Food Chemistry 275 (2019) 390–396

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Quantification of aflatoxin B_1 in vegetable oils using low temperature clean-up followed by immuno-magnetic solid phase extraction

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ARTICLE INFO

Keywords:
Vegetable oil
Food hazard
Aflatoxin
Low temperature cleanup
Nanoparticle
Immuno magnetic solid phase extraction
Fluorometric analysis
Rapid detection

ABSTRACT

In the present study, a convenient and highly efficient method was developed to quantify aflatoxin B_1 (AFB_1) in oil samples. Low temperature clean-up (LTC) followed by immuno magnetic solid phase extraction (IMSPE) was used to clean up oil samples. LTC assisted in freezing out the interference from the oil matrix while IMSPE further helped to preconcentrate the targeted analyte. For IMSPE, we synthesised and characterised anti-AFB_1 monoclonal antibody-functionalised magnetic nanoparticles. Oil sample extraction was then carried out using LTC-IMSPE. The proposed method showed satisfactory efficiency and reproducibility with recovery rates being within the range of 79.6–117.9%, with a relative standard deviation below 11.48%. The sensitivity of the method was satisfactory with the limits of detection and quantification being as low as 0.0048 and 0.0126 $ng \cdot g^{-1}$, respectively. Real sample analysis was carried out for five kinds of different vegetable oils. The results suggest that the method developed is very sensitive and accurate.

1. Introduction

Aflatoxins are secondary metabolites predominately produced by fungi such as *Aspergillus parasiticus* and *A. flavus*, which widely contaminate agricultural produce (McCullum, Tchounwou, Ding, Liao, & Liu, 2014). These aflatoxins have potent carcinogenic, teratogenic, mutagenic, immunosuppressive and estrogenic effects, making them harmful to humans if consumed (Delmulle, De Saeger, Sibanda, Barna-Vetro, & Van Peteghem, 2005). The International Agency for Research on Cancer classified aflatoxins in 1987, and later in 1993 classified aflatoxin B_1 (AFB_1) as a Group I carcinogen (Li, Zhang, & Zhang, 2009). Among the eighteen types of aflatoxins, AFB_1 is the most abundant and widespread in the world and accounts for 75% of aflatoxin contamination of food and feed (Ayub & Sachan, 1997). In the European Union, the maximum limit (ML) of 2 $\mu g \cdot kg^{-1}$ is set for AFB_1 in peanuts. In Japan, ML for AFB_1 is $\leq 10\ \mu g \cdot kg^{-1}$ in all foods. The ML for AFB_1 in foods is set at 5 $\mu g \cdot kg^{-1}$ in Singapore. Fungal growth is encouraged by a warm and humid environment, which suggests that crops grown in subtropical and tropical areas are more susceptible to AFB_1 contamination compared to cooler areas (Delmulle et al., 2005). In China, the ML for peanuts and peanut-derived products is at 20 $\mu g \cdot kg^{-1}$ (Ding, Li, Bai, & Zhou, 2012).

The occurrence of AFB_1 in sunflower seeds, cottonseeds, and soybeans is frequently reported. These oil seeds are used in the production of vegetable oils and because AFB_1 is lipophilic, it could be present in oils extracted from contaminated oil seeds (Wang et al., 2015). Processing steps during the production of these oils, such as manual selection and deacidification of the product, could be used to lower their AFB_1 contamination levels (Li et al., 2016). However, not all the AFB_1 can be removed. Around 10–20% of the AFB_1 could be carried over to the crude oil (Bordin, Sawada, Rodrigues, da Fonseca, & Oliveira, 2014). According to public reports, high incidence of AFB_1 contamination was observed for vegetable oils produced in regions of China, India, Sri Lanka and Sudan (Lim, Yoshinari, Layne, & Chan, 2015). The toxic and carcinogenic effect of AFB_1 has been verified by many studies, the analysis of AFB_1 in edible vegetable oils is highly necessary.

Food matrices are complex because of the existence of various nutritious components such as proteins, fats, minerals and carbohydrates (Bordin et al., 2014; Delmulle et al., 2005). Oil samples are especially notorious for being hard to deal with (Sinthusamran, Benjakul, & Kishimura, 2014). Their high viscosity and rich fat contents will

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https://doi.org/10.1016/j.foodchem.2018.09.132
Received 1 April 2018; Received in revised form 31 August 2018; Accepted 21 September 2018
Available online 22 September 2018
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inevitably cause severe interference during analysis (Yu, Ang, Yang, Zheng, & Zhang, 2017). Therefore, sample preparation techniques to get rid of the interference caused by fat play an important role in the determination of trace chemical hazards such as AFB_1 in oil samples. Various sample clean-up methods have been developed by analytical scientists, including liquid-liquid extraction (LLE), solid phase extraction (SPE), gel permeation chromatography, immuno chromatography, quick, easy, cheap, effective, rugged and safe (QuEChERS) methods, immune assay extraction and low temperature cleanup (LTC) (Wang et al., 2014; Xie et al., 2015; Zhao, Chen, Shen, & Qu, 2017).

