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# Quantitative analysis and health risk assessment of polycyclic aromatic hydrocarbons in edible vegetable oils marketed in Shandong of China



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#### ABSTRACT

This work studies on the quantitative analysis and health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in edible vegetable oils in Shandong, China. The concentrations of 15 PAHs in 242 samples were determined by high performance liquid chromatography coupled with fluorescence detection. The results indicated that the mean concentration of 15 PAHs in oil samples was 54.37  $\mu g \ kg^{-1}$ . Low molecular weight PAH compounds were the predominant contamination. Especially, the carcinogenic benzo(a)pyrene (BaP) was detected at a mean concentration of 1.28  $\mu g \ kg^{-1}$ , which was lower than the limit of European Union and China. A preliminary evaluation of human health risk assessment for PAHs was accomplished using BaP toxic equivalency factors and the incremental lifetime cancer risk (ILCR). The ILCR values for children, adolescents, adults, and seniors were all larger than 1  $\times$  10<sup>-6</sup>, indicating a high potential carcinogenic risk on the dietary exposed populations.

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#### 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds containing two or more fused aromatic rings, which are formed and released during the incomplete combustion of the organic materials and geochemical processes (Moret and Conte, 2000; Simon et al., 2008). PAHs are known to have carcinogenic and mutagenic effects, which depend on their molecular structures (Durant et al., 1999; Jung et al., 2010; Nisbet and Lagoy, 1992). Therefore, 16 PAHs are included in the United States Environmental Protection Agency (U.S.EPA) and the European Food Safety Authority (EFSA) priority pollutant list (Ferey et al., 2014). Seven of them including benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), chrysene (Chr), dibenzo[a,h]anthracene (DahA), and indeno[1,2,3-c,d]pyrene (IcdP) are probable human carcinogens according to the U.S.EPA (2002). Nowadays, PAHs and their derivatives are omnipresent in

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the environment and are found in water, air, soil and foods (Algarra et al., 2005; Kuosmanen et al., 2003; Lourenço et al., 2014; Ni and Guo, 2013; Shen et al., 2013; Sofowote et al., 2010). Due to their lipophilic nature, PAHs distribute widely, especially, the edible vegetable oils often suffer from the contamination of PAHs. The PAHs contamination to vegetable oils could be generated by environmental pollution of the vegetable raw material, and by contamination from seed drying, solvent extraction, soil burn, package material, mineral oils residues and migration from contaminated water or soils (Dennis et al., 1991; Ergönül and Sánchez, 2013; Larsson et al., 1987; Moret and Conte, 2000; Teixeira et al., 2007). Among these different routes, the migration from contaminated vegetables and soils and the refining process of vegetable oils might be responsible for major PAHs contamination of vegetable oils (Kang et al., 2014: Moret and Conte, 2000: Rojo Camargo et al., 2012; Shen et al., 2013; Teixeira et al., 2007; Wang et al., 2011).

Food safety and quality are desirable throughout the world. Although for most people the edible vegetable oils represent only a small part of their total diet, it is still considered as one route of human exposure to PAHs. The PAHs contamination to edible vegetable oils may cause a public health hazard, since some of them

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have been showed to be highly carcinogenic in laboratory and have been associated with lungs, bladder, and skin cancer (Boffetta et al., 1997; Martorell et al., 2010). Therefore, it is necessary to detect the content and to assess health risk of PAHs in edible vegetable oils. Many analytical methods have been reported for the detection of PAHs in real samples (Ding et al., 2007; Ferey et al., 2014; Garcia Londono et al., 2014; Ghislain et al., 2012; Moret and Conte, 2000). Among them, high performance liquid chromatogram (HPLC) coupled with a fluorescence detection represents one of the most powerful technique in what concerns sensitivity and selectivity, owing to the strong fluorescence of many PAHs (Ergönül and Sánchez, 2013; Girelli et al., 2014; Moret et al., 2005).

Due to the difficulty of extrapolating toxicity data from animals to humans, there is not a standard procedure for the health risk assessment of PAHs. Toxic equivalency factor (TEF) is widely adopted for the assessment of the potential risk of a PAH mixture (Essumang et al., 2013; Fisher et al., 2011; Nisbet and Lagoy, 1992). Especially, benzo(a)pyrene (BaP) is usually used as an indicator for PAHs contamination. The factor for each PAH often evaluates its potency relative to BaP, which has a TEF of unity (Essumang et al., 2013). The TEFs were used to estimate BaP equivalent concentration and further for the incremental lifetime cancer risk (ILCR) calculation (Zhao et al., 2014).

