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Fatty acid profiles based adulteration detection for flaxseed oil by gas chromatography mass spectrometry

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ABSTRACT

Flaxseed oil is popular edible oil and an important additive in functional foods and feeds. Recently, economically motivated adulteration as a type of oil fraud becomes emerging risk. In this study, the fatty acid profiles of flaxseed oil were analyzed by gas chromatography-mass spectrometry operating in selected ion monitoring mode and then used to detect adulterated flaxseed oil with the help of multivariate statistical methods including principle component analysis (PCA), and recursive support vector machine (R-SVM). The detection results indicate that the discriminant model built with 28 fatty acids can identify adulterated flaxseed oil samples (10%) with high accuracy of 95.6%. Therefore, fatty acid profiles based adulteration detection for flaxseed oil is an important strategy for preventing customers far from adulterated flaxseed oil.

1. Introduction

Flaxseed oil, also known as linseed oil, is made from the seeds of the *Linum usitatissimum* plants (Linaceae family), which contain rich content of essential fatty acids (especially omega-3 fatty acids) and phytoestrogen lignans (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014). Recently, flaxseed oil has been the focus of increased interest in its potential health benefits associated with the biologically active components. With high content of Omega-3 fatty acids and flaxseed lignans, the flaxseed oil play an important role in reducing the risks associated with inflammatory and autoimmune diseases, cardiovascular diseases, diabetes, breast, colon, ovary and prostate cancers (Allman, Pena, & Pang, 1995; Bassett, Rodriguez-Leyva, & Pierce, 2009; Demark-Wahnefried et al., 2008; Goyal et al., 2014; Paschos, Magkos, Panagiotakos, Votteas, & Zampelas, 2007). In addition, flaxseed oil contains all essential amino acids crucial for the synthesis of the proteins that regulate and maintain proper cellular functions (Oomah & Mazza, 1993; Shuai et al., 2014). Therefore, during the last two decades, flaxseed oil has gained popularity in the human diet for improving the nutritional and health status (Oomah, 2001; Wahid et al., 2011). Furthermore, flaxseed oil is usually employed to be an important ingredient in functional foods (Oomah, 2001) and feeds for livestock such as cows to enhance the nutritional quality of related products (Catherine, Dutreuil, Coppa, Agabriel, & Martin, 2014; Gómez-Cortés, Bach, Luna, Juárez, & Fuente, 2009; Wang, Zhu, Ahmad, Zhang, & Wang, 2013).

With the potential health benefits, the flaxseed oil is more expensive than other edible oils. The economically motivated adulteration in flaxseed oil becomes an emerging risk that infringes the rights and interests of consumers and might be harmful to human health. Authenticity assessment of edible vegetable oils is a tough nut to crack worldwide. Though it is not hard to discriminate the pure flaxseed oil from other edible oils, adulteration detection of flaxseed oil with low-price oils is still hot but problematic as the

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same as olive oil adulteration in Western countries. Therefore, there is a great demand for reliable detection methods for oil adulteration (Raquel, Martins, & Cabrita, 2013). The most common instrumental detection methods include liquid chromatography (Rachid, Armbruster, & Schwack, 2014), gas chromatography (Monfreda, Gobbi, & Grippa, 2014) and hyphenated mass spectrometry (Mümtaz, Dıraman, & Özdemir, 2013; Zhang et al., 2010), infrared spectroscopy (Kuriakose & Joe, 2013), fluorescence spectroscopy (Ge, Chen, Liu, & Zhao, 2014), Raman spectroscopy (Wei, Zhang, Zhang, & Wang, 2013). Fatty acids are the dominant components of edible oils, whose composition is relatively stable in the oilseeds from different producing areas or edible oils produced by different processing methods. Moreover, polyunsaturated fatty acids (PUFA) in flaxseed oil, especially omega-3 PUFA, are greatly important in contributing to nutritional functions (Débora & Jorge, 2013; Rajiv, Indrani, Prabhasankar, & Rao, 2012). Intuitively, α-linolenic acid (ALA) might be a good choice for detecting adulteration of flaxseed oil in consideration of the fact that flaxseed oil has a relatively higher content of α-linolenic acid (ALA) than most of other edible oils. However, according to the Chinese standard of flaxseed oil, the relative content of ALA in flaxseed oil generally ranges from 39% to 62%. High linolenic varieties have about 73% of ALA (Dinushika et al., 2013). It indicates that relative content of ALA of the high linolenic flaxseed oils is still higher than 39% even if they are adulterated with about 40% low-price edible oils (such as cotton-seed oil). Thus, the univariate adulteration method is ineffective and multivariate data analysis of the fatty acid profiles is required to detect flaxseed oil adulteration.

