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Direct analysis of fatty acid profile from milk by thermochemolysis–gas chromatography–mass spectrometry

Mariza Gomes Reis[a,*], Marlon Martins dos Reis[a], Shane Leath[a], Kerst Stelwagen[b]

[a] Agri-Foods & Health Section, AgResearch, Ruakura Research Centre, PB 3123, Hamilton, New Zealand
[b] Agri-Search Ltd, Hamilton, New Zealand

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ABSTRACT

The fatty acid composition of milk is of considerable interest due to their nutritional and functional properties. Although rapid milk fat separation and transesterification procedures have been developed, the overall procedure remains time consuming, specially, for the analysis of a large number of samples. In this work, a fast and simple method for direct profiling of fatty acids from milk using thermochemolysis has been developed. This method has the capability of directly analyse fatty acids from one drop of milk without fat extraction or cleanup. Our approach for thermochemolysis is based on thermal desorption integrated with a cold trap inlet. The optimized method does not present isomerisation/degradation of polyunsaturated fatty acid and shows milk fatty acid profiles comparable to the conventional method based on fat extraction and alkaline transesterification. Overall, this method has demonstrated significant potential for high throughput analysis of fatty acids in milk.

1. Introduction

Milk fat confers taste, smell and texture to dairy products [1] and has been associated with positive or negative factors that affect the health of consumers [2]. For these reasons the ability to modulate the milk fatty acid composition has been claimed as a feasible way to transform milk into a high-value product [3]. The modulation of fatty acid composition is achievable on-farm since in ruminant milk the fatty acid composition is related to intrinsic factors (such as animal species, breed, genotype, pregnancy and stage-of-lactation) and extrinsic (environmental) factors [4]. The presence of genetically linked components in fatty acid composition has been considered as a strategy to enhance the modulation of fatty acid composition on-farm and has motivated phenotypic investigations of dairy cows based on fatty acid composition [3,4]. Technologies that allow identification of special individuals with traits associated with the production of premium milk (i.e. rich in high-value components such as conjugate linoleic acid 'CLA') have become increasingly important [5]. Phenotypic investigations involve the analysis of large number of samples [5], thus an ideal technology would have high throughput; relatively low cost per analysis; be easily automated; be robust; produce highly informative data; and allow detection of unknown traits without bias. Gas chromatographic (GC) analysis is a robust and well established technology that meets most of these requirements, however, procedures for GC analysis of milk fatty acid profiles remain time consuming [6–11], specially for large number of samples such as phenotypic investigations [5].

Over recent years, the use of thermochemolysis has gained importance in analytical chemistry because it provides high sensitivity using a minimal amounts of sample and has been successfully applied for characterization of different materials [12–16]. Thermochemolysis is a thermally assisted derivatisation [16,17] of polar groups using methyl donors such as Tetramethylammonium hydroxide (TMAH) [18], Trimethylsulfonium hydroxide (TMSH) [19], Tetramethylammonium acetate (TMAAc) [20], and Trimethylphenylammonium hydroxide (TMPAH) [21].

Thermochemolysis has been implemented using pyrolysis devices such as the pyrolysis microfurnace [22], Curie-point [23], heated filament [24,25], by direct thermal desorption interface [26], programmed temperature vaporization injection [27], and also off line [28,29]. However, limitations have been pointed out during the analysis of lipids. The strong alkalinity of methyl donors causes undesirable isomerisation and/or degradation of polyunsaturated fatty acid (PUFA) components. Different strategies have been adopted for the detection of PUFA components without appreciable side reactions by replacing strongly alkaline conditions by lower reagents concentration or using other reagents such as TMAAc and TMSH [21,30,31].

This work presents a new analytical methodology for the rapid characterization of milk fat without the need for fat extraction and uses direct transesterification by thermochemolysis

* Corresponding author. Tel.: +64 7 8385532; fax: +64 7 838 5625.
E-mail address: mariza.gomesreis@agresearch.co.nz (M.G. Reis).

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Table 1
Linearity of 17 triglycerides commercially available and evaluated under optimized conditions for milk thermochemolysis.

FAME	Ions (m/z) used	Range ($\mu g\,mL^{-1}$)	Equation $n=2$	r^2	RSD (%) $n=4$
6:0	74	19–600	$y=9588.6x+109{,}718$	0.9854	5.162
8:0	74	19–600	$y=21{,}532x-148{,}633$	0.9900	5.78
10:0	74	19–600	$y=28{,}275x-531{,}30$	0.9904	6.30
12:0	74	19–600	$y=30{,}874x-200{,}373$	0.9908	6.15
14:0	74	19–1000	$y=25{,}003x+110{,}425$	0.9899	6.48
16:0	74	19–2000	$y=20{,}104x+1E+06$	0.9851	7.13
16:1	55	19–600	$y=35{,}667x-309{,}188$	0.9893	6.15
18:0	74	19–1000	$y=23{,}359x-33{,}358$	0.9846	8.13
18:1	55	19–1000	$y=29{,}705x+521{,}320$	0.9878	6.27
18:2	67	19–600	$y=16{,}296x-238{,}137$	0.9901	6.13
18:3	79	19–600	$y=27{,}164x-350{,}261$	0.9927	12.30
18:2 (9*c*,11*t*)	67	19–600	$y=31{,}459x-642{,}043$	0.9847	6.41
18:2 (10*t*,12*c*)	67	19–600	$y=31{,}466x-562{,}969$	0.9890	6.78
20:4	79	19–600	$y=26{,}908x-390{,}584$	0.9983	16.96
20:5*n*−3	79	19–600	$y=27{,}378x-486{,}717$	0.9961	20.34
22:6*n*−3	79	19–600	$y=25{,}254x-753{,}793$	0.9907	16.69

