk products. *Journal of Near Infrared Spectroscopy, 21*, 311–322.

Hourant, P., Baeten, V., Morales, M. T., Meurens, M., & Aparicio, R. (2000). Oil and fat classification by selected bands of NIRS. *Applied Spectroscopy, 54*, 1168–1174.

Juárez, M., Polvillo, O., Contò, M., Ficco, A., Ballico, S., & Failla, S. (2008). Comparison of four extraction/methylation analytical methods to measure fatty acid composition by gas chromatography in meat. *Journal of Chromatography A, 1190*, 327–332.

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

Meurens, M., Van Den Eynde, O., & Vanbelle, M. (1987). Fine analysis of liquids by NIR reflectance spectroscopy of dry extract on solid support (DESIR). In J. Hollo, K. J. Kaffka, & J. L. Gonczy (Eds.), *Near infrared diffuse reflectance/transmittance spectroscopy* (pp. 297–302). Budapest, Hungary: Akademiai Kiado.

Murray, I., & Williams, P. C. (1987). Chemical principles of near-infrared technology. In P. C. Williams & K. Norris (Eds.), *Near infrared technology in the agricultural and food industries* (pp. 17–34). St. Paul, Minnesota, USA: American Association of Cereal Chemists Inc.

Núñez-Sánchez, N., Serradilla, J. M., Ares, J. L., & Garrido-Varo, A. (2008). Effect of moisture uptake on the repeatability of near infrared spectroscopy analyses of ewe milk using the DESIR method. *Journal of Near Infrared Spectroscopy, 16*, 381–388.

Šašić, S., & Ozaki, Y. (2000). Band assignment of near-infrared spectra of milk by use of partial least-squares regression. *Applied Spectroscopy, 54*, 1327–1338.

Shenk, J. S., & Westerhaus, M. O. (1991a). Population definition sample selection and calibration procedures for near infrared spectra and modified partial least squares regression. *Crop Science, 31*, 469–474.

Shenk, J. S., & Westerhaus, M. O. (1991b). Population structuring of near infrared spectra and modified partial least squares regression. *Crop Science, 31*, 1548–1555.

Shenk, J. S., & Westerhaus, M. O. (1995a). *Analysis of agriculture and food products by near infrared reflectance spectroscopy. Monograph*. 12101 Tech Road, Silver Spring, MD 20904, USA: NIRSystems Inc.

Shenk, J. S., & Westerhaus, M. O. (1995b). *Routine operation, calibration, development and network system management manual*. 12101 Tech Road, Silver Spring, MD 20904, USA: NIRSystems Inc.

Shenk, J. S., & Westerhaus, M. O. (1996). Calibration the ISI way. In A. M. C. Davies & P. C. Williams (Eds.), *Near infrared spectroscopy: The future waves* (pp. 198–202). Chichester, UK: NIR Publications.

Shenk, J. S., Westerhaus, M. O., & Abrams, S. M. (1989). Protocol for NIR calibrations: Monitoring analysis results and recalibration. In G. C. Martens, J. S. Shenk, & F. E. Barton II (Eds.), *Near infrared spectroscopy (NIRS): Analysis of forage quality. USDA-ARS agriculture handbook*, N 643 (pp. 104–110). Washington, DC: US Government Printing Office.

Shingfield, K. J., Bonnet, M., & Scollan, N. D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal, 7*, 132–162.

Shingfield, K. J., Chilliard, Y., Toivonen, V., Kairenius, P., & Givens, D. I. (2008). *Trans* fatty acids and bioactive lipids in ruminant milk. *Advances in Experimental Medicine and Biology, 606*, 3–65.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine Pharmacotherapy, 56*, 365–379.

Soyeurt, H., Dehareng, F., Gengler, N., McParland, S., Wall, E., Berry, D. P., et al. (2011). Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. *Journal of Dairy Science, 94*, 1657–1667.

Sukhija, P. S., & Palmquist, D. L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry, 36*, 1202–1206.

Thyholt, K., & Isaksson, T. (1997). Near infrared spectroscopy of dry extracts from high moisture food products on solid support – A review. *Journal of Near Infrared Spectroscopy, 5*, 179–193.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet, 338*, 985–992.

Westad, F., Schmidt, A., & Kermit, M. (2008). Incorporating chemical band-assignment in near infrared spectroscopy regression models. *Journal of Near Infrared Spectroscopy, 16*, 265–273.

Williams, C. M. (2000). Dietary fatty acids and human health. *Annales De Zootechnie, 49*, 165–180.

Williams, P. C. (2001). Implementation of near-infrared technology. In P. C. Williams & K. H. Norris (Eds.), *Near-infrared technology in the agricultural and food industries* (pp. 145–171). St. Paul, Minnesota: AACC Inc.

Zidi, A., Fernández-Cabanás, V. M., Urrutia, B., Carrizosa, J., Polvillo, O., González-Redondo, P., et al. (2010). Association between the polymorphism of the goat stearoyl-CoA desaturase 1 (SCD1) gene and milk fatty acid composition in Murciano-Granadina goats. *Journal of Dairy Science, 93*, 4332–4339.