Journal of Chromatography A, 1218 (2011) 7499–7510

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Characterization of fatty acid and triacylglycerol composition in animal fats using silver-ion and non-aqueous reversed-phase high-performance liquid chromatography/mass spectrometry and gas chromatography/flame ionization detection

Miroslav Lísa[a], Kateřina Netušilová[a], Lukáš Franěk[a], Hana Dvořáková[a], Vladimír Vrkoslav[b], Michal Holčapek[a,*]

[a] University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 53210 Pardubice, Czech Republic
[b] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v. v. i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Available online 20 July 2011

Keywords:
Silver-ion HPLC
Non-aqueous reversed-phase HPLC
Triacylglycerol
Ruminant
Trans-fatty acid
Branched fatty acid

ABSTRACT

Fatty acid (FA) and triacylglycerol (TG) composition of natural oils and fats intake in the diet has a strong influence on the human health and chronic diseases. In this work, non-aqueous reversed-phase (NARP) and silver-ion high-performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry detection and gas chromatography with flame-ionization detection (GC/FID) and mass spectrometry detection are used for the characterization of FA and TG composition in complex samples of animal fats from fallow deer, red deer, sheep, moufflon, wild boar, cock, duck and rabbit. The FA composition of samples is determined based on the GC/FID analysis of FA methyl esters. In total, 81 FAs of different acyl chain length, double bond (DB) number, branched/linear, *cis*-/*trans*- and DB positional isomers are identified. TGs in animal fats contain mainly monounsaturated and saturated FAs. High amounts of branched and *trans*-FAs are observed in the samples of ruminants. In NARP mode, individual TG species are separated including the separation of *trans*- and branched TGs. Silver-ion mode provides the separation of TG regioisomers, which enables the determination of their ratios. Great differences in the preference of unsaturated and saturated FAs in the *sn*-2 position on the glycerol skeleton are observed among individual animal fats. Unsaturated FAs are preferentially occupied in the *sn*-2 position in all animal samples except for wild boar with the strong preference of saturated FAs in the *sn*-2 position.

1. Introduction

The composition of dietary triacylglycerols (TGs) and their fatty acids (FAs) has significant effects on the prevention or genesis of serious chronic diseases, such as cardiovascular diseases, diabetes, and cancer [1–3]. The correct ratio of saturated and polyunsaturated fatty acids in plant or animal tissues in our diet is especially important, as reflected by recommended daily income of these FAs by some national health organizations, *e.g.*, National Institute of Health in USA. The presence of unusual FAs with special biochemical properties should be also taken into account [4,5]. Wide differences in the FA composition can be found among various types of animal adipose tissues, *e.g.*, ruminants, poultry or fish. Animal fats are characterized by a high content of saturated FAs that have higher temperature and oxidation stability in comparison to unsaturated FAs, but on the other hand their higher content in the diet increases the risk of coronary heart diseases. On the other hand, fish oils are a predominant source of ω-3 polyunsaturated FAs, mainly eicosapentaenoic (Δ*5,8,11,14,17*-C20:5, EPA) and docosahexaenoic (Δ*4,7,10,13,16,19*-C22:6, DHA) acids, precursors of eicosanoids that reduce the inflammation in the body and they also play a crucial role in the prevention of atherosclerosis or heart attack [6]. In the natural samples, *cis*-configuration of double bonds (DBs) is predominant, but small amounts of FAs (<5%) with *trans*-configuration (*trans*-FAs) are present in ruminant meats and milk as products of rumen bacteria. Other products of rumen bacteria are odd- and branched-chain FAs having the important role to maintain an optimal fluidity of the microbial cell membrane or FAs with conjugated DBs as an intermediate in the biohydrogenation of unsaturated acids [4,7].