These methods all have their own advantages and limitations. LLE is a traditional and easy method for preconcentration (Wang et al., 2014; Xie et al., 2015). However, it requires a large amount of organic solvent because of its low selectivity and is time consuming (Yu & Yang, 2017). The same issue of low selectivity also affects LTC, although it solves the problem of getting rid of fat interference in an easy way by simply using a freezer (Jiang, Li, Jiang, Li, & Pan, 2012; Payanan, Leepipatpiboon, & Varanusupakul, 2013). SPE and immune assays are becoming more and more popular because of their convenience and high selectivity; however, their long and tedious operation procedures affect their wider applicability (Xie et al., 2015; Yu et al., 2017). With the rapid development of nanotechnology, various nano materials grafted with different functional groups become available for analytical scientists. Among these nano-materials, magnetic nano-materials have become popular because of their highly manoeuvrable properties and special response to both magnetic fields and photo stimulation (Yu, Li, Ng, Yang, & Wang, 2018). Recently, immuno-magnetic solid phase extraction (IMSPE) has become popular because it combines the convenience and high selectivity of SPE and immune assays by functionalising of nano scale magnetic solid phase adsorbents with antibodies (Urusov, Petrakova, Vozniak, Zherdev, & Dzantiev, 2014). Moreover, through the addition of a magnetic core into the solid phase adsorbent particle, it enables the adsorbents to be separated from the complex sample matrix easily and rapidly, thus saving handling time during extraction (Yu et al., 2018).

A large array of detection methods for AFB_1 have also been developed, namely, high performance liquid chromatography (HPLC), enzyme-linked immuno-sorbent assays, thin layer chromatography, and lateral flow dipsticks among many others (Xie et al., 2015; Delmulle et al., 2005; Li et al., 2009). However, many of these methods are plagued by problems such as long analysis time, low sensitivity, need for high performance equipment, highly trained personnel, high cost, and harmful solvents. In recent years, research attention has shifted towards fluorescence spectroscopy (FL), which has a short analysis time, low cost, and high sensitivity (Wang et al., 2015). Removal of possible interferences during clean-up can contribute to lowering the background interference before FL analysis, thus improving the accuracy of the results. Furthermore, to enhance the FL signal, AFB_1 can be derivatised through an easy and fast chemical reaction with bromite before the fluorometric measurement.

In this study, we hypothesised that a method combining LTC with IMSPE followed by FL detection could be used to analyse trace AFB_1 in vegetable oils. Through the LTC process, the mass fat interference of the vegetable oil sample was removed. Thereafter, IMSPE helped to enhance the selectivity and efficiency of extraction using the specific interaction of antibody-antigen binding. The IMSPE was based on a conventional enzyme-linked immunosorbent assay (ELISA) method, and used immuno-magnetic adsorbents where an antibody was added to increase the extraction ability. Through the joint application of traditional LTC and modern IMSPE, the sensitivity and selectivity of the extraction process were enhanced, while the time and cost were reduced. LTC can be easily carried out using a domestic freezer, which greatly reduced the technical difficulty of the method (Delmulle et al., 2005; Yu et al., 2017). The final quantitative analysis via FL is rapid and accurate, thus making the method a good choice for on-site food safety analysis.

2. Materials and methods

2.1. Oil samples

Vegetable oil samples were obtained from Singapore local markets, including canola, soybean, corn, olive and peanut oils. One bottle of canola oil of a specific brand was determined to be free of any AFB_1 using a standard approach modified from Association of Official Analytical Chemists (AOAC) method. This oil was used for the preliminary tests, as well as to build a calibration curve (Li et al., 2014). Spiked samples with different concentrations of AFB_1 were prepared by adding a specific volume of 1 $\mu g \cdot g^{-1}$ AFB_1 standard stock solution into blank oil samples.

2.2. Chemicals and standards

AFB_1 antibodies (produced in rabbits, 100 mg in 1 mL of 0.01 M phosphate buffered saline, pH = 7.4), AFB_1 (produced from *Aspergillus flavus*), Iron (II) chloride tetra-hydrate ($FeCl_2{\cdot}4H_2O$), (3-aminopropyl) triethoxysilane (APTES), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), glutaric dialdehyde ($C_5H_8O_2$, 50% w/w in water), ammonia water ($NH_3{\cdot}H_2O$) and bromine reagent (99.5%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) pellets were purchased from Chemi-con (Singapore). Iron (III) chloride hexa-hydrate ($FeCl_3{\cdot}6H_2O$) was purchased from EMSURE®, Merck (Kenilworth, NJ, USA). Hydrochloric acid (HCl, 37%) was obtained from Schedeko (Singapore). HPLC grade acetonitrile, ethyl alcohol and methanol were purchased from VWR chemicals (Singapore). Phosphate buffered saline (PBS) at different pH values were prepared using NaH_2PO_4 and Na_2HPO_4 dissolved in deionised (DI) water. The bromine derivatisantion reagent was prepared using 1% bromine reagent in DI water, which were then further diluted in methanol (1: 50, v/v) for use. It is important to note that fresh bromine is required daily as the reagent was usually not dependable after 8 h of use.

2.3. Instruments

Flurometer-Fluorolog-3 from Horiba Scientific (Kyoto, Kyoto Prefecture, Japan) equipped with FluroEssence software was used for the FL analysis. A quartz glass micro-fluorometer cuvette (wavelength range: 190–2500 nm; light path: 10 mm; inside width: 1 mm; volume: 0.35 mL) equipped with a polytetrafluoroethylene stopper was used as the sample well (26F-Q-10 Fusion Technology, Singapore).

2.4. Synthesis of immuno-magnetic adsorbents

As shown in Fig. 1a, the Fe_3O_4 nanoparticles (NPs) obtained via chemical coprecipitation were first functionalised with NH_2 groups, which were then activated using glutaric aldehyde. The anti-AFB_1 antibody was then grafted onto the particle surface. The antibody-functionalised NPs, via their magnetic core, can be easily separated from the system using a permanent magnet (Urusov et al., 2014; Xie et al., 2014).

