Food Chemistry 156 (2014) 170–175

Contents lists available at ScienceDirect

Food Chemistry

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

Short communication

Concordance analysis between estimation methods of milk fatty acid content

Mary Ana Petersen Rodriguez, Juliana Petrini, Evandro Maia Ferreira, Luciana Regina Mangeti Barreto Mourão, Mayara Salvian, Laerte Dagher Cassoli, Alexandre Vaz Pires, Paulo Fernando Machado, Gerson Barreto Mourão *

University of São Paulo, PO Box 9, CEP 13418-900 Piracicaba, SP, Brazil

ARTICLE INFO

Article history:
Received 20 May 2013
Received in revised form 9 January 2014
Accepted 26 January 2014
Available online 6 February 2014

Keywords:
Concordance analysis
Conjugated linoleic acid
Gas chromatography
Fourier transform infrared spectroscopy
Milk quality

ABSTRACT

Considering the milk fatty acid influence on human health, the aim of this study was to compare gas chromatography (GC) and Fourier transform infrared (FTIR) spectroscopy for the determination of these compounds. Fatty acid content (g/100 g of fat) were obtained by both methods and compared through Pearson's correlation, linear Bayesian regression, and the Bland–Altman method. Despite the high correlations between the measurements ($r = 0.60–0.92$), the regression coefficient values indicated higher measures for palmitic acid, oleic acid, unsaturated and monounsaturated fatty acids and lower values for stearic acid, saturated and polyunsaturated fatty acids estimated by GC in comparison to FTIR results. This inequality was confirmed in the Bland–Altman test, with an average bias varying from −8.65 to 6.91 g/100 g of fat. However, the inclusion of 94% of the samples into the concordance limits suggested that the variability of the differences between the methods was constant throughout the range of measurement. Therefore, despite the inequality between the estimates, the methods displayed the same pattern of milk fat composition, allowing similar conclusions about the milk samples under evaluation.

1. Introduction

Nowadays, there is an increased concern about food composition, and search for healthier food has become very important. It is known that bovine milk is characterised by the predominance of saturated fatty acids (70%), which are associated with high levels of low density cholesterol (LDL) and, therefore, with cardiovascular diseases (Kromhout, Menotti, Kestleloot, & Sans, 2002). Among them, lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) are the major fatty acids related to the increase of blood cholesterol (Bonanome & Grundy, 1988). However, milk also has many beneficial components, such as unsaturated fatty acids (Mensink, Zock, Kester, & Katan, 2003), especially oleic acid (C18:1*cis*-9) and conjugated linoleic acid (CLA). Thus, the knowledge about the composition of milk and, consequently, about the environmental and genetic factors that may influence or change the profile of fatty acids (FA), is very important to improve the nutritional quality of this product (Soyeurt, Dehareng, Mayeres, Bertozzi, & Gengler, 2008; Soyeurt et al., 2006; Soyeurt et al., 2007; Stoop, Van Arendonk, Heck, Van Valenberg, & Bovenhuis, 2008). Given this, the use of a fast, inexpensive and accurate way to quantify the levels of fatty acids in milk is a significant issue to be considered.

The determination of the fatty acid proportion in milk is performed by gas chromatography (GC) and Fourier transform infrared (FTIR) spectroscopy methods. Commonly used (Collomb & Buhler, 2000; Dorey, Brodin, Le Querler, & Kuzdzalsavoie, 1988; Soyeurt et al., 2006) due to its efficiency, GC allows the quantification of each FA. However, with the disadvantage of requiring the preparation of an esterified compound, this method is time consuming and requires specialized skills. In turn, the FTIR is an alternative method to GC, allowing the analysis of a higher number of samples, nearly 500 samples per hour (Foss, 2008; Soyeurt et al., 2006) compared to GC.

FTIR analyzes the vibrational motions of molecules and can be used for determination of FA in different ways. As there is no need for pre-preparation of the sample for analysis, this method becomes advantageous because of the low cost of reagents, time and specialized labour skills. Furthermore, FTIR is important for studies involving cellular responses, and it can be used as biochemical screening technique for explorative research, it requires

* Corresponding author. Address: University of São Paulo, Animal Science Department, PO Box 9, CEP 13418-900, Piracicaba, SP, Brazil. Tel.: +55 19 34294009; fax: +55 19 34294215.
E-mail address: gbmourao@usp.br (G.B. Mourão).

http://dx.doi.org/10.1016/j.foodchem.2014.01.092
0308-8146/

minimal sample preparation and preserves the components in their natural environment (Najbjerg et al., 2011). Recently, a novel approach for FTIR characterization of the milk fatty acid composition based on dried film measurements has been presented and compared to a standard FTIR approach based on liquid milk measurements (Afseth et al., 2010; Najbjerg et al., 2011). However, despite of the potential for using this approach in routine measurements the dried film approach has not yet found industrial use (Najbjerg et al., 2011).