Nutrition properties of TG mixtures are given by their FA composition differing in acyl chain lengths and number, position and

* Corresponding author. Tel.: +420 466 037 087; fax: +420 466 037 068.
E-mail address: Michal.Holcapek@upce.cz (M. Holčapek).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.07.032

configuration (*cis*-/*trans*-) of DBs. The most widespread method for the analysis of FA profiles is gas chromatography with flame ionization detection (GC/FID) of fatty acid methyl esters (FAMEs) after the catalyzed transesterification of TGs. This method enables fast, simple and reliable characterization of very complex samples based on FA profiles. High-performance liquid chromatography (HPLC) with mass spectrometry (MS) detection is a powerful tool for the characterization of TGs in complex natural mixtures. Two chromatographic techniques are most widespread in the analysis of TGs in natural samples, *i.e.*, non-aqueous reversed-phase (NARP) HPLC and silver-ion chromatography. In NARP-HPLC [8–22], retention times of TGs increase with the increasing equivalent carbon number (ECN) defined as the carbon number (CN) in all acyl chains minus twice the number of DBs (ECN = CN − 2DB). The separations of TGs differing in the position [15,17,22] and configuration [20–23] of DBs or the partial resolution of regioisomers [24] and TGs containing linear and branched FAs [25] have been reported as well. The retention times of TGs can be significantly reduced without the loss of resolution by the use of ultra-high performance liquid chromatography with sub-two micron particles column [26,27].

Silver-ion chromatography [28–34] is based on the formation of weak reversible complexes of silver ions impregnated on the silica or mostly bounded to the ion-exchange stationary phase with π electrons of DBs of unsaturated TGs. The mixture of hexane/acetonitrile is a typical mobile phase used in silver-ion chromatography but with a poor reproducibility due to the low miscibility of these solvents. The addition of 2-propanol into the mobile phase improves the miscibility of these solvents which provides a better reproducibility of retention times among analyses within one or more days [33]. Chlorinated mobile phases are also frequently used in silver-ion HPLC using dichloromethane or dichloroethane with the addition of other polar modifiers at low concentration, typically acetonitrile, acetone, and methanol [31,32,35]. In silver-ion chromatography, separation of TGs is governed mainly by the number of DBs. Double bond positional isomers [32,33], *cis*-/*trans*-isomers [23,28,30] or regioisomers ($R_1R_1R_2$ vs. $R_1R_2R_1$) [23,28,33] can be also separated. The orthogonality of silver-ion and NARP modes can be demonstrated in two dimensional separation for very complex samples in on-line [36–38] or off-line [23,39,40] setup.

The main goal of this work is the characterization of FA and TG composition of selected animal samples important in the nutrition and foodomics. NARP-HPLC and silver-ion HPLC with APCI-MS detection are used for the analysis of TGs according to acyl chain lengths and the number of DBs and FA composition of samples is determined using GC/FID analysis of FAMEs after the transesterification of TGs. The composition of TGs and unusual FAs in analyzed animal fats is discussed.

2. Materials and methods

2.1. Materials

Acetonitrile (HPLC gradient grade), 2-propanol, hexane (both HPLC grade) and sodium methoxide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Model mixtures of FAME standards GLC#617 (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, Δ*9*-C14:1, C15:0, Δ*10*-C15:1, C16:0, Δ*9*-C16:1, C17:0, Δ*10*-C17:1, C18:0, Δ*9*-C18:1, Δ*9t*-C18:1, Δ*9,12*-C18:2, Δ*9t,12t*-C18:2, Δ*6,9,12*-C18:3, Δ*9,12,15*-C18:3, C20:0, Δ*11*-C20:1, Δ*11,14*-C20:2, Δ*8,11,14*-C20:3, Δ*11,14,17*-C20:3, Δ*5,8,11,14*-C20:4, Δ*5,8,11,14,17*-C20:5, C21:0, C22:0, Δ*13*-C22:1, Δ*13,16*-C22:2, Δ*7,10,13,16*-C22:4, Δ*4,7,10,13,16*-C22:5, Δ*7,10,13,16,19*-C22:5, Δ*4,7,10,13,16,19*-C22:6, C23:0, C24:0, Δ*15*-C24:1) and GLC#566 (C8:0, C10:0, C11:0, Δ*10*-C11:1, C12:0, C13:0, Δ*12*-C13:1, C14:0, Δ*9*-C14:1, C15:0, C16:0, Δ*9*-C16:1, C17:0, Δ*10*-C17:1, C18:0, Δ*9*-C18:1, Δ*11t*-C18:1, Δ*9,12*-C18:2, Δ*6,9,12*-C18:3, Δ*9,12,15*-C18:3, C19:0, C20:0, Δ*11*-C20:1, Δ*11,14*-C20:2, Δ*8,11,14*-C20:3, Δ*11,14,17*-C20:3, Δ*5,8,11,14*-C20:4, Δ*5,8,11,14,17*-C20:5, C21:0, C22:0, Δ*13*-C22:1, Δ*13,16*-C22:2, Δ*7,10,13,16*-C22:4, Δ*4,7,10,13,16*-C22:5, Δ*7,10,13,16,19*-C22:5, Δ*4,7,10,13,16,19*-C22:6, C23:0, C24:0, Δ*15*-C24:1) were purchased from Nu-Chek-Prep (Elysian, MN, USA). Standards of methyl 15-methylheptadecanoate (*ai*C18:0) and methyl 16-methylheptadecanoate (*i*C18:0) were purchased from Larodan Fine Chemicals (Mälmo, Sweden). Samples of adipose tissues from fallow deer (*Dama dama*), red deer (*Cervus elaphus*), sheep (*Ovis aries*), moufflon (*Ovis musimon*), wild boar (*Sus scrofa*), cock (*Gallus gallus*), duck (*Anas platyrhynchos domesticus*) and rabbit (*Oryctolagus cuniculus*) were obtained from the local veterinarian. The amount of 20 g of the sample was crushed in a homogenizer with 20 mL of hexane for 10 min. The mixture was filtered and hexane was evaporated under a mild stream of nitrogen yielding the pure animal fat.