So far, the studies concentrated on dietary health risk assessment of PAHs are rather limited (Xia et al., 2010; Zhao et al., 2014). Especially, the health risk of human exposure to PAHs in edible vegetable oils has not been studied extensively to date (Kang et al., 2014). Shandong is located on the east coast of China, spanning from 115°08′ E to 122°41′ E, and from 34°76′ N to 37°91′ N, with an area of 157,800 km<sup>2</sup> (Wei et al., 2013). This region, mainly including the Yellow River Delta (YRD), the Shandong Peninsula, the Huanghuai plain of Shandong and the Yimeng mountainous area, has become a major area for the development of oil crops and one of the most important provinces for vegetable oils production and export. With the rapid economic growth and development of industry, this region has suffered from the considerable increasing load of PAHs (Yang et al., 2014; Yuan et al., 2014; Zhang et al., 2015). However, most previous studies mainly focused on the PAHs pollution in the soils, being scarce information available in vegetable oils (Dai et al., 2008; Yang et al., 2014; Yuan et al., 2014). This study provides the first report on the quantitative analysis and human health risk assessment of PAHs in edible vegetable oils of Shandong Province. The results allow an improved assessment of population exposure to PAHs and hopefully, are essential for human health protection.

#### 2. Materials and methods

#### 2.1. Chemicals and standards

A standard solution of 15 U.S.EPA priority PAHs were supplied by Sigma—Aldrich (St. Louis, MO, USA). They were naphthalene (Nap), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DahA), indeno(1,2,3-cd)pyrene (IcdP) and benzo(g,h,i)perylene (BghiP). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Deionized water (resistivity 18.2 M $\Omega$  cm-1) was produced by a Milli-Q water purification system ((Millipore Co., USA), and was used for sample preparation, standard solutions, and mobile phase in HPLC. Other chemicals and reagents used were analytical grade.

#### 2.2. Standard preparations and calibration curve

The stock solutions of PAHs were stored in the dark ( $-20\,^{\circ}\text{C}$ ) and the intermediate dilutions (1000 ng/mL) were weekly prepared by appropriated dilution in acetonitrile. The seven-point calibration curves corresponding to PAHs compounds were prepared, ranging from 2 to 400 ng/mL. Each point was the average of three injections and the target analytes were quantified by using the external calibration method.

#### 2.3. Sample collection and pretreatment

Oil samples were collected based on the food safety risk assessment, which was undertaken to find the contamination levels of PAHs in Shandong Province. This area is one of the major oil-producing regions of China. A total of 242 samples, representing 75 species of peanut oils, 77 species of soybean oils, 44 species of blend oils and 46 species of corn oils commonly consumed by local residents, were randomly collected from local markets and supermarkets in 17 cities of Shandong Province, China. According to the labels of samples, these oil samples were all from China and mostly from Shandong Province. The types of vegetable oils were chosen because they are the most important staple oils in this region. The number of samples represents the different brands and batches of available commercial vegetable oils in the Shandong market at the time of sampling. They were stored in a dark room at room temperature.

To analyze PAHs from edible vegetable oils, a rapid solid-phase extraction (SPE) procedure was used based on the previously established methods (Ergönül and Sánchez, 2013; Teixeira et al., 2007). Firstly, 2.00 g of an oil sample was diluted with 5 mL acetonitrile-acetone (v/v = 1:1), shaked for 30 s with vortex, sonicated 5 min and centrifuged 5 min at 5000 rpm. The top layer was transferred into a conical tube. Then the solvent was evaporated under the nitrogen gas in a water bath at 35 °C. This extraction process was repeated three times. The SPE was performed in a 12port (or 24-port) Visiprep SPE Vacuum Manifold (Supelco, USA). The SPE cartridges were Oasis HLB (WAT106202, 6cc/200 mg) and Sep-Pak Florisil (WAT043390, 6 cc/1 g), both purchased from Waters (USA). The Oasis HLB phase cartridges were activated with 10 mL dichlormethane, 10 mL methanol and 10 mL acetonitrile, and the Florisil with 15 mL dichloromethane and 15 mL n-hexane. The residue from the liquid-liquid extraction was dissolved in 3 mL of acetonitrile-acetone (v/v = 1:1) solvent, shaken during 15 s with vortex and centrifuged at 5000 rpm for 1 min. The top layer was then transferred to the HLB phase cartridge. Five milliliters acetonitrile-acetone (v/v = 1:1) and 5 mL dichlormethane were eluted through the cartridge under vacuum, respectively. The solvents were evaporated under a nitrogen stream at 35 °C. The residue was then dissolved in 2 mL of n-hexane and then transferred to the previously conditioned Florisil bonded phase cartridge. The cartridge was eluted with 5 mL of n-hexane-dichloromethane (v/ v = 2:1). This procedure was repeated two times. The collected extract was evaporated under a flow of nitrogen at 35 °C until 1 mL of residue left over, then 1 mL of toluene was added, and the evaporation continued until 0.2 mL of residue left over. This residue was diluted with acetonitrile until 1 mL, and then passed through a 0.45-µm membrane filter and transferred into a vial for the HPLC analysis.

#### 2.4. HPLC detection

A high-resolution liquid chromatography consisting of a module separations Alliance 2695 (Waters, Made in Singapore), and a fluorescence detector (Waters 2475, Made in Ireland) was used for

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