To prepare the Fe_3O_4 NPs, an NaOH solution (6%, w/v) was first prepared using 15 g of NaOH dissolved in 250 mL of DI water, which was placed in a three-neck round bottom flask in an 80 °C water bath. A mixture of 5.2 g of $FeCl_3{\cdot}6H_2O$, 2.0 g of $FeCl_2{\cdot}4H_2O$, 850 μL of HCl (37%), and 25 mL of DI water was prepared in a 100 mL beaker and placed in ultrasonic machine for 1 min. The as-prepared homogenous solution was transferred to a dropping funnel and slowly added into the NaOH solution under vigorous stirring. The mixture was allowed to reflux for 1 h under vigorous stirring while a N_2 atmosphere was used to protect the product from oxidation. Magnets were used to separate the Fe_3O_4 NPs product. The product was washed with ethanol and water in turn for three times each. The final black magnetic NPs were stored in the 100 mL ethanol-water mixture (3:1, v/v).

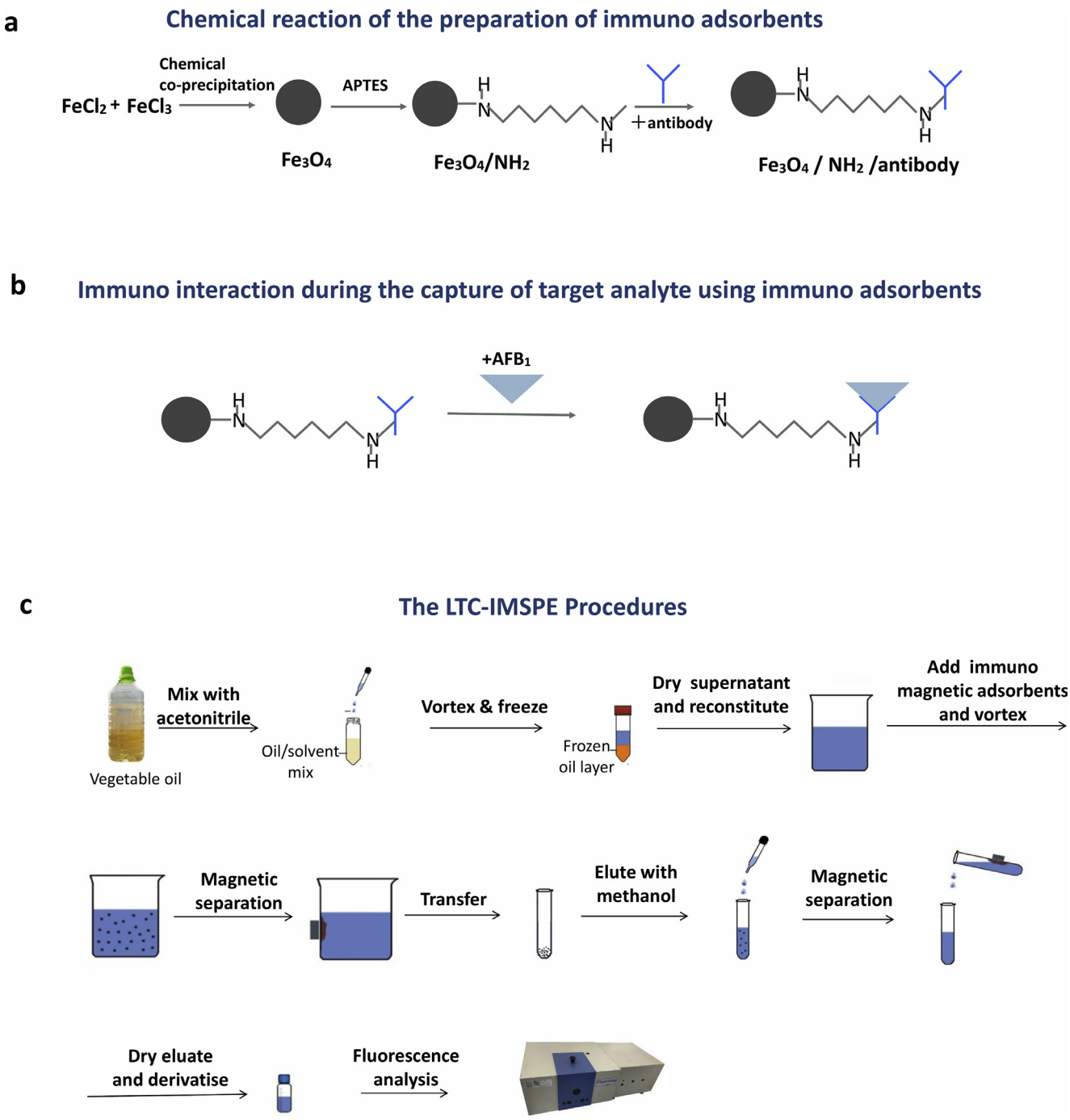

Fig. 1. Illustrative schemes of: (a) Chemical reaction of the preparation of the immuno-magnetic adsorbents; (b) Immuno interaction during the capture of the target analyte using immuno-magnetic adsorbents; and (c) The low temperature clean-up (LTC)- immuno-magnetic solid phase extraction (IMSPE) procedures.

To graft the NH_2 groups, the prepared concentrated Fe_3O_4 NPs fluid (12 mL) was added into ethanol (288 mL) and the obtained mixture was homogenised using ultrasound for 30 min. APTES (18 mL) was then added and the pH value of the reaction mixture was adjusted to 11 using ammonia water. The solution was allowed to reflux for 5 h in a 60 °C water bath. The functionalised Fe_3O_4 NPs were collected via magnetic separation and washed with ethanol for five times. The product was stored in 10 mL ethanol until use.

