Journal of Food Composition and Analysis 32 (2013) 131–140

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

Interlaboratory evaluation of milk fatty acid composition by using different GC operating conditions☆

Giovanna Contarini [a,*], Milena Povolo [a], Valeria Pelizzola [a], Lucia Monti [a], Giovanni Lercker [b]

[a] Consiglio per la Ricerca e la Sperimentazione in Agricoltura-Centro di ricerca per le Produzioni Foraggere e Lattiero-Casearie (CRA-FLC), Via A. Lombardo 11, 26900 Lodi, Italy
[b] Dipartimento di Scienze e Tecnologie Agro-Alimentari Alma Mater Studiorum, Università di Bologna, Via Fanin 40, 40127 Bologna, Italy

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 6 August 2013
Accepted 28 August 2013

Keywords:
Milk fatty acid composition
Gas chromatography
100 m capillary column
Interlaboratory study
Repeatability
Reproducibility
Laboratory performance
Precision
Validation
Uncertainty
Food analysis
Food composition

ABSTRACT

The aim of this work was to test the effectiveness of an analytical protocol, including the calibration with a reference milk fat and the internal standard method, on milk fatty acid (FA) composition. The performances of both different types of stationary phases and column length (30–100 m), together with the elution order of some isomers, were verified. This latter was dependent not only on the polarity and length of the GC column, but also on the temperature gradient. The calibration of the instrument, using a natural reference material, affected the precision of the ISO 15885 standard in a positive way, lowering both the repeatability (from 0.5 to 0.15%, for the range of FA concentration varying from 1 to 5%; from 1 to 0.75% for the range of FA concentration varying from 5 to 10%) and the reproducibility (from 1 to 0.24% for the range of FA concentration varying from 1 to 5%; from 4 to 0.8% for the range of FA concentration varying from 5 to 10%). Moreover, the application of the response factors provided a correct and precise quantification of the short-chain FAs (4–8 carbon atoms). The protocol applied can be considered a useful example for further collaborative studies aiming to standardise the milk FA analysis with long polar GC columns.

1. Introduction

In certain research projects, different laboratories are asked to evaluate the same constituents on different sets of samples. Moreover, if no common parameters must be determined, then no validated methods can be applied. In such cases, even if the laboratories participating in the project are highly experienced, the reliability of the results will have to be evaluated in order to render the comparison between the different sets of data statistically significant. This paper reports both the methodology applied and the results of a collaborative study performed within a research project studying the influence of different cow feeding systems on the fatty acid (FA) composition of bovine, ovine and caprine milk. Seven partners were involved in the project and they had to provide results on FA composition of milk of different species.

Even though the FA composition of milk fat is a very popular gas chromatographic (GC) determination in the dairy field, it still involves problems related to the presence of a wide range of FAs having different concentrations and physical–chemical characteristics (Jensen, 2002). Some critical points have to be taken into account when the GC analysis of milk FAs is performed, the first of which is the presence of short-chain FAs, which are difficult to separate from the solvent and require the application of a response factor to obtain a correct quantification. Ackman and Sipos (1964) demonstrated that the carbonyl C-atom does not contribute in general to the FID response of fatty acid methyl esters (FAME), and that short-chain FAMEs, in particular, have a carbon atom deficiency >1. Moreover the concept of theoretical response factors is not directly applicable to methyl esters of short-chain FAs, since their carbon deficiency is larger than expected from the

☆ Collaborators: Luisito Cercaci and Mara Mandrioli (Dipartimento di Scienze e Tecnologie Agro-Alimentari Alma Mater Studiorum, Università di Bologna, Italy), Marcello Mele and Andrea Serra (Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Italy), Arianna Buccioni (Dipartimento di Scienze delle Produzioni Agroalimentari dell'Ambiente Università di Firenze, Italy) Aldo Prandini (Istituto di Scienze degli alimenti e della nutrizione, Università Cattolica del S. Cuore di Piacenza, Italy), Nico Brogna (Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Italy), Anna Nudda (Dipartimento di Agraria, Sezione di Scienze Zootecniche, Università di Sassari, Italy).

