Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(10):409-414

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Rapid assay of polycyclic aromatic hydrocarbons in edible oils by HPLC with FLD detection without clean-up

Ye YU[1,2,3], Qinze Jin[1,2] and Xing Guo Wang[1,2]*

[1]*School of Food, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu, PR China*
[2]*State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu, PR China*
[3]*Food Inspection Authority of Zhangjiagang Entry-Exit Inspection and Quarantine Bureau Zhangjiagang, PR China*

ABSTRACT

This study describes a simple and rapid analytical method for the determination and quantification of polycyclic aromatic hydrocarbons (PAHs) in edible oils. After dissolution in tetrahydrofuran, edible oil samples were detected by reverse-phase high-performance liquid chromatography (HPLC). PAHs had a linear response at 0.5–50 μg/L with correlation coefficients >0.999. The PAH recoveries were 87.6–98.7%. The detection limits and quantification limits were 0.07–0.61 μg/kg and 0.23–2.04 μg/kg, respectively. The developed method was used for the determination of PAH content in 116 edible oils. Compared to the traditional method, this HPLC-based method is fast and reduces solvent waste. The recoveries of low molecular mass PAHs were significantly improved without any evaporation or concentration steps.

Keywords: HPLC, polycyclic aromatic hydrocarbons, edible oils, FLD detection

---

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) comprise a large group of organic compounds with two or more fused aromatic rings. Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in the environment; some of the PAHs proved to be mutagenic, carcinogenic and teratogenic, and are listed as priority pollutants in the U.S. EPA and the European Community environmental regulations. PAHs are lipid-soluble and can be absorbed via the lungs, gut, and skin. PAHs are converted to dihydrodiols by the hepatic enzyme hydrocarbon hydroxylase. Dihydrodiols and their epoxide derivatives, which bind to DNA and protein molecules, are highly mutagenic [1, 2]. Exposure to PAHs is a major concern for human health; individuals who have been exposed to PAHs for long periods of time have developed cancer.

In certain European countries, e.g., Germany, Austria, and Poland, the maximum permitted levels of benzo (a) and pyrine (BaP) is 1 ppb in smoked foods [3]. In China, the maximum permitted levels of BaP is 10 ppb in edible oils; however, there is no maximum limit of PAHs in oils and fats [4]. The German Society for Fat Science has set maximum limits of 25 ppb for total PAHs and 5 ppb for heavy PAHs [5–7].

There are several PAH determination methods, which rely on different extraction, purification, and detection techniques [7–10]. However, these methods involve time-consuming and complex extraction and clean-up procedures. Liquid–liquid extraction (LLE)[7], caffeine complexation[8] and saponification [19]are mostly used for extracting PAHs from the oil. The three procedures are very useful, but they are tedious, time-consuming and requires large amounts of toxic and flammable solvents. SPE is a commonly used technique, but it also has some disadvantages, such as particle blockage and slow sample processing rate. Light PAHs are often lost during the evaporation and

concentration step [10]. Cloud point extraction(CPE) is another technique that has been developed for extraction of PAHs from oil samples. Despite many benefits of using CPE, the main disadvantage is not compatibility of extraction phase with instrumental analysis such as HPLC–FLD method. A fast, simple, and accurate method would greatly facilitate the determination of PAHs in edible oils.

In this study, we developed an HPLC method for the determination of PAHs in edible oils using direct solvent dissolution and fluorescent detection. Several analytical parameters, including sensitivity, linearity, accuracy, precision, and limits of detection (LODs), were assessed. Due to its fluorescence, PAHs can be accurately quantified with a fluorimetric detector avoiding any interferences from complex components present in edible oils. The HPLC-based method developed in this study can be used to quantify PAHs in most edible oils. Compared to the traditional method, this method is fast and reduces solvent waste.

