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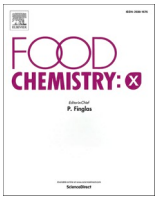

High-throughput detection and dietary exposure risk assessment of 44 mycotoxins in Mango, Litchi, Longan, and their products in South China

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ABSTRACT

Mycotoxins exposure from food can trigger serious health hazards. This study aimed to establish an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the simultaneous detection of 44 mycotoxins in fruits and their products, followed by dietary exposure risk assessment. The optimized UPLC-MS/MS method exhibited a good linear relationship with correlation coefficients $\geq$ 0.99041. The limits of detection (LOD) and the limits of quantification (LOQ) were within the range of 0.003 ~ 0.700 μg/kg and 0.01 ~ 2.00 μg/kg, respectively. The three fruits and their corresponding value-added products, with a total sampling size of 42, were subjected to analysis and detected with mycotoxins. Further dietary exposure risk assessment revealed that the hazard quotient (HQ) and hazard index (HI) of mycotoxins were 1.213 ~ 60.032 % and 5.573 ~ 93.750 %, indicating a low risk for Chinese consumers. However, we still need be cautious about 15-acetyl-deoxynivalenol (15-ADON), as it had 78.6 % occurrence among all samples. This work provides an accurate analysis strategy for 44 mycotoxins and contributes to mycotoxins supervision.

1. Introduction

Mycotoxins, a diverse group of secondary metabolites primarily produced by various fungal species, are a worldwide threat to public health (Yang, et al., 2020). According to Food and Agriculture Organization (FAO), about 25 % of the world's grains are contaminated with mycotoxins. Recently, over 400 mycotoxins have been identified (Huong, et al., 2016; Sun, et al., 2020), and the toxicologically important ones including deoxynivalenol (DON), aflatoxins (AFs), ochratoxin A (OTA), patulin (PAT), zearalenone (ZEN), and fusarenone-X (FUS-X). Mycotoxins can cause a range of diseases, such as cancer (Ahmed Adam, Tabana, Musa, & Sandai, 2017), alimentary toxic aleukia (Kepinska-Pacelik & Biel, 2021), immune and neurological disorders (Ratnaseelan, Tsilioni, & Theoharides, 2018) for humans. Several mycotoxins contamination outbreaks have been reported in Kenya, India, and Malaysia (Shephard, 2008), causing hundreds of humans death worldwide.

Mango, litchi, and longan are important commercial tropical fruits in South China. These fruits and their products are highly susceptible to fungal infection and mycotoxin contamination due to their high levels of water, sugars, and other nutrients. Previous studies revealed that 90.5 % of dried longan samples in China had PAT contamination with the maximum of 194.3 μg/kg, which exceeds the maximum limit of 50 μg/kg set by EU regulation (Ji, et al., 2017). *Aspergillus*, *Penicillium*, and *Fusarium* species were the primary mycotoxigenic fungi found on the surface of mango (Chatha, Anjum, & Zahoor, 2014). The aflatoxins AFB1, AFB2, AFG1, and AFG2 were detected in both mangoes and their products (Yaguibou A G, et al., 2022). The warm and humid climatic conditions in the tropical and subtropical regions of South China (Guangdong, Guangxi, Hainan) are very suitable for fungal growth during fruit processing and storage. Thus, it is essential to accurately detect mycotoxin contamination and estimate exposure risk from these tropical fruits and their products.

High-throughput and sensitive analysis of mycotoxin involves

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appropriate purification, detection, and qualification methods, because mycotoxins can exert their toxicity even at ultra-low levels (Agriopoulou, Stamatelopoulou, & Varzakas, 2020). In addition, complex food matrices are another urgent challenge to trace detection. Recently, ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has achieved much attention and widely used to simultaneous detection of mycotoxins in foodstuffs at trace levels. For example, UPLC-MS/MS method was successfully applied to detect 6 *Alternaria* mycotoxins in grape (Guo, et al., 2019), 12 *Fusarium* mycotoxins in beer (Habler, Gotthardt, Schuler, & Rychlik, 2017), and 16 mycotoxins in vegetable oils (Zhao, Chen, Shen, & Qu, 2017). To date, comprehensive information regarding the composition and concentration of mycotoxins in mango, litchi, longan, and their products is still limited. Consequently, the dietary risk assessment of mycotoxins in these foodstuffs remains unclear.

In this study, we developed an UPLC-MS/MS method for high-throughput analyses of 44 mycotoxins. Furthermore, 42 fruits and their products were determined by the method, followed by the dietary exposure risk assessment. This study could provide valuable information for the rapid pretreatment, simultaneous detection, and enaction of food-safety standards for mycotoxins in tropical fruits and their products.

