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# Quantitative determination of epoxy stearic acids derived from oxidized frying oil based on solid-phase extraction and gas chromatography

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ABSTRACT

An improved preparation method based on solid phase extraction (SPE) for determination of epoxy fatty acids derived from oleic acid in frying oils was developed. A three-step separation of fatty acid methyl esters (FAMEs) by SPE on a silica gel column was proposed, and quantification by gas chromatograph with a fiame ionization detector (GC-FID) was performed. The limits of detection (LODs) of *trans*- and *cis*-epoxy stearic acid (ESA) were 6.32 and 13.09 μg/mL, respectively. And the limits of quantification (LOQs) ranged from 20.87 to 43.19 μg/mL with recoveries ranging from 89.32% to 107.08%. Good reproducibility was observed, with an intraday relative standard deviations of 0.93–2.35%, and an interday relative standard deviations of 2.44–11.68%. Results showed total levels of ESAs in different oil samples were in the range of 235.65–5916.05 mg/kg, and the contents of ESAs increased with the increase of frying time. Thus, combining the improved sample preparation method for ESAs analysis with the separation on a fused-silica capillary column using GC is highly useful for determination of ESAs in frying oils.

## 1. Introduction

Frying is a popular and convenient process for the preparation of foods. Fried foods are welcome owing to their pleasant flavor, color, taste and crispy texture (Bou, Navas, Tres, & Guardiola, 2012; Lim, Jeong, Oh, & Lee, 2017). However, lipid oxidation takes place during frying process and causes adverse changes in the sensory, physical, chemical and nutritional aspects of food and oil (Juárez, Osawa, Acuña, Sammán, & Gonçalves, 2011). Besides, oxidized lipids have been claimed to exert negative biological effects, such as causing CNS traumas (Adibhatla & Hatcher, 2010), stimulating the endoplasmic reticulum stress response (Feldstein et al., 2010), inducing nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (Haberzettl & Hill, 2013).

Lipid oxidation process involves a series of reactions including degradation, hydrolysis, polymerization and so on. Meanwhile dimers and polymers of TAGs, as well as oxidized TAG monomers, diacylglycerols and free fatty acids are formed. Among them, oxidized TAG monomers are relatively stable compounds and have the highest content of oxidation compounds, and have shown a high absorption in both animal and humans. Oxidized TAG monomers contain fatty acids with keto, epoxy, hydroxyl and aldehyde groups (Kmiecik, Kobus-Cisowska, & Korczak, 2017; Márquez-Ruiz & Dobarganes, 2006; Zhang, Saleh, Chen, & Shen, 2012). In general, these oxygenated fatty acids have shown toxicity and some other detrimental effects (Wilson, Lyall, Smyth, Fernie, & Riemersma, 2002; Wilson, Fernie, Scrimgeour, Lyall, Smyth & Riemersma, 2002). Epoxy-TAGs are formed by reaction of hydroperoxy radicals with alkenyl groups of fatty acids, which leads to the formation of an epoxy compound and an alkoxyl radical. Furthermore, the position of epoxy group may exist in the original C = C position of TAG with high unsaturated degree. The contents of such compounds in frying oil at different fried intervals are difficult to be quantified exactly owing to the complexity of frying system.

Previous studies focused on high-performance size-exclusion chromatography (HPSEC) for separation and detection of lipid oxidation products according to the molecular size, normally related to the molecular weight (Márquez-Ruiz & Dobarganes, 2005; Sánchez-Muniz, Cuesta, & Garrido-Polonio, 1993; Arroyo, Cuesta, Sánchez-Montero, & Sánchez-Muniz, 1995; Romero et al., 1995), but there is little information on isolation and identification of oxidized TAG monomers, especially TAG molecules linking with extra oxygen-containing groups such as ubiquitous epoxy-TAGs. Considering the existence of epoxy-TAGs, which may mostly be shown in one or more fatty acyl chains of TAG molecules in oil, the separation and quantitation of oxidized fatty acids with oxygen-containing functional groups needs to transform TAGs into simple and easily detectable fatty acid methyl esters (FAMEs)

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(Marmesat, Velasco, & Dobarganes, 2008). Generally, the analysis of FAMEs is performed by gas chromatography with flame ionization detection (GC-FID). So fatty acids should be methylated first by base-catalyzed transmethylation, which made it possible to evaluate precisely fatty acids in thermoxidized lipids and oils (Berdeaux, Velasco, Márquez-Ruiz, & Dobarganes, 2002; Velasco, Marmesat, Berdeaux, Márquez-Ruiz, & Dobarganes, 2005). Similarly, the determination of epoxy fatty acids in frying oils could be carried out by GC-FID after base-catalyzed transmethylation.

Solid phase extraction (SPE) is a pre-concentration technique and has been widely used due to its small amount of solvent, simple operation, high selectivity and good reproducibility (Pullen & Hock, 2006). Epoxy fatty acids have higher polarity than ordinary fatty acids, and their content is much lower than ordinary fatty acids, so SPE cartridge is used to isolate polar FAMEs from non-polar FAMEs in order to improve the sensitivity and accuracy of detection in epoxy fatty acids (Marmesat et al., 2008). A few studies focused on determination of epoxy fatty acids in food matrices (Mubiru, Shrestha, Papastergiadis, & De Meulenaer, 2014) and plants (Borch-Jensen & Mollerup, 1996; Hu, Mendoza, Buchs, & Gülaçar, 1988), but changes of epoxy fatty acids in frying oil with different frying moments have not been extensively studied.

