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# Simultaneous determination of 13 mycotoxins in feedstuffs using QuEChERS extraction

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## Abstract

Mycotoxins are secondary metabolites produced by various fungi and are known to have a significant negative impact on human and animal health. When feedstuffs are contaminated with mycotoxins, their toxicities may be caused a variety of diseases. In this study, the residual mycotoxins in feedstuffs were analyzed using LC–MS/MS incorporated with QuEChERS extraction. Analytical method validation was performed for LOD, LOQ, linearity, and recoveries with consideration of matrix effects prior to the residual analysis. They were all reached to the accepted range of validation level. Using 39 feedstuff samples (5 g) for mycotoxin analysis, nine samples were contaminated by four major mycotoxins such as fumonisin B1 (FB1), deoxynivalenol, fumonisin B2, and zearalenone. Among them, FB1 was detected at the highest concentration as 18.0943 mg/kg. The total sum of fumonisins in 39 samples did not exceed the maximum residual level (MRL) criterion set by Korean Food and Drug Administration. Altogether, intensive management of mycotoxins in Korean feedstuffs should be implemented with proper and routine monitoring, even their residual concentrations are not exceeded over the MRL levels because of high frequent detection found in this study.

**Keywords:** Mycotoxins, QuEChERS, LC–MS/MS, Feedstuffs, Fumonisines

## Introduction

Mycotoxins are natural compounds produced by various fungi and that cause various diseases in humans and livestock [1–4]. Aflatoxins (AFs) and ochratoxins (OTs) are representative examples produced with well-known fungi and their biosynthetic mechanisms are well understood

[5, 6]. Three primary strategies are used in agricultural and food industries to properly manage such mycotoxins, and they are physical-based control method, chemical-supporting control procedures, and biologically suppressed methods on the fungi growth.

Physical strategies typically use irradiation, such as gamma-rays [7, 8]. Chemical controls include the application of fungicides [9, 10], use of chemical preservatives [11, 12], and new treatments with natural products [11, 13, 14]. Direct chemical degradation of mycotoxins is reported [15]. As an example, the decomposition of aflatoxin using ammonia is a well-known method to remove the toxin from peanuts and corn [16, 17]. Efficient biological controls use competition with atoxigenic fungi as biocontrol agents [18], suppression of mycotoxin production with other microbial strains [19], and decomposition of mycotoxins with microbes [20].

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A second critical aspect of mycotoxin management is adequate monitoring through multi-component simultaneous analysis to quickly and inexpensively analyze toxins in agricultural products and foods [21, 22]. Such analysis has recently been developed and introduced in various countries and is directly used for mycotoxin monitoring [21, 22]. Simultaneous analysis of mycotoxins needs to be accompanied with an effective mycotoxin extraction method with procedures, such as immunoaffinity or QuEChERS [21–23].

The third aspect of management is the development of adequate standards for acceptable mycotoxin limits for humans and livestock [24]. Various issues must be considered at the national level since each country establishes its own standards for tolerable intake levels [25]. Food types consumed are diverse, and quantities consumed of different foods vary widely, especially between Asian countries and European countries [24, 25].

Mycotoxin contamination analysis in agricultural products and feedstuffs is regularly conducted at the national level to check actual contamination levels, predict temporal variation, and understand relationships between mycotoxin generation and climatic conditions. Significant differences in the regional occurrence of mycotoxins are observed [26, 27]. With this regard, a simultaneous analysis method was developed for faster and cheaper determination of multiple mycotoxins in feedstuffs because single compound analysis took several disadvantages including analytical time consumption and expensive analytical cost. In this study, an extraction method was employed as quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction for lowering analytical cost, and thirteen different mycotoxins in feedstuffs analyzed using LC–MS/MS were developed to reduce analytical time consumption. Successful analytical procedures on the multiple mycotoxins at the same time were introduced with acceptable method validation results and higher efficient QuEChERS extraction level.

## Materials and methods

### Chemical and reagents

Methanol (MeOH) and acetonitrile (MeCN) were purchased from Honeywell (Muskegon, MI, USA). Ammonium formate ( $\geq 99.0\%$ ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary-Secondary-Amine (PSA bonded silica) was purchased from Supelco (Bellefonte, PA, USA).  $C_{18}$  end-capped resin was purchased from Agilent (Santa Clara, CA, USA). Formic acid (99.0%, GR) was purchased from Samchun Chemical Co. (Gyeonggi-do, Korea). Formic acid (LiChropur<sup>®</sup>) was purchased from Merck (Darmstadt, Germany). Other chemicals used in this study were analytical grades.