2. Materials and methods

2.1. Standards and reagents

Acetonitrile, methanol, and formic acid were supplied by Merck (Darmstadt, Germany). 44 mycotoxins and 18 internal standards were supplied by Achemtek Co., Ltd. (Worcester, MA, USA), o2si Co., Ltd. (North Charleston, SC, USA), and CATO Research Chemicals Inc. (Eugene, OR, USA). The detail information was shown in Table S1.

2.2. Preparation of standard solution

All 44 mycotoxin and 18 internal standards were prepared using acetonitrile with the concentration ranging from 10 to 400 ng/mL according to Table S1.

2.3. Sample preparation

A total of 42 samples, included 2 mangoes, 6 dried mangoes, 6 mango jams, 2 litchis, 3 dried litchis, 6 litchi jams, 2 longans, 11 dried longans, and 4 longan jams were purchased from Taobao online store (https://www.taobao.com). All samples were produced in Hainan (10), Guangxi (10), Guangdong (12), and Fujian (10) Province, South China.

One kilogram fresh fruit samples, 500 g dried fruits, and 500 g jams were homogenized using a blender for two minutes for each. Two grams of the homogenized sample were transferred into a 50 mL centrifuge tube and mixed with 0.4 mL of 18 internal standards and 10 mL extraction solutions. The extraction efficiency of four solutions, including solution 1 (1 % formic acid acetonitrile solution), solution 2 (80 % acetonitrile aqueous solution with 1 % acetic acid), solution 3 (80 % acetonitrile aqueous solution with 0.1 % formic acid), and solution 4 (80 % acetonitrile aqueous solution with 1 % formic acid) were compared.

The sample was vortexed and sonicated in a water bath for 10 min before being centrifuged at 10,000 r/min for 5 min. The supernatant was transferred into a 15 mL tube containing 200 mg of primary secondary amine (PSA) and 200 mg of C18 (particle size: 40 ~ 60 μm). Then, the tube was vortexed for 10 min and centrifuged at 10,000 r/min for 5 min again. Five milliliters supernatant of the tube was dried by nitrogen at 40℃. One milliliter 10 % acetonitrile solution was used to dissolve the residue in the tube, followed by vortexing for 30 s. Finally, the solution was analysed by the UPLC-MS/MS after being filtered by SCAA-104 (0.22 μm).

2.4. UPLC-MS/MS analysis

UPLC: Analyses were performed using the AB4500 QTRAP UPLC-MS/MS system (AB Sciex, MA, USA), equipped with five columns: Waters ACQUITY UPLC BEH C18 (1.7 μm, 2.1 mm × 100 mm), Phenomenex-Luna C18 (1.7 μm, 2.1 mm × 100 mm), Waters BEH HILIC (2.7 μm, 2.1 mm × 100 mm), Waters HSS T3 (1.7 μm, 2.1 mm × 100 mm), Phenomenex-Kinetex XB C18 (1.7 μm, 2.1 mm × 100 mm). The injection volume was 5.0 μL, and the flow rate was 0.3 mL/min with a column temperature set at 35 °C. The gradient elution programs were set as Table 1.

MS: ion source: ESI source, positive and negative ion modes; scan mode: selected reaction monitoring (SRM) mode; The MS parameters of 44 mycotoxins and 18 internal standards were shown in Table 2.

2.5. Recovery of mycotoxins

Six negative samples, including mango, dried mango, litchi, dried lichi, longan, and dried longan, were spiked with 44 mycotoxins at low, medium, and high concentration. Specifically, 1.25, 2.5, and 5 μg/kg AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2 were added to mango, litchi, longan, and their products. 2.5, 5.0, and 10.0 μg/kg OTA, OTB, OTC, and OTα were added to three fruits and their products. Similarly, other 34 mycotoxins were spiked at 5.0, 10.0, and 20.0 μg/kg concentrations. Recovery (in percentage) was calculated as the ratio between the mean concentration obtained by UPLC-MS/MS method in each sample and additional concentration.