Thus, considering the particularities of each method, the aim of this study was to compare the measurements obtained by gas chromatography and Fourier transform infrared spectroscopy using validation methodologies, such as Pearson correlation, Bland–Altman and Bayesian linear regression, in order to verify the equivalence of both methods in fatty acids determination.

2. Materials and methods

2.1. Dataset

89 milk samples were collected from Holstein cows with lactations ranging between one and six. These samples, preserved with bronopol, were analysed by GC and FTIR to determine the concentration of FA, expressed as grams per 100 g of milk fat.

In GC analysis, 35 mL of bovine milk were centrifuged at 12,000 rpm (17,800×*g*) for 30 min at 4 °C to separate the fat from whey. Fat was transferred to a 1.5 mL eppendorf and centrifuged at 12,000 rpm (17,800×*g*) for 20 min at 20 °C (Feng, Lock, & Garnsworthy, 2004). After centrifugation, the fat had separated into three layers: the top layer of lipids; the middle layer of protein, fat and other water-insoluble solids; and the bottom layer of water. Then, an aliquot of the lipid extract was methylated in two steps with 2 mL of 0.5 M sodium methoxide (10 min at 50 °C), followed by addition of methanoic HCl (10 min at 80 °C), according to Kramer et al. (1997) and was stored at −20 °C in amber vials containing 1.5 mL of nitrogen to avoid possible oxidation.

After this step, a gas chromatography system (Agilent Technologies 7890A) was used equipped with a flame ionisation detector for the quantification and determination of FA. 10 μL of the sample were injected into the system with a 10 μL syringe. The identification of the FA in the samples was done by comparing the retention time of fatty acid methyl esters with a standard. The standard used was the Supelco® mix of 37 compounds (Sigma Aldrich) and individual patterns for the identification of C18:1 *trans*-11 (vaccenic acid), C18:2 *cis*-9 *trans*-11, C18:2 *trans*-10 *cis*-12 (Nu-CheckPrep) and C18:1-OH (Sigma Aldrich) were obtained. The dataset acquisition was performed using the software Chem Station (Agilent Technologies).

FTIR spectra were taken using a Delta Instruments Combi-Scope™ Filter equipment, Advanced Instruments, Inc., Norwood, USA. Based on these analyses, the samples concentrations of the FA palmitic (C16:0), stearic (C18:0), oleic (C18:1*cis*-9), groups of saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated (MONO), polyunsaturated (POLY) were determined. Extreme values, identified as values larger or smaller than three standard deviations from the mean of each fatty acid, were considered to be outliers and not considered (Table 1).

2.2. Concordance analysis

The comparison of the results obtained by both methods was carried out using Pearson's correlation, Bland–Altman analysis and Bayesian linear regression.

The Pearson's correlation (r) quantifies the degree of linear relationship between two variables (x and y). Values of r near to −1 represents an inverse relationship between two variables, and values near to 1 indicates a positive relationship between them, while r equal to zero means that the variables are not correlated (Gasparini, Barbieri, & Mazzer, 2007; Polit & Hungler, 1995).

Table 1
Number of observations (N), mean, standard deviation, maximum and minimum (in g/100 g of fat) of fatty acids measures obtained by gas chromatography and Fourier transform infrared (FTIR) spectroscopy.

Fatty acid	*N*	Mean	SD	CV	Maximum	Minimum
Gas chromatography						
C16:0	87	30.24	4.707	15.6	30.85	22.35
C18:0	86	11.25	2.795	24.8	20.07	4.82
C18:1*cis*-9	86	23.68	4.480	19.0	37.16	16.66
SFA	87	66.67	5.016	7.5	75.58	52.97
UFA	86	31.66	5.209	16.5	44.70	22.45
MONO	86	28.50	4.749	16.7	41.08	19.67
POLY	87	3.09	0.695	22.5	4.85	1.75
Fourier transform infrared spectroscopy						
C16:0	87	25.81	3.157	12.2	32.91	16.32
C18:0	86	18.17	3.587	19.7	32.56	11.49
C18:1*cis*-9	86	15.02	3.771	25.1	26.17	7.60
SFA	87	70.96	4.453	6.3	79.41	58.94
UFA	86	25.83	5.227	20.2	40.57	15.18
MONO	86	21.35	4.336	20.3	32.75	11.63
POLY	87	4.28	0.967	22.6	7.10	2.01