### Preparation of standard solution

The certified standards of mycotoxins were also purchased from Cfm Oskar Tropitzsch GmbH (Marktredwitz, Germany). Standard stock solutions of mycotoxins were prepared by dissolving each mycotoxin in distilled water/MeOH (50/50, v/v). Working standard solutions were then diluted with MeCN for the calibration curve. Solutions were then kept in the dark at  $-20\text{ }^{\circ}\text{C}$  in amber glass vials before use.

### Samples

For sample preparation, bean and maize were purchased from local markets in Ansung (Gyeonggi-do, Korea). Beans were blended and homogenized to a powder-like consistency. Maize as raw kernels were dried at  $50\text{ }^{\circ}\text{C}$  for one day, then blended and homogenized. All samples were kept at  $-20\text{ }^{\circ}\text{C}$  until use.

### QuEChERS sample preparation

#### Extraction

For all tested samples, five grams ( $5.0\text{ g}\pm 0.1\text{ g}$ ) of the freeze-dried samples were accurately weighed and transferred into 50 mL conical tubes. The weighed sample was extracted in 10 mL of a solution of 10% formic acid in  $\text{H}_2\text{O}$  (10/90, v/v), and 10 mL MeCN by shaking on a mechanical wrist shaker for 30 min. After extraction, samples were treated with salt 1 (4 g anhydrous magnesium sulfate and 1 g sodium chloride), salt 2 (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodiumcitrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate), or with salt 3 (6 g anhydrous magnesium sulfate and 1.5 g sodium acetate), and shaken for 1 min. Extracted samples were then centrifuged at 4,000 rpm for 10 min.

#### Cleanup

One mL of supernatant from extract after centrifugation was vortexed with 25 mg  $C_{18}$  (powder type) and 25 mg PSA (powder type) for 1 min. Then, it was centrifuged at 10,000 rpm for 5 min at  $4\text{ }^{\circ}\text{C}$ . Four hundred  $\mu\text{L}$  aliquots of supernatant were transferred to a microtube and mixed with 500  $\mu\text{L}$  distilled water and 100  $\mu\text{L}$  MeCN. This solution was stored at  $4\text{ }^{\circ}\text{C}$  for 30 min. Samples solutions were passed through a 0.20  $\mu\text{m}$  and 4 mm polytetrafluoroethylene (PTFE) syringe filter (Hyundai Micro, Seoul, Korea), before analysis using the LC–MS/MS system.

#### LC–MS/MS analysis

An Agilent ultrahigh-performance liquid chromatography (UPLC) system (1290 Infinity, 1290 high-speed pump; G7120A, 1290 multisampler; G7167B, MCT; G71166B, Agilent, Santa Clara, CA, USA) was used to perform reverse-phase chromatographic separation of 13 mycotoxins. The separation used a Zorbax Eclipse Plus  $C_{18}$  column

(50 mm × 2.1 mm i.d., 1.8 μm particle size, Agilent, Santa Clara, CA, USA). The mobile phase employed a time-programmed gradient system using H<sub>2</sub>O (solvent A) and MeOH (solvent B); both solvents contained 5 mmol/L ammonium formate and 0.1%(v/v) formic acid. Gradient elution was initiated with 95% A for 1.5 min. Solvent B was then linearly increased to 30% over 2.5 min, and 60% within 4 min. B was further gradually increased to 80% within 5 min, 99% within 6 min, then kept constant for 8.5 min. Finally, B was decreased linearly to 5% over 8.6 min and equilibrated for 4.4 min. The flow rate was 350 μL/min.

The LC system was coupled with an Agilent 6470 TQ mass spectrometer (Santa Clara, CA, USA) equipped with an AJS ESI source operation in positive and negative ionization mode. ESI–MS/MS was performed with dynamic multiple reaction monitoring. MassHunter Version 10.0 (Santa Clara, CA, USA) was used to control the UPLC-MS/MS system. Gas temperature, gas flow, nebulizer, sheath gas temperature, sheath gas flow, and capillary voltage were set at 300 °C, 7 L/min, 35 psi, 350 °C, 11

L/min, and 3500 V, respectively. Fragmentation and collision energy levels were optimized for each precursor ion and different product ions. For each compound, at least one precursor, and one product ion for both identification and quantification purposes were determined, selecting the most abundant product ion for quantification and the second one for confirmation. The precursor ion and optimized MS/MS parameters (fragment and collision energy) for each mycotoxin are summarized in Table 1.