The last step to prepare the immune adsorbents was the coupling of the antibodies. Fe_3O_4/NH_2 NPs were blown to dryness using nitrogen gas. Fe_3O_4/NH_2 NPs (50 mg) were dispersed in 4 mL of PBS (pH = 7.0). After 30 min of ultrasonic dispersion, 16 mL of glutaric dialdehyde (25%) was added to the solution. The mixture was incubated at 29 °C with constant stirring for 3 h. NPs were washed three times with PBS (pH = 5.0) and dispersed in 5 mL of PBS (pH = 5.0). The anti-AFB_1 antibodies were coupled to the Fe_3O_4/NH_2 NPs by adding the antibody (500 μL, 1 mg·mL^{-1}) to the prepared activated Fe_3O_4/NH_2 NPs (5 mL), and stirring overnight at 4 °C. The obtained final products were kept in 10 mL of PBS (pH = 7.0) (Urusov et al., 2014).

2.5. Characterisation of the immuno-magnetic adsorbents

The magnetic strength, shape, and size as well as the functional group composition of the synthesised Fe_3O_4 and Fe_3O_4/NH_2 NPs were analysed using a vibrating sample magnetometer (VSM), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy

(FT-IR), respectively. For the FT-IR analysis, samples were prepared by grinding NPs together with KBr and pressing the mixture into a thin pellet. The pellet was then analysed using a Perkin Elmer Spectrum One FT-IR spectrometer (Waltham, MA, USA). For VSM, a Lakeshore 7404 Vibrating Sample Magnetometer (Westerville, OH, USA) was used. Finally, TEM imaging was conducted using a JEOL 3010 microscope (Akishima, Tokyo, Japan) at an acceleration voltage of 200 kV.

2.6. Extraction of AFB_1 from oil samples

As shown in Fig. 1b, firstly, edible vegetable oil sample (5 mL) were added to 20 mL of acetonitrile in a 50 mL centrifuge tube and vortexed for 5 min. The sample mixture was then placed into a −20 °C freezer for 20 h to allow low temperature clean-up. The supernatant was then taken and dried using a reduced pressure evaporator. The blown dried extract was reconstituted by adding 800 μL of acetonitrile, and 2200 μL of DI water. One aliquot (1 mL) of the immune-adsorbents was then added into the sample mixture before it was vortexed for 5 min, followed by magnetic separation of the adsorbents. Methanol (2 mL) was then added to elute the AFB_1 from the adsorbents. The eluate was then dried and reconstituted in 120 μL of methanol.

2.7. FL analysis

Chemical derivatisation of AFB_1 was carried out to enhance the sensitivity of the FL signal because the natural fluorescence activity of AFB_1 was not sufficient for detection. In the present study, 240 μL of bromine derivatisation reagent was added to the extract. The obtained solution was mixed well before being placed into the quartz cuvette for FL analysis. The excitation wavelength was fixed at 365–380 nm, while the emission wavelength was fixed at 450–550 nm.

2.8. Statistical analysis

The optimisation experiments and real sample analysis were performed in triplicate to make sure that reliable results were collected. For the optimisation experiments, the significance of the observed difference was determined by analysis of variance (ANOVA) using SPSS software (IBM Statistics 19, Armonk, NY, USA) to determine the optimum antibody-nanoparticle ratio for synthesis. The results were displayed in a solid line chart to achieve intuitive perception, with capital letters showing the significant difference between different data points. A paired-samples T test was carried out using SPSS software (IBM Statistics 19) to compare the precisions of different methods.

Table 1
FT-IR absorption bands assignment for the self-synthesised Fe_3O_4 NPs and Fe_3O_4-NH_2 NPs.

Absorption band (cm^{-1})	Functional groups in Fe_3O_4 NPs	Functional groups in Fe_3O_4-NH_2 NPs
570	Fe–O	–
572	–	Fe–O
1000	–	SiO–H
1082	–	Si–O–Si
1565	HO–H	–
1652	–	N–H
3312	HO–H	–
3320	–	N–H

*Note: NP: Nanoparticles.

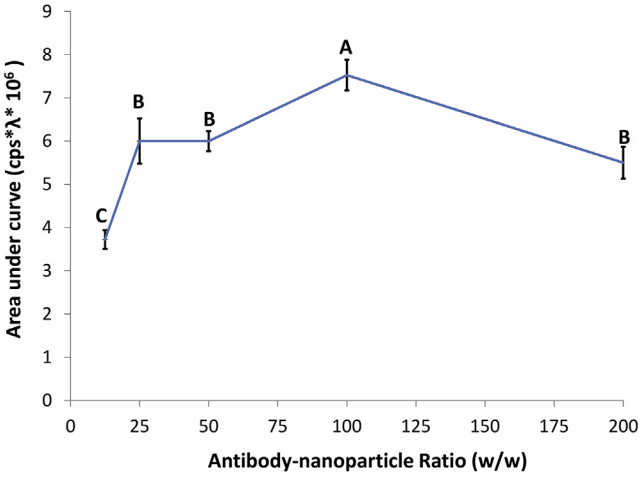

Fig. 3. Optimisation curve of the antibody-nanoparticle ratio during the preparation of immuno-magnetic adsorbents (same capital letters suggest that there was no significant difference between the data points while different capital letters show that there was significant difference between the data points).

