Determination of benzo[a]pyrene in camellia oil via vortex-assisted extraction using the UPLC-FLD method

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Abstract In this paper, vortex-assisted extraction using the ultraperformance liquid chromatography analysis method was performed to determine benzo[a]pyrene in camellia oil. Optimum results were obtained when 0.5 g of oil sample was used followed by vortex-assisted extraction for 10 min with 25 mL of acetonitrile. Chromatographic separation was performed on an Agilent ZORBAX Eclipse Plus C₁₈ column (2.1 mm×100 mm, particle size 1.8 μm). The optimum mobile phase comprised 70% acetone and 30% water. The detection limit of benzo[a]pyrene was 0.2 μg/kg. The recoveries were in the range of 81.0–97.0%. The proposed method was simple and fast, and it provided high throughput in the determination of benzo[a]pyrene in an oil matrix sample.

Keywords: benzo[a]pyrene, vortex-assisted extraction, UPLC, camellia oil

Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds containing two or more fused aromatic rings composed of carbon and hydrogen atoms, is a widespread contaminant in water, soil, and air (1). In foodstuffs, PAHs usually form because of charcoal grilling, roasting, and smoke-drying. They remain in the environment for a significant period of time because of their stable chemical structure. Benzo[a]pyrene is one of the most familiar PAHs and is widely found in sea foods, smoking foods, and edible oils (2,3). In recent years, researchers have reported the toxic effects of benzo[a]pyrene in low-concentration levels on humans as well as its carcinogenic and mutagenic effects (4–6).

The maximum permitted level of benzo[a]pyrene in edible oil differs from country to country. In China, the maximum permitted level of benzo[a]pyrene is 10 μg/kg in edible oil. The International Olive Oil Council recommended a value of 2 μg/kg as a maximum tolerable concentration of benzo[a]pyrene in olive oil. In the European Union (EU), the maximum permitted levels of benzo[a]pyrene is 2 μg/kg in oil and fat and 5 μg/kg in smoked food (7). Camellia oil, produced from the seeds of the *Camellia oleifera* tree (*C. oleifera* seeds), is widely in food processing and cooking in China as well as some other Asian countries. The production of camellia seed oil reached almost 200,000 tons in 2011 (8). Camellia oil contains rich unsaturated fatty acids and antioxidative substances (9), similar to olive oil. Many studies have shown that camellia oil is beneficial to human health; moreover, it is increasingly favored by East Asian

consumers (10).

Hot press extraction is a traditional processing method in China for obtaining camellia oil, and this method always needs high temperature. However, high-temperature processing techniques such as hot pressing are considered to be the main cause of excessive benzo[a]pyrene (11). Chinese media reported that the concentration of benzo[a]pyrene was found to be more than 10 μg/kg (the maximum residue limit (MRL) of benzo[a]pyrene in vegetable oil is 10 μg/kg in China) in some camellia oil samples in Hunan Province; this news gained large public awareness that benzo[a]pyrene played as a negative role in oil. Considering the fact that camellia oil is widely consumed in daily diets in China, it is necessary to develop a reliable, fast method to monitor the concentration of benzo[a]pyrene in camellia oil.

Triglyceride is a major matrix interference associated with the determination of benzo[a]pyrene (12). Saponification and solid-phase extraction (SPE) are the most widely used cleanup methods. However, saponification is time-consuming because of the extensive manipulations involved after sampling (13). In addition, SPE using special materials such as styrene-divinylbenzene copolymer sorbent is expensive (14,15). More recently, a new extraction technique named vortex-assisted liquid–liquid extraction has been used for the determination of pesticides, plasticizers, and food additives (16,17). This extraction method is based on high-speed vortex extraction, which can achieve equilibrium within a very short period. Compared with saponification and SPE methods, multitube vortex-assisted liquid–liquid extraction is simpler, faster, and less expensive. However, no study has reported the use of multitube vortex-assisted

liquid–liquid extraction for benzo[a]pyrene extraction.

Various analytical techniques such as GC, GC-MS, HPLC-FLD, and HPLC-MS have been applied to benzo[a]pyrene analysis, and the advantages and disadvantages of these methods have been thoroughly discussed in many papers (18–21).

In this study, a simple, rapid method was developed to process camellia oil via vortex-assisted liquid–liquid extraction for benzo[a]pyrene using employing UPLC-FLD.

Materials and Methods

Materials and reagents Camellia oil samples were purchased from a local market in the province of Zhejiang, China. Acetonitrile, *n*-hexane, methanol, acetone, and dichloromethane (Merck, Darmstadt, Germany) were all of HPLC purity grade. Benzo[a]pyrene (20 mg/kg) was obtained from National Research Center for Certified Reference Materials, China (NRCCRM). The stock solution (100 µg/L) of benzo[a]pyrene was prepared in acetonitrile, and the working standard solutions (0.5, 1.0, 2.0, 5.0, and 10.0 µg/L) were prepared by diluting the stock standard solution with acetonitrile. Purified water (Milli-Q-Plus ultrapure water system; Merck Millipore, Billerica, MA, USA) was used in the mobile phase.

LC conditions Benzo[a]pyrene analysis was performed using an ultraperformance liquid chromatography (UPLC) system (1290; Agilent Technologies, Santa Clara, CA, USA) equipped with a scanning fluorescence detector (FLD) (1260; Agilent Technologies). Benzo[a]pyrene was separated using a C18 column (2.1 mm×100 mm, particle size 1.8 µm, Agilent ZORBAX Eclipse Plus C₁₈). The mobile phase comprised 70% acetone and 30% water at a flow rate of 0.3 mL/min. FLD was used to monitor and quantitate benzo[a]pyrene using 384-nm excitation and 406-nm emission wavelengths. The column temperature was maintained at 25°C, and the injection volume was 20 µL.

Sample extraction An aliquot (0.5 g) of the oil sample was weighed in a 50 mL plastic centrifuge tube. The sample was extracted once with 25 mL of acetonitrile using a multitube vortex agitator (DMT-2500; Shengke Instruments Inc., Hangzhou, China) for 10 min at 2,500 rpm and then centrifuged for 10 min at 4,600×g at 4°C. After removing 20 mL of supernatant, the sample was concentrated using a rotatory evaporator (RV10; IKA, Staufen im Breisgau, Germany) at 50°C. The dry residue was redissolved in 1 mL of acetonitrile. Before UPLC analysis, the oil extracts were filtered using a 0.22-µm tetrafluoroethylene membrane filter.

Results and Discussion

Optimization of extraction Selecting the organic solvents for the extraction of benzo[a]pyrene from the oil sample was important.

Fig. 1. Effect of the extraction solvent/oil ratio on the extraction efficiency (vortex speed: 2,500 rpm, extraction time: 15 min)

Organic solvents were selected on the basis of lipophobicity, extraction capability of target compound, and good liquid-chromatography behavior.

Considering these factors, acetonitrile and methanol could be suitable extractants because of their good extraction capability and delamination with oils. Compared these two solvents, we found more matrix interferences in the chromatogram when using methanol. Hence, acetonitrile was chosen as the extraction reagent.

In this experiment, different ratios of oil/solvent were used to evaluate the extraction recovery. As shown in Fig. 1, the extraction efficiency increased with the ratio of oil/solvent and the extraction duration. However, when the oil/solvent ratio decreased to 1:2, it was very difficult to extract benzo[a]pyrene (recovery less than 80.0%) even if extraction was performed thrice. Using other ratios of oil/solvent (1/5, 1/10, 1/25, and 1/50), all recoveries exceeded 85.0%. When the ratio of oil/solvent was 1:50, the extraction rate was 98.3%. Therefore, in terms of the extraction efficiency, the final ratio of oil/solvent was selected as 1:50.

In order to shorten the extraction time, we used the blank oil sample by adding a known volume of the standard solution (the spiked level was 10 µg/kg). Extraction was performed once with 25 mL of acetonitrile, and different extracting durations were investigated. According to the extraction-efficiency values, the results show that the recovery exceeded 95.0% and that there was no significant difference ($p > 0.05$) from 10 to 20 min.

Chromatographic conditions Most researchers have employed the acetonitrile/water mobile phase for benzo[a]pyrene determination via HPLC-FLD (22–25). However, if oil is injected into the LC system with an acetonitrile/water mobile phase, the lifetime of the chromatographic column is shortened. In this experiment, we found that the column pressure increased by 20% when using the acetonitrile/water mobile phase with continued inletting of the

Fig. 2. Effects of different mobile-phase concentrations on benzo[a]pyrene separation

sample for 50 times. We also found that the pressure can be kept constant using the acetone/water mobile phase. Additionally, compared with the acetonitrile/water mobile phase, the acetone/water mobile phase more easily separated oil from the chromatographic column. In this study, different ratios of acetone/water (90/10, 80/20, and 70/30, v/v) were tested. As shown in Fig. 2, when the mobile phase of acetone/water was used (90/10 and 80/20), oil cannot be separated from the interferent. The best mobile phase in terms of sensitivity and separation was the acetone/water mobile phase (70/30, v/v) at a flow rate of 0.3 mL/min. Under the described chromatographic conditions, benzo[a]pyrene was separated in 5 min.

Method validation Validation of the selected method was performed by evaluating several parameters, including the limit of detection (LOD) and quantification (LOQ), linear correlation coefficient, precision, recovery, and stability.

Linearity is the ability of a method to provide a response proportional to the concentration of the analyte. In this case, linearity was assessed using 5 standard concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 $\mu\text{g/L}$) under the chromatographic conditions described above and using the peak area as a calibration parameter plotted against the concentrations with an equation $y=114.702X+18.804$.

The linearity agreed well with a linear fit for the analyte, with a reliable coefficient of regression of 0.9999.

LOD and LOQ were expressed as the concentration of benzo[a]pyrene, which gave a signal $3r$ and $10r$ at the mean blank signal (where r is the standard deviation of the blank signal), and the measured values were 0.2 and 0.5 $\mu\text{g/kg}$. The LOD values are adequate for the determination of benzo[a]pyrene in camellia oil at concentrations as low as 1/10 of the maximum permitted concentration in the EU (26).

A known volume of the standard solution with the concentrations of 2.0, 5.0, and 10.0 $\mu\text{g/kg}$ to 0.5 g of the sample was added to the oil sample to evaluate the recovery and precision. The results (listed in Table 1) indicate that three replicates were performed at each spiking level, the mean recoveries of intraday precisions ranged from 81.0 ± 4.2 to $97.0\pm1.2\%$, and the mean recoveries of intraday precisions ranged from 85.5 ± 3.4 to $96.6\pm1.8\%$; this verifies that the determination method has a good accuracy and a high precision for the determination of benzo[a]pyrene in oil. The chromatograms of the standard solution and camellia oil sample with 2.0 $\mu\text{g/L}$ benzo[a]pyrene standard solution are shown in Fig. 3.

Sample determination The reference sample provided by the China CFAPA Testing Technology Company Limited of Dalian, which was tested by 23 labs with known reference values of $27.52\pm2.15 \mu\text{g/kg}$, was also tested to verify the accuracy of the whole procedure. The mean value obtained via the optimized method was $26.10\pm0.28 \mu\text{g/kg}$, and the mean recovery value was 94.8%.

The optimized method was applied to the analysis of benzo[a]pyrene in refined and virgin camellia oil samples purchased from a local market. The results are shown in Table 2. The benzo[a]pyrene concentration in virgin camellia oils ranged from 1.52 to 4.51 $\mu\text{g/kg}$. The benzo[a]pyrene concentration in refined camellia oil ranged from ND to 2.01 $\mu\text{g/kg}$. All the measured camellia samples were in compliance with the China regulation (10 $\mu\text{g/kg}$); however, five virgin camellia oils and one refined camellia oil samples were in noncompliance with the EU regulation (2 $\mu\text{g/kg}$). The benzo[a]pyrene concentration in other measured vegetable oil samples was lower than LOD value. The government should pay more attention toward supervising the safety standards for camellia oil.

In our study, the concentration of benzo[a]pyrene in refined camellia oil was significantly lower than those in virgin camellia oil. This is due to some cleanup procedures which absorbed benzo[a]pyrene during the refining process (27). In addition, we applied the

Table 1. Precisions and recoveries for different added concentrations of benzo[a]pyrene ($n=3$)

Sample	Added ($\mu\text{g/kg}$)	Intraday precisions			Interday precisions		
		Recovered mean ($\mu\text{g/kg}$)	Recovery (%)	RSD	Recovered mean ($\mu\text{g/kg}$)	Recovery (%)	RSD
Camellia oil	2.0	1.62	81.0	4.2	1.71	85.5	3.4
	5.0	4.72	94.4	3.1	4.63	92.6	2.5
	10.0	9.70	97.0	1.2	9.66	96.6	1.8

Fig. 3. (A) Chromatogram of the benzo[a]pyrene (5 µg/L) standard solution; (B) Chromatogram of the camellia oil samples (Black line) with 2 µg/L benzo[a]pyrene standard solution (Grey line)

optimized method to olive oils, peanut oils, and maize oils, and the results indicated that the method also adapted to other edible oils.

In this study, vortex extraction using the UPLC-FLD method was performed for the determination of benzo[a]pyrene in camellia oil. The sample pretreatment process described herein offers a faster and milder alternative to the traditional methods. This method may be employed in routine analysis to monitor benzo[a]pyrene in camellia oils because it enables the achievement of high analytical frequency and less reagent consumption. Refined and virgin camellia oils were analyzed herein using UPLC-FLD. The content of benzo[a]pyrene in virgin camellia oil was higher than that in refined camellia oil.

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Disclosure The authors declare no conflict of interest.

Table 2. Content of benzo[a]pyrene in refined and virgin camellia oils

	Sample No.	Content (µg/kg)
Refined camellia oils	Sample 1	1.12±0.02
	Sample 2	1.51±0.01
	Sample 3	ND ¹⁾
	Sample 4	ND
	Sample 5	0.82±0.21
	Sample 6	ND
	Sample 7	2.01±0.03
	Sample 8	ND
Virgin camellia oils	Sample 9	3.20±0.04
	Sample 10	4.51±0.08
	Sample 11	2.83±0.01
	Sample 12	2.32±0.03
	Sample 13	1.52±0.13
	Sample 14	2.05±0.06
Virgin olive oils	Sample 15	ND
	Sample 16	ND
Refined peanut oils	Sample 17	ND
	Sample 18	ND
Refined maize oils	Sample 19	ND
	Sample 20	ND

¹⁾ND, not detected, LOD=0.2 µg/kg

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