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Analytical Methods

Optimization of the GC method for routine analysis of the fatty acid profile in several food samples

Marinko Petrović *, Nataša Kezić, Vesna Bolanča

Faculty of Food Technology and Biotechnology, Food Control Center, Jagićeva 31, Zagreb, Croatia

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ABSTRACT

The fatty acids profile of food samples was determined by gas chromatography (GC). The fat was extracted from different food samples using Soxhlet technique. Extracted triglycerides were converted to corresponding methyl esters using methanolic solution of potassium hydroxide (*trans*-esterification). GC method for the analysis of obtained methyl esters was optimized on two different cyanopropyl capillary columns. Good resolution of all fatty acids commonly found in foodstuffs was achieved. After optimization, the method was validated and the results for linearity, precision, limit of quantitation (LOQ), limit of detection (LOD), robustness and stability were presented. The method has been applied to the quantitative determination of the fatty acid content in different food samples: edible oil, dairy products rich with omega-3 fatty acids, different food supplements, baby food, etc.

1. Introduction

The fatty acids (FAs) composition of food is very important because lipids are one of the three major constituents of food. Their roles in biological tissues are: (1) source of energy, (2) components of biological membranes, (3) precursor for many different molecules and (4) transport vehicle for vitamin A, D, E and K. The composition of fatty acids plays an important role in the transport of substances in and out of the cell because of their impact on the fluidity of the cell membrane (Chow, 2000; O'Keefe, 2000). Also each essential fatty acid has been subjected to its own metabolic path. The elongation and desaturation of linoleic acid leads to arachidonic acid, which is the precursor of prostaglandines while linolenic acid is metabolized eventually to provide eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Major causes of death in Western societies such as degenerative cardiovascular diseases and cancer are linked to aspects of dietary fat intake (Kritchevsky, 2002). A lot of attention is given to dietary fats as an important factor human health. Due to that, the content of different kinds of FAs having bad effects on health is significant even to the everyday consumer. The content of FAs with potential benefits on health also attracts the attention of dietary supplement consumers. Nutritional labeling laws in many countries require all processed foods to be analyzed for various kinds of saturated, mono and polyunsaturated fatty acids (PUFA) and reporting the results to the consumers. Consumption of *trans* FAs is linked with a higher level of cholesterol and low-density-lipoprotein (LDL) cholesterol and lower level of high-density cholesterol in plasma (Kritchevsky, 2002; Vijver et al., 2000).

The main sources of lipids in our diet are vegetable oils and different foodstuffs of animal source e.g. meat, eggs, milk and milk products. The authenticity of vegetable oil has been analyzed for over 50 years. Lessening the quality of oil with proven health benefit by adding certain oils of poor quality can make significant financial gain (Aparicio & Aparicio-Ruiz, 2000; Hajimahmoodi et al., 2005). The ratio between omega-6 and omega-3 FAs in the western diet is in the range of 10–30:1, which is recognized by many nutritionists as insufficient and responsible for health disorders (Chapkin, 2000; McKenzie, 2001). It is possible to distinguish the FA profile in foodstuffs of animal source by changing the diet (Bell et al., 1997; McKenzie, 2001). Enriching animal diet with omega-3 FAs resulted in a higher amount of omega-3 FAs in lipids in comparison with the control group of animals, which were fed the classical diet. Also, many omega-3 products of animal origin with different content and types of omega-3 FA have been marketed the few last years. That emphasizes the importance of determining FA profile of food for correct nutrition labeling and also for control of labeling authenticity.

Prior to the analysis, lipids were extracted by the standard procedure (ISO 1443, 1973). The lipid mixture obtained by solvent extraction consists of a variety of lipid classes of different polarity: triacylglycerols, phospholipids, free fatty acids, free sterols, steryl esters, etc. All of them are extracted when using chloroform/methanol mixture. In the case of petrol ether only non-polar lipids are extracted. The obtained lipids are then converted into fatty acid methyl esters (FAMEs) for GC analysis. Many different methylation methods are described in literature and four of them are

* Corresponding author. Tel.: +385 1 3740 090; fax: +385 1 3744 044.
E-mail address: mpetrovic@ckn.pbf.hr (M. Petrović).

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commonly used: acid or base-catalyzed methylation, borontrifluoride methylation, methylation with diazomethane and silylation (ISO 5509, 2000; Mendez Antolin, Marrero Delange, & Gonzalez Canavaciolo, 2008; Seppanen-Laakso, Laakso, & Hiltunen, 2002). The first method is more acceptable then other methods because it uses less aggressive reagents then other methods.

GC is the most widely used technique for determining the FA profile (AOAC, 2000; ISO 5508, 1999; Lehotay & Hajšlova, 2002; Mendez Antolin et al., 2008; Seppanen-Laakso et al., 2002). A wide range of data is available on the fatty acid composition of vegetable oils (Hajimahmoodi et al., 2005; Ramadan, Sharanabasappa, Seetharam, Seshagiri, & Moersel, 2006; Stransky, Zarevucka, & Wimmer, 2005) and animal origin food products (Dayhuff & Wells, 2005; Huang, Wang, & Crenshow, 2006; Jalali-Heravi & Vosough, 2004; Maduko & Park, 2007). The flame ionization detector is sufficient for a majority of analytical applications of this kind. GC/MS has been used for the analysis of trace amounts of FAs especially for identifying FAs when no standards are available. Electron impact ionization may give us useful information on a number of double bonds in analyzed FAs but for isomer determination various deconvolution software has been used (Ramadan et al., 2006). GC × GC technique is the most useful technique for identification of odd carbon number FAs (de Geus, Aidos, de Boer, Luten, & Brinkman, 2001; Mondello, Tranchida, Dugo, & Dugo, 2006).