2.6. Dietary exposure risk assessment

In order to estimate the exposure risk of mycotoxins from mango, litchi, longan, and their products, an assessment based on food consumption, mycotoxin contamination, and body weight was performed. According to The Chinese Food Guide Pagoda, derived from the Chinese Dietary Guidelines (2022), 200 ~ 350 g of fresh fruit per day is recommended for Chinese residents. Dried fruits and jam are also important supplements when fresh fruits are insufficient. In this study, the consumption of fresh fruit (mango, litchi, and longan) is 200 g/d, and the consumption of fruit products (dried fruit and jam) is 20 g/d accordingly. The average weight of adult Chinese is 60 kg (Lozowicka, et al., 2014). Mycotoxin intake and HQ (Hazard quotient, %) were calculated by the following formulas (1) and (2) as described by Ji et al. (2017):

$$Mycotoxin\ intake = \frac{Mycotoxin\ mean\ concetration\ \times\ consumption}{Mean\ body\ weight\ \times\ 1000} \quad (1)$$

$$HQ\ (Hazard\ quotient,\ \%) = \frac{Mycotoxin\ \ intake \times 100}{PMTDI} \quad (2)$$

PMTDI (provisional maximum tolerable daily intake) of mycotoxins are set at 0.4 and 1.0 μg/kg bw/d. HI (Hazard index, %) is the sum of all HQs per sample.

Table 1
Gradient elution program of UPLC.

Elution Program 1			Elution Program 2		
Time/ min	A: water/ %	B: acetonitrile/ %	Time/ min	A: 0.2 % formic acid/ %	B: acetonitrile/ %
0	95	5	0	95	5
1	95	5	1	95	5
5.5	20	80	4.5	5	95
9	20	80	9	5	95
10	95	5	10	95	5
12	95	5	12	95	5

Table 2
MS/MS spectrometry parameters for 44 mycotoxins and 18 internal standards.

Compound	Abbreviation	Time/min	Parent Ion(*m/z*)	Ion Pair /(*m/z*)	Collision Energy /(eV)	Elution Program
Fumonisin B1	FB1	4.54	722.0	334.0/352.0	50/50	2
Fumonisin B2	FB2	4.76	706.3	336.2/354.2	45/44	2
Fumonisin B3	FB3	4.76	706.4	336.2/318.4	48/47	2
13C34-Fumonisin B1	13C34-FB1	4.54	756.6	374.0	50	2
13C34-Fumonisin B2	13C34-FB2	4.73	740.5	358.4	50	2
13C34-Fumonisin B3	13C34-FB3	4.73	740.5	358.4	50	2
Aflatoxin B1	AFB1	5.59	313.0	241.0/269.0	47/40	1
Aflatoxin B2	AFB2	5.43	315.0	287.0/259.0	35/40	1
Aflatoxin G1	AFG1	4.43	329.0	243.1/311.0	35/30	1
Aflatoxin G2	AFG2	5.27	331.1	313.0/245.0	32/40	1
Aflatoxin M1	AFM1	5.08	329.0	273.1/259.1	35/30	1
Aflatoxin M2	AFM2	4.88	331.0	313.1/285.0	23/33	1
13C17-Aflatoxin B1	13C17-AFB1	5.58	330.2	301.1	30	1
13C17-Aflatoxin B2	13C17-AFB2	5.42	332.2	303.2	33	1
13C17-Aflatoxin G1	13C17-AFG1	5.42	346.1	257.2	36	1
13C17-Aflatoxin G2	13C17-AFG2	4.57	348.2	313.1	17	1
13C17-Aflatoxin M1	13C17-AFM1	5.07	346.1	288.0	30	1
13C17-Aflatoxin M2	13C17-AFM2	4.55	348.2	313.1	17	1
Ochratoxine A	OTA	7.13	404.0	358.0/239.0	20/33	1
Ochratoxine B	OTB	6.54	370.0	205.0/187.0	28/48	1
Ochratoxine C	OTC	7.41	432.0	358.0/239.0	25/36	1
Ochratoxine α	OTα	4.74	255.0	167.0/211.0	–33/-20	2
13C20-Ochratoxine A	13C20-OTA	7.11	424.0	377.2	23	1
Deoxynivalenol	DON	3.89	297	249.2/231.0	13/18	1
Deepoxy-deoxynivalenol	DOM	4.28	279.1	249.1/231.1	−14/–22	1
15-Acetyl-deoxynivalenol	15-ADON	4.83	339	137.0/321.0	14/13	1
3-Acetyl-deoxynivalenol	3-ADON	4.87	339	231.0/203.0	15/17	1
Deoxynivalenol-3-glucoside	D3G	3.88	503.1	427.1/457.1	−29/-19	1
Fusarenone-X	FUS-X	4.34	354.9	136.9/174.9	31/19	1
13C15-Deoxynivalenol	13C15-DON	3.90	312	263.0	14	1
13C17-3-Acetyldeoxynivalenol	13C17-3-DON	4.87	356	245.0	13	1
T-2 Toxin	T-2	6.38	489.2	327.2/387.1	29/29	1
HT-2 Toxin	HT-2	5.82	447.1	345.1/285.1	27/28	1
13C24-T-2 Toxin	13C24-T-2	6.37	513.2	260.1	36	1
Patulin	PAT	2.61	152.8	108.9/53	−11/-25	1
13C7-Patulin-	13C7-PAT	2.60	160	115	−11	1
Sterigmatocystin	SMC	6.70	325.1	310.0/281.1	32/50	1
13C18-Sterigmatocystin	13C18-SGM	6.69	343.2	327.0	37	1
(-)-Citrinin	CIT	5.26	249.0	205.0/176.9	–22/-31	2
Penicillic acid	PCA	4.63	171.0	125.1/153.1	17/12	1
Virginiamycin M1	VGM M1	5.95	526.1	508.0/355.3	14/26	1
Tentoxin	TEN	5.80	415.1	312.2/256.1	23/45	1
Tenuazonic	TEA	4.92	196.0	112.0/139.0	−28/-25	2
Alternariol	ALTL	5.68	256.9	214.8/146.9	−35/-42	1
Altenuene	ALTE	5.32	291.2	202.9/248	−43/-34	1
Alternariol monomethyl ether	AME	6.52	270.8	255.9/228	−30/-38	1
13C10-Tenuazonic acid	13C10-TEA	4.91	205.9	144.9	−26	2
α-Zearalanol	α-ZAL	6.08	321.1	277.2/303.2	−30/-28	1
β-Zearalanol	β-ZAL	5.85	321.1	277.2/303.2	−30/-28	1
α-Zearalenol	α-ZOL	6.14	319.1	275.1/301.1	−27/-27	1
β-Zearalenol	β-ZOL	5.89	319.1	275.1/301.1	−27/-27	1
Zearalanone	ZAN	6.51	319.1	275.1/301.1	−27/-27	1
Zearalenone	ZEN	6.55	317.1	174.9/273.1	−30/-27	1
13C18-Zearalenone	13C18-ZEN	6.54	335.2	185.1	–32	1
Diacetoxyscirpenol	DAS	5.58	384.2	307/107	14/25	1
Neosolaniol	NEO	4.52	400.2	305/185.1	16/23	1
Gliotoxin	GLI	4.74	327	245/215	23/30	2
Cyclopiazonic acid	CPA	5.77	335	154/180	−39/-35	2
13C20-Cyclopiazonic acid	13C20-CPA	5.77	355	145.9	−35	2
Verruculogen	VER	5.64	534.3	392.1/360	18/33	2
Destruxin A	DA	5.13	578.4	465.3/437.2	28/39	2
Destruxin B	DB	5.43	594.4	481.3/453.2	27/39	2

