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Analytical Methods

Direct detection of free fatty acids in edible oils using supercritical fluid chromatography coupled with mass spectrometry

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ABSTRACT

Determination of free fatty acids (FFAs) in food products is of enormous interest mainly because they are related to the quality and authenticity of the oils. In this study, supercritical fluid chromatography (SFC), followed by an electrospray ionisation triple–quadrupole mass spectrometry (ESI-MS), is shown to provide a novel method for the separation and detection of FFAs in edible oils without any pretreatment. Eight FFAs were separated on a HSS C18 SB column with gradient elution within 3 min. Effects of different columns, modifiers and column temperature changes were evaluated. The results indicated the feasibility of this method for the high-throughput determination of individual FFAs with satisfactory correlation coefficients ($R^2 > 0.994$) and good reproducibility of RSD < 13.5% (intraday) and <15.0% (interday). By combined with principal component analysis (PCA), different types of edible oil were successfully distinguished into several categories, showing a potential application for the determination of oil quality or authenticity.

1. Introduction

Free fatty acids (FFAs), as a class of lipids, appearing both in living organisms and in food, oil and other industrial products, play a significant rule with many biological functions (Clapperton, 1978; Prentki & Murthy Madiraju, 2012; Soto Vaca, Losso, McDonough, & Finley, 2013). FFAs are triacylglycerols (TAGs, the main constituents of oils) hydrolysis products during manufacturing process and storage. FFAs are less stable than TAGs and can cause rancidity, which results in lower oil quality and functionality (Wei et al., 2012). Hence, TAGs degradation in edible oils is undesirable, and FFAs content is one of the most frequently determined quality indices during production, trading, and storage of edible oils (Saad et al., 2007). The analysis of FFAs can be used to determine the authenticity of oils, optimise the edible oil refining and control the oil degradation produced during storage under different conditions (Díaz & Borges, 2012). In light of the significant role in quality control or biological system played by FFAs, it is essential to develop a rapid and reliable analytical method to identify and quantify FFAs in edible oils or other matrices.

The separation and determination of FFAs is a challenging and complicated task because of their relatively low concentration in the highly complex matrix. FFAs are difficult to be directly separated by gas chromatography (GC) due to their high polarity and low volatility. With derivatization or esterification of fatty acids, GC has been a good choice for analysing FFAs (as methyl esters) with higher resolution ability in the last few years (Güler & Gürsoy Balcı, 2011; Pereira, Valentão, Teixeira, & Andrade, 2013; Sindhu Kanya, Jaganmohan Rao, & Shamanthaka Sastry, 2007; Van Vleet & Quinn, 1978; Wei et al., 2012). However, sample preparation for analysis of trace FFAs in edible oils is complicated, including the efficient separation for FFAs from complex matrix and the time-consuming derivatization procedure. These procedures have to face the problems associated to an incomplete derivatization and the residual TAGs hydrolysis when used in real samples (Kohn, Van der Ploeg, Möbius, & Sawatzki, 1996; Wei et al., 2012). Separation of fatty acid derivatives can also be achieved by liquid chromatography (LC) techniques with different separation modes and detection methods (Chu & Nagy, 2013; Li et al., 2011; Lin, McKeon, & Stafford, 1995). Although attempts have been made for the direct separation of FFAs using HPLC, pre- or post-column derivatizations of FFAs such as esterification or introduction of other suitable chromophores are carried out in order to gain efficient separation or enhance the detection sensitivity of ultraviolet–visible (UV–Vis) adsorption, evaporative light scattering detection (ELSD), fluorescence detection (FLD), etc. (Chen & Chuang, 2002). The low sensitivity of photometric detection, widely used for the detection of fatty acids, limits the

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applicable sample volume. Mass spectrometry can be used to detect fatty acids, in which way highly sensitive and selective detection of targets can be performed by selected ion recording (SIR).

In contrast with GC and HPLC, supercritical fluid chromatography (SFC) is a promising technique for the analysis of FFAs without previous sample extraction and derivatization. SFC is characterised by high resolution and flow rate owing to the properties of supercritical fluids (Li et al., 2013). Considerable attempts have been made to describe the retention mechanisms of SFC due to the rapid developments in instrumentation and applications of this alternative analytical technique (Abrahamsson & Sandahl, 2013; Khater, West, & Lesellier, 2013; Lesellier, 2009). The application of SFC to analyse hydrophobic compounds such as lipids and their fatty acid esters has been reported previously (Bernal, Martín, & Toribio, 2013; Lee et al., 2012). Important advances have been achieved in recent years, especially dealing with real food samples, even with direct injection of oils onto the SFC column, demonstrating that the SFC method is of great use to check the quality of edible oils and detect oil adulteration or spoilage (Señoráns & Ibañez, 2002; Staby, Borch Jensen, Balchen, & Mollerup, 1994a,b).

In this study, a rapid and reliable separation and determination method based on SFC coupled with triple quadrupole mass spectrometry for the direct detection and accurate quantification of FFAs in edible oils without any pretreatment was developed. Compared to the standard method of the American Oil Chemists' Society (AOCS) which involves the titration procedure, our study can accurately determine not only total FFAs but also individual FFAs in edible oils and give us a FFAs profiling of various oils. All FFAs could be eluted rapidly within 3 min, and positional isomers (α/γ-linolenic acid) were successfully resolved.