Determination coefficients (r^2) of calibration curve and relative standard deviation (RSD). n is the number of replicates.

within a thermodesorption–gas chromatography/mass spectrometry (TDS–GC–MS) platform. Thermochemolysis for qualitative milk fat determination was primarily applied on milk fat extract [32] but not for analysis of milk. We propose thermochemolysis for direct analysis of milk using thermal desorption integrated with a cold trap inlet aiming to: reduce the size of the milk sample needed for analysis (~1 μL); facilitate faster chromatographic analysis and obtain better peak shapes [33] by pre-focussing analytes into a cryo trap prior injection into the column; avoid disposal issues by using only the sample required for injection into GC; protect analytical column integrity by trapping low-volatile compounds in a replaceable inlet liner and avoid the transference of the thermochemolysis reagent to the analytical column.

Thus the proposed method can allow the reduction of the cost per analysis (in terms of time of analysis, use of chemicals and long term integrity of the system), be easily automated (extraction of the fat is not required) and suitable for GC high throughput fatty acid profiling of milk.

2. Materials and methods

2.1. Materials

Tetramethyl ammonium acetate (TMAAc), Trimethylphenylammonium hydroxide solution 0.5 M in methanol (TMPAH), Tetramethylammonium hydroxide solution 25 M in methanol (TMAH), trimethylsulfonium hydroxide solution 0.25 M (TMSH) and adsorbent 10% SP™ 2330 on 100/120 Chromosorb® were obtained from Aldrich (Sigma–Aldrich, New South Wales, Australia). Most of the standards (Table 1) used in the method optimization were purchased from Sigma–Aldrich Chemie (Sigma–Aldrich, New South Wales, Australia), except the triarachidonin (5,8,11,14-all cis), 10*t*, 12*c*-octadecanoyl triglyceride (CLA 10*t*, 12*c*), 9*c*, 11*t*-octadecanoyl triglyceride (CLA 9*c*, 11*t*), trieicosapentaenoin (5, 8, 11, 14, 17 all cis) triglyceride and tridocosahexanoin (4, 7, 10, 13, 16, 19 all cis) triglyceride standards, which were purchased from Larodan Fine Chemicals (Malmö, Sweden). Standard FAME 37 mixture and *cis/trans* mixture were obtained from Restek (Shimadzu –Auckland, New Zealand). The stock solution used to evaluate the derivatisation efficiencies were prepared in dichloromethane (tricaprylin, tributyrin, tripalmitolein, triolein, triarachidonin, trieicosapentaenoin, tridocosahexanoin), chloroform (tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin), and hexane (trilinolein, CLA 10*t*, 12*c* and CLA 9*c*, 11*t* triglycerides). Stock solutions of the mixture of various components were prepared in dichloromethane. Milk samples were collected from the Ruakura No. 1 Dairy farm located at the Ruakura Research Centre, Hamilton, NZ in April 2009 and homogenized milk from local commercial supplier.

2.2. Instrumentation

The thermochemolysis reaction was performed with a Gerstel automated thermal desorption system (TDS) with programmable cooled injection system (CIS) coupled to a Shimadzu QP2010 GC–MS equipped with a fused-silica RT-2330 capillary column (10 m × 0.18 nm I.D., 0.1 μm film thickness; Restek) except, in the investigation of the reagents stability (see Section 2.6) and in the analysis of homogenized milk samples. In these cases, a fused-silica RT-2330 capillary column (20 m × 0.18 nm I.D., 0.1 μm film thickness; Restek) was used. The TDS platform was used as the injector in the splitless/split mode. The temperature of the TDS was 30 °C during the insertion of the TDS tube, and increased to 250 °C at rate of 720 °C/min and held at 250 °C for 0.40 min and then increased to 255 °C/min held for 0.40 min in the splitless mode. Analytes were cold trapped (−30 °C) in the CIS inlet packed with approximately 6 mg of 10% SP™ 2330 on 100/120 Chromosorb®. The CIS was ramped to 250 °C at 12 °C/s with hold of 2 min in a split mode (50:1). The analytes were transferred to the column of GC–MS system. The Mass spectrometry detection system was operating at 70 eV full scan m/z 40 to 400 a.m.u. Oven temperature programming was 50 °C isotherm for 1 min, increased to 175 at 50 °C/min, then increased to 195 °C at 8 °C/min and then to 250 °C at 150 °C/min and isotherm for 0.5 min (total run time 6.9 min). When using fused-silica RT-2330 capillary column (20 m × 0.18 nm I.D., 0.1 μm film thickness; Restek), oven temperature programming was 50 °C isotherm for 1 min, increased to 175 °C at 50 °C/min, then increased to 195 °C at 8 °C/min and then to 250 °C at 150 °C/min and held isothermally for 4 min (total run time 10.37 min). The carrier gas (He) flow was maintained at a constant velocity of 74 cm/s.

2.3. Method optimization

The derivatisation and TDS–GC–MS analysis were optimized using a representative set of test compounds including food standard mixture, methyl ester of CLA, triglycerides (long and short chain, saturated, monounsaturated and polyunsaturated fatty acids) and milk samples. Several parameters were optimized, i.e.: GC–MS parameters to achieve a rapid run with reduced effect on peak resolution; methylation reagent (TMAH, TMSH, TMAAc, TMPAH), reagent concentration (TMAH 10, 5, 2, 0.25, 0.1 M; TMSH 5, 0.25 and 0.05 M); reagent volume (1 μL and 2 μL); temperature