### Validation of method performance

#### Linearity

Linearity was evaluated by preparing calibration curve with five concentrations. Concentration ranges for mycotoxins are provided in Additional file 1: Table S1. Matrix-matched calibration curves were prepared for all two validated matrices, and solutions for matrix-matched calibration is reported in Additional file 1: Table S2.

**Table 1** Optimized LC–MS/MS parameters for the determination of mycotoxins in feedstuffs

Mycotoxin	Retention Time (min)	Ionization	Precursor ion (m/z)	Product Ion (m/z)	Fragment voltage (V)	Collision energy (V)	Cell accelerator voltage (V)
Aflatoxin B <sub>1</sub>	4.53	[M+H] <sup>+</sup>	313.0	285.0 241.0	175	25 40	5
Aflatoxin B <sub>2</sub>	4.42	[M+H] <sup>+</sup>	315.0	259.0 287.0	185	35 35	5
Aflatoxin G <sub>1</sub>	4.29	[M+H] <sup>+</sup>	329.0	243.0 311.0	130	30 20	5
Aflatoxin G <sub>2</sub>	4.16	[M+H] <sup>+</sup>	330.9	313.0 245.0	160	25 35	5
Deoxynivalenol	3.04	[M+H] <sup>+</sup>	297.3	249.1 191.0	109	10 58	5
Fumonisin B <sub>1</sub>	5.08	[M+H] <sup>+</sup>	722.0	352.3 704.2	180	45 40	5
Fumonisin B <sub>2</sub>	5.53	[M+H] <sup>+</sup>	706.4	335.9 354.3	180	35 30	5
Ochratoxin A	5.49	[M+H] <sup>+</sup>	404.1	238.9 192.7	100	25 50	5
T-2 toxin	5.31	[M+NH <sub>4</sub> ] <sup>+</sup>	483.8	215.0 263.1	140	20 10	5
HT-2 toxin	5.05	[M+NH <sub>4</sub> ] <sup>+</sup>	442.5	263.1 215.1	120	10 10	5
Zearalenone	5.55	[M–H] <sup>–</sup>	317.0	175.1 186.9	120	25 15	5
α-zearalenol	5.38	[M–H] <sup>–</sup>	319.0	275.2 301.1	145	25 25	5
β-zearalenol	5.16	[M–H] <sup>–</sup>	319.0	275.2 301.1	145	25 25	5

### Matrix effect

The evaluation of signal suppression/enhancement due to matrix effects was calculated with the following equation from five point calibration curves.

$$\begin{aligned} \text{Matrix effect (\%)} \\ &= 100 \times \frac{\text{slope}_{\text{matrix-matched standard calibration}}}{\text{slope}_{\text{solvent standard calibration}}} \end{aligned}$$

### Recovery

Recovery was assessed following SANTE/11813/2017 and CODEX guidelines with replicated spiking experiments ( $n=5$ ) with 2MLOQ, 4MLOQ, and 20MLOQ levels for each mycotoxin. Average recovery values were calculated, and standard deviations for repeatability from five replicates were obtained as a measure of precision.

Retention times of analytes in sample extracts that correspond to the average of the calibration standards measured in same analytical sequence with a tolerance of  $\pm 0.1$  min; ion ratios (defined as the response of the peak with the lower area divided by the response of the peak with the higher area) was calculated to be within  $\pm 30\%$  (relative) to that obtained from the average of the calibration standards from the same sequence. The employed sample preparation for the analysis of mycotoxins are summarized in Additional file 1: Figure S1.

## Results and discussion

### LC-MS/MS optimization

The optimization of the LC-MS/MS parameters used the optimizer program. Formation of a protonated precursor ion or ammonium adducts formic acid and ammonium formate was added to mobile phases. For T-2 toxin and HT-2 toxin, ammonium adducts were selected as precursor ions. For other components, protonated  $m/z$  were chosen as precursor ions (Table 1). Ten mycotoxins were optimized in positive ESI mode and three mycotoxins, zearalenone (ZEN),  $\alpha$ -zearalenol ( $\alpha$ -ZEA), and  $\beta$ -zearalenol ( $\beta$ -ZEA), in negative mode (Fig. 1). These ionization products are quite different from a previous report [21] since these authors used negative mode for nivalenol (NIV), deoxynivalenol (DON), and deoxynivalenol-3-glucoside (D3G).