EXPERIMENTAL SECTION

Materials and Reagents

A poly-nuclear aromatic hydrocarbon mixture from (O2SI smart solutions. Charleston,USA) was used for the identification and quantification of PAHs. The hydrocarbon mixture consisted of 200 µg/mL of naphthalene (Na), 200 µg/mL of acenaphthene (Ace), 200 µg/mL of fluorene (F), 200 µg/mL of phenanthrene (Phe), 200 µg/mL of anthracene (Ant), 200 µg/mL of fluoranthene (Flu), 200 µg/mL of pyrene (Pyr), 200 µg/mL of benz[*a*]anthracene (BaA), 200 µg/mL of chrysene (Chr), 200 µg/mL of benzo[*b*]fluoranthene (BbF), 200 µg/mL of benzo[*k*]fluoranthene (BkF), and 200 µg/mL of benzo[*a*]pyrene (BaP). This hydrocarbon mixture was stored at -20°C in the darkness to avoid volatilization and photodegradation. Standard solutions (200 µg/L) were prepared by diluting the hydrocarbon mixture with tetrahydrofuran and acetonitrile (1:1, v/v). HPLC-grade acetonitrile and tetrahydrofuran were purchased from Fisher (Fisher Scientific, USA). Purified water was used throughout the experiments (Milli-Q Ultrapure Water Purification System, Millipore, Bedford, MA, USA).

Instruments

The following instruments were used during sample preparation: a vortex mixer (WH-866, Huamei Corporation,Wuxi, China), an electronic balance (AL 204, Mettler Toledo, Shanghai ,China), and Supelco nylon SCAA-104 (13 mm × 0.22 µm) membrane filters (ANPEL Scientific Instrument Co. Ltd., Shanghai ,China).

Samples

Different brands of vegetable oils (crude and refined coconut oil, sunflower oil, olive oil, soybean oil, grape-kernel oil, rapeseed oil, peanut oil, and sesame oil) were purchased from local supermarkets in 2012.

Oil Sample Preparation

Aliquots (0.5 g) of vegetable oils were dissolved in tetrahydrofuran (1 mL) and diluted to 2 mL with tetrahydrofuran:acetonitrile (1:1, v/v). The solutions were filtered and injected into an HPLC system.

PAHs in Organic Solvents

In this experiment, 10 mL of organic solvents (methanol, ethanol, hexane, ethyl acetate, pentane, and cyclohexane) were drawn in a 15 mL-centrifuge tube. The residues were dissolved in 1 mL of tetrahydrofuran:acetonitrile (1:1, v/v) under a constant stream of nitrogen gas. The solution was filtered through a 0.45 µm-membrane and injected into an HPLC system.

HPLC Conditions

Selected analytes were separated with an HPLC system (Agilent series 1200, Agilent Technologies, Santa Clara, CA), which consisted of a vacuum degasser, autosampler, column thermostat, binary pump, diode array, and fluorescence detector. The HPLC system was equipped with a reverse phase C18 column (250 mm × 4.6 mm, 5 µm particle size, SupelcosilTM LC-PAH). The column temperature was maintained at 25°C. The injection volume was 20 µL and the flow rate was 1 mL/min. The mobile phase consisted of solvents A (acetonitrile) and B (water). The elution conditions consisted of 50% A for 0–5 min; 50–100% A for 5–30 min; and 100% A for 30–50 min. The column was re-equilibrated for 10 min between injections. Peaks were identified by comparing their retention times with those of PAH standards. A PAH standard plot was used for PAH quantification.

RESULTS AND DISCUSSION

Optimized Detection Wavelengths

Excitation and emission wavelengths were scanned. Considering that changes in detection wavelengths during the elution gradient might cause baseline drifts, the same detection wavelengths were used for analytes whose peak times

were similar to ensure accuracy in the measurements. Optimized detection wavelengths were 0–13.5 min of 212 and 336 nm; 13.5–18 min of 260 and 336 nm; 18.0–19.6 min of 230 and 420 nm; and 26.0–50.0 min of 260 and 420 nm. The 12 PAHs studied were identified and quantified by comparing their retention times with those of PAH standards. The chromatogram of a 5 µg/L standard mixture is shown in Figure 1.