3. Results and discussion

3.1. FT-IR spectrometry

Fig. 2a shows the FTIR spectra of Fe_3O_4 NPs and Fe_3O_4/NH_2 NPs, where the –NH_2 functional groups came from the addition of APTES. For clarity, the results of peak assignments are summarised in Table 1 (Jaiswal, Jha, Kaur, & Borah, 2017; Wang, et al., 2010; Saif, Wang, Chuan, & Shuang, 2015). The peaks at 570 and 572 cm^{-1} on the Fe_3O_4 and Fe_3O_4/NH_2 spectra, respectively, were attributed to Fe–O bonding, showing that the magnetic NPs were successfully prepared (Mahdavi

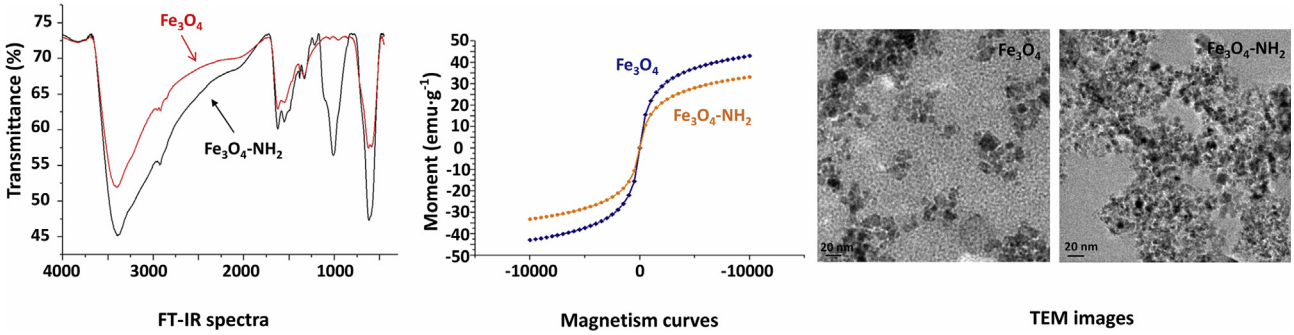

Fig. 2. Characterisation of the Fe_3O_4 and the Fe_3O_4-NH_2 nanoparticles (NPs): (a) Fourier transform infrared spectroscopy (FT-IR) spectra of the Fe_3O_4 and the Fe_3O_4-NH_2 NPs; (b) Room temperature magnetism curves of the Fe_3O_4 and the Fe_3O_4-NH_2 NPs; and (c) Transmission electron microscopy (TEM) images of Fe_3O_4 and the Fe_3O_4-NH_2 NPs.

Table 2
Recovery rates of AFB_1 in vegetable oil samples spiked at different concentration levels and intra- and inter-day precisions (n = 3).

Sample	Spiked concentration ($ng \cdot g^{-1}$)	Intra-day		Inter-day	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Corn oil	0.5	96.3	4.4	103.2	9.6
	20.0	117.9	3.1	104.0	2.3
Canola oil	0.5	104.2	6.4	101.0	6.8
	20.0	94.9	5.6	101.1	7.4
Olive oil	0.5	97.7	4.5	95.7	5.8
	20.0	111.4	8.4	100.7	8.5
Soybean oil	0.5	92.7	7.7	89.9	6.7
	20.0	101.4	7.3	99.4	8.1
Peanut oil	0.5	103.0	6.1	105.4	3.7
	20.0	94.2	6.6	100.3	6.0

*Note: RSD: Relative standard deviation.

et al., 2013). In the Fe_3O_4 spectrum, the peak at 3312 cm^{-1} was associated with HO–H stretching and the peak at 1565 cm^{-1} was associated with HO–H bending stretching which was possibly caused by the presence of water molecules that were not totally removed before analysis. In the Fe_3O_4/NH_2 spectrum, absorption bands at 1000 and 1082 cm^{-1} corresponded to SiO–H and Si–O–Si stretching, which meant that APTES was successfully attached to the NPs (Saif et al., 2015; Jaiswal et al., 2017). The peaks at 3320 and 1652 cm^{-1} were attributed to N–H stretching and bending of NH_2 groups brought in by APTES, respectively (Can, Ozmen, & Ersoz, 2009).

The attachment of APTES to Fe_3O_4 NPs, which was confirmed by the FT-IR spectrum, produced an important intermediary product: amino group functionalised Fe_3O_4 NPs. The amino groups would then form a covalent bond with the carbonyl group on one end of glutaric dialdehyde and the carbonyl group on the other end would cross-link with the $–NH_2$ group on the AFB_1 antibodies, forming an amine-glutaraldehyde-amine covalent bond (Wang et al., 2010; Jha, Jaiswal, Borah, Gautam, & Srivastava, 2015). The presence of the antibodies on the NPs allowed AFB_1 to be attached to them via antibody-antigen binding, and this binding was important for subsequent analysis.

3.2. VSM

The hysteresis curves of Fe_3O_4 and Fe_3O_4/NH_2 which were collected at 300 K are shown in Fig. 2b using different colours.

The Fe_3O_4 NPs and Fe_3O_4/NH_2 NPs had no coercivity and remanence, which meant that both were superparamagnetic. The saturation magnetic moments of Fe_3O_4 NPs and functionalised NPs were 48.9 and 33.1 $emu \cdot g^{-1}$, respectively. Both of them could be magnetised when applied to the external magnetic field; nevertheless, the magnetic property would disappear once the external magnetic field was removed (Peng et al., 2014). Thus, these magnetic properties meant that the separation of the NPs by magnets was feasible. After extraction using the antibody-linked NPs, the NPs could be gathered together using a magnet. Under the same intensity of the external magnetic field, the Fe_3O_4 NPs showed bigger moment compared with Fe_3O_4/NH_2 NPs, which meant the magnetism of the Fe_3O_4 NPs was higher than that of the Fe_3O_4/NH_2 NPs. The amino-group functionalised to the surface of the magnetic NPs which was non-magnetic, accounted for the decrease in the moment.