Peak intensity of the final dilution solution was tested using different values in distilled water, 5% HCOOH in distilled water, 10% HCOOH in distilled water, and HCOOH/ $\text{NH}_4\text{COOH}$ . No significant differences were observed (Additional file 1: Table S3). For identification purposes, the two most intense transition ions among precursor ions were selected; for mycotoxin quantification, only the most intense peak (quantifier) was used. Also, retention time (RT) and ion ratio (IR) variations measured in the samples

met requested criteria ( $\pm 0.1$  min for RT and  $\pm 30\%$  for IR), when compared with values obtained for calibration standards.

Another study reported similar findings for optimization of mycotoxin determination using LC-MS/MS, though retention times of mycotoxins were somewhat late compared our current result (Table 1) [21]. These authors used different eluting solutions with lower formic acid content. Different studies often develop their own optimal analytical system for determining mycotoxins.

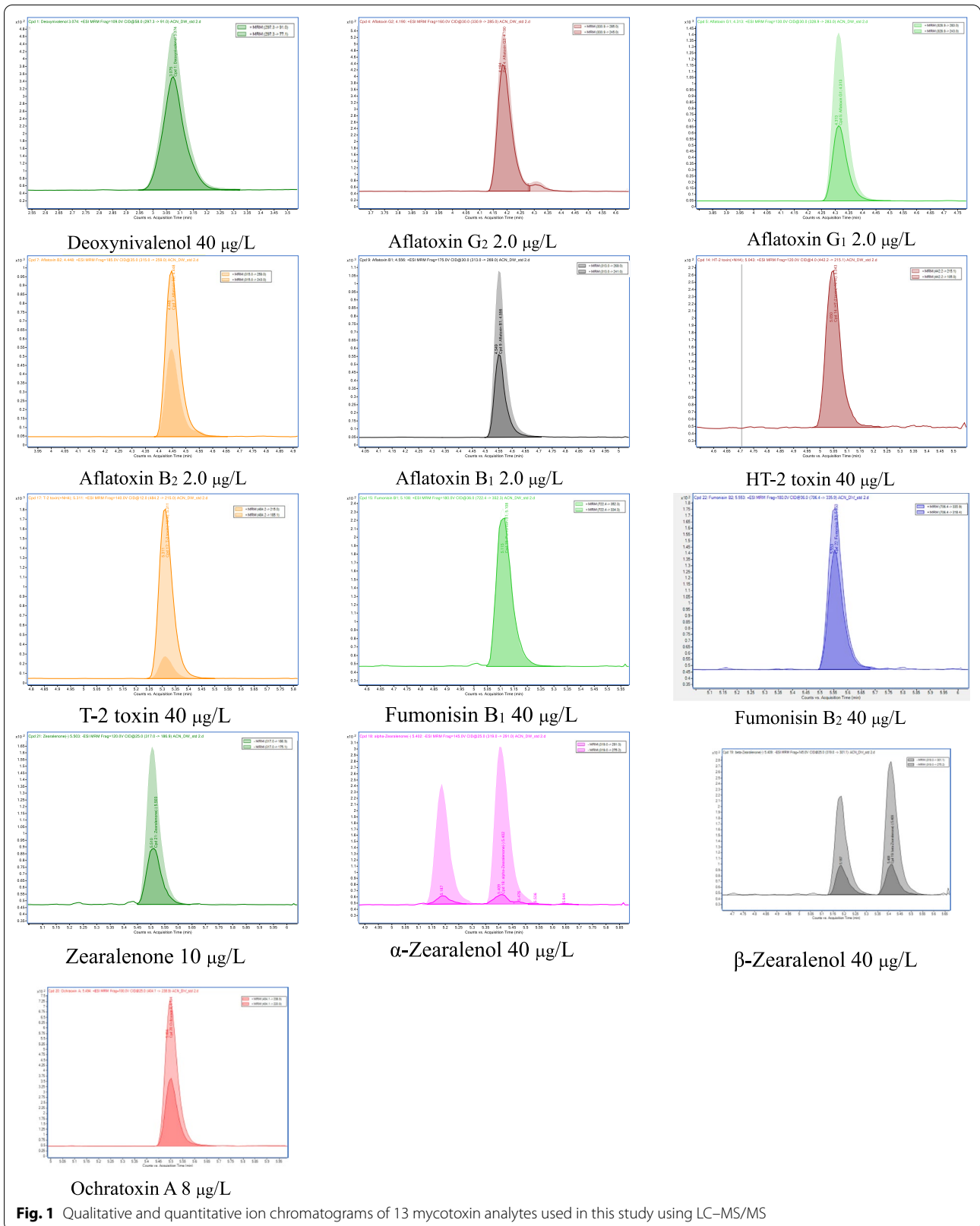
### Extraction and cleanup step

To obtain high extraction efficiency, three extraction solutions, Salt 1, 2, and 3, were compared, and extraction with Salt 1 and 2 showed similar extraction effectiveness, but Salt 3 was less efficient (Table 2). For fumonisins (FUMs) and ochratoxin A that include a carboxylic acid moiety, 10% formic acid was used in distilled water to decrease pH. The pH of extraction solutions (Salt 1; pH 1.89, Salt 2; pH 2.24, and Salt 3; 2.86) was compared. Salt 1 solution, displaying the lowest pH, was selected for extracting mycotoxins in this study.