In the multivariate data analysis, Chemometrics is a multivariate data analysis tool, which is a powerful tool against oil fraud when used qualitatively for classifying unknown samples with similar characteristics and quantitatively for determining adulterant analytes in samples (Moore, Lipp, & Griffiths, 2011). In recent reports, chemometric methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), self-organizing maps based on chaotic parameters, and cluster discriminant analysis (CDA) were used to distinguish edible oils from refined recycled cooking oils, identify edible oils from different regions, and detect adulteration of extra virgin olive oil with inferior edible oils (Liu, Zhou, Chen, Li, & Shi, 2013; Mümtaz et al., 2013; Torrecilla, Cancilla, Matute, Díaz-Rodríguez, Flores, & 2013), respectively. Generally, chemometric data analysis for oil fraud detection increases (Moore et al., 2011).

Obviously, more fatty acids involved in a model indicate a higher probability of detecting edible oils adulterated with other oils. In this study, the fatty acid profiles of flaxseed oil were analyzed by gas chromatography mass spectrometry in selected ion monitoring mode to obtain more information about fatty acids. Subsequently, a classification model for the flaxseed oil and other 5 types of edible oils and a discriminant model for the flaxseed oils and their simulated adulterated oils were built by PCA, and recursive support vector machine (R-SVM). After recognition of serial adulterated oils, the model built in this study could be used to effectively detect adulterated flaxseed oil.

2. Materials and methods

2.1. Oil samples and reagents

A total of 20 flaxseed samples were purchased from different regions and the seed oils were prepared by oil mill machinery (TZC-0502, Brand of TEN GUARD, Foshan, China). Meanwhile, 22 flaxseed oil samples representing almost all types of flaxseed oil in the Chinese market were purchased from different companies. Supelco 37 Component FAME Mix (No. 47885-U), 11-octadecenoic acid (C18:1n-7, >97.0 purity), and 7-hexadecenoic acid methyl ester were purchased from Sigma (St. Louis, MO, USA).

2.2. Derivatization

As descried in the previous studies (Zhang, et al., 2014a; Zhang, et al., 2014b), 0.06 g seed oil was diluted with 2 mL diethyl ether/petroleum ether (1:1 v/v). Then, 1 mL 0.4 M KOH–CH_3OH was added to the dilution, vortexed, and kept at room temperature for 2.5 h. After that, 2 mL redistilled water was added, vortexed, and centrifuged at 4500 rpm for 2 min. Finally, 50 μL organic phase containing fatty acid methyl esters (FAMEs) was collected and diluted by 950 μL petroleum ether prior to GC–MS analysis.

2.3. GC–MS analysis

GC–MS analysis was conducted by an Agilent GC 7890 gas chromatograph interfaced to an Agilent 5973 mass spectrometer.

In the gas chromatography system equipped with a fused silica capillary column DB-23 (30 m × 0.25 mm × 0.25 μm), helium (99.999% purity) was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The temperature of the column was first set at 100 °C and held for 0.2 min, which was subsequently increased to 215 °C at a rate of 10 °C/min and held for 0.1 min, until the final temperature reached 224 °C at a rate of 2 °C/min and was held for additional 0.2 min. The total run time was 16.5 min. This is the optimum temperature-programming condition for both the separation and the run time. The mass spectrometric conditions were as follows: the electron ionization (EI) mode was at 70 eV; the temperatures of the injector, ion-source, and detector were 220 °C, 250 °C, and 150 °C, respectively; the solvent cut time was 3 min; the split ratio was 20:1; the selected ion monitoring (SIM) mode were at *m*/*z* 55, 67, 74, and 79.

2.4. Identification of FAMEs

Fatty acids in seed oil samples were identified by both retention time and mass spectral characteristics (Zhang et al., 2010; Zhang, Tan, Zeng, Lu, & Liang, 2012). This identification was performed in the following three steps: (1) An automated mass spectral search was conducted to identify all expected straight saturated FAMEs in each chromatogram; (2) The retention time of straight saturated FAMEs was used to calculate the electrochemical luminescence (ECL) values of the unsaturated FAMEs (Christie, 1988). If the retention time of interest was out of the straight saturated FAMEs, extrapolation was employed using the retention time of two nearest straight saturated FAMEs; (3) The ECL values of the fatty acids in the samples were compared with those of the fatty acids in our database (Wasta & Mjøs, 2013; Zhang et al., 2012) to identify unsaturated FAMEs.

2.5. Multivariate analysis

The data matrix includes the relative contents of the fatty acids in edible oils. The fatty acid database for edible oils consists of 17 soybean oil samples, 75 peanut oil samples collected from major producing areas, 57 sunflower seed oil samples, 76 rapeseed oil samples, and 73 sesame oil samples purchased from different production areas. Data simulation for adulterated oils was implemented in Matlab 2011a for Windows (The Mathworks, Natick, MA). To establish a precise discriminant model for flaxseed oil and its adulterated oils, 42 adulterated oil samples was simulated by blending the preceding five types of oil with flaxseed oil until the contents of those oils reached 10% in random proportions.