3.3. TEM imaging

As shown in Fig. 2c, the average diameter of the Fe_3O_4 NPs was determined as 11.4 ± 3.5 nm. Their anisotropic dipolar attraction property meant that the NPs had a tendency to aggregate, making it difficult to separate the boundaries between the aggregated NPs (Tartaj, Gonzalez-Carreno, & Serna, 2001). The chemical stability of the magnetic NPs was improved after amine-group functionalisation (Lu, Dai, Song, Wang, & Yang, 2008). Theoretically, the size of the functionalised NPs should be increased compared with the non-functionalised Fe_3O_4 NPs because of the functional group attached to the surface of the magnetic NPs (Saif et al., 2015). However, the surface of the NPs was modified, but the aggregation was not alleviated, causing the boundaries to be obscure, which made the measurement of the Fe_3O_4/NH_2 NPs' size impractical. In general, the size of the particles was within the nano-meter range, which provided a high surface ratio, which would be beneficial for carrying out the extraction procedures.

3.4. Optimisation

Optimisation of the antibody-nanoparticle ratio during coupling synthesis was carried out by varying the antibody-nanoparticle ratio (w/w) within the range of 12.5:1, 25:1, 50:1, 100:1 and 200:1. According to the results obtained in Fig. 3, 100:1 was selected as the optimum ratio for coupling synthesis.

3.5. Calibration curve and analytical performances

A calibration curve for the quantitative analysis was established using extracts of canola oil samples spiked with different amounts of AFB_1. The extracts were produced using the developed LTC-IMSPE method. The spike concentrations were in the range of 0.02–200 $ng \cdot g^{-1}$ (0.02, 0.2, 2, 20, 100, 200 $ng \cdot g^{-1}$). Creating the calibration curve helped balance out the deviation caused by the extraction method itself.

According to the obtained calibration curve, the equation for quantification was $y = 0.0244x + 0.2016$. The obtained calibration line with $R^2 = 0.9957$ showed a strong linear correlation between the signal response and the concentration of AFB_1 spiked in the oil sample, which suggest good accuracy of the extraction method.

The limit of detection (LOD) was determined as the lowest spike concentration detectable using the proposed method with signal-noise ratio above 3, which was determined as 0.0048 $ng \cdot g^{-1}$ (Li et al., 2015). Likewise, limit of quantification (LOQ) was determined as the lowest spike concentration detectable using the proposed method with signal-noise ratio above 10, which was determined as 0.0126 $ng \cdot g^{-1}$ (Yu & Yang, 2017). To obtain the accurate LOD and LOQ, different concentrations of spiked samples were tested and ten assays were performed for each concentration tested to ensure the reliability of the obtained results.

Table 3
Comparison of the proposed method and other methods based on pretreatment protocols, detection approaches, usage of organic solvents LODs and recoveries.

Method	Samples	Pretreatment	Detection	Type of organic solvent	Amount of organic solvent (mL)	LOD ($ng \cdot g^{-1}$)	Recovery (%)	References
1	Vegetable oils (corn, canola, peanut, soybean, olive)	LTC; IMSPE	FL	Acetonitrile; methanol	25	0.0048	79.6–117.9	This method
2	Vegetable oils (peanut, soybean, corn, olive, sunflower, linseed)	QuEChERS	HPLC-MS	methanol	9	0.05	72.8–105.8	Zhao et al. (2017)
3	Vegetable oils (peanut, sunflower, olive)	LLE; immuno affinity column clean-up	UPLC-MS/MS	Acetonitrile; methanol	74	0.04	72–98	Xie et al. (2015)
4	Vegetable oils (not specified on type of oils)	LLE; gel permeation chromatography	HPLC-FLD	Ethyl acetate; cyclohexane	113.4	1.0	82.6–101.6	Wang et al. (2014)

*Note: LOD: Limit of detection; LTC: Low temperature cleanup; FL: Fluorescence spectroscopy; QueEChERS: Quick, easy, cheap, effective, rugged and safe method; LLE: Liquid-liquid extraction; UPLC-MS: Ultra performance liquid chromatography tandem mass spectrometry.

3.6. Recovery rates of AFB_1 in spiked vegetable oil samples and detection of in natural samples

To validate the proposed method further, various oil samples spiked at different concentration levels (0.5 and 20.0 $ng \cdot g^{-1}$) were analysed (Li et al., 2015; Zhang et al., 2015). Triplicates of each analysis were carried out to obtain the relative standard deviations. According to the results displayed in Table 2, the proposed method showed satisfactory accuracy and reproducibility for all the samples with the recovery rates of inter-day and intra-day assays being in the range of 92.7 ± 7.7–117.9 ± 3.1% and 89.9 ± 6.7–104.0 ± 2.3% (± relative standard deviation (RSD)), respectively.

Five oil samples collected from local supermarkets in Singapore were analysed using the proposed method, including corn, canola, olive, soybean and peanut oil. The results showed that no contamination of AFB_1 was detected for the five samples tested, suggesting that it is generally safe to consume vegetable oils in Singapore.