For adequate cleanup efficiency, three absorbents, anhydrous  $\text{MgSO}_4$ ,  $\text{C}_{18}$ , and PSA were compared. Each absorbent was used at the amount of 25 and 50 mg. In Table 3, when only acetonitrile was used, anhydrous  $\text{MgSO}_4$  and  $\text{C}_{18}$  were absorbed and were properly detected. As it contains carboxylic moiety, it is likely to absorb mycotoxins. However, fumonisin  $\text{B}_1$  and  $\text{B}_2$  were not absorbed by these two absorbents, while  $\text{C}_{18}$  efficiently absorbed all tested mycotoxins. For zearalenone,  $\alpha$ -zearalenol, and  $\beta$ -zearalenol, PSA absorbents showed a recovery lower than the acceptable range of 70–120% (Table 3).

Conversely, adsorption rate for each mycotoxin varied between extraction steps using  $\text{MgSO}_4$  and PSA (Additional file 1: Table S4). However, adsorption pattern by these two absorbents towards mycotoxins were so similar. However, anhydrous  $\text{MgSO}_4$  was used often to remove  $\text{H}_2\text{O}$ , and thus would be better to be excluded from cleanup procedure. Therefore, 25 mg PSA was selected for the cleanup step as an adsorbent.

Three purification and extraction methods have been applied for mycotoxins and the first method is to use a solid phase extraction (SPE) column [21]. These authors used SPE column (Isolute column, Myco, Biotage, Sweden) to separate and purify mycotoxins from Korean soybean paste, and this method is easy to handle, inexpensive, and does not require any special storage condition. The second method is related to use QuEChERS materials [21, 23, 28], and this method successfully separates and extracts mycotoxins from various matrices



**Fig. 1** Qualitative and quantitative ion chromatograms of 13 mycotoxin analytes used in this study using LC-MS/MS

**Table 2** Efficiency of different extraction salt system towards mycotoxins

Mycotoxin	Fortification Level (µg/kg)	Recovery (%)					
		Salt 1		Salt 2		Salt 3	
		Average	%RSD	Average	%RSD	Average	%RSD
Aflatoxin B <sub>1</sub>	5	92.62	1.20	96.16	2.39	88.09	1.06
Aflatoxin B <sub>2</sub>	5	80.21	1.65	82.05	0.87	75.51	2.11
Aflatoxin G <sub>1</sub>	5	78.40	1.12	81.40	0.42	73.15	1.82
Aflatoxin G <sub>2</sub>	5	79.30	0.77	80.43	2.08	74.05	3.06
Deoxynivalenol	100	77.68	1.49	82.74	0.49	74.95	0.69
Fumonisin B <sub>1</sub>	100	74.76	3.40	79.32	3.20	72.13	0.45
Fumonisin B <sub>2</sub>	100	78.59	0.95	82.27	0.72	71.87	1.47
Ochratoxin A	20	78.86	1.73	80.71	1.95	71.81	2.54
T-2 toxin	100	78.73	1.91	81.65	2.27	73.90	2.51
HT-2 toxin	100	78.73	1.91	81.65	2.27	73.90	2.51
Zeralenone	25	82.19	1.28	83.99	1.13	77.30	5.57
α-zearalenol	100	79.09	1.90	82.70	1.83	74.43	3.72
β-zearalenol	100	75.34	3.66	78.88	5.47	68.81	10.76