The aim of this work was to establish the extraction method for a sensitive and accurate quantitation of epoxy stearic acids (ESAs) formed in frying oil through the detection of GC-FID after base-catalyzed transmethylation. To achieve this aim, the factors affecting SPE conditions have been extensively studied. Identification of ESAs in frying oils was carried out by comparison with the standards of epoxy stearic acid methyl esters. The extraction method after optimization was applied to analyze epoxy fatty acids in fried palm oil, sunflower oil and soybean oil.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Methyl *trans*-9,10-epoxystearate and methyl *cis*-9,10-epoxystearate were purchased from Toronto Research Chemicals (Toronto, Canada). Methyl heneicosanoate (C21:0) and 37 component FAME mix analytical standards (C4-C24) were supplied by Supelco (Bellefonte, PA, USA). Hexane (99% purity) was purchased from J&K Chemical Technology (Shanghai, China). All the other chemical reagents were of analytical grade and were obtained from Sinopharm Chemical Reagent Company. Florisil SPE cartridge (200 μm, 60 Å, 6 mL, 1000 mg), alumina-N SPE cartridge (150 μm, 60 Å, 6 mL, 1000 mg), diol SPE cartridge (60 μm, 100 Å, 6 mL, 1000 mg) and silica SPE cartridge (50 μm, 60 Å, 6 mL, 1000 mg) were obtained from ANPEL Laboratory Technologies (Shanghai, China).

### 2.2. Samples

Palm oil, soybean oil and sunflower oil were purchased from a local market.

### 2.3. Standard preparations

Stock solution (2000 μg/mL) of each standard was prepared by dissolving the pure substance in hexane. Working standards were prepared by diluting the standard stock solutions to concentrations of 25, 50, 100, 200, 400, 800, 1000 and 2000 μg/mL with hexane.

### 2.4. Frying procedure

5 L of oil was poured into a commercial electrical fryer (24 × 30 × 14 cm) with a maximum oil capacity of 10 L. The frying temperature was maintained at 180 ± 5 °C, then a batch of 100 ± 1 g fresh potato sticks (40 × 7.2 × 7.2 mm) was added and fried for 4 min, and the frying cycle was 20 min. Frying oil (50 mL) was collected at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 h of frying process. All the oil samples were stored at −20 °C in darkness.

### 2.5. Determination of epoxy FAMEs

#### 2.5.1. Preparation of FAMEs

Base-catalyzed transmethylation of accurate quantity and high analytical sensitivity for ESAs was chosen to obtain FAMEs from frying oils. Transmethylation followed Mubiru et al. (Mubiru, Shrestha, Papastergiadis, & De Meulenaer, 2013) method with modifications. Briefly, 500 mg oil sample and 5 mL n-hexane were added into a screw-capped centrifuge tube (10 cm × 10 mm I.D.). Then 2.5 mL volume of 0.5 M NaOMe in methanol solution was added, the mixture was vortexed for 1 min and allowed to stand at room temperature for 2 min. Then the tube was heated in water bath at 65 °C for 30 min. After cooling down, 0.2 mL of 0.5 M $H_2SO_4$ was added, then vortexed for 30 s, and centrifuged at 1311 g for 3 min. The organic layer was separated and evaporated to dryness under a steam of nitrogen.

#### 2.5.2. Separation of FAMEs by SPE

SPE cartridges (6 mL) (Anpel, Shanghai, China) were filled with different materials and sorbent amounts. Prior to extraction, 2 mL *n*-hexane/diethyl ether (98:2, v/v) was added to wash the column. Then the resultant FAMEs were dissolved into 2 mL *n*-hexane/diethyl ether (98:2, v/v) and loaded onto the SPE cartridges. The non-altered FAMEs (non-polar fraction) were eluted with 15 mL *n*-hexane/diethyl ether (98:2, v/v), and *n*-hexane/diethyl ether with other different proportions and volumes was added to elute the altered FAMEs (polar fraction).

#### 2.5.3. Qualitative analysis of ESAs by GC-MS

Qualitative identification of epoxystearates was conducted by GCMS-QP2010 Ultra (SHIMADZU, Japan), which is equipped with a TR-FAME fused-silica capillary column (60 m × 0.25 mm I.D. × 0.25 μm thickness) and a FID detector (FID-14C, SHIMADZU, Japan). The analysis was performed in splitting mode with a 100:1 split ratio and the injector temperature was 250 °C. The temperature program was set as follows: initial 60 °C for 3 min, raised to 175 °C at ramp rate of 5 °C $min^{-1}$, held for 15 min, then increased to 220 °C at 2 °C $min^{-1}$ and final temperature holding for 10 min. The fiame ionization detector temperature was set at 250 °C. The flow rate of the helium used as carrier gas was 1 mL $min^{-1}$. The samples were dissolved in diethyl ether, and injection volume of analytical solution was 1 μL. The mass spectrometer was operated in the electron impact mode at a voltage of 70 eV, the ion source temperature was set at 200 °C, and mass spectra performed in the scan range of 30–550 amu.

Identification of epoxystearates detected by GC-MS analysis was based on computer matching with the reference mass spectra of the MS library of NIST 14 and Wiley 8.0.

#### 2.5.4. Quantitative analysis of ESAs by GC

Quantitative determination of epoxystearates was conducted by GC-FID using a GC-2010 PLUS (SHIMADZU, Japan). Chromatographic conditions and column were the same as those applied on GC-MS. Identification of individual epoxy FAMEs was carried out by comparison of retention times of sample with those of authentic standards.

### 2.6. Fatty acid composition

Fatty acid composition was determined by GC-2010 PLUS (SHIMADZU, Japan) after derivatization to FAMEs according to Firestone (Firestone, 1994). Chromatographic conditions and column were the same as those applied on detection of epoxystearates by GC-FID. Methyl C21:0 fatty acid methyl ester (purity > 99%) was used as the internal standard.