* Corresponding author. Tel.: +39 037145011; fax: +39 037135579.
E-mail address: giovanna.contarini@entecra.it (G. Contarini).

0889-1575/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2013.08.008

theory (Ulberth et al., 1999). Secondly, milk FA composition includes a high number of *cis*/*trans* isomers, in particular for octadecenoic acid (18:1) (Destaillats et al., 2007; Ledoux et al., 2000). Finally, if this determination is performed on milk samples deriving from experimental trials aiming to enrich the fat composition with essential FAs, the content of polyunsaturated fatty acids (PUFA), belonging to the omega-3 chemical class, should also be evaluated (Dewhurst et al., 2006; Wright et al., 2003).

ISO 15885 (ISO, 2002b) is the validated method usually applied to evaluate the milk FA composition. This method includes the procedure for the calibration of the gas chromatographic system by using a reference fat of known composition, but it does not provide operating conditions which are useful to separate both the *trans* isomers and PUFA omega-3.

Over the past decade, due to the increasing importance of conjugated linoleic acid (CLA) on human health and its principal precursor, *trans*-vaccenic acid (VA), studies on milk fat *trans* isomers have undergone much development (Chilliard et al., 2007; Mosley et al., 2006). New and very long (up to 200 m) GC columns coated with high polar stationary phases have been developed to separate *cis*/*trans* isomers and several authors have investigated the performance of these columns for the evaluation of FA composition of milk fat (Delmonte et al., 2011, 2012; Kramer et al., 2002; Ratnayake et al., 2006). The results showed that no column was able to carry out the complete separation of all the milk FAs including *trans* isomers, even when testing a wide number of different operating conditions. The most complete separation was achieved only by applying pre-separation techniques, such as Ag-TLC, Ag-SPE or Ag-HPLC (Cruz-Hernandez et al., 2004; Delmonte and Rader, 2007; Kramer et al., 2001, 2002, 2004; Martin et al., 2008; Ratnayake, 2004), even though satisfactory results were obtained by analysing the same sample under two different GC operating conditions and by using the same column (Kramer et al., 2008).

The aim of this work was to test the effectiveness of the application of an analytical protocol on the results of milk FA composition among different laboratories working within the same research project on samples of different milk species. Because of the large scope of the project, which required both the quantitative evaluations of all the milk FAs-including VA and CLA–and the analysis of a large number of milk samples, a simple and rapid analytical method was required.

The study was divided into two steps: a pilot and a collaborative study. In the first part the effectiveness of the calibration procedure made by using a reference fat of known composition, expressed as percentage on the total FAs, was tested on a milk fat sample, leaving the laboratories the choice of the GC operating conditions (column, temperature and integration parameters). In the second step, three different milk fat samples in blind duplicate were distributed, together with a reference fat calibrated in g/100 g of fat by using the internal standard method. In addition, more well-defined methodological conditions of operation were requested. This second step, for clarity of explanation in this paper, is called collaborative study, even though it did not involve the minimum number of laboratories (i.e. 8) requested by a full collaborative study as defined in the ISO 5725 standard (ISO, 1994).

2. Materials and methods

2.1. Chemicals and reagents

Methanol, hexane, diethyl ether (all GC analytical grade, >99%) and potassium hydroxide (purity 99%) were purchased from Sigma–Aldrich Chemical Co., St. Louis, MO, USA. The following reference triacylglycerol and FAME standards, at purity 99% (Sigma–Aldrich Chemical Co., St. Louis, MO, USA), were used: glycerol tributanoate, glycerol trihexanoate, methyl octanoate, methyl nonanoate, methyl decanoate, methyl dodecanoate, methyl tetradecanoate, methyl hexadecanoate, methyl octadecanoate, methyl *cis*-9-octadecenoate, methyl *trans*-11-octadecenoate (VA), methyl *cis*,*cis*-9,12-octadeca-dienoate, methyl *cis*,*cis*,*cis*-9,12,15-octadeca-trienoate, methyl all *cis*-5,8,11,14,17-icosapentaenoate (EPA), methyl all *cis*-4,7,10,13,16,19 docosahexaenoate (DHA), methyl tricosanoate and conjugated methyl octadeca-dienoate (CLA-FAME). The CLA-FAME standard was a mixture of isomers of which *c*9,*t*11 and *c*10,*t*12 were the most abundant ones. The sum of the areas of the different isomers was used to calculate the correction factor.