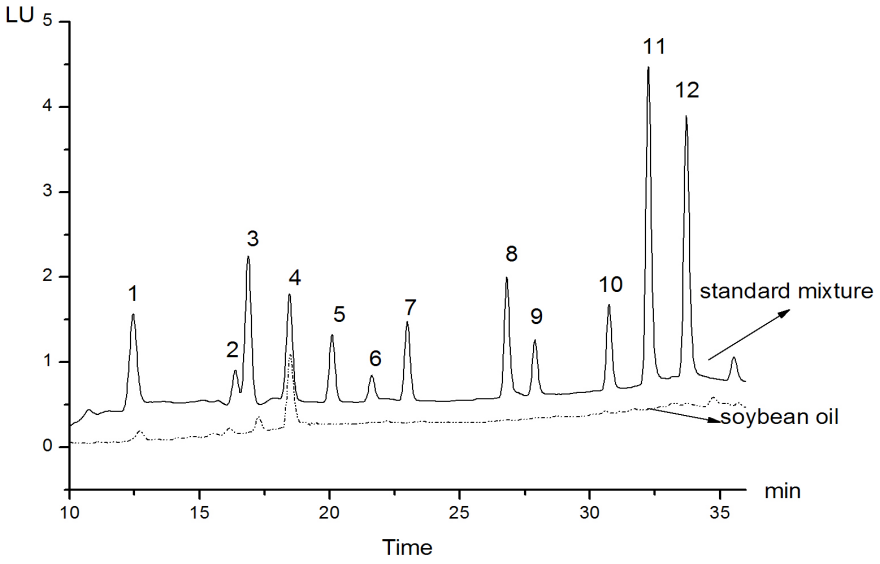

Fig.3. Chromatogram of CRM 459 and of CRM 459 spiked with 2 µg/kg of PAH (equivalent to the 0.5 µg/L PAH standard). 1, Na; 2, Ace; 3, F; 4, Phe; 5, Ant; 6, Flu; 7, Pyr; 8, BaA; 9, Chr; 10, BbF; 11, BkF;12, BaP

PAH Interference from Solvent

Oils have PAH content in the µg/kg level (trace level); therefore, any interference from solvents should be minimized. Solvent interferences arise during oil pre-treatment. The PAH content in commonly used solvents are shown in the Table 1.

Table 1. PAH content in commonly used solvents

	PAHs[µg/L]												
Solvents	Na	Ace	F	Phe	Ant	Flu	Pyr	BaA	Chy	BbF	BkF	BaP	Total PAHs
Methanol	1.96	---	0.21	0.69	0.05	0.09	0.04	---	0.02	0.01	0.01	0.01	3.09
Tetrahydrofuran	0.44	---	0.05	0.17	0.01	0.04	0.07	---	---	0.01	0.01	0.01	0.81
Acetonitrile	1.60	---	0.16	0.48	0.05	0.05	0.03	---	0.01	0.01	0.01	0.01	2.41
Dichloromethane	1.20	---	0.17	0.45	0.07	0.05	0.06	0.2	0.04	0.03	0.01	0.03	2.31
Petroleum	1.88	0.03	0.48	1.57	0.09	0.02	0.12	0.04	0.03	0.01	0.10	0.15	4.52
n-hexane	0.98	---	0.14	0.40	0.05	0.07	0.02	---	---	---	0.01	0.01	1.68
ethyl acetate	0.77	---	0.08	0.23	0.07	0.06	0.03	---	---	---	---	0.01	1.25
cyclohexane	10.18	---	0.84	6.21	2.39	3.83	3.07	1.55	1.21	0.8	0.43	1.83	32.34
Pentane	1.76	---	0.48	1.92	0.18	0.36	0.21	---	---	---	---	---	4.91
Acetone	5.66	---	1.08	0.49	1.94	0.67	0.50	---	---	---	---	---	10.34
Isopropanol	6.51	0.30	0.60	1.35	---	0.16	0.14	---	---	---	---	---	9.06