2. Materials and methods

2.1. Standards and reagents

Myristic acid (C14:0, ⩾99%), palmitic acid (C16:0, ⩾99%), α-linolenic acid (α-C18:3, ⩾99%), γ-linolenic acid (γ-C18:3, ⩾99%), linoleic acid (C18:2, ⩾99%), oleic acid (C18:1, ⩾99%), stearic acid (C18:0, ⩾99%) and arachidic acid (C20:0, ⩾99%) standards were purchased from Nu-Chek Prep, Inc. (Minnesota, USA). CO_2 (purity ⩾99.999%) was obtained from Shiyuanjingye Co., Ltd. (Beijing, China). HPLC-grade methanol, *n*-hexane, acetonitrile, formic acid were purchased from Fisher Scientific (Fair Lawn, USA). Stock solutions were prepared in *n*-hexane at a concentration of 5.0 mg/mL. All stock solutions were kept at 4 °C. Mixed standard solution was prepared by mixing the individual fatty acid stock solutions and then diluted to the desired concentration for the following experiments.

2.2. Oil material

A total of 35 vegetable oil samples, including 11 soybean oils, 7 olive oils, 5 peanut oils, 5 corn oils, 4 sesame oils, and 3 sunflower oils of different brands, were purchased from local supermarkets. Animal fats were obtained from local markets and rendered. 10 mg aliquot of each sample was dissolved in *n*-hexane to make a final total volume of 1 mL. Then the vial was vigorously shaken for 1 min and filtered with 0.22 μm organic microporous membrane. Finally, 1 μL aliquot of the filtrate was directly injected into the SFC instrument for analysis.

2.3. Instrumentation

An Acquity ultra performance convergence chromatography system (Waters, USA) coupled to a Micromass Quattro Premier triple quadrupole mass spectrometer (Waters, USA) was used. SFC separation of fatty acids was carried out on a Waters Acquity UPC^2 HSS C18 SB column (1.8 μm, 3.0 mm × 100 mm i.d.) with a gradient elution. Back pressure was set at 1500 psi. Eluent A was supercritical CO_2, eluent B was the modifier, a mixed solvent of methanol/acetonitrile (50:50, v/v) with 0.1% formic acid. The flow rate was constant at 1.6 mL/min and the column temperature was set at 40 °C. The gradient was as follows: isocratic to 1 min with 97.0% (A), 97.0–96.5% (A) from 1 to 2 min, then held isocratic for 1 min, 96.5–93.0% (A) from 3 to 6 min. The column was returned to its original condition with a liner elution in 0.2 min and equilibrated for 0.8 min before starting the next run.

The mass spectrometer was equipped with an electrospray ionisation (ESI) source, and selected ion recording (SIR) in negative ion mode was employed to perform the analysis (detailed information described in Supplementary material 1). The MS parameters were set as follows, cone voltage 25 V, source temperature 120 °C, capillary voltage 3.5 kV, desolvation gas (N_2) rate 600 L/h, desolvation gas temperature 350 °C, cone gas (N_2) rate 50 L/h.

Masslynx version 4.1 software (Waters, USA) was used for instrument control, data acquisition and processing. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 16.0.

3. Results and discussion

3.1. Optimisation of chromatographic conditions

3.1.1. Screening the columns

Selecting suitable chromatographic column is of great importance for the acceptable separation of the analysed components. In this study, two columns were selected to perform the experiments including Waters Acquity UPC^2 BEH 2-EP (1.7 μm, 3.0 mm × 100 mm i.d.) and HSS C18 SB (1.8 μm, 3.0 mm × 100 mm i.d.). Fig. 1A shows that all FFAs cannot be baseline separated on BEH 2-EP column, especially the positional isomers (α-C18:3 and γ-C18:3, which stand for (6Z,9Z,12Z)-6,9,12-octadecatrienoic acid and (9Z,12Z,15Z)-9,12,15-octadecatrienoic acid, respectively). Even if 0.1% formic acid was added to the mobile phase, it still exhibited slightly tailing peaks, probably due to the strong hydrogen bonding between the polar FFAs (proton donors) and the polar sites (pyridine N-atoms) at the surface of this stationary phase. Otherwise on the HSS C18 SB column a promising separation with almost baseline separated α/γ-C18:3, saturated and unsaturated FFAs were obtained. Fig. 1B shows the greater separation of eight FFAs observed under optimised chromatographic conditions on HSS C18 SB. It is clear that all FFAs exhibit symmetrical peaks with relatively shorter retention times. As shown, saturated FFAs were eluted with a good baseline resolution according to the alkyl chain length, and FFAs with shorter chain length were eluted earlier. This could be explained by the mechanism that long alkyl chain of the FFAs has stronger interaction with the C18-bonded phase. Elution order of saturated FFAs was the same with that on BEH 2-EP. However, it is interesting to note that retention times of unsaturated FFAs got longer with the decrease of the number of double bonds on HSS C18 SB, while the opposite elution order of unsaturated FFAs was observed on BEH 2-EP. In addition, positional isomers (α-C18:3 and γ-C18:3) were baseline separated on HSS C18 SB.

3.1.2. Effect of modifiers

Although FFAs are weakly polar compounds, the acid groups exhibit strong interactions with the silanol groups at the surface of the packing materials and produce peak tailing. In order to improve the shape of peaks, organic modifiers such as methanol and formic acid are classically added to CO_2 mobile phase to change the polarity of the mobile phase and prevent the interactions. Here,