2.2. Samples

Different brands of concentrated butter (fat > 98%) were purchased locally and filtered through anhydrous sodium sulphate at 45 °C to obtain anhydrous milk fat samples to prepare the reference fat samples and the samples for the pilot test and the collaborative study.

2.2.1. Pilot test

The content of the most important FAs of Reference fat T (Ref. T) was determined by using the Certified anhydrous milk fat Reference Material CRM164 purchased from IRMM (Institute for Reference Materials and Measurements, European Commission, Geel, Belgium), and it was expressed as percentage on the total FAs. Mean and standard deviation values obtained from the analysis of CRM164 (4:0 = 3.54 ± 0.050; 6:0 = 2.36 ± 0.017; 8:0 = 1.37 ± 0.020; 10:0 = 2.87 ± 0.006; 12:0 = 4.09 ± 0.008; C14:0 = 10.73 ± 0.111; 16:0 = 26.98 ± 0.080; 18:0 = 10.49 ± 0.020; 18:1 = 24.91 ± 0.110; 18:2 = 1.83 ± 0.004; 18:3 = 0.51 ± 0.004) were not significantly different from the certified values ± the uncertainty (4:0 = 3.49 ± 0.06; 6:0 = 2.36 ± 0.019; 8:0 = 1.36 ± 0.10; 10:0 = 2.89 ± 0.12; 12:0 = 4.03 ± 0.10; C14:0 = 10.79 ± 0.35; 16:0 = 26.91 ± 0.84; 18:0 = 10.51 ± 0.40; 18:1 = 24.82 ± 0.61; 18:2 = 1.80 ± 0.40; 18:3 = 0.51 ± 0.04).

For the pilot test, two samples were distributed: Ref. T and one milk fat sample (Sample 1) of unknown composition. Vials of 2 mL of capacity were filled with the fat maintained at 45 °C, by using an automatic pipette, and immediately closed and stored at −20 °C, up to the delivery date.

2.2.2. Collaborative study

The reference fat for the second step of the harmonisation study (Ref. W) was required to contain significant amount of PUFA belonging to the omega-3 class. To that purpose, 92 g of anhydrous milk fat were mixed with 8 g of fish oil (ROPUFA® n-3 Food Oil, Roche S.p.A., Milan, Italy).

In order to obtain samples of unknown composition having a suitable FA composition in terms of VA, CLA and omega-3 content, three mixtures were prepared by weighing the following amounts of anhydrous milk fat (*X*), sheep milk fat (*Y*) and fish oil (*Z*): sample A: 96.9 g (*X*) and 3.1 g (*Z*); sample B: 98.5 g (*X*) and 1.5 g (*Z*); sample C: 47.6 g (*X*), 47.5 g (*Y*) and 4.9 g (*Z*).

Sheep milk fat was chosen because of its higher content of CLA and VA in comparison with cow milk fat (Tsiplakou and Zervas, 2008). It was extracted from bulk raw sheep milk collected on a local farm, by applying ISO 14156 procedure (ISO, 2001). The mixtures were maintained at 45 °C for 1 h in a water bath using magnetic stirring agitation to assure a complete homogenisation. Then the vials were prepared and stored as previously described, up to the delivery date. Six unknown samples, A, B, and C in blind duplicate, labelled with numeric random codes, and two samples of Ref. W were distributed to the seven laboratories involved in the project.