Different PAHs were found in the solvents. Cyclohexane had the highest PAH content (32.34 µg/kg) whereas tetrahydrofuran had the lowest PAH content (0.81 µg/kg). The detected PAHs were mainly light PAHs such as Na, F, Phe, and Pyr because most organic solvents were distilled by cracking and fractionating crude petroleum. The polarities of certain PAHs were similar to those of the organic solvents. Sometimes azeotropes are formed between organic solvents, which are very difficult to remove and thus affect PAH determination. To remove triglycerides present in oil and fat, 50–100mL of organic solvent was used during oil pre-treatment (SPE, liquid-liquid extraction, LC-LC extraction). After a series of extraction, purification, and concentration steps, the solvent is concentrated, thereby affecting PAH determination. The developed method, which relies on the direct dissolution of edible oils, can greatly reduce interferences from organic solvents.

Tetrahydrofuran, acetone, and isopropanol are efficient at dissolving oils and can also be used for the liquid phase. Tetrahydrofuran has been used to dissolve edible oils for HPLC analysis because it has the lowest PAH content.

Interference by Vitamin E

Oils are rich sources of vitamin E. Vitamin E, which has strong fluorescent properties, affects PAH determination. Figure 2 shows the chromatogram of α-vitamin E, β-vitamin E, γ-vitamin E, and δ-vitamin E. The polarity of vitamin E is weaker than that of PAHs. As a result, vitamin E elutes later than PAHs in a reverse phase column. The four vitamin E forms eluted at 40–45 min; PAHs eluted before 36 min. Consequently, vitamin E does not interfere with PAH determination.

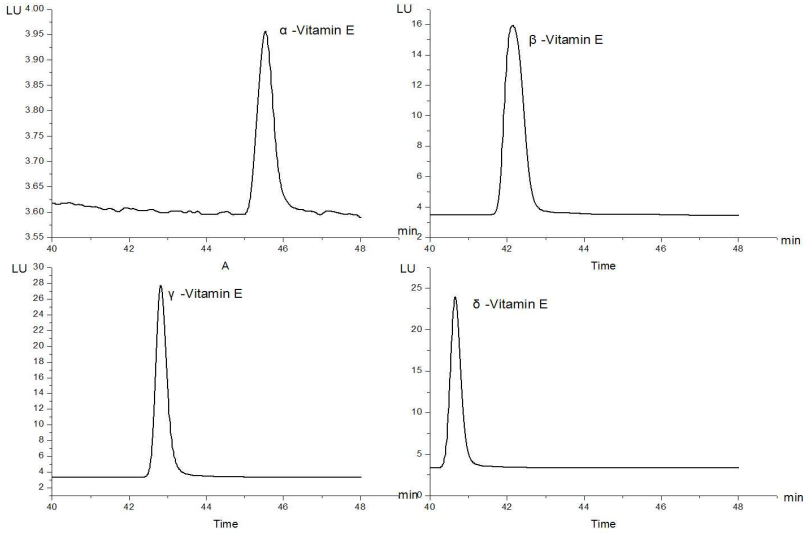

Fig.2. chromatogram of Vitamin E

Validation Study

Calibration, LOD, and Limit of Quantification

Calibration was performed using standard PAH mixtures at six different concentrations: 0.5, 1, 2, 5, 10, 20, and 50 μg/L（conversion to the samples of 2, 4, 8, 20, 40 and 200μg /kg）for all 12 PAHs. The linearity of the calibration curves was assessed by the peak area. Good linearity was observed in the concentration range studied with correlation coefficients >0.999.

Detection and quantification limits were calculated from the concentrations corresponding to 3× and 10×, respectively, of the standard deviation of the blank noise. The results are shown in Table 2.

The limits of quantification were <1μg/kg for most PAHs. The concentrations of PAHs in the oil samples were within the linear range, except for the crude coconut oil and virgin olive oil, which had high PAH contents; therefore, it was necessary to dilute these samples.

