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Analytical Methods

Rapid and sensitive detection of free fatty acids in edible oils based on chemical derivatization coupled with electrospray ionization tandem mass spectrometry

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ABSTRACT

In this study, a strategy based on chemical derivatization coupled with electrospray ionizationtandem quadrupole mass spectrometry (ESI-MS/MS) for rapid and sensitive detection of FFAs in edible oils was developed. A derivative reagent (N,N-diethyl-1,2-ethanediamine, DEEA) was employed to selectively label carboxyl groups of FFAs to form an amino compound with a tertiary amino group. The DEEA derivative products could lose a characteristic neutral loss fragment of 73 Da in collision-induced dissociation (CID), which enabled to discriminate and analyze the DEEA derived FFAs with neutral loss scan (NLS 73 Da)under the positive ion mode of mass spectrometry. The assay was linear over the concentration range 0.5–200 nmol/L with satisfactory correlation coefficients ($R^2 \geq 0.9942$), whilst the limit of detection and quantitation were 0.1–0.3 nmol/L and 0.3–1.0 nmol/L, respectively. Finally, the established method was applied to determine dynamic FFA formation in seven types of edible oils subjected to a microwave heating treatment test.

1. Introduction

As a primary constituent of the human diet, edible oils serve as an important source of energy, essential fatty acids and fat-soluble vitamins, etc. Triacylglycerols (TAGs) are the main component of edible oils (95–98%). Free fatty acids (FFAs) are common TAG hydrolysis products in oils during manufacturing process and storage as a result of oxidation or TAG degradation, impairing oil quality and functionality (O'Brien, 2008). Therefore, FFA content is one of the most concerned indexes to characterize high-quality oils and to evaluate oil damage (Hamm & Hamilton, 2000; Señoráns & Ibañez, 2002).

Traditionally, total FFAs in edible oil can be detected by titrimetry recommended by the American Oil Chemists' Society (AOCS) (Berner, 1989), which is simple, but the sensitivity is insufficient for catering to the wide range of edible oil quality and only total FFAs can be determined. Gas chromatography (GC) and high-performance liquid chromatography (HPLC), provide useful information on the fatty acid composition and its content in edible oils (Kotani, Kusu, & Takamura, 2002; Rosenfeld, 2002). However, due to the complex matrix of oil, the complex fatty matrices usually require tedious sample purification for the enrichment of FFAs before analysis by using liquid-liquid extraction (LLE), solid-phase extraction (SPE) or magnetic solid-phase extraction (Wei et al., 2013). Recently, supercritical fluid chromatography (SFC) has been used for the determination of FFAs in edible oils (Ashraf-Khorassani, Isaac, Rainville, Fountain, & Taylor, 2015; Lee et al., 2011; Qu, Du, & Yun, 2015). However, SFC methods are limited by their expensive instruments and are unsuitable for in-plant quality control or centralized commercial laboratories.

Alternatively, determination of FFAs by direct infusion "shotgun" MS equipped with an electrospray ionization (ESI) source, in which extracts can be analyzed directly without separation, holds most promise (Leavens, Lane, Carr, Lockie, & Waterhouse, 2002; Leng et al., 2013). However, the direct employment of MS for FFAs detection is arduous in their native form due to their poor ionization, and the non-specific loss of either carbon dioxide or water. In addition, the identification of FFAs is also easily affected by matrix effects. Chemical derivatization of the analyte is often used to enhance the ionization efficiency and detection sensitivity (Atabani et al., 2013; Jiang et al., 2016; Villaverde, Sevilla-Morán, López-Goti, Alonso-Prados, & Sandín-España, 2016). Selective derivatization carboxyl groups of FFAs will be in favor of providing characteristic fragment ions, allowing the detection of derived FFAs can be performed by using either neutral loss scan (NLS)

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or precursor ion scan (PIS), which can provide better detection selectivity and sensitivity compared with full scan mode. This strategy has been successfully used for analysis of FFAs in biological samples such as serum, urine, tissue, etc (Bollinger et al., 2010; Dupuy et al., 2016; Hao et al., 2015; Wang, Han, & Han, 2015), and also used for the analysis of fatty acids in eggs (Smith, Lamos, Shortreed, Frey, & Belshaw, 2007).

In this study, a strategy based on simple chemical derivatization coupled with NLS-ESI-MS/MS for rapid and sensitive detection of FFAs in edible oils without complicated sample purification and enrichment was developed. Finally, the established method was applied to determine dynamic FFA formation in seven types of edible oils subjected to a microwave heating treatment test.

2. Materials and methods

2.1. Chemicals and reagents

Triethylamine (TEA) and N,N-diethyl-1,2-ethanediamine (DEEA) were purchased from Shanghai Aladdin reagent Co., Ltd. 2-chloro-1-methylpyridinium iodide (CMPI) were purchased from Sinopharm Chemical Reagent Co., Ltd. HPLC-grade Acetonitrile (ACN), chloroform ($CHCl_3$), methanol, acetic acid and formic acid were purchased from CNW (Düsseldorf, Germany). The water used throughout the study was purified on a Milli-Q apparatus (Millipore, Bedford, MA, USA). All other solvents and chemicals were of analytical grade.

Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), and eicosenoic acid (C20:1) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Margaric acid (C17:0) standard, used as an internal standard (I.S.) for GC-FID quantification, was also purchased from Sigma-Aldrich. 7,7,8,8-palmitic acid-d4 (C16:0-d4) used as an internal standard (I.S.) for ESI-MS quantification was a product of Cambridge Isotope Laboratories (Andover, MA). The individual fatty acid stock solutions and the I.S. stock solution were prepared in *n*-hexane (HPLC grade) at a concentration of 5 mg/mL. Mixed fatty acids stock solution containing 1 mg/mL of each fatty acid was prepared by mixing the individual fatty acid stock solutions. All stock solutions were kept at 4 °C in the dark. The stock solutions were diluted to the desired concentration for the following experiments.