2.7. Statistical analysis

SPSS software (version 22.0, IBM Corp. Armonk, NY, US) was used to perform statistical analysis and calculate the correlation coefficient ($R^2 \geq 0.99$). OriginPro software (2019b, OriginLab Inc., Northampton, USA) was used to draw the spectral graphs and boxplot graphs.

3. Results and discussion

3.1. Optimization of extraction solutions

The majority of mycotoxins are extremely soluble in organic solutions, except for fumonisins and patulin (PAT) which are soluble in water (Liu, et al., 2019). Since most mycotoxins contain –COOH and –OH groups, the pH of the extract can also considerably affect the stable ionization of mycotoxins. The addition of water facilitates the penetration of organic solution into the foodstuff. In addition, organic acids can

destroy the tight bonds between the analyzed substances and other food nutrients, i.e., protein and sugar, thereby enhancing the extraction of mycotoxins (Rahmani, Jinap, & Soleimany, 2009). In this study, the extraction efficacy of acetonitrile solutions containing acetic or formic acid ranged from 0.1 % to 1 % was compared. As shown in Fig. 1, the average recoveries of the 44 mycotoxins extracted by four solutions were within the range of 12.0 ~ 178.8 %, 67.8 ~ 128.2 %, 7.7 ~ 383.1 %, and 0 ~ 299.5 %, with center lines of 97.1 %, 102.8 %, 100.9 %, and 68.6 %, respectively. Notably, the majority of the 44 mycotoxins extracted by solution 2 were closely scattered in the box plot, with median quantile (Q2) 92.1 % and third quantile (Q3) 112.3 %, exhibiting the optimal extraction efficacy. Thus, an 80 % acetonitrile aqueous solution (with 1 % acetic acid) was selected as the optimal extraction solvent for further analyses.