The CV values (the coefficients of variation) are expressed as (SD/Mean) × 100 (%). *Abbreviations:* C16:0, palmitic acid; C18:0, stearic acid; C18:1*cis*-9, oleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MONO, monounsaturated fatty acids; POLY, polyunsaturated fatty acids; SD, standard deviation.

The Bland–Altman analysis (Bland & Altman, 1999) is based on the construction of a scatter plot linking the average of results obtained by the two methods (abscissa axis) with the bias (ordinate axis), in order to evaluate the dimension of the differences between the methods, the dispersion of these differences around the mean, and possible outliers and trends. The average of the measurements was calculated by $(x_i + y_i)/2$, where x_i is the concentration of FA (in g/100 g fat) determined by the FTIR method for the ith milk sample and y_i is the concentration of the same FA measured by GC in the ith milk sample analyzed. Similarly, the bias was given by the difference between the measurements of each method on the same sample by the following equation: $(x_i - y_i)$. From the bias mean $(\overline{d})$ and its standard deviation (s_d), the limits of agreement (LA) were estimated using the equation $LA = \overline{d} \pm 1.96\, s_d$, which indicates the area where 95% of the differences in the studied cases can be found, considering a normal distribution of the data. The accuracy of the bias and the limits of agreement values were calculated using the standard error $(SE_{\overline{d}})$ and confidence interval (CI), the former being given by $SE_{\overline{d}} = s_d/\sqrt{n}$ where n is the sample size, and the latter estimated by $CI = \overline{d} \pm t \times EP_{\overline{d}}$, where t is the tabulated value of t distribution for $n - 1$ degrees of freedom.

The third method used was the simple linear regression with a Bayesian approach based in the model: $\boldsymbol{y} = \alpha + \beta\boldsymbol{x} + \boldsymbol{\varepsilon}$, where $\boldsymbol{y}$ is the vector of the observed values of fatty acid concentration estimated by GC, α is the regression intercept, β is the angular coefficient of regression, $\boldsymbol{x}$ is the vector of observed values of concentration of the same fatty acid found in the vector $\boldsymbol{y}$, but measured by FTIR, and $\boldsymbol{\varepsilon}$ is the residual vector ($\boldsymbol{\varepsilon} \sim N(\boldsymbol{0}, \boldsymbol{I}\sigma^2)$). For the vectors $\boldsymbol{y}$ and $\boldsymbol{x}$, the C16:0, C18:0, C18:1*cis*-9 FA as well as SFA, UFA, MONO and POLY FA groups were analyzed. For each linear regression, analyses were performed considering non-informative and informative *priors* for α (intercept) and β (inclination), and non-informative *priors* for τ (precision, $\tau = 1/\sigma^2$). For the α and β non-informative *priors*, normal distributions with mean equal to zero and precision equal to 10^6 were used, while for τ the *prior* the gamma distribution was used with shape and scale parameters equal to 10^{-3}. The informative *priors* for α and β were chosen assuming concordance between $\boldsymbol{y}$ and $\boldsymbol{x}$ in each regression, and therefore, $\alpha \sim N(0, 100)$, $\beta \sim N(1, 100)$. The range of 0 and 1 values by the

credibility intervals of the intercept and its inclination, respectively, allows the conclusion that both methods studied produce similar results.

The analysis involving Bayesian linear regression was performed by the Markov Chain Monte Carlo method associated with Gibbs algorithm (Casella & George, 1992; Sorensen & Gianola, 2002) through the program WinBUGS (Lunn, Thomas, Best, & Piegelhalter, 2000). Three Markov chains of 10,000 samples with sampling intervals of one were conducted. The first thousand samples were discarded. The lack of convergence in each chain was verified by the Geweke (1992), Heidelberger and Welch (1983), Gelman and Rubin (1992), and Raftery and Lewis (1992) tests.