**Table 3** Efficiency of different absorbents towards mycotoxins

Mycotoxin	Fortification level (µg/kg)	Recovery (%)											
		25 mg anh. MgSO <sub>4</sub>		50 mg anh. MgSO <sub>4</sub>		25 mg PSA		50 mg PSA		25 mg C <sub>18</sub>		50 mg C <sub>18</sub>	
		Aver	%RSD	Aver	%RSD	Aver	%RSD	Aver	%RSD	Aver	%RSD	Aver	%RSD
Aflatoxin B <sub>1</sub>	5	106.06	1.22	111.84	1.17	104.15	4.11	100.26	2.47	120.74	1.23	125.24	1.18
Aflatoxin B <sub>2</sub>	5	103.37	2.31	103.88	2.23	95.57	1.81	89.05	1.94	103.32	0.67	103.93	2.87
Aflatoxin G <sub>1</sub>	5	102.95	1.32	102.16	2.12	90.29	1.08	81.60	2.30	104.02	1.13	103.96	1.72
Aflatoxin G <sub>2</sub>	5	103.90	2.01	104.22	3.34	96.54	2.01	89.49	1.25	105.28	0.79	105.32	2.81
Deoxynivalenol	100	92.81	0.77	92.48	1.74	87.51	1.53	80.54	1.35	92.03	1.01	92.65	0.37
Fumonisin B <sub>1</sub>	100	N.D	N.C	N.D	N.C	N.D	N.C	N.D	N.C	101.38	3.47	95.54	1.31
Fumonisin B <sub>2</sub>	100	N.D	N.C	N.D	N.C	N.D	N.C	N.D	N.C	104.51	6.66	96.74	4.17
Ochratoxin A	20	110.67	2.97	106.19	1.67	14.88	7.65	15.25	19.65	112.30	2.95	112.29	3.79
T-2 toxin	100	100.20	1.07	100.99	0.44	102.26	0.89	97.40	3.02	101.81	0.55	102.00	0.53
HT-2 toxin	100	95.73	1.18	98.29	1.46	89.20	3.11	84.03	3.86	98.20	2.07	97.10	0.88
Zeralenone	25	104.73	2.88	103.42	1.21	62.75	4.82	46.25	7.96	104.47	2.37	102.41	2.05
α-zearalenol	100	101.53	2.46	102.72	2.63	60.04	4.04	39.36	4.45	103.14	1.57	102.33	1.39
β-zearalenol	100	99.26	1.18	99.41	3.05	52.71	8.64	36.99	20.95	96.41	1.79	98.72	1.05

N.D Not detected, N.C not calculated

such as cereals, fish products, and Korean soybean paste. Finally, the immunoaffinity column (IAC) is the last purification and extraction method for mycotoxins, which has been superior to the other two methods in establishing the analytical conditions under the same purification conditions [21]. However, this method did not react with OTB, sterigmatocystin, and 15-acetyldeoxynivalenol. Therefore, the IAC method has disadvantages that must be used after confirming the cross-reactivity with

each mycotoxin to be analyzed, and it is difficult to use because of the high purchase price. With these results, in this study, we decided to analyze 13 different kinds of mycotoxins from Korean feedstuffs using the QuEChERS method.

#### Method validation

According to the CODEX and SANTE guideline, the limit of detection (LOD) is defined as the minimum analyte



concentration based on a signal-to-noise (S/N) ratio of 3:1, and the limit of quantitation (LOQ) on an S/N ratio of 9:1. These parameters were calculated by injection of neat solvent standard solution at different concentration levels. MLOD and MLOQ, applied to the pre-treatment method, are shown in Additional file 1: Table S5.

Linearity was tested by the evaluation of determination coefficients ( $R^2$ ). Linear range was estimated for curves prepared a neat solvent, as well as for spiked extracts and spiked samples (Additional file 1: Table S6). The target value for acceptability of mycotoxin curves was an  $R^2 > 0.99$ , and recovery tests were all matrix-matched calibration curves prepared for two analyzed matrices. Matrix effects (ME%) compared curves for neat solvent ESTD calibration, and matrix-matched ESTD calibration were obtained using five concentration levels of standard mycotoxin solutions (Additional file 1: Figure S2). Almost all target mycotoxins showed signal enhancement, and fumonisin B1 (FB1) reacted differently depending on the matrix. However, fumonisin B2 (FB2) displayed signal suppression.

Finally, control samples (5.0 g  $\pm$  0.1 g) of freeze-dried bean and maize were analyzed and results were within the acceptable range of recoveries (70–120%) (Additional file 1: Figure S3a and b, Table 4).

#### Measurement of 13 mycotoxins in Korean feedstuff samples

Measurements of the 13 residual mycotoxins in 39 Korean feedstuff samples are shown in Table 5. All measurements showed an RSD of less than 20%, confirming the reliability of the analysis (Table 5). The most frequently detected mycotoxins were FB1, DON, FB2, and ZEN with detections in 17, 16, 16, and 14 of 39 samples, respectively. In addition, AFB2, T-2, and HT-2 were detected in one of the 39 samples. Nine samples showed detectable amounts of mycotoxins, and four samples displayed three 3 mycotoxins. In the sample that showed AFB2, DON was simultaneously detected. Interestingly, DON, FB1, FB2, and ZEN were detected at the same time. Therefore, DON, FB1, FB2, and ZEN appear to be more important contaminants in feedstuff in Korea than contamination with aflatoxins.