2.2. NARP-HPLC/APCI-MS

NARP-HPLC experiments were performed on a chromatographic apparatus consisting of a Model 616 pump with a quaternary gradient system, a Model 996 diode-array UV detector, a Model 717+ autosampler, a thermostated column compartment and a Millennium chromatography manager (all from Waters, Milford, MA, USA). Samples were analyzed using the following HPLC conditions: two chromatographic columns Nova-Pak C_{18} (300 mm × 3.9 mm and 150 mm × 3.9 mm, 4 μm, Waters) connected in series, a flow rate of 1 mL/min, an injection volume of 10 μL, column temperature of 25 °C and the mobile phase gradient according to Ref. [23]: 0 min – 80% A + 20% B, 121 min – 40% A + 60% B, 122 min – 80% A + 20% B, where A is acetonitrile and B is a mixture of hexane–2-propanol (1:1, v/v). The injector needle was washed with the mobile phase before each injection. The column hold-up volume, t_M, was 3.2 min for the system with 300 + 150 mm Nova-Pak C_{18} columns.

The Esquire 3000 ion trap analyzer (Bruker Daltonics, Bremen, Germany) with positive-ion APCI was used in the mass range *m/z* 50–1200 with the following setting of tuning parameters: pressure of the nebulizing gas 70 psi, drying gas flow rate 3 L/min, temperatures of the drying gas and APCI heater were 350 °C and 400 °C, respectively. Individual reconstructed ion current chromatograms were used to support the identification of coeluting peaks.

2.3. Silver-ion HPLC/APCI-MS

Silver-ion HPLC experiments were performed on a liquid chromatograph Agilent 1200 Series (Agilent Technology, Waldbronn, Germany). Samples were analyzed using the following HPLC conditions according to Ref. [33]: three silver-ion chromatographic columns ChromSpher Lipids (250 mm × 4.6 mm, 5 μm, Varian, Palo Alto, CA, USA) connected in series, the flow rate of 1 mL/min, the injection volume of 1 μL, column temperature of 25 °C, and the mobile phase gradient: 0 min – 100% A, 140 min – 61% A + 39% B, where A is the mixture of hexane–2-propanol–acetonitrile (99.8:0.1:0.1, v/v/v) and B is the mixture of hexane–2-propanol–acetonitrile (96:2:2, v/v/v). The mobile phase was prepared freshly every day. Silver-ion columns were conditioned at 50 μL/min using the initial mobile phase composition overnight and at 1 mL/min for 1 h before the first analysis. The injector needle was washed with the mobile phase after each injection. The chromatographic system was equilibrated between injections for 45 min.

The hybrid quadrupole time-of-flight (QqTOF) analyzer micrOTOF-Q (Bruker Daltonics, Bremen, Germany) with positive-