3. Results and discussion

In the samples, a high share of SFA (approximately 51% and 58% for GC and FTIR, respectively, Table 1) was observed, with a low content of POLY (about 2% and 3.5% for GC and FTIR, respectively). A low content of POLY in milk occurs due to the biohydrogenation of about 90% to vaccenic acid (C18:1 *trans*-11) with a subsequently biohydrogenation to stearic acid (C18:0) by rumen bacteria (Van Knegsel et al., 2007). The C16:0 was the most abundant FA found in milk samples (30.24 and 25.81 g/100 g of fat to GC and FTIR, respectively). Similar results were obtained by Afseth et al. (2010), whose study compared the approaches of FTIR based on dried film measurements with FTIR based on liquid milk measurements. As also evidenced by Afseth et al. (2010), many of the fatty acids were present high sample-to-sample variation (Table 1), which denotes the set of data to be suitable for further studies calibration and evaluation of prediction equations.

With respect to the linear relationship between the measurements obtained by GC and FTIR, this was verified by the high values for the Pearson correlation (r = 0.60–0.92 – Table 2, Figs. 1 and 2). The groups of SFA (r = 0.86), UFA (r = 0.92) and MONO (r = 0.89) showed a concentration dispersion nearest to the diagonal line of concordance, indicating a high degree of linear relationship between the two methodologies (Fig. 1). The closer to 1 the value of r is (regardless of sign) the greater the degree of linear statistical dependence between variables. Thus, it can be said that the increase or decrease of one unit in the content of these FA obtained by GC generates the same effect on the same FA obtained by FTIR. In other words, the higher the value of r, the greater is the shared variance between the same fatty acids obtained by the two methods. In graphic terms, the Pearson correlation requires a sharing of variance and this variation is distributed linearly. Despite of the high correlation (r = 0.60) between the measures in both methods studied, POLY also showed a high dispersion of points, which can be attributed to the instability and difficulty quantification of this FA in milk generated by the action of rumen microorganisms in C18:0 conversion, as mentioned above.

It should be emphasised that the Pearson correlation coefficient quantifies the degree of linear association between the two methods used to obtain the concentration of fatty acids and not the agreement between them. According to Bland and Altman (1986), there is a perfect agreement only if the points are in the equality line ("line zero" – graphical display obtained in the Bland–Altman methodology) and a perfect correlation is found if the points are in any straight line. Thus, a high correlation does not necessarily indicate that the measurements obtained by GC and FTIR are similar.

Bayesian methods are increasingly being used in several fields due to advantages such as the inclusion of uncertainty in the probability model, yielding more realistic predictions. This gives the opportunity to compare models with different methods including hierarchical models, and identify of inferences on the data, without dependence on asymptotic approximation (Wasserman, 2004). Herein, the adoption of Bayesian inference aimed to exploit mainly the combination of *prior* information with the data. This way, two *priors* were employed: a non-informative *prior*, which has minimal impact on the posterior distribution of the parameters, assigning equal likelihood on all possible values of the parameters, and an informative *prior* assuming the hypothesis of equality in the methods in the mensuration of fatty acids concentration, since the same samples were analyzed for both methodologies. However, the similar results between these two analyses suggested that in both cases the information provided by the data had more effect on the posterior distribution compared to the prior information. This can be probably due to the large sample data used in the analyses and also because the prior distribution established was not aligned with the actual distribution of the parameters considered.

Therefore, in the simple Bayesian linear regression analyses, independent of the *prior* used, it was verified through the values

Table 2
Pearson correlation (r), and values of mean (M), standard deviation (SD), credibility interval (CI) for the intercept (α) and slope (β) in Bayesian linear regression, according with the prior used.

Fatty acid	r		Bayesian linear regression					
			Non informative prior			Informative prior		
			M	SD	CI	M	SD	CI
C16:0	0.75	α	1.29	2.799	(−4.20, 6.82)	1.20	2.692	(−4.09, 6.51)
		β	1.12	0.108	(0.91, 1.33)	1.13	0.104	(0.92, 1.33)
C18:0	0.75	α	0.66	1.055	(−1.42, 2.74)	0.65	1.049	(−1.41, 2.72)
		β	0.58	0.057	(0.47, 0.70)	0.58	0.057	(0.47, 0.70)
C18:1*cis*-9	0.83	α	8.90	1.138	(6.67, 11.16)	8.79	1.130	(6.57, 11.02)
		β	0.98	0.073	(0.84, 1.13)	0.99	0.073	(0.85, 1.13)
SFA	0.86	α	−1.65	4.555	(−10.61, 7.36)	−1.37	4.132	(−9.48, 6.79)
		β	0.96	0.064	(0.84, 1.09)	0.96	0.058	(0.84, 1.07)
UFA	0.92	α	8.08	1.16	(5.80, 10.37)	7.97	1.152	(5.70, 10.25)
		β	0.91	0.044	(0.83, 1.00)	0.92	0.044	(0.83, 1.00)
MONO	0.89	α	7.61	1.184	(5.29, 9.96)	7.51	1.175	(5.19, 9.83)
		β	0.98	0.054	(0.87, 1.09)	0.98	0.054	(0.88, 1.09)
POLY	0.60	α	1.25	0.278	(0.71, 1.80)	1.25	0.278	(0.71, 1.80)
		β	0.43	0.063	(0.30, 0.55)	0.43	0.063	(0.31, 0.55)