3.2. Optimization of chromatographic column and mobile phase

Five chromatographic columns and six mobile phases were used for the UPLC separation of the 44 mycotoxins and 18 internal standards. Three C18 chromatographic columns exhibited the better performance than the Waters BEH HILIC and Waters HSS T3 columns. Notably, 44 mycotoxins and 18 internal standards were clearly separated by Waters BEH C18 with symmetrical peak shapes, high signal responses, and good stability (Fig. S1). Six mobile phases were carefully compared using Waters BEH C18 column for separation of 44 mycotoxins and 18 internal standards. Eleven mycotoxins, including FB1, FB2, FB3, Otα, CIT, TEA, GLI, CPA, VER, DA, DB, and corresponding internal standards were clearly separated by 0.2 % formic acid aqueous as mobile phase. While, other 33 mycotoxins and corresponding internal standards were clearly separated by acetonitrile as the mobile phase. To separate each mycotoxin and its corresponding internal standard, we developed two gradient elution programs using mobile phases composed of water, 0.2 % formic acid, and acetonitrile (Table 1 and 2).

The best separation of 44 mycotoxins and 18 internal standards were achieved using the Waters BEH C18 column with water/acetonitrile and 0.2 % formic acid aqueous/acetonitrile as the mobile phases. The C18 column was widely used for separation of 15 mycotoxins in milk (Flores-Flores & Gonzalez-Penas, 2017), 6 mycotoxins in vegetable oil (Hidalgo-Ruiz, Romero-Gonzalez, Martinez Vidal, & Garrido Frenich, 2019), and 13 mycotoxins in cereal grains (Kim, et al., 2017) in previous studies.

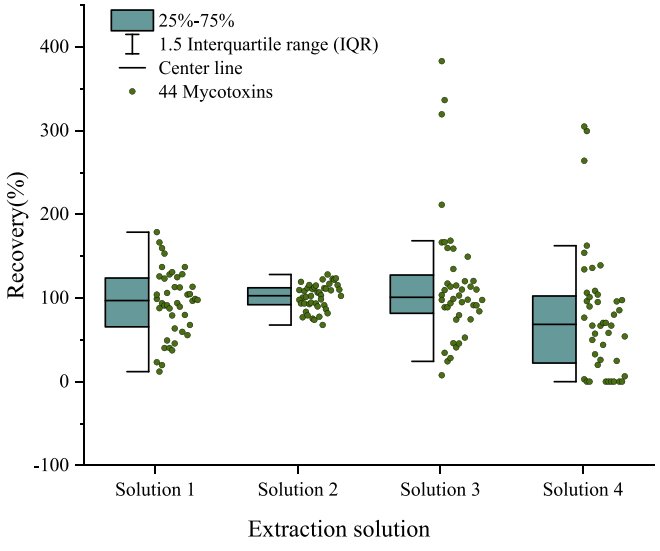

Fig. 1. Average recoveries of 44 mycotoxins extracted by 4 solutions. Note: solution 1: 1% formic acid acetonitrile solution; solution 2: 80% acetonitrile aqueous solution (with 1% acetic acid); solution 3: 80% acetonitrile aqueous solution (with 0.1% formic acid); solution 4: 80% acetonitrile aqueous solution (with 1% formic acid).

When 0.2 % formic acid added, the 44 mycotoxins and 18 internal standards were better separated by the C18 column with high signal response. This probably because the H^+ provided by formic acid in mobile phase make 44 mycotoxins more stable.

3.3. Sensitivity of UPLC-MS/MS

UPLC-MS/MS was performed to detect 44 mycotoxin residues under optimal conditions. As shown in Table 3, all 44 mycotoxins can be quantified in the linear range from 0.2 to 320 ng/mL, with the correlation coefficient R^2 above 0.99041. The limits of detection (LOD) and the limits of quantification (LOQ) of the method were defined by instrumental signal-to-noise ratios of 3 and 10, respectively. The LOD and LOQ were within the range of 0.003 ~ 0.8 μg/kg and 0.01 ~ 2.0 μg/kg. The limit for penicillin in fruit products, fruit and vegetable juices is 50 μg/kg, and the limit for aflatoxin B1 (AFB1) in cereal grains is 5 μg/kg according to GB2761-2017 (National Food Safety Standards: Limits of Mycotoxins in Foods, 2017). However, no regulation about maximum residue limits of other mycotoxins in either tropical fruits or their products has been set in China till now. The LOD and LOQ of this UPLC-MS/MS method were much lower than 5 μg/kg, indicating high analytical sensitivity.