Further, amounts of these mycotoxins were in the range of 0.0447–4.8073 mg/kg for DON, 0.0418–18.0943 mg/kg for FB1, 0.0181–5.3227 mg/kg for FB2 and 0.0200–4.5617 mg/kg for ZEN. Stall food samples showed the highest levels of DON, FB1, FB2, and ZEN were 4.8073, 18.0943, 5.3227, and 4.5617 mg/kg, respectively. Also, AFB2 was detected at 0.0018 mg/kg, T-2 at 0.0236 mg/kg, and HT-2 at 0.0168 mg/kg. AFB2 concentrations did not reach the current Korean MRLs of 10  $\mu$ g/kg, which is for the total amount of aflatoxin in compound feed, and

50  $\mu$ g/kg, the total amount of aflatoxin for single constituents of feedstuffs.

However, the Korean government currently applies MRLs as recommended levels, and feed producers are not obligated to meet these levels. Currently, Korean recommended MRLs are the same for T-2 and HT-2 and did not exceed this value in any tested sample. In contrast, the Korean recommended MRL for feedstuffs for FB1 and FB2 is defined as the sum of the two toxins. This MRL is 60 mg/kg for a single ingredient. MRLs for formulated feedstuffs for rearing livestock and pets, young ruminants, and ruminants are 5, 20, and 50 mg/kg, respectively. Thus, our results for the sum of FB1 and FB2 show that three samples in 39 samples were exceeded MRLs (Table 5).

Similarly, MRLs for Korean feedstuffs for DON are 0.9 mg/kg for rearing livestock and pets, 2.0 mg/kg for young livestock, and 10.0 mg/kg for single materials for feedstuff. Our results did not exceed these levels. MRL values of for ZEN in formulated feedstuffs for rearing pigs and ruminants, and for single materials for feedstuffs are not exceeded at the levels of 0.25, 0.5, and 3.0 mg/kg, respectively. One of 39 samples in the current study exceeded MRLs. Appropriate management of mycotoxins, FUMs and DON, in Korean feedstuffs should include proper monitoring.

Results of monitoring mycotoxins at the national level are continuously reported [29]. Eight mycotoxins (four AFs, FB1 and FB2, OTA and ZEN) were analyzed and the sum of FB1 and FB2 in 35 of 127 samples ranged from 4.8–738.5  $\mu$ g/kg. The highest concentrations were observed in maize (738  $\mu$ g/kg) and adlay (103  $\mu$ g/kg) [29].

In another report, AFs, OTA, ZEN, and FUM were frequently detected in Korean soybean paste (Doenjang), and FUM was detected in eight of 30 commercially available foodstuffs [21]. FB1 and FB2 were detected in 16 of 30 homemade Doenjang [21]. Thus, when consuming such Doenjang proper analysis of mycotoxins should be considered. In the present study, AFB1 was detected at a level of 4.45  $\mu$ g/kg, which may be unhealthy. Also, detection of AFG1 and AFG2 is not reported, and *Aspergillus flavus* rather than *A. parasiticus* are likely involved in contamination of aflatoxin in Korean Doenjang. *A. flavus* appears to produce more AFB1 and AFB2 than AFG1 and AFG2 [30, 31].

In China, a total of 160 feedstuff samples, consisting of 76 maize and 84 soybean meals, were analyzed using an LC–MS/MS analytical system for 26 mycotoxins and none of these samples showed concentrations of AFB1, T-2, and ZEN that exceeded MRLs [32]. Maize (13.1%) and soybean (2.4%) meal samples exceeded the maximum tolerance of DON for formulated pig feed of

