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Use of an extremely polar 100-m column in combination with a cyanoalkyl polysiloxane column to complement the study of milk fats with different fatty acid profiles

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ABSTRACT

A detailed dairy fat fatty acid (FA) profile can generally be achieved by gas chromatography using capillary columns with polar cyanoalkyl polysiloxane stationary phases (CPSP). However some compounds overlap and cannot be discriminated after a single run. In this work a new, extremely polar phase column (SLB-IL111) was used to complement the analysis carried out by a CPSP column. Separation of the FAs in a variety of milk fats using both a 100-m SLB-IL111 column and a 100-m CP-Sil 88 column was carried out and a more complete profile was established. The combination of results was useful to discriminate FA pairs, i.e., 10:1/11:0, *cis*-10 12:1/13:0, iso 17:0/*trans*-9 16:1, *anteiso* 17:0/*cis*-9 16:1, *trans*-16 18:1/*cis*-14 18:1, *trans*-11 *cis*-15 18:2/*trans*-10 *cis*-15 18:2 and *trans*-7 *cis*-9 18:2/*cis*-9 *trans*-11 18:2. Furthermore *trans*-8 and *trans*-13/14 18:1 isomers were also well discriminated. The use of both columns allowed detection in milk of some lactone and keto FAs.

1. Introduction

Dairy fat is regarded as one of the most complex naturally occurring fats and oils because of the large number of fatty acids (FAs) and the variety of molecular structures. Schröder and Vetter (2013) identified approximately 430 FAs in butter, although the vast majority are present in extremely small quantities (<0.01% of the total FAs). These can have 4 to 24 carbons and be saturated (branched or unbranched) or unsaturated (1–6 double bonds), and all geometric (*cis* or *trans*) and positional isomers are possible. Milk fat can also contain small amounts of FAs with cyclic, keto and other functional groups.

Because it is so complex, analysis of milk FAs is a challenging task and is one of the most important analytical fields for scientists working on the physiological aspects of milk fat production and modification. By far the most common procedure for analysing such a mixture of FAs is by gas chromatography (GC) of the fatty acid methyl esters (FAMEs). There is general agreement that 100-m polar cyanoalkyl polysiloxane stationary phase (CPSP) columns are necessary to help to analyse this fat (Contarini, Povolo, Pelizzola, Monti, & Lercker, 2013; De la Fuente & Juárez, 2009; Kramer, Blackadar, & Zhou, 2002). However, despite the success of these columns for the separation of milk FAs, there are limitations pertaining mainly to the monounsaturated and conjugated linoleic acid (CLA) regions. Some of these limitations were overcome by using two oven temperature programs to resolve many of the *trans*- and *cis*- 16:1, 18:1 and 20:1 FAs or by using a prior silver ion solid phase extraction (Ag^+-SPE) (Kramer, Hernandez, Cruz-Hernandez, Kraft, & Dugan, 2008) as well as argentation thin layer chromatography fractionation (Ag^+-TLC) (Precht & Molkentin, 2000, 2003). However, even so, there are some overlapping FAMEs that cannot be resolved under any conditions using CPSP columns of this kind.

The recent availability of capillary columns coated with ionic liquids of extreme polarity has provided new selectivity for FA analysis. Delmonte et al. (2011) used reference materials and characterised FAME mixtures to show that, relative to CPSP columns, an extremely polar 100-m SLB-IL111 can provide enhanced separation of geometric and positional isomers of the methyl esters of unsaturated FAs. A longer (200-m) capillary column with the same stationary phase was later successfully applied to resolve FAs contained in a variety of foods (Delmonte et al., 2012; Fardin-Kia et al., 2013; Tyburczy et al., 2012).

The aim of this study was provide a tutorial for the analysis of the most relevant FAMEs (about 100) from various milk fat samples

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with different fatty acid profiles using complementary GC separations involving 100-m CP-Sil 88 and SLB-IL111 columns. CP-Sil 88 should supply the most important part of the FAME whereas SLB-IL111 could discriminate some compounds not previously resolved on CP-Sil 88. Thus the role of SLB-IL111 would be complementary to CP-Sil 88 allowing for a more comprehensive FAs analysis of total milk fat. Four milk fats differing in their FA composition, mainly in the total content and isomer distribution of *trans* 18:1 and CLA profile, were selected to demonstrate the effectiveness of resolving FAME pairs in different chromatographic regions, particularly when adjacent isomers had asymmetric distributions. Ag^{+}-SPE, mass spectrometry (MS) detection and FAME commercial synthetic mixtures were used as tools to identify FAs.

2. Materials and methods

2.1. Sample selection and standards

This work was carried out with four milk fat samples reported in previous studies. All of them were stored at −17 °C. The samples were selected to cover the effects of different types of dietary lipid supplements on FA profiles. Briefly, the first milk fat dealt with the impact of a diet supplemented with 6% olive oil (OLI) on the composition of bulk ewe milk (Gómez-Cortés et al., 2008). The second evaluated the effect of diet supplementation with 6% of extruded linseed (LIN) on individual ewe milk fats (Gómez-Cortés, Tyburczy, Brenna, Juárez, & De la Fuente, 2009). The third sample (ALG) dealt with the effect of a combination of sunflower (2.5%) and microalgae (1.5%), a source of long-chain omega-3 FAs, on bulk ewe milk (Toral et al., 2010). Finally, a control butter sample (CON) from cows fed without lipid supplementation was also analysed. This fat was used for comparison with samples from supplemented diets.

Seven FAME mixtures (GLC-403, GLC-409, GLC-411, GLC-423, GLC-461, GLC-481B and GLC-643) were purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). A mixture of CLA FAME isomers (*trans*-8 *cis*-10, *cis*-9 *trans*-11, *cis*-11 *trans*-13 and *trans*-10 *cis*-12), also from Nu-Chek, was used in the CLA region analysis. To form other CLA isomers, this mixture was isomerised using I_2 according to Eulitz et al. (1999). Additionally, linoleic (Ref. 7792) and linolenic (Ref. 7793) acid methyl ester mixtures covering the entire geometric *cis*/*trans* spectrum were purchased from Sigma–Aldrich (Madrid, Spain).

2.2. Sample preparation

Milk fats were defrosted and derivatised to FAMEs. The derivatives were prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) in accordance with the ISO-IDF (2002a) procedure. To isolate different milk FA groups according to the number and geometry of the double bonds, Ag^{+}-SPE was applied as Kramer et al. (2008) described. Ag^{+}-SPE cartridges were purchased from Supelco (Bellefonte, PA, USA). All the milk fat FAMEs were applied to the column and eluted with hexane containing increasing amounts of acetone: 99:1 (v/v) eluted saturated FAMEs; 96:4 (v/v) eluted mono-*trans* FAMEs; 90:10 (v/v) eluted mono-*cis* and *trans*/*trans* dienes. Lastly, 0:100 (v/v) eluted *cis*/*cis* and *cis*/*trans* dienoic FAMEs. All fractions were taken to dryness in a nitrogen stream and then reconstituted in an appropriate volume of hexane for analysis by GC.

2.3. Gas chromatography analysis

Two gas chromatographs were used. An Agilent model 6890 N Network System (Palo Alto, CA, USA) equipped with auto injector, fitted with a flame ionisation detector (FID) on a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., Varian, Middelburg, The Netherlands). Injector and detector temperature was 250 °C. Helium was the carrier gas, at an inlet pressure of 193.9 kPa and a split ratio of 1:100. Sample volume injected was 1 μL. Total time of the chromatographic program was 90.73 min, including two ramps. Initial oven temperature was 45 °C. After 4 min, oven temperature was raised at 13 °C min^{-1} to 165 °C and held for 35 min, then increased to 215 °C at 4 °C min^{-1} and maintained for 30 min.

Another Agilent gas chromatograph, model 7820A GC System equipped with auto-injector and FID, was fitted with an SLB-IL111 capillary column (100 m × 0.25 mm i.d., Supelco). Injector and detector temperature was 250 °C. The column inlet pressure was set at 241 kPa, resulting in helium gas flow rates of 0.8 mL min^{-1}. Sample (1 μL) was injected at a split ratio of 1:100. Total time of the chromatographic program was 90 min, including two ramps. Initial oven temperature was isothermal (168 °C), and after 45 min it was raised at 5 °C min^{-1} to 210 °C and held for 36.6 min.

2.4. Identification of fatty acids

Identification of common FAs was firstly accomplished by comparing sample peak retention times with standard mixtures as enumerated in Section 2.1. When no commercial standards were available, FAMEs were analysed on an Agilent chromatograph (model 7890A) with an MS detector (5975C inert MSD). The filament trap current was 400 μA at 70 eV. Injections were under data system control with an auto-injector. Chromatographic conditions were similar to those described in the previous paragraph for CP-Sil 88 and SLB-IL111 columns but with a split ratio of 1:20. The injection volume was 1.0 μL. Wiley 275 and NIST 05 libraries were used to identify the mass spectra. Finally when previous methodologies were not enough, identification of unknown compounds was based on published chromatograms obtained under similar analytical conditions.

2.5. Combining separations from the two columns

A more comprehensive FA analysis could be obtained by combining the results of the CP-Sil 88 and 100-m SLB-IL111 columns. The CP-Sil 88 results were used as the main reference, whereas the SLB-IL111 results complemented the FA spectrum. The CP-Sil 88 results would allow calculation as a relative percentage of total FAMEs according to ISO-IDF (2002b), whereas only the integrated values from the SLB-IL111 chromatogram allow calculation of the corrected composition. If a specific FA was resolved in both columns, the CP-Sil 88 was chosen. Sample calculations are provided in Table 1.

3. Results and discussion

3.1. Region 4:0–16:0

The oven GC temperature program with the CP-Sil 88 column starting at 45 °C was selected to resolve the short-chain FAs, followed by a plateau temperature at 165 °C during elution of the 18:1 and 18:2 isomers. The separations shown in Fig. 1 (region 4:0–16:0) were obtained using those operating conditions. The shortest chain FAs (from 4:0 to 8:0) were well separated from the hexane peak. Between 10:0 and 12:0, a peak comprising 10:1 and 11:0 emerged. These FAs eluted separately when SLB-IL111 was applied (Fig. 2). The double bond structure of 10:1 mostly present in dairy fat was previously identified (Ackman & Macpherson, 1994) as n-1.