3.4. Accuracy of UPLC-MS/MS

To estimate the accuracy of UPLC-MS/MS method, a recovery test was performed by addition of three concentrations of 44 mycotoxins to 6 negative fruit samples. As shown in Table S2, the average recovery was ranged from 71.3 to 123.6 % ($n = 3$), with relative standard deviations (RSD) of 0.1 ~ 8.2 %. All the results indicated the good sensitivity and accuracy of the UPLC-MS/MS method. The recovery of FB1 in dried longan sample was the lowest of 71.3 %. The 67 % recoveries of FB1 were also found in corn by established liquid chromatographic method (Stack, & Eppley, 1992). FB1 ($C_{34}H_{59}NO_{15}$) has a straight chain skeleton containing 20 carbon atoms, and various carboxyl, hydroxyl, and ester bonds distributed on both sides of the skeleton. This unique skeleton structure may make it difficult to separate from fruit components, resulting in low recovery.

3.5. Detection of mycotoxins from market samples

A total of 42 samples from the South China market were collected and analyzed using UPLC-MS/MS for the residues of 44 mycotoxins. The results from Table 4 indicated that all samples in the South China market were detected as positive for mycotoxins, with dried longan having the highest concentration of 15-ADON (7473.0 μg/kg). Except for mango jam, 15-ADON was one of the top three mycotoxins with the highest concentration among all market samples. Less than three mycotoxins were found in fresh mango, litchi, and longan fruit indicated that the fungal infection might be minimal. The M1 (mean total concentration of 44 mycotoxins in each product) of dried longan, dried litchi, and dried mango were 2735.1, 1190.7, and 482.9 μg/kg, respectively. The number of mycotoxins with a concentration higher than 100 μg/kg in dried longan, dried litchi, and dried mango were 23, 8, and 7, respectively. The potential high-risk mycotoxins in the fruits and their products were 15-ADON, FUS-X, PAT, DOM, 3-ADON, and TEA. Our findings also demonstrated that fruit products, especially dried fruits were more susceptible to fungal infection and mycotoxin contamination compared to fresh ones.

3.6. Dietary exposure risk assessment

In order to evaluate the potential risk of top three mycotoxins in fruits and their products, the hazard quotient (HQ) and hazard index (HI) were calculated using the methods recommended by World Health Organization (WHO) (GEMS/FOOD, 2012). The PMTDI (provisional

Table 3
Linear equation, limits of detection, and quantification of 44 mycotoxins.