C16:0, palmitic acid; C18:0, stearic acid; C18:1*cis*-9, oleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MONO, monounsaturated fatty acids; POLY, polyunsaturated fatty acids.

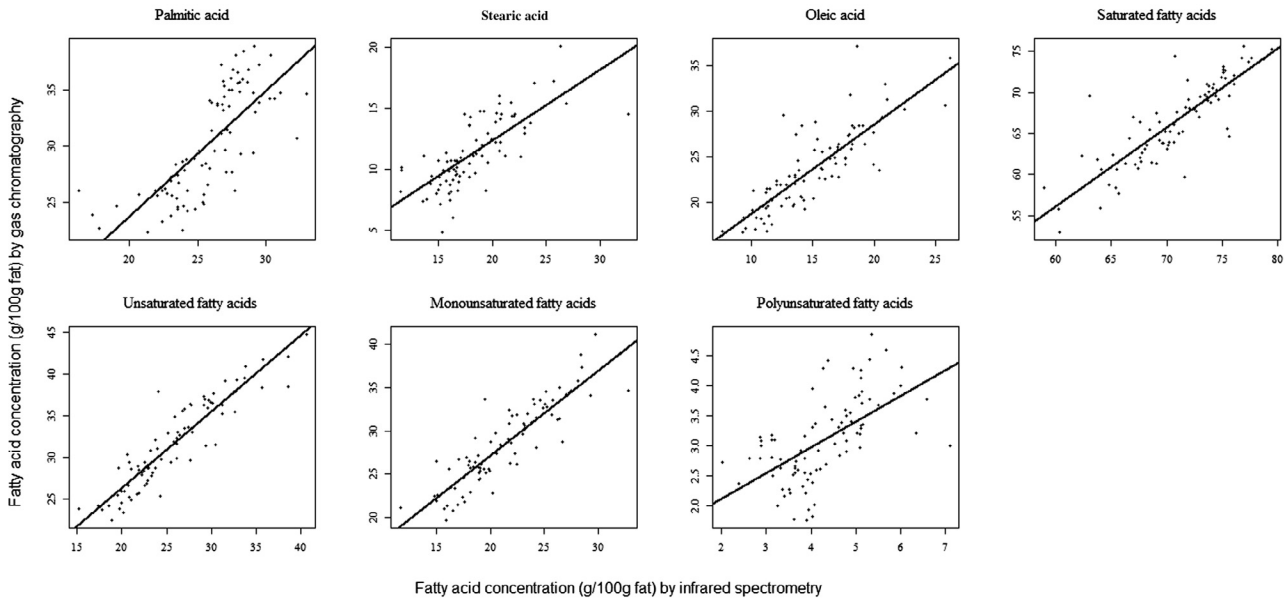

Fig. 1. Dispersion of fatty acids concentration (g/100 g of fat) measures determined by gas chromatography and infrared spectrometry (the black line represents the diagonal line of concordance).

