Determination of Benzo[a]pyrene in Edible Oil by High Performance Liquid Chromatography-Fluorescence Detector (HPLC-FL)

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Abstract In this study, an optimized high performance liquid chromatography-fluorescence detector (HPLC-FL) method for the determination of benzo[a]pyrene in edible oil was established. HPLC was performed with Thermo Fisher Scientific C18 column (250 mm×4.6 mm, 5 μm) as the chromatographic column and acetonitrile and water as the mobile phase, and the excitation wavelength and emission wavelength of fluorescence detector were 286 and 430 nm, respectively. The response was high, and the linear range was 0.5-10.0 ng/ml. The lowest limit of detection was 0.11 ng/ml, and the average recovery was 92.5%. This method is suitable for quantitative analysis of benzo[a]pyrene content in edible oil.

Key words Benzo [a] pyrene; High performance liquid chromatography; Fluorescence detector

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Benzopyrene is a kind of polycyclic aromatic hydrocarbon[1] formed by the fusion of benzene and pyrene, abbreviated as BaP. According to the different positions of fusion, there can be two isomers of benzo[a]pyrene and benzo[e]pyrene, which are derived from the numbering of chemical bonds in pyrene molecular ring in IUPAC nomenclature. The most common benzopyrene is benzo[a] pyrene. Benzo[a]pyrene is a kind of organic compound with obvious carcinogenic effect. It is a polycyclic aromatic hydrocarbon compound composed of a benzene ring and a pyrene molecule. At present, more than 400 main carcinogens have been detected, more than half of which belong to polycyclic aromatic hydrocarbons. Among them, benzo[a] pyrene is a strong carcinogen. Smoking smoke, high-temperature vegetable oil that has been used many times, burnt food and fried food will all produce benzopyrene[2-5]. GB 2762-2022 stipulates that the limit of benzo[a]pyrene in edible oil is 10 μg/kg. In this study, benzo[a] pyrene[6-8] in edible oil was extracted by solid phase extraction, and the content of benzo[a]pyrene in edible oil was quantitatively determined by high performance liquid chromatography-fluorescence detector (HPLC-FL) under optimized excitation wavelength and emission wavelength.

Materials and Methods

Materials and Instruments

Benzo[a]pyrene standard (Tianjin Alta Scientific Co., Ltd.); edible oil (commercially available); acetonitrile (chromatographically pure); dichloromethane (chromatographically pure); N-hexane (chromatographic purity); experimental water, ultrapure water; SPE column for MIP-BAP benzo[a]pyrene (Anpel Laboratory Technologies (Shanghai) Inc.); ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); nitrogen Blower (Guangzhou Detelogy Technology Co., Ltd.); electronic balance (Shunyu Hengping); Vanquish Core liquid chromatography-fluorescence detector (Thermo Fisher Scientific).

Experimental methods

Selection of chromatographic conditions Chromatographic column: Thermo Fisher Scientific C18 column (250 mm×4.6 mm, 5 μm); mobile phase: acetonitrile : water = 88 : 12; injection volume 10 μl; flow rate 1.0 ml/min; fluorescence detector: excitation wavelength 286 nm and emission wavelength 430 nm.

Standard solution preparation First, 1 ml of benzo[a]pyrene standard (100 g/ml) was accurately transferred, dissolved in acetonitrile, and diluted to constant volume in a 10 ml volumetric flask, and the solution obtained after shaking well was used as a standard reserve solution. Next, the above solution was accurately transferred and diluted with acetonitrile to prepare 0.5, 1.0, 2.5, 5 and 10 ng/ml standard series points.

Sample preparation First, 1 g of sample was weighed, and added with 12 ml of n-hexane. After vortex-mixing evenly, the solution was loaded to a column for purification. An activated BAP solid-phase extraction column was adopted for the purification, and the purified liquid was collected, and blown dry with nitrogen. Next, 1 ml of acetonitrile was accurately transferred, and after vortex-mixing evenly, and ultrasonic treatment was performed in an ultrasonic cleaner to ensure that the reconstitution solution was completely dissolved. Finally, the reconstitution solution was filtered with a microfiltration membrane for testing.

Results and Analysis

Selection of excitation wavelength and emission wavelength of fluorescence detector

In this study, two groups of excitation wavelengths and

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emission wavelengths were selected as the fluorescence detection conditions for comparison, and the standard point response value at the same concentration of benzo[a]pyrene (1.0 ng/ml) was taken as the index. The results showed that the response of benzo[a]pyrene with excitation wavelength of 286 nm and emission wavelength of 430 nm was obviously better than that with excitation wavelength of 384 nm and emission wavelength of 406 nm, as shown in Fig. 1. The detection limit, linear relationship, precision, repeatability and recovery were investigated at the selected wavelength.

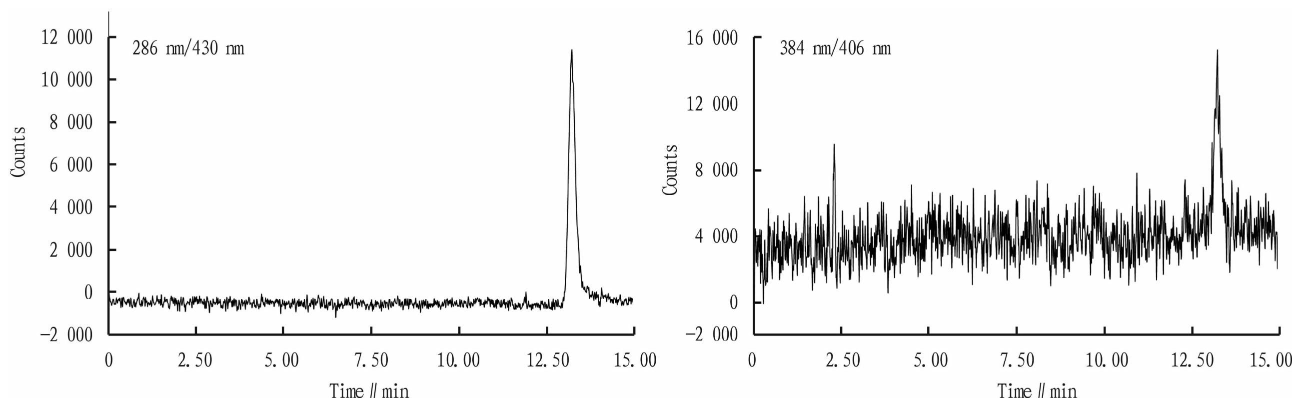

Fig. 1 Comparison of response values of benzo[a]pyrene under two groups of wavelengths conditions

Determination of detection limit

With three times of the signal-to-noise ratio (S/N) as the standard, the corresponding concentration was the detection limit. Accordingly, the detection limit was 0.11 ng/ml.

Investigation of linear relationship

A standard curve with peak area as the ordinate and concentration as the abscissa was drawn, as shown in Fig. 2. The regression equation was obtained as $y = 3\ 853.7x - 877.69$, $R^2 = 0.999\ 6$, and the linear relationship was good in the range of 0.5 - 10.0 ng/ml.

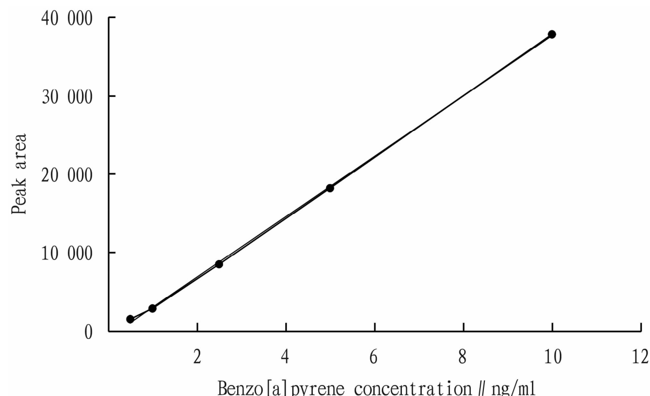

Fig. 2 Benzo[a]pyrene standard curve

Precision test

The standard solution (10 ng/ml) was selected for six times of repeated injection, and the peak areas of chromatograms were recorded (37 031; 37 265; 38 014; 37 047; 37 383; 37 472). The average value (37 369) and relative standard deviation were calculated (1.0%). The relative standard deviation of this precision test was 1.0%, indicating that the precision of benzopyrene determination was good.

Reproducibility test

For the same edible oil sample, the extraction and determination were repeated for six times. The determination results were 0.589, 0.564, 0.576, 0.556, 0.576, and 0.566, with an average value of 0.571 μg/kg, and the relative standard deviation was 2.0%, indicating that this method had good reproducibility in determining the content of benzopyrene in edible oil.

Recovery test

The sample was accurately weighed for six times, and benzopyrene was accurately added at an amount of 0.5 μg/kg. The solution to be detected was obtained according to the above sample extraction process, and quantified by the external standard method. The results are shown in Table 1.

Table 1 Determination results of recovery

No.	Background value of sample μg/kg	Addition amount of standard μg/kg	Determined results μg/kg	Recovery %	Average recovery %
1	0.571	0.5	1.041	94.0	92.5
2			1.068	99.4	
3			1.042	94.2	
4			1.025	90.8	
5			1.014	88.6	
6			1.012	88.2	

Table 1 shows that the recovery values of this test were in the range of 88.2% - 99.4%, and the average recovery was 92.5%. It indicated that this method had good accuracy in detecting benzopyrene content in edible oil.

Conclusions

In this study, the detection limit, linear relationship, precision, reproducibility and recovery were investigated under the optimized wavelength conditions, and the results were good. It shows that this method is suitable for the determination of benzopyrene in edible oil.

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Conclusions

Chicken muscle growth is important traits to evaluate the production of poultry meat. miRNAs play a vital role in the growth and development[13-14].

The calpain system controls the degradation of myofibrillar proteins. Myofibers are the basic units that make up muscles, and the state of myofibers will directly affect the quality of chicken meat. In this study, we found 128 DEGs that probably regulated tenderness trait were selected from 16 significantly enriched GO terms and 13 significantly enriched pathways.

The expression changes of the *CAPN*1 and *CAST* genes in the pectoral and leg muscle tissues mainly manifest in the degradation of muscle proteins and the growth and development changes of myofibers. It is known that the development of the comb can directly reflect the sexual maturity of chickens. The early-maturing MC chicken has a small body weight. Therefore, comb weight can also reflect the growth and development of chickens to a certain extent. Meanwhile, this study found that the developmental changes in the expression levels of the *CAPN*1 gene in the pectoral and leg muscles were significantly positively correlated ($P < 0.05$) with the cumulative growth values of live weight and comb weight. The developmental changes in the expression levels of the *CAST* gene in the pectoral and leg muscles were not significantly correlated with the cumulative growth values of live weight and comb weight.

In summary, it is hypothesized that the expression level of the *CAPN*1 gene in muscle tissues may be closely related to the growth and development of myofibers and plays an important regulatory role in the metabolism of myofibrillar proteins. The research on *CAST* genes as candidate genes for meat quality has just begun. In general, the specific regulatory mechanism of the calpain system is not very clear and requires further in-depth research.

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