Triacylglycerols (TAGs) are the main constituents (95–98%) of plant oils, which are esters composed primarily of three medium or long-chain fatty acids linked to a glycerol molecule. Names of TAGs are abbreviated by three letters corresponding to the fatty acids bound to the glycerol backbone. In this paper, TAG 15:0/18:1/15:0-D5 (1,3(d5)-dipentadecanoyl-2-octadecenoyl)-glycerol) and TAG 14:0/14:0/14:0 (1,2,3-tritetradecanoyl-glycerol) were used to evaluate the stability of TAGs in oil during chemical derivatization of free fatty acids in oil. The following abbreviations are used: myristic acid (14:0); pentadecanoic acid (15:0); D5-pentadecanoic acid (15:0-D5); oleic acid (18:1). All TAG standards were purchased from Larodan Fine Chemicals (Malmö, Sweden) and were dissolved in hexane to prepare a 5 mg/mL stock solution at 4 °C in the dark.

2.2. Oil samples

Perilla oil (PO), sesame oil (SO), corn oil (CO), linseed oil (LS), rapeseed oil (RO), olive oil (OO) and lard oil (LO), all purchased from local markets in Wuhan (China) and stored at room temperature, were used for this study. Microwave heating oil test, two milliliters of each kind of oil was placed into a 12 mL round bottom glass-bottle positioned right in the middle of microwave turntable and kept for up to 10 min microwave heating at 800 W. In order to prepare the edible oil samples with different microwave heating treatment time, 12 mL of each kind of oil were separated into six bottles (2 mL in each bottle), all of the bottles were placed in the middle of microwave turntable for microwave heating at the same time, and at each microwave heating

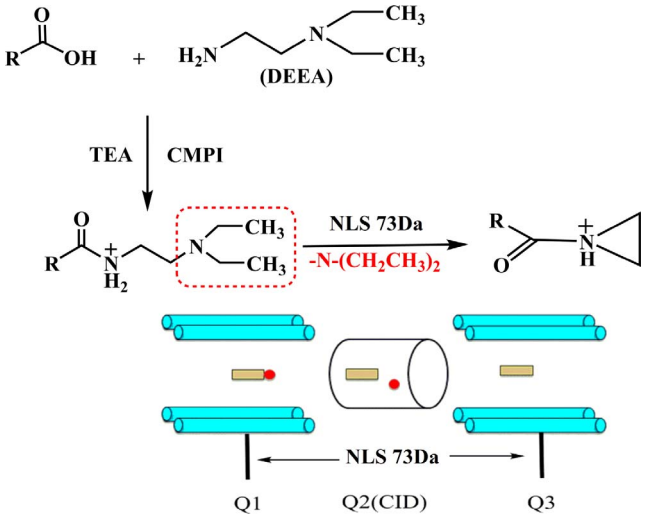

Fig. 1. Schematic diagram of the reaction of fatty acids with DEEA to form a product with a tertiary amino group.

treatment time point (i.e. minutes 0, 2, 4, 6, 8 and 10 min), one bottle was taken out from the microwave oven. After the microwave heating treatment, all the oil samples in glass-bottles were stored at −4 °C until analysis. All experiments were carried out independently in triplicate, and average values were used for quantization.

2.3. Chemical derivatization of free fatty acids

DEEA was used to derivatize carboxyl groups of FFAs and the reaction schematic was shown in Fig. 1. Briefly, 2 mg aliquot of each oil samples was dissolved in *n*-hexane and diluted to 0.2 mg/mL. Thus 50 μL aliquot of diluted oil sample was transferred to a glass culture tube for directly DEEA derivative reaction. 10 μL internal standard 7,7,8,8-palmitic acid-d4 (C16:0-d4, 10 μmol/L in ACN) for quantification of FFAs was added into the tube. Then, 840 μL ACN, 30 μL of TEA (20 μmol/mL, in ACN) and 20 μL of CMPI (20 μmol/mL, in ACN) were sequentially added and then vortex for 1 min. After that, 50 μL of DEEA (20 μmol/mL, in ACN) was added to label FFAs. The derivatizing reaction was performed at 40 °C for ultrasonic 5 min. The resulting solution was evaporated under nitrogen stream and 1 mL chloroform was added to the residue. After that, liquid-liquid extraction was used to remove the unreacted CMPI and DEEA, which will interfere with the ionization process of mass spectrometry, resulting in lower peak intensity of analyte and contamination of ion source. 2 mL formic acid-water (v/v, 10:90) was added into the chloroform solution mentioned above, the mixture was then vortexed for 2 min and allowed to separate into layers, then the upper formic acid-water phase including unreacted CMPI and DEEA were removed. This step was repeated for 3 times to completely get rid of the remaining catalyst and DEEA. Finally the chloroform phase was evaporated under a nitrogen gas. The residue was redissolved in 1 mL acetonitrile and subjected to mass spectrometric analysis. And the developed method was applied for determining the dynamics of FFAs formation in oil microwave heating treatment tests.

2.4. Shotgun-ESI-MS/MS

Analysis of FFAs and its derivatives were performed on shotgun-ESI-MS/MS system consisting of a 4000 Q-Trap mass spectrometer (AB Sciex, Toronto, ON, Canada) with an electrospray ionization source which was equipped with a syringe pump. Data acquisition and processing were performed using AB SCIEX Analyst1.5 Software (Applied Biosystems, Foster City, CA, USA). Fatty acids standards without derivatization were detected using full scan in negative-ion mode; fatty acids derivatives were detected using neutral loss scan (NLS 73 Da) in