Compound	Linear Range (ng/mL)	Regression Equation	Correlation Coefficient(R^2)	Limit of Detection (μg/kg)	Limits of Quantification (μg/kg)
FB1	4 ~ 320	y = 1.0563x-0.02604	0.99748	0.5	2.0
FB2	4 ~ 320	y = 0.65233x + 0.00385	0.99734	0.5	2.0
FB3	4 ~ 320	y = 0.83486x-0.000673578	0.99823	0.5	2.0
AFB1	0.2 ~ 16	y = 0.76723x-0.038	0.99843	0.004	0.01
AFB2	0.2 ~ 16	y = 2.03792x-0.09126	0.99833	0.01	0.02
AFG1	0.2 ~ 16	y = 0.7136x + 0.00464	0.99747	0.004	0.01
AFG2	0.2 ~ 16	y = 5.78744x + 0.43183	0.99872	0.003	0.01
AFM1	0.2 ~ 16	y = 1.41503x-0.13763	0.99784	0.01	0.02
AFM2	0.2 ~ 16	y = 0.90581x + 0.01911	0.99847	0.004	0.01
OTA	1 ~ 80	y = 1.45605x-0.00696	0.99700	0.05	0.2
OTB	1 ~ 80	y = 2.32821x-0.01158	0.99765	0.02	0.1
OTC	1 ~ 80	y = 4.88874x + 0.03794	0.99800	0.01	0.1
DON	4 ~ 320	y = 0.84113x-0.00358	0.99925	0.05	0.5
15-ADON	4 ~ 320	y = 0.56344x-0.03106	0.99750	0.7	2.0
3-ADON	4 ~ 320	y = 1.38273x + 0.04757	0.99649	0.1	0.5
T-2	2 ~ 160	y = 0.49771x + 0.0169	0.99674	0.4	1.0
HT-2	2 ~ 160	y = 0.14817x + 0.01441	0.99630	0.7	2.0
PCA	2 ~ 160	y = 34023.4x-718.65588	0.99985	0.06	0.2
VGM M1	2 ~ 160	y = 49172.2x-20035.6452	0.99862	0.04	0.1
TEN	2 ~ 160	y = 40006.7x-22160.63199	0.99733	0.01	0.04
SMC	2 ~ 160	y = 1.83628x-0.01154	0.99913	0.01	0.04
FUS-X	4 ~ 320	y = 598.736x + 408.1768	0.99630	0.6	2.0
Otα	1 ~ 80	y = 5171.82095x-3244.49999	0.99859	0.1	0.5
D3G	4 ~ 320	y = 1372.40684x + 1546.02674	0.99691	0.2	1.0
CIT	2 ~ 160	y = 2903.27821x + 782.86696	0.99966	0.2	1.0
TEA	2 ~ 160	y = 1886.31943x-867.96367	0.99904	0.2	1.0
α-ZAL	2 ~ 160	y = 1.59819x-0.00809	0.99796	0.03	0.1
β-ZAL	2 ~ 160	y = 0.99938x + 0.00394	0.99929	0.03	0.1
α-ZOL	2 ~ 160	y = 0.31806x + 0.00467	0.99660	0.05	0.1
β-ZOL	2 ~ 160	y = 0.18387x + 0.00260	0.99764	0.04	0.1
ZAN	2 ~ 160	y = 0.41593x-0.00244	0.99558	0.03	0.1
ZEN	2 ~ 160	y = 0.22137x + 0.00178	0.99531	0.06	0.2
ALTL	2 ~ 160	y = 7034.72777x-6589.9603	0.99567	0.04	0.1
ALTE	2 ~ 160	y = 1913.78969x + 1026.31562	0.99604	0.1	0.5
DOM	4 ~ 320	y = 322.86660x-633.13514	0.99637	0.2	0.5
PAT	4 ~ 320	y = 0.54421x + 0.06271	0.99041	0.05	0.2
DAS	20–400	y = 67425.9x + 133925	0.99745	0.02	0.1
NEO	20–400	y = 44287.1x + 910539	0.99643	0.02	0.1
AME	10–200	y = 95501.5x + 828367	0.99685	0.01	0.05
GLI	20–400	y = 3599.21589x + 20462.28761	0.99770	0.8	2.0
VER	20–400	y = 1906.57801x-28146.47177	0.99556	0.5	2.0
DA	5–100	y = 50.69429x + 3.84344	0.99759	0.005	0.02
DB	5–100	y = 51.85117x + 1.52632	0.99826	0.005	0.02
CPA	20–400	y = 0.5733x-0.08402	0.99863	0.04	0.1

Table 4
Overview of the occurrence of 44 mycotoxins in fruits and their products in South China.

Fruit and Product	Sample	Positive (%)	Numbers of Mycotoxins				Maximum (μg/kg)	M1 (μg/kg)	Top 3
			<LOQ (μg/kg)	0.01 ~ 9.99 (μg/kg)	10 ~ 99.99 (μg/kg)	>100 (μg/kg)			
Fresh fruit	Mango	100	85	0	3	0	58.6	56.0	15-ADON, 3-ADON
	Litchi	100	86	0	1	1	176.8	129.6	15-ADON
	Longan	100	86	0	1	1	364.0	224.0	15-ADON
Dried fruit	Dried mango	100	197	41	19	7	679.0	482.9	DOM, 15-ADON, FUS-X
	Dried litchi	100	81	26	17	8	1213.4	1190.7	15-ADON, CIT, PAT
	Dried longan	100	356	73	32	23	7473.0	2735.1	15-ADON, FUS-X, PAT
Fruit jam	Mango jam	100	197	37	19	11	508.4	594.4	PAT, DOM, FUS-X
	Litchi jam	100	46	61	22	3	166.7	386.4	15-ADON, PAT, DOM,
	Longan jam	100	130	25	17	4	241.0	387.1	FUS-X, 15-ADON, TEA

Note: M1: mean total concentration of 44 mycotoxins in each product. Top 3: the three mycotoxins of highest concentrations.

maximum tolerable daily intakes) of 15-ADON, 3-ADON and PAT were 1.0, 1.0, and 0.4 μg/kg bw/d, respectively, according to WHO (JECFA, 2011). The PMTDI of DOM, FUS-X were calculated as a hypothetic value of 1.0 μg/kg·bw/d according to previous studies. As showed in Table 5, the HQs of mycotoxins in fruits and their products ranged from 1.213 to 60.032 %, and HIs ranged from 5.573 to 93.750 %, both of which were

Table 5
Risk assessment of dietary exposure in Chinese adults.