**Table 4** Recovery (n = 5) of mycotoxins obtained from QuEChRES sample preparation

Mycotoxin	Fortification level ( $\mu\text{g}/\text{kg}$ )	Recovery (%)					
		Maize			Bean		
		Aver	STDEV	%RSD	Aver	STDEV	%RSD
Aflatoxin B <sub>1</sub>	5	83.7	2.1	2.5	83.7	3.2	3.8
	10	86.3	2.5	2.9	78.8	1.5	1.9
	50	86.3	3.3	3.8	85.5	1.0	1.2
Aflatoxin B <sub>2</sub>	5	77.0	5.2	6.8	85.3	3.3	3.9
	10	81.5	1.1	1.3	87.0	0.7	0.8
	50	83.7	1.9	2.2	86.6	0.7	0.9
Aflatoxin G <sub>1</sub>	5	71.0	2.4	3.4	77.8	2.0	2.5
	10	79.9	0.6	0.8	85.4	1.2	1.4
	50	86.1	1.8	2.1	87.2	0.1	0.2
Aflatoxin G <sub>2</sub>	5	87.4	6.2	7.1	82.8	1.0	1.3
	10	88.9	3.0	3.3	88.8	2.1	2.4
	50	88.4	2.0	2.3	83.1	0.9	1.1
Deoxynivalenol	100	89.0	10.0	11.3	100.5	2.1	2.1
	200	76.7	2.7	3.5	87.0	3.4	3.9
	1000	78.5	5.5	6.9	74.9	3.9	5.2
Fumonisin B <sub>1</sub>	100	99.0	3.3	3.4	86.7	3.2	3.6
	200	73.9	1.3	1.8	78.1	2.2	1.5
	1000	75.0	1.1	1.4	72.1	1.1	1.5
Fumonisin B <sub>2</sub>	100	112.3	2.9	2.6	115.6	4.1	3.5
	200	80.9	7.5	9.3	88.0	2.6	2.9
	1000	73.3	1.5	2.0	73.1	1.5	2.0
Ochratoxin A	20	106.3	1.8	1.7	96.4	1.8	1.9
	40	76.6	1.3	1.7	89.3	1.7	1.8
	200	88.1	0.6	0.7	82.5	1.0	1.2
T-2 toxin	100	86.7	2.9	3.3	89.3	2.3	2.6
	200	90.0	1.2	1.3	94.7	1.2	1.3
	1000	88.7	1.6	1.8	93.7	1.9	2.0
HT-2 toxin	100	72.8	3.2	4.4	86.5	2.96	3.1
	200	83.2	1.7	2.0	92.2	3.9	4.2
	1000	89.6	2.4	2.7	90.2	3.9	1.2
Zeralenone	25	70.1	4.2	5.9	88.5	2.8	3.2
	50	83.3	3.2	3.8	84.1	3.6	4.3
	100	89.4	1.9	2.1	80.5	1.9	2.4
$\alpha$ -zearalenol	100	75.2	3.4	4.5	111.6	2.7	2.4
	200	82.8	2.5	3.0	97.8	7.0	7.1
	1000	86.3	3.3	3.8	88.7	2.0	2.2
$\beta$ -zearalenol	100	94.1	6.0	6.4	83.6	2.5	3.0
	200	86.3	1.0	1.1	84.0	3.5	4.1
	1000	84.4	3.4	4.0	84.4	1.7	2.0



**Table 5** Measured contents of mycotoxins in 39 Korean feedstuffs

No	Type of feedstuffs	Contents (average ± standard deviation, mg/kg)												
		AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	DON	FMB <sub>1</sub>	FMB <sub>2</sub>	OTA	T-2 toxin	HT-2 toxin	ZON	α-ZEL	β-ZEL
1	Formula animal food for dogs	-	-	-	-	-	0.0951 ± 0.0122	-	-	-	-	-	-	-
2	Foodstuffs for calves	-	-	-	0.6626 ± 0.0266	-	1.7590 ± 0.0629	0.3965 ± 0.0272	-	-	-	0.1441 ± 0.0025	-	-
3	Fish soluble from fish processing offal	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Cat food	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Feed ingredients for animal	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Cat food	-	-	-	-	-	0.1691 ± 0.0097	0.0793 ± 0.0036	-	-	-	0.0200 ± 0.0011	-	-
7	Formula animal feed for dogs	-	-	-	0.2823 ± 0.0140	0.3927 ± 0.0093	0.1228 ± 0.0155	-	-	-	-	0.0249 ± 0.0011	-	-
8	Dog food	-	-	-	0.2362 ± 0.0043	0.4797 ± 0.0340	0.1458 ± 0.0251	-	-	-	-	0.0312 ± 0.0020	-	-
9	Cat food	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Feed supplements	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Product from <i>Bacillus subtilis</i> rich in protein	-	-	-	0.5422 ± 0.0687	-	-	-	-	-	-	-	-	-
12	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Formula animal food for dogs	-	-	-	-	-	0.3957 ± 0.0310	0.2514 ± 0.0059	-	-	-	-	-	-
14	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Cat food	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Formula animal food for dogs	-	-	-	0.1284 ± 0.0066	-	-	0.1417 