The aim of this work was to optimize a simple and reliable method for the determination of FAMEs and to prove its applicability for determining the fatty acid profile in different food samples. Different stationary phases were used for this purpose and we chose two cyanopropyl stationary phases: DB-23 column with 50%-cyanopropyl-methylpolysiloxane and DB-225ms column with 50%-cyanopropylphenyl-dimethylpolysiloxane stationary phase. Their main advantage is the ability to separate *cis* and *trans* isomers. The resolution of elaidic and oleic acid was used as criterion during method optimization. The method validation included linearity, precision, limit of quantification (LOQ), limit of detection (LOD), robustness and stability of 37 FAMEs included in analytical standard for food industry.

2. Materials and methods

2.1. Chemicals and reagents

Isooctane and methanol gradient grade was purchased from J.T. Backer, (Devanter, Holland). Petrol ether, p.a., hydrochloric acid, p.a., fluted filter paper, quantitative, potassium hydroxide, p.a., and sodium hydrogen sulfate monohydrate, p.a., were purchased from Kemika (Zagreb, Croatia). Analytical standard of fatty acid methyl esters were: Food Industry FAME, 37 components in methylene chloride, *cis*/*trans* FAME Mix, 8 components in methylene chloride and AOCS#1, 6 components, all purchased from Restek (Bellefonte, PA, USA) and Linolenic acid, 94% mixture of isomers, obtained from Roth (Karlsruhe, Germany).

2.2. Samples description

Central Science Laboratory, Sand Hutton, York, UK provided the test material and calculate the results for FAPAS proficiency test 1455 and 1467. Test material for test 1455 was vegetable (rapeseeds) oil and for test 1467 was breakfast cereals. Pumpkin seeds oil, olive oil, sunflower oil samples were supplied by local producer in Croatia. Butter cake sample was obtained for nutritional labeling from Koestlin, Bjelovar, Croatia and canned pilchard sample was also obtained for nutritional labeling from Mirna, Rovinj, Croatia. Flaxseed, evening primrose and borage oil capsules together with CLA food supplements were provided by dm-drogerie market, Karlsruhe, Germany and Biofarm, Zagreb Croatia. Sample of infant formula was provided by Vivera, Glina, Croatia and tuna fish samples were obtained from Tuna-Kali, Kali, Croatia.

2.3. Extraction of lipids

Lipids were extracted by standard procedure (ISO 1443, 1973). Approximately 5 g of homogenized sample was weighted into a conical flask and dried for 1 h at 105 °C. The flask was cooled to room temperature, and 50 mL of 4 M hydrochloric acid was added. The solution was boiled for 1 h. Then 150 mL of water was added, the solution was filtered through fluted filter paper and washed until neutral reaction on litmus paper. Filter paper was dried for 1 h at 105 °C and inserted in extraction thimble of Soxhlet apparatus. Lipids were extracted with petrol ether into weighted round bottom flask for 4 h on the sand bath. After extraction, petrol ether was evaporated, the flask was dried at 105 °C and weighted.

2.4. Preparation of fatty acid methyl esters (FAMEs)

Lipids obtained after extraction of vegetable oil and fat samples were converted to corresponding FAMEs by *trans*-esterification with potassium hydroxide (ISO 5509, 2000). Approximately 60 mg of sample was dissolved in 4 mL of isooctane in test tube and 200 μL of methanolic potassium hydroxide solution (2 mol/L) was added. Solution was shaken vigorously for about 30 s. The solution was neutralized by addition of 1 g of sodium hydrogen sulfate monohydrate. After the salt has settled, 1 mL of upper phase was transferred into 2 mL vial and analyzed.

2.5. GC analysis

GC analyses were performed on CP-3800 (Varian, Palo Alto, USA) equipped with flame ionization detector and split/splitless injector. Injector temperature was at 250 °C and samples were injected manually (1 μL) with split ratio of 1:30. In the case of small amounts of lipids split injections were performed with a split ratio of 1:5. Two different cyanopropyl silicone capillary columns were used: DB-225ms 30 m × 0.25 mm, with film thickness of 0.25 μm and DB-23 60 m × 0.25 mm, with film thickness of 0.25 μm. The temperature program 60 °C rising to 220 °C at rate of 7 °C/min was the same for both columns. Helium was used as carrier gas at a flow rate of 1 mL/min in case of DB-225ms column and at 1.5 mL/min in case of DB-23 column. Detector temperature was at 280 °C. Star GC Workstation Version 6.4 chromatographic software was used for data collection, and calculation of all parameters.

2.6. Validation of test procedure

GC method for determining fatty acid methyl esters was subjected to validation following recommendations of the International Conference on Harmonization (ICH, 1996). Quantification of individual fatty acids was based on the obtained peak area, results were normalized and no correction factor was used (ISO 5508, 1999). Criteria used for evaluation of obtained results were established according to literature (AOAC, 2000; European Pharmacopoeia, 2005) in two different concentration ranges: for major constituents present in excess if 5% and for minor constituent present in smaller quantities. Relative standard deviation (RSD%) of 3% with a maximum mass deviation of 1% (m/m) for major constituents and 0.2% (m/m) for minor constituents were used as criteria for evaluation of linearity, precision (repeatability and reproducibility), stability of prepared test sample. Accuracy of the method was tested by participation in proficiency tests on vegetable oils