3.7. Method comparison

To obtain an objective evaluation of its merits, the proposed method was compared with several other recently published methods for analysing AFB_1 in vegetable oils based on a few important aspects. The comparison is shown in Table 3, which shows data from the present study compared with data extracted from previous studies for the other methods. The methods used for the comparative study include LLE, immune affinity column clean-up, QuEChERS, UPLC-MS/MS, HPLC-MS, and HPLC-FLD. Paired-samples T test was carried out between the proposed method (method 1) and other methods (methods 2–4) to evaluate the precisions of different methods. According to the results obtained from the two-tailed T test, the *P* (sig) values were 0.000, 0.000 and 0.042, respectively, which are less than 0.05, indicating that there was no significant difference between the precisions of the proposed method and methods 2–4. The data in Table 3 showed that our proposed method had obviously better sensitivity compared with the other methods, as proved by its low detection limits. After the thorough and convenient clean-up of our proposed method, the quantitative analysis was completed via simple, cheap and easily accessible FL detector instead of other sophisticated and high-maintenance instruments such as HPLC-MS/MS, without compromising sensitivity. By contrast, the high sensitivity of the proposed technique was attributed to the effective removal of fat interference by LTC and the highly selective and efficient preconcentration of IMSPE using the nanoparticles with high surface ratio and extraction power. In addition, only a limited amount of low toxicity organic solvent was used in our proposed method, making it environmentally-friendly (Xie et al., 2015; Zhao et al., 2017; Wang et al., 2014).

4. Conclusion

In this study, we hypothesised that a method combining LTC with IMSPE followed by FL detection could be used to analyse trace AFB_1 in vegetable oils. The hypothesis was supported by the results. We successfully developed LTC-IMSPE for the highly efficient preconcentration of AFB_1 in vegetable oils. LTC was employed to freeze out fat interference conveniently using a domestic freezer while IMSPE helped further capture the trace AFB_1 from the sample mixture. The laboratory prepared antibody-functionalised magnetic NPs were capable of carrying out highly targeted, rapid, and efficient extraction of AFB_1. Reliable results and desirable sensitivity were obtained as evidenced by the satisfactory linear correlation results, low RSD, LOD, and LOQ. Compared with other methods to detect AFB_1 in oil samples, our proposed method showed significantly higher sensitivity and required less toxic organic solvents. Our proposed method is an ideal way to detect trace amounts of AFB_1 in vegetable oils without harming the environment. Considering that the analysis can be completed using cheap and

easily accessible equipment, such as a freezer and an FL detector, thus lowering the threshold of running such analysis, the proposed method is also promising for the convenient screening of vegetable oils in poverty-stricken areas where AFB_1 contamination is underestimated.

Conflict of interest

The authors declare no conflict of interest associated with this study.

Acknowledgements

This work was supported by Arawana Nutrition and Safety Research Grant. Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-A40-114), projects 31371851 and 31471605 supported by NSFC also contributed to this research.

References

Ayub, M., & Sachan, D. (1997). Dietary factors affecting aflatoxin B_1 carcinogenicity. *Malaysian Journal of Nutrition, 3*, 161–179.

Bordin, K., Sawada, M., Rodrigues, C. C., da Fonseca, C., & Oliveira, C. (2014). Incidence of aflatoxins in oil seeds and possible transfer to oil: A review. *Food Engineering Reviews, 6*(1–2), 20–28.

Can, K., Ozmen, M., & Ersoz, M. (2009). Immobilization of albumin on aminosilane modified superparamagnetic magnetite nanoparticles and its characterization. *Colloids and Surfaces B: Biointerfaces, 71*(1), 154–159.

Delmulle, B. S., De Saeger, S. M., Sibanda, L., Barna-Vetro, I., & Van Peteghem, C. H. (2005). Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B_1 in pig feed. *Journal of Agricultural and Food Chemistry, 53*(9), 3364–3368.

Ding, X., Li, P., Bai, Y., & Zhou, H. (2012). Aflatoxin B_1 in post-harvest peanuts and dietary risk in China. *Food Control, 23*(1), 143–148.

Jaiswal, P., Jha, S. N., Kaur, J., & Borah, A. (2017). Detection and quantification of anionic detergent (lissapol) in milk using attenuated total reflectance-Fourier transform infrared spectroscopy. *Food Chemistry, 221*, 815–821.

Jha, S. N., Jaiswal, P., Borah, A., Gautam, A. K., & Srivastava, N. (2015). Detection and quantification of urea in milk using attenuated total reflectance-Fourier transform infrared spectroscopy. *Food and Bioprocess Technology, 8*(4), 926–933.

Jiang, Y., Li, Y., Jiang, Y., Li, J., & Pan, C. (2012). Determination of multiresidues in rapeseed, rapeseed oil, and rapeseed meal by acetonitrile extraction, low-temperature cleanup, and detection by liquid chromatography with tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 60*(20), 5089–5098.

Li, D., Wang, W., Durrani, R., Li, X., Yang, B., & Wang, Y. (2016). Simplified enzymatic upgrading of high-acid rice bran oil using ethanol as a novel acyl acceptor. *Journal of Agricultural and Food Chemistry, 64*(35), 6730–6737.

Li, R., Wang, X., Zhou, T., Yang, D., Wang, Q., & Zhou, Y. (2014). Occurrence of four mycotoxins in cereal and oil products in Yangtze Delta region of China and their food safety risks. *Food Control, 35*(1), 117–122.

Li, N., Zhang, L., Nian, L., Cao, B., Wang, Z., Lei, L., ... Yu, A. (2015). Dispersive micro-solid-phase extraction of herbicides in vegetable oil with metal–organic framework MIL-101. *Journal of Agricultural and Food Chemistry, 63*(8), 2154–2161.

Li, P., Zhang, Q., & Zhang, W. (2009). Immunoassays for aflatoxins. *TrAC Trends in Analytical Chemistry, 28*(9), 1115–1126.