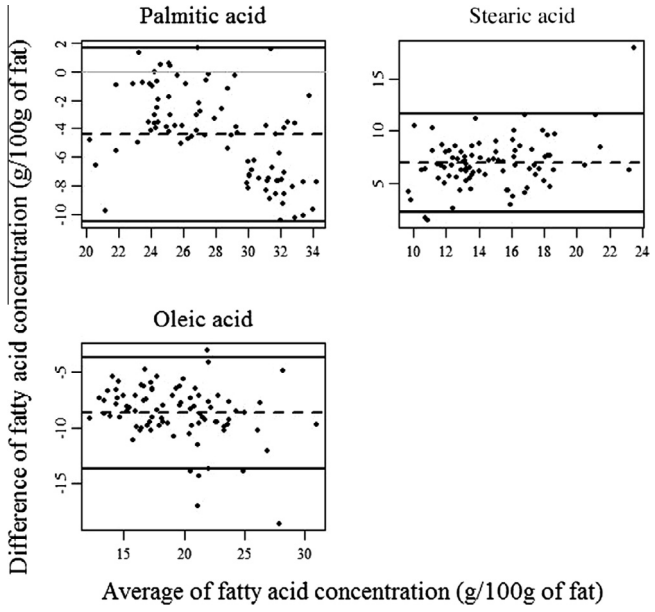

Fig. 2. Dispersion for the difference and the mean between the values obtained by gas chromatography and infrared spectrometry (the black lines represent concordance thresholds and the dotted line represents the average bias).

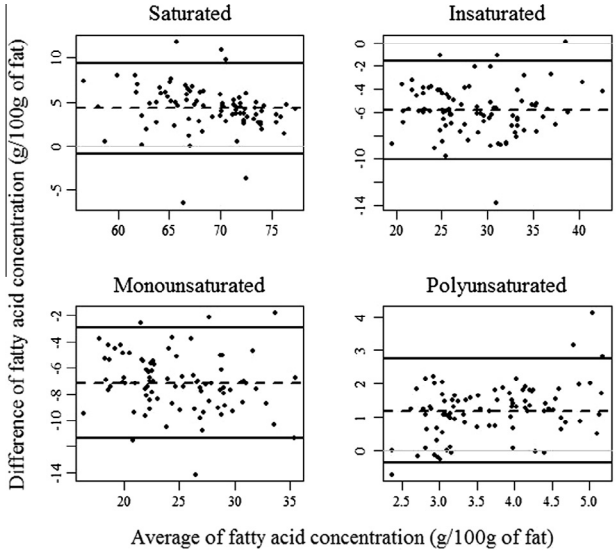

Fig. 3. Dispersion for the difference and the mean between the values obtained by gas chromatography and infrared spectrometry (the black lines represent concordance thresholds and the dotted line represents the average bias).

estimated for the intercept (α) and the slope (β) that there was no similarity between the methods of determining the content of fatty acids in milk (Table 2). Only for the C16:0 and SFA group confidence intervals included zero and one values for the intercept and slope, respectively. However, it should be considered that, despite including zero, the confidence interval for the intercept in SFA concordance analysis was wide, indicating low accuracy for the estimate and consequently making it impossible to firm conclusions about these results.

Also, by the simple Bayesian linear regression analysis, it was observed that for C16:0, C18:1*cis*-9, UFA and MONO, the measurements provided by GC were higher than those obtained by FTIR while for C18:0, SFA and POLY, these were smaller for chromatography, which was also verified by the means of FA concentration in the evaluated samples (Tables 1 and 2). Thus, considering the predominance of C16:0 (20–32%) and C18:1*cis*-9 (15–30%) fatty acids in bovine milk (Grummer, 1991), the highest values of these FA measured by GC can be explained by the greater amount of fat (3.81 g/100 g milk) in analyzed samples by this approach as compared to milk fat obtained by FTIR analysis (3.69 g/100 g milk).

Bland–Altman analyses showed a certain correlation between the estimates provided by GC and FTIR (Figs. 2 and 3). There were discrepancies between FA concentrations in three to five samples evaluated, which corresponded to approximately 3.5–5.8% of the total number of samples, values very close of the tolerated threshold of 5%. However, the levels of FA obtained by the two methods are not equal, since the bias values estimated in the Bland–Altman

Table 3
Standard-deviation (SD), standard error (SE), concordance thresholds and confidence interval of average bias (g/100 g of fat) from Bland–Altman analyses for fatty acids.

Fatty acid	Average bias	SD	SE	Concordance threshold	Confidence interval
C16:0	−4.42	3.123	0.334	−10.54, 1.69	−5.09, −3.75
C18:0	6.91	2.380	0.256	2.20, 11.62	6.40, 7.42
C18:1*cis*-9	−8.65	2.511	0.270	−13.63, −3.68	−9.19, −8.12
SFA	4.29	2.606	0.279	−0.87, 9.45	3.73, 4.84
UFA	−5.83	2.134	0.230	−10.05, −1.60	−6.28, −5.37
MONO	−7.14	2.136	0.230	−11.38, −2.91	−7.60, −6.69
POLY	1.19	0.784	0.084	−0.35, 2.74	1.02, 1.36

C16:0, palmitic acid; C18:0, stearic acid; C18:1*cis*-9, oleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MONO, monounsaturated fatty acids; POLY, polyunsaturated fatty acids.

methodology are not close to zero, one of the necessary conditions to assume similarity between the methods (Table 3).