Fruit and Product	Sample	Mycotoxin	M2(μg/kg)	PMTDI(μg/kg bw/day)	Mycotoxin intake(μg/kg bw/day)	HQ(%)	HI(%)
Fresh fruit	Mango	15-ADON	50.800	1.0	0.1693333	16.933	18.658
		3-ADON	5.175	1.0	0.0172500	1.725	
	Litchi	15-ADON	129.620	1.0	0.4320667	43.207	43.207
	Logan	15-ADON	224.030	1.0	0.7467667	74.677	74.677
Dried fruit	Dried mango	15-ADON	128.450	1.0	0.0428167	4.282	12.998
		DOM	133.858	1.0	0.0446194	4.462	
		FUS-X	127.642	1.0	0.0425472	4.255	
	Dried litchi	15-ADON	777.433	1.0	0.2591444	25.914	34.845
		PAT	107.167	0.4	0.0357222	8.931	
	Dried logan	15-ADON	1800.968	1.0	0.6003227	60.032	93.750
		FUS-X	649.559	1.0	0.2165197	21.652	
		PAT	144.793	0.4	0.0482644	12.066	
Fruit jam	Mango jam	DOM	204.417	1.0	0.0681389	6.814	22.165
		FUS-X	108.083	1.0	0.0360278	3.603	
		PAT	140.975	0.4	0.0469917	11.748	
	Litchi jam	15-ADON	51.750	1.0	0.0172500	1.725	5.573
		DOM	36.400	1.0	0.0121333	1.213	
		PAT	31.621	0.4	0.0105403	2.635	
	Logan jam	15-ADON	128.875	1.0	0.0429583	4.296	8.064
		FUS-X	113.050	1.0	0.0376833	3.768	

Note: Fresh fruits consumption is 200 g/d, and dried fruit and jam consumption is 20 g/d. M2: mean concentration of mycotoxin in samples. Mean body weight is 60 kg. Mycotoxin intake = (Fruits and their products consumption × M2)/(mean body weight × 1000). HQ (Hazard Quotient, %) = mycotoxin intake × 100/PMTDI. HI (Hazard Index, %) = $\sum HQ$.

below 100 %. The accumulation of mycotoxins in the analyzed samples was less than PMTD, indicating a low risk for Chinese consumers. Notably, the HQs of 15-ADON in fresh longan and dried longan reached the maximum of 74.677 and 60.032, respectively, which were the highest among the majority of other samples.

15-ADON is a frequently detected mycotoxin in wheat (Wang, et al., 2021), maize (Han, et al., 2014), and rice (Xu, et al., 2016), particularly in grains that are infected by the toxigenic molds. Acetylated deoxynivalenol (ADON) shows a stronger toxicity than DON, because they are absorbed more rapidly into the intestine (Pinton, et al., 2012). A previous study indicated that human intestinal cells exhibited the highest levels of permeability and IL-8 secretion when exposed to 15-ADON (Kadota, et al., 2013). The results of this study indicate that 15-ADON was the most prevalent mycotoxin in mango, litchi, longan, and their products, with 78.6 % occurrence (33/42). Nevertheless, this study examined only 42 fresh fruit, dry fruit, and jam samples, and multiple mycotoxins in other foodstuffs, including grains, vegetables, and water were ignored. Therefore, further investigations are necessary to confirm these findings.

4. Conclusions

In this study, we established a highly sensitive, accurate, and reliable UPLC-MS/MS method for detection of 44 mycotoxin residues in mango, litchi, longan, and their products. The process involved sample extraction with acetonitrile (containing 1 % acetic acid), purification by PSA and C18, and separation by an ACQUITY UPLC BEH C18 (1.7 μm, 2.1 mm × 100 mm) column. The LOD and LOQ were 0.003 ~ 0.700 μg/kg, and 0.01 ~ 2.00 μg/kg, respectively. The average recoveries ranged from 71.3 % to 123.6 % with RSD ranging from 0.1 to 8.2 %. All 42 market samples were positive for mycotoxins, with the maximum concentration (7.47 mg/kg) of 15-ADON detected in dried longan. Further exposure risk assessment from these foods did not reveal significant threat to Chinese customers. However, it is till necessary to pay special attention to 15-ADON due to its 78.6 % highest positive occurrence.

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CRediT authorship contribution statement

Hao Deng: Conceptualization, Software, Writing – original draft. **Zhenlin Xu:** Conceptualization, Methodology. **Lin Luo:** Software, Writing – original draft. **Yunkai Gao:** Writing – review & editing. **Lingyu Zhou:** Software. **Xiaomei Chen:** Writing – review & editing. **Chunquan Chen:** Methodology. **Bei Li:** Writing – review & editing. **Qingchun Yin:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101002.

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