Lim, C. W., Yoshinari, T., Layne, J., & Chan, S. H. (2015). Multi-mycotoxin screening reveals separate occurrence of aflatoxins and ochratoxin a in Asian rice. *Journal of Agricultural and Food Chemistry, 63*(12), 3104–3113.

Lu, Z., Dai, J., Song, X., Wang, G., & Yang, W. (2008). Facile synthesis of Fe_3O_4/SiO_2 composite nanoparticles from primary silica particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 317*(1), 450–456.

Mahdavi, M., Ahmad, M. B., Haron, M. J., Gharayebi, Y., Shameli, K., & Nadi, B. (2013). Fabrication and characterization of SiO_2/(3-aminopropyl) triethoxysilane-coated magnetite nanoparticles for lead (II) removal from aqueous solution. *Journal of Inorganic and Organometallic Polymers and Materials, 23*(3), 599–607.

McCullum, C., Tchounwou, P., Ding, L.-S., Liao, X., & Liu, Y.-M. (2014). Extraction of aflatoxins from liquid foodstuff samples with polydopamine-coated super-paramagnetic nanoparticles for HPLC-MS/MS analysis. *Journal of Agricultural and Food Chemistry, 62*(19), 4261–4267.

Payanan, T., Leepipatpiboon, N., & Varanusupakul, P. (2013). Low-temperature cleanup with solid-phase extraction for the determination of polycyclic aromatic hydrocarbons in edible oils by reversed phase liquid chromatography with fluorescence detection. *Food Chemistry, 141*(3), 2720–2726.

Peng, G., Ding, D., Xiao, F., Wang, X., Hun, N., Wang, Y., ... Cao, Z. (2014). Adsorption of uranium ions from aqueous solution by amine-group functionalized magnetic Fe_3O_4 nanoparticle. *Journal of Radioanalytical and Nuclear Chemistry, 301*(3), 781–788.

Saif, B., Wang, C., Chuan, D., & Shuang, S. (2015). Synthesis and characterization of Fe_3O_4 coated on APTES as carriers for morin-anticancer drug. *Journal of Biomaterials and Nanobiotechnology, 6*(04), 267.

Sinthusamran, S., Benjakul, S., & Kishimura, H. (2014). Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. *Food Chemistry, 152*, 276–284.

Tartaj, P., Gonzalez-Carreno, T., & Serna, C. J. (2001). Single-Step nanoengineering of silica coated maghemite hollow spheres with tunable magnetic properties. *Advanced Materials, 13*(21), 1620–1624.

Urusov, A. E., Petrakova, A. V., Vozniak, M. V., Zherdev, A. V., & Dzantiev, B. B. (2014). Rapid immunoenzyme assay of aflatoxin B1 using magnetic nanoparticles. *Sensors, 14*(11), 21843–21857.

Wang, D., Zhang, Z., Li, P., Zhang, Q., Ding, X., & Zhang, W. (2015). Europium nanospheres-based time-resolved fluorescence for rapid and ultrasensitive determination of total aflatoxin in feed. *Journal of Agricultural and Food Chemistry, 63*(47), 10313–10318.

Wang, H., Zhao, L., Yang, H., Guo, Q., Shi, H., Pan, H., ... Qian, C. (2014). Determination of benzo(a)pyrene and aflatoxins (B1, B2, G1, G2) in vegetable oil by GPC-HPLC-FLD. *Analytical Methods, 6*(5), 1545–1549.

Wang, J. H., Zheng, S. R., Shao, Y., Liu, J. L., Xu, Z. Y., & Zhu, D. Q. (2010). Amino-functionalized Fe_3O_4@SiO_2 core-shell magnetic nanomaterial as a novel adsorbent for aqueous heavy metals removal. *Journal of Colloid and Interface Science, 349*(1), 293–299.

Xie, F., Lai, W., Saini, J., Shan, S., Cui, X., & Liu, D. (2014). Rapid pretreatment and detection of trace aflatoxin B_1 in traditional soybean sauce. *Food Chemistry, 150*, 99–105.

Xie, J., Peng, T., He, J. L., Shao, Y., Fan, C. L., Chen, Y., ... Jiang, H. Y. (2015). Preparation and characterization of an immunoaffinity column for the selective extraction of aflatoxin B1 in 13 kinds of foodstuffs. *Journal of Chromatography B, 998–999*, 50–56.

Yu, X., Ang, H. C., Yang, H., Zheng, C., & Zhang, Y. (2017). Low temperature cleanup combined with magnetic nanoparticle extraction to determine pyrethroids residue in vegetables oils. *Food Control, 74*, 112–120.

Yu, X., Li, Y., Ng, M., Yang, H., & Wang, S. (2018). Comparative study of pyrethroids residue in fruit peels and fleshes using polystyrene-coated magnetic nanoparticles based clean-up techniques. *Food Control, 85*, 300–307.

Yu, X., & Yang, H. (2017). Pyrethroid residue determination in organic and conventional vegetables using liquid-solid extraction coupled with magnetic solid phase extraction based on polystyrene-coated magnetic nanoparticles. *Food Chemistry, 217*, 303–310.

Zhang, X., Wang, X., Sun, M. J., Zhang, X. F., Song, H. H., Yan, Y. X., ... Fang, W. H. (2015). A Magnetic nanoparticle based enzyme-linked immunosorbent assay for sensitive quantification of zearalenone in cereal and feed samples. *Toxins, 7*(10), 4216–4231.

Zhao, H., Chen, X., Shen, C., & Qu, B. (2017). Determination of 16 mycotoxins in vegetable oils using a QuEChERS method combined with high-performance liquid chromatography-tandem mass spectrometry. *Food Additives & Contaminants: Part A, 34*(2), 255–264.