Talanta 112 (2013) 101–110

Contents lists available at SciVerse ScienceDirect

Talanta

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

Towards combinatorial spectroscopy: The case of minor milk fatty acids determination

I. Stefanov [a,*], V. Baeten [b], B. De Baets [c], V. Fievez [a]

[a] Laboratory for Animal Nutrition and Animal Product Quality (LANUPRO), Faculty of Bioscience Engineering, Ghent University, Proefhoevestraat 10, B-9090 Melle, Belgium
[b] Food and Feed Quality Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre, Chaussée de Namur, 24. B-5030 Gembloux, Belgium
[c] Department of Mathematical Modeling, Statistics and Bioinformatics, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 1 October 2012
Received in revised form
13 February 2013
Accepted 17 February 2013
Available online 27 February 2013

Keywords:
Trans MUFA
CLA
OBCFA
Combinatorial spectroscopy
ATR/FT-IR
Raman
Concatenation
Chemometrics

ABSTRACT

Chemometrical models for determination of milk fatty acids (FA) are typically developed using spectral data from a single spectroscopy technique, e.g., mid-infrared spectroscopy in milk control. Such models perform poorly in determining minor components and are highly dependent on the spectral data source and on the type of matrix. In milk fat, the unsuccessful determination of minor (fatty acids lower than 1.0 g/100 g in total fat) FA is often the result of: (1) the molecular structure similarity between the minor and the major FA within the milk fat matrix (thus the chemical signature specific to individual fatty acids has restricted specificity), and (2) the low signal intensity (detection limit) for specific vibrational modes. To overcome these limitations, data from different types of spectroscopy techniques, which brings additional chemical information in relation to the variation of the FA, could be included in the regression models to improve quantification. Here, Fourier transform (FT) Raman spectra were concatenated with attenuated total reflectance FT infrared (ATR/FTIR) spectra. The new combinatorial models showed up to 25% decrease in the root mean squared error of cross-validation (RMSECV) values, accompanied with a higher R^2_{cv} for most individual FA or sums of FA groups, as compared to regression models based on Raman only or ATR/FTIR only spectra. In addition, improved models included less PLS components indicating an increased robustness. Interpretation of the most contributing regression coefficients indicated the value of newly combined spectral regions as carriers of specific chemical information. Although requiring additional spectroscopy instrumentation and prolonged acquisition time, this new combinatorial approach can be automated and is sufficient for semi-routine determination of the milk FA profile.

1. Introduction

The animal production and food control fields are continuously evolving via advancements in analytical methodologies. Spectroscopy-based analytical techniques are particularly useful for disease and health condition screening and manufacturing process control, which when combined with accurate reference data and appropriate statistical methods can provide fast answers to the composition of various types of samples. The spectral bands not only depend on the types of chemical bonds, but are also correlated to their amounts present in the sample, which makes them very useful in quantification tasks [1]. Quantifications of fatty acids in different oil and food matrices via vibrational spectroscopy techniques have been intensely studied [2]. Knowledge of the fatty acids composition in milk is of importance to human health [3] and could provide useful information on the health status of dairy animals [4]. But milk has a complex fat matrix containing over 400 different fatty acids [5]. Due to their large number and structural variety, determination of milk fatty acids is considered a very difficult task not just by vibrational spectroscopy techniques, but also by conventional chromatography methods. Quantification of minor ($\leq$10 g/kg milk fat) milk fatty acids using vibrational spectroscopy becomes especially difficult in raw milk, due to the presence of other milk components, which interfere with fatty acid specific signals [6]. Minor fatty acid quantifications might also be difficult even when only the spectrum of milk fat is used for determinations [7]. In such cases, as well as when the constituent of interest is very minor ($<$0.5 g/100 g) and exhibits weak molecular vibration signals with hard to detect variations in concentration, new approaches

Abbreviations: ATR/FTIR, attenuated total reflectance Fourier transform infrared; CLA, conjugated linoleic acid; FA, fatty acid; GC, gas chromatography; IR, infrared; MIR, mid-infrared; MUFA, monounsaturated fatty acids; NIR, near-infrared; OBCFA, odd and branched chain fatty acids; PC, principal component; RMSECV, root-mean squared error of cross-validation; TFA, trans fatty acids; TLC, thin-layer chromatography

* Corresponding author. Tel.: +32 479196013; fax: +32 1 8605402525.
E-mail addresses: ivan@ivanstefanov.com, ivan_stefanov@yahoo.com (I. Stefanov).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.02.034

dealing with determinations of the milk fatty acid profile are required. One such novel approach would be to combine the spectrum of a milk fat sample from two different spectroscopy techniques and use the concatenated spectra for construction of better minor fatty acid determination models.