Table 2. **Limit of quantification, working linear range, and precision of the developed method**

PAHs	Limit of detection [μg/kg]	Limit of quantification [μg/kg]	Linear range [μg/kg]	Intra-day precision%	Inter-day precision%
Na	0.12	0.41	2-200	3.26	2.12
Ace	0.38	1.27	2-200	5.14	4.00
F	0.11	0.36	2-200	0.75	3.90
Phe	0.14	0.47	2-200	1.20	1.93
Ant	0.26	0.87	2-200	6.61	10.40
Flu	0.61	2.04	2-200	7.69	5.44
Pyr	0.24	0.79	2-200	7.51	11.59
BaA	0.15	0.50	2-200	8.91	7.76
Chr	0.27	0.90	2-200	8.96	5.84
BbF	0.20	0.66	2-200	4.25	4.62
BkF	0.07	0.24	2-200	1.49	3.14
BaP	0.07	0.23	2-200	2.97	6.02

Intra-day precision of a 0.5 μg/L standard solution (n = 6); inter-day precision of a 0.5 μg/L standard solution (n = 6). Linear range of 2–200 μg/kg (equivalent to the 0.5-50 μg/L standard)

The precision of the assay was assessed during three days by intra- and inter-day analysis of six replicates of a 0.5 μg/L standard solution. The results are shown in Table 2. The intra-day precision was 0.75–8.96% and the inter-day precision was 1.93–11.59%. The results revealed that the developed method had good reproducibility and precision. Additionally, the results revealed that the method was satisfactory for the determination of PAHs at the μg/L level [17]. These results were similar to those obtained by other researchers.

Recovery and Precision

Recovery experiments were carried out by spiking edible oils with three PAH concentrations (0.5, 2.5, and 5 μg/L); the PAH content in the oils were 2, 10, and 20 μg/kg, respectively. The spiked samples and blank were analyzed in duplicate. Figure 3 shows the chromatogram obtained from the blank spiked with 2 μg/kg of PAH.

Recoveries were calculated from the differences in the PAH content between the spiked samples and the blank. The recovery results are shown in Table 3.

Table 3. Recovery of the method

PAHs	Recoveries [%]		
	Fortified at 2 μg/kg	Fortified at 10 μg/kg	Fortified at 20 μg/kg
Na	---	---	90.7
Ace	90.5	95.4	93.6
F	90.6	93.6	94.0
Phe	93.8	95.2	89.6
Ant	88.9	88.6	92.6
Flu	87.6	91.5	92.5
Pyr	94.3	97.4	88.8
BaA	93.8	94.0	93.0
Chr	95.7	97.3	92.4
BbF	90.2	96.2	95.7
BkF	96.5	89.8	97.6
BaP	98.7	94.5	98.4

The recoveries were 87.6–98.7%. The recoveries of low molecular mass PAHs (e.g., Na, Ace, F, Phe, and Ant) were 88.6–95.2%, which were higher than those obtained by other analytical methods [14, 16]. The recoveries of these PAHs were higher because there is no evaporation or concentration steps in the developed HPLC-based method.