Another point assessed was the limits of concordance determined in this methodology. It can be seen that the differences given by the limits are considered acceptable (Table 3). Therefore, despite the unequal value, the variability of the differences between the methods was constant throughout the range of measurement.

The approach in the present study is somewhat different than that applied by Afseth et al. (2010). In their study, all spectra were pre-treated mathematically prior to calibration in order to remove scaling and offset variations from spectrum to spectrum. In this paper, even though the milk samples were homogenised prior to liquid milk analysis there was no other kind of pre-treatment. This may have contributed to the lack of similarity between the content of fatty acids obtained by GC and FTIR in this study. Thus, the content of FA obtained here showed greater variability (CV ranged from 6.3 to 25.1 for the SFA and C18:1*cis*-9, respectively) when compared to FA analyzed by Afseth et al. (2010) (CV ranged from 5.5 to 17.0 for the SFA and C18:1*cis*-9, respectively).

It is noteworthy that the FTIR has been widely used for the study of lipids, increasingly replacing the GC (Rutten, Bovenhuis, Hettinga, van Valenberg, & van Arendonk, 2009; Soyeurt et al., 2006). According to Flåtten, Bryhni, Kohler, Egelandsdal, and Isaksson (2005), results showed that marine fatty acids and fatty acid composition in pork fat can be measured with FTIR spectroscopy with good precision and the classification of the samples on the basis of these measurements gives the opportunity for useful implementations of the method in commercial situations, with less labour and time required than alternative chromatographic methods.

The majority of the studies use FTIR data associated with multivariate statistical methods in FA prediction. However, it is important to consider that studies involving FA concordance and prediction analyses, despite of the methodology employed, are still rare. Thus, this study is relevant as an initial evaluation of some of these approaches, with the aim to increase the amount and the quality of FA data for researches involving food nutritional quality. Herein, among the statistical methodologies employed, the Bland–Altman concordance analysis showed to be more appropriate to the dataset and to the objectives of this study, since it allowed a visualisation of the dispersion and magnitude of the differences.

4. Conclusions

There is no equivalency between the measurements of milk FA yielded by GC and FTIR. However, it was observed that both methods indicate a similar pattern of milk composition. Therefore, independently of the method employed, the conclusions taken by comparing samples will be probably the same.

Moreover, given the constant variability of the differences in measures obtained by these two methodologies, it may be possible to establish an association between the concentrations of FA through linear regression, in order to predict the concentration of certain fatty acids in milk only estimated by GC, for samples subjected to FTIR using equations that involve the fatty acids determined by both methods. This way, the number of analyzed samples could be expanded and consequently, more studies involving milk nutritional quality could be realised.

Acknowledgements

This research was supported by FAPESP, CNPq and CAPES.

The authors acknowledge the "Clínica do Leite" and Department of Animal Science, Escola Superior de Agricultura "Luiz de Queiroz" (Universidade de São Paulo, Piracicaba – SP, Brazil) for its support and providing the database.

References

Afseth, N. K., Martens, H., Randby, A., Gidskehaug, L., Narum, B., Jørgensen, K., et al. (2010). Predicting the fatty acid composition of milk: A comparison of two Fourier transform infrared sampling techniques. *Applied Spectroscopy, 64*(7), 700–707.

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet, 327*, 307–310.

Bland, J. M., & Altman, D. G. (1999). Measuring agreement in method comparison studies. *Statistical Methods in Medical Research, 8*, 135–160.

Bonanome, A., & Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *The New England Journal of Medicine, 318*, 1244–1248.

Casella, G., & George, E. I. (1992). Explaining the Gibbs sampler. *American Statistician, 46*, 167–174.

Collomb, M., & Buhler, T. (2000). Analyse de la composition en acides gras de la graisse de lait. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 91*, 306–332.

Dorey, F., Brodin, D., Le Querler, J. F., & Kuzdzalsavoie, S. (1988). Analyse des acides gras du beurre par chromatographie en phase gazeuse couplée avec la spectrometrie de masse. *Industries Alimentaires et Agricoles, 10*, 437–442.

Feng, S., Lock, A. L., & Garnsworthy, P. C. (2004). A rapid method for determining fatty acid composition of milk. *Journal of Dairy Science, 87*, 3785–3788.

Flåtten, A., Bryhni, E. A., Kohler, A., Egelandsdal, B., & Isaksson, T. (2005). Determination of C22:5 and C22:6 marine fatty acids in pork fat with Fourier transform mid-infrared spectroscopy. *Meat Science, 69*(3), 433–440.

Foss (2008). *Foss Analytical Milkoscan 6000 ft+: Operator's manual*. Hillerød: Foss Analytical.

Gasparini, A. L. P., Barbieri, C. H., & Mazzer, N. (2007). Correlação entre diferentes métodos de avaliação funcional da marcha de ratos com lesão por esmagamento do nervo isquiático. *Acta Ortopédica Brasileira, 15*, 285–289.

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science, 7*, 457–472.

Geweke, J. (1992). Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In J. M. Bernardo, J. O. Berger, A. P. Dawid, & A. F. M. Smith (Eds.). *Bayesian statistics* (Vol. 4, pp. 163–190). Oxford: Clarendon Press.

Grummer, R. R. (1991). Effect of feed on the composition of milk fat. *Journal of Dairy Science, 74*, 3244–3257.

Heidelberger, P., & Welch, P. D. (1983). Simulation run length control in the presence of an initial transient. *Operations Research, 31*, 1109–1144.

Kramer, J. K. C., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossobia, M. M., & Yurawecz, M. P. (1997). Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids, 32*, 1219–1228.

Kromhout, D., Menotti, A., Kestleloot, H., & Sans, S. (2002). Prevention of coronary heart disease by diet and lifestyle: Evidence from prospective cross-cultural, cohort, and interventional studies. *Circulation, 105*, 893–898.

Lunn, D. J., Thomas, A., Best, N., & Piegelhalter, D. (2000). WinBUGS – A Bayesian modeling framework: Concepts, structure, and extensibility. *Statistics and Computing, 10*, 325–337.

Mensink, R. P., Zock, P. L., Kester, A. D., & Katan, M. B. (2003). Effects of dietary fatty acids and carbohydrates on the ration of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *The American Journal of Clinical Nutrition, 77*, 1146–1155.

Najbjerg, H., Afseth, N. K., Young, J. F., Bertram, H. C., Pedersen, M. E., Grimmer, S., et al. (2011). Monitoring cellular responses upon fatty acid exposure by Fourier transform infrared spectroscopy and Raman spectroscopy. *Analyst, 136*, 1649–1658.

Polit, D. F., & Hungler, B. P. (1995). *Fundamentos de pesquisa em enfermagem* (3rd ed.). Porto Alegre: Artes Médicas.

Raftery, A. E., & Lewis, S. M. (1992). One long run with diagnostics: Implementation strategies for Markov chain Monte Carlo. *Statistical Science, 7*, 493–497.

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.

Sorensen, D., & Gianola, D. (2002). *Likelihood, Bayesian, and MCMC methods in quantitative genetics*. London: Springer-Verlag.

Soyeurt, H., Dehareng, F., Mayeres, P., Bertozzi, C., & Gengler, N. (2008). Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. *Journal of Dairy Science, 91*, 3611–3626.

Soyeurt, H., Gillon, A., Vanderick, S., Mayeres, P., Bertozzi, C., & Gengler, N. (2007). Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. *Journal of Dairy Science, 90*, 4435–4442.

Soyeurt, H., Dardenne, P., Dehareng, F., Lognay, G., Veselko, D., Marlier, M., et al. (2006). Estimating fatty acid content in cow milk using mid-infrared spectrometry. *Journal of Dairy Science, 89*, 3690–3695.

Stoop, W. M., Van Arendonk, J. A. M., Heck, J. M. L., Van Valenberg, H. J. F., & Bovenhuis, H. (2008). Genetic parameters for major fatty acids and milk production traits of Dutch Holstein-Friesians. *Journal of Dairy Science, 91*, 385–394.

Van Knegsel, A. T. M., Van Den Brand, H., Dijkstra, J., Van Straalen, W. M., Heetkamp, M. J. W., Tamminga, S., et al. (2007). Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. *Journal of Dairy Science, 90*, 1467–1476.

Wasserman, L. (2004). *All of statistics: A concise course in statistical inference*. New York: Springer-Verlag.