Infrared [IR, near-IR (NIR) and mid-IR (MIR)] and Raman spectroscopy are the main vibrational spectroscopy techniques proven useful in various routine and screening tasks, but there is a limit to the amount and the specificity of the information a particular technique provides [2]. The difference lies in the phenomenon behind each spectroscopy technique. IR represents a direct light absorption process and Raman spectroscopy is a light scattering process. In NIR, the absorbance bands are the overtones of the fundamental bands occurring in the MIR region. NIR bands are relatively weak, overlapped and are not clearly delineated. NIR is considered the least progressive of all vibrational spectroscopy techniques for the current task. In contrast, attenuated total reflectance Fourier transform mid-IR (ATR/FTIR) and Raman spectroscopy methods both measure the energy required to change fundamental vibrational and rotational energy states of chemical bonds. However, the absorption (ATR/FTIR) and scattering (Raman) phenomena have different rules governing their occurrence and thus offer differing sensitivities to the same functional groups, e.g. some vibrational transitions that are observed in IR spectroscopy are not observed in Raman spectroscopy. This results in different amounts of chemical information for the same compound by each technique, which makes the techniques complementary. Nevertheless, organic functional groups exhibit characteristic and well delineated bands when analyzed using ATR/FTIR and Raman spectroscopy. For example, the fatty acids' C=O carbonyl group is the most intensive MIR active absorption bands, whereas carbon double bonds C=C have strong isolated Raman scattering bands [8]. As a result of the sensitivity for various functional groups and the fundamental nature of the signal, Raman and ATR/FTIR can readily provide more useful analytical data as compared to NIR. All these features make Raman and ATR/FTIR ideal candidates in the new concatenation approach. Thus, here the spectra from both methods were combined for the first time in order to construct regression models for the determination of milk fatty acids.

2. Materials and methods

2.1. Sample selection

The sample storage and selection methodology was previously described [7]. Briefly, a total of 100 milk samples were selected from a sample bank ($n=1033$) of six different cow feeding experiments [7]. The sample subset was selected using a genetic algorithm applied to cover the naturally occurring concentration range of several milk fatty acids of interest, in particular odd and branched chain saturated fatty acids and several *trans*-C18:1 and *cis*/*trans*-C18:2 unsaturated isomers [9]. The milk fat was extracted using a previously described methodology involving dichloromethane–ethanol [10].

2.2. GC reference data

Quantification of *trans* fatty acids (TFA) and fatty acid groups using spectral data requires precise Gas Liquid Chromatography (GC) reference data for the construction of mathematical models. Identification and quantification of TFA through GC have been greatly improved with new highly polar, long capillary columns, but direct GC without prior fractionation could show overlapping between different *trans-n* and *cis-n* C18:1 positional isomers and *trans*-n C16:1 coelution with specific branched chain saturated and *cis-n* C16:1 mono-unsaturated FAs, which might result in an underestimation of the total TFA content [7,11]. Here, we used the temperature dependency of the polarity of cyanopropyl phases [12] to mathematically deduce concentrations of overlapping fatty acids using two different temperature programs without prior fractionation. A similar approach was described before [7,13,14]. After extraction, all samples were methylated [10] and fatty acid methyl esters (FAME) were analyzed by GC according to Vlaeminck et al. [15] (first temperature program) and by an isothermal ($T=180$ °C) (second) temperature program. Implementation of both temperature programs without prior separation on silver ion thin-layer chromatography (Ag^+ TLC), allowed quantification of individual *trans* monounsaturated FA, which coelute with branched chain saturated and specific *cis-n* monounsaturated FAs when only one GC temperature program is used. Due to a different separation with the second temperature program, most FA could be quantified individually as previously described [7,13]. As coelution of FA also depends on the column status, the identity of the FA and coeluting bands regularly requires confirmation by injections of Ag^+ TLC fractions. Due to a limited sample quantity for 8 of the 100 selected samples, GC profile reference data was available for 92 milk fat samples only.

2.3. Vibrational spectroscopy analysis

2.3.1. Fourier transform Raman spectroscopy

All Raman spectra were acquired on a Vertex 70—RAM II Bruker Fourier transform Raman spectrometer (Bruker Analytical, Madison, WI). The instrument is equipped with a Nd:YAG laser (yttrium aluminum garnet crystal doped with triply ionized neodymium) with an output at 1064 nm (9398.5 cm^{-1}). The maximum of the laser power is 1.5 W. The measurement accessory is pre-aligned, only the *Z*-axis of the backscattered light was adjusted to set the sample in the appropriate position regarding the local point and to maximize the scattering intensity. The 180° backscattering refractive geometry, CaF_2 beam splitter, and liquid nitrogen-cooled Ge diode array detector have been used. The OPUS 6.5 software for Windows of Bruker Instruments was used for the instrument management, spectral acquisition and file transformation. The spectral data were obtained with a resolution of 4 cm^{-1} and a nominal laser power of 600 mW. Milk fat samples and pure FA standards (~0.1–0.5 g/sample) were analyzed in vials selected by CRA-W in previous Fourier transform Raman analysis [8] with PE-caps (Klaus Ziemer GmbH, Mannheim, Germany), at room temperature (~25 °C) (RT) and immediately after freezing at −80 °C (FT). To ensure homogenization of the milk fat, all samples were melted at 38 ± 1 °C in a water bath, at a minimum of 1 h prior to temperature treatment. For each spectrum, 64 scans were co-added and averaged to obtain a good signal-to-noise ratio. A total of 3734 data points were recorded from 0 to 3599 cm^{-1}. Because of very low milk fat quantity in 14 and extraction solvent contamination in 3 of all 92 selected samples, Fourier transform Raman spectroscopy data in RT and FT were available for 75 milk fat samples.

2.4. ATR/FTIR spectroscopy

All attenuated total reflectance Fourier transform mid-infrared (ATR/FTIR) spectra were acquired on a Vertex 70RAM II Bruker spectrometer (Bruker Analytical, Madison, WI) operating with a Golden Gate ™ diamond ATR accessory (Specac Ltd., Slough, UK). The internal reflection element was a small, non-temperature-controlled Type IIa diamond prism allowing a sampled diameter of approximately 2.0 mm. The optically dense medium was in contact with two ZnSe focusing lenses, one used to focus the incident infrared radiation and the second one to collect the reflected infrared radiation. The optical bench included an interferometer with a RockSolid configuration, KBr substrate beam