Table 4. PAH content (μg/kg) in different edible oils

oil sample	Na	Ace	F	Phe	Ant	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	Total PAHs	light PAHs	heavy PAHs
peanut oil	6.56	nd	4.52	32.08	3.48	20.28	22.32	13.36	3.08	4.96	2.72	4.92	118.28	105.68	12.60
peanut oil	3.96	nd	nd	3.60	nd	nd	4.32	nd	4.40	nd	nd	0.60	16.88	16.28	0.60
cotton oil	5.04	0.84	nd	10.32	1.64	3.24	5.72	1.52	1.20	1.40	nd	1.04	31.96	29.52	2.44
cotton oil	4.20	nd	nd	4.12	nd	nd	2.20	nd	nd	nd	nd	5.76	16.28	10.52	5.76
cotton oil	0.76	nd	nd	3.48	nd	nd	nd	1.68	nd	nd	nd	2.92	8.84	5.92	2.92
virgin olive oil	386.92	nd	2.52	16.16	nd	nd	nd	nd	nd	nd	nd	nd	405.60	405.60	nd
virgin olive oil	125.32	nd	0.68	11.88	nd	nd	2.80	1.96	nd	nd	nd	nd	142.64	142.64	nd
virgin olive oil	4.44	nd	nd	8.64	nd	3.36	2.80	nd	nd	nd	nd	nd	19.24	19.24	nd
virgin olive oil	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
virgin olive oil	40.48	nd	18.24	85.24	4.04	13.24	7.56	2.80	nd	1.08	1.68	0.28	174.64	171.60	3.04
rapeseed oil	4.84	nd	nd	4.84	nd	nd	5.88	nd	nd	nd	nd	2.16	17.72	15.56	2.16
rapeseed oil	nd	nd	nd	3.12	nd	10.52	1.28	nd	nd	nd	nd	nd	14.92	14.92	nd
rapeseed oil	nd	nd	nd	6.24	nd	nd	1.72	0.36	nd	nd	nd	nd	8.32	8.32	nd
rapeseed oil	nd	nd	nd	2.00	nd	nd	3.20	2.40	nd	1.48	0.32	1.56	10.96	7.60	3.36
sunflower oil	nd	nd	nd	4.24	nd	nd	1.28	1.04	1.40	nd	nd	nd	7.96	7.96	nd
sunflower oil	1.36	nd	nd	2.36	nd	nd	1.48	2.60	nd	1.12	1.60	0.84	11.36	7.80	3.56
sunflower oil	2.56	1.44	nd	5.00	4.68	nd	2.44	1.56	nd	1.64	3.48	1.72	24.52	17.68	6.84
sesame oil	nd	8.16	nd	17.00	nd	nd	11.00	nd	3.52	3.08	1.20	1.56	45.52	39.68	5.84
sesame oil	nd	nd	92.72	206.88	nd	61.08	131.40	126.96	nd	nd	8.28	11.32	638.64	619.04	19.60
sesame oil	nd	nd	nd	23.32	nd	8.32	33.16	1.44	18.44	3.04	0.72	3.32	91.76	84.68	7.08
safflower oil	1.28	18.60	nd	2.48	7.00	nd	1.36	nd	nd	nd	nd	nd	30.72	30.72	nd
safflower oil	5.52	nd	nd	7.80	nd	10.72	11.60	10.16	4.96	13.16	nd	15.56	79.48	50.76	28.72
grape-kernel oil	0.96	1.88	nd	2.12	nd	nd	nd	nd	nd	1.48	nd	nd	8.28	4.96	1.48
grape-kernel oil	nd	nd	nd	1.36	nd	4.20	nd	nd	0.92	nd	0.44	nd	6.92	6.48	0.44
rice bran oil	3.88	1.44	nd	7.52	nd	nd	nd	nd	1.84	nd	3.40	0.96	19.04	14.68	4.36
linseed oil	25.08	nd	3.92	29.32	2.72	19.00	15.84	nd	nd	nd	1.32	2.92	100.12	95.88	4.24
fish oil	16.08	nd	11.04	10.40	1.12	4.72	5.96	1.84	nd	nd	0.44	2.04	53.20	51.16	2.04
camellia japonica seed oil	2.84	nd	nd	1.00	nd	nd	nd	nd	nd	nd	nd	0.56	4.40	3.84	0.56

nd:not detected

Sample Determination

The developed method was used to determine the PAH content in 29 edible oils to assess its effectiveness. The results were shown in Table 4. Different PAHs were detected in the edible oils.

The results revealed that 3.44% of the oils were devoid of PAH, 96.56% of the oils contained different types of PAHs, and 72.41% of the oils contained heavy PAHs. The oils with a total PAH content above 25 μg/kg were 41.38% and heavy PAHs exceed 5μg/kg were 24.14%.

CONCLUSION

In this study, a simple and rapid method for the determination of PAHs in edible oils was described. Samples were dissolved in solvents and separated by HPLC. PAHs were detected by fluorescence. Compared to traditional methods, the developed HPLC-based method significantly reduces the amount of solvent used. Furthermore, the method is fast: the developed method requires <60 min whereas traditional methods require 10 h.

The developed method exhibits excellent precision and sensitivity and satisfactory recoveries. The method improved recoveries of PAHs, particularly the lighter PAHs. The recoveries of PAHs at the tested concentrations were 87.6–98.7%.The PAHs showed a linear response at 0.5–50 μg/L with correlation coefficients >0.999. All of these features make this method suitable for routine PAH determination and analysis.

The developed method has been used for PAH determination in vegetable oils. Rapeseed oil, peanut oil, olive oil, cotton oil, sesame oil, and safflower oil contained variable levels of total PAHs. Similar to the findings of Balenoic et al. [11] and Pandey M.K et al. [18], some oil samples had a total PAH content >25 ppb. PAHs pollution in edible oils become a common problem with the development of industrial production, we must take some necessary measures to control its further generation and deterioration.

Acknowledgements

This study was supported by the Research Funds for Jiangsu Entry-Exit Inspection & Quarantine Bureau (grant No. 2012KJ44) and the National Key Technology R&D Program in the 12th Five-Year Plan of China (grant No. 2012BAK08B03). The manuscript has been edited by an international scientific editing company. We are grateful to the staff of the Technology Center of Zhangjiagang Entry-Exit Inspection & Quarantine Bureau of P.R. China for their technical assistance.

REFERENCES

[1] Bartle, K.;C. Creaser.;R. Purchase. Food contaminants: sources and surveillance,**1991**.
[2] Larsen, J.;E. Poulsen.Toxicological aspects of food. London: Elsevier Applied Science,**1987**.
[3] Bories, G. Tossicità degli idrocarburi policiclici aromatici e dei prodotti di pirolisi. *Tossicologia e Sicurezza degli Alimenti*,**1988**,359-372.
[4] Hygienic standard fore eible vegetable oil. China Standard Press, **2005**.
[5] Gertz, C. ;H. Kogelheide . *Fett Wissenschaft Technologie*. **1994**,96(5),175-179.
[6] Swetman, T.;S. Head; D. Evans.Inform-International News on Fats Oils and Related Materials. **1999,**10(7),06-712.
[7] Speer K;Steeg E;Horstmann P et al.*hrc-journal of high resolution chromatography*, **1990**, 13(2), 104-111.
[8] Sagredos A N;Sinha-Roy D;Thomas A. *Fett wiss. Technol.*, **1988**, 90,76-81.
[9] Menichini E; Bocca A; Merli F. et al. *Food Addit Contam A*, **1991**,8(3),363-369.
[10] Moret, S.;L.S. Conte. *hrc-journal of high resolution chromatography*, 1998,21(4),253-257.
[11] Balenovic, J.; I. Petrovic; M. Perkovac.in Proceedings of the European Conference on Food Chemistry. **1995**.
[12] Bartle, K.D.; M.L. Lee; S.A. Wise. *chemical society reviews*, **1981**,10(1),113-158.
[13] Stijve, T.;H. Diserens.*Deut Lebensm-Rundsch*.,**1987**, 83(6),183-185.
[14] Yusty L; Cortizo Daviña J L. *Food Control*, **2005**, 16(1): 59-64.
[15] Ballesteros, E., García Sánchez, A., & Ramos Martos, N.. *Journal of Chromatography A*, **2006**,1111(1), 89-96.
[16] Arens, M.; C.R. Gertz, *Lipid/Fett*, **1996**. 98(6),216-220.
[17] Horwitz, W., Kamps, L. R., & Boyer, K. W.. *Journal of Association of Official Analytical Chemists*, **1980**,63(6), 1344.
[18] Pandey, M. K., Mishra, K. K., Khanna, S. K., & Das, M.. *Journal of the American Oil Chemists' Society*, **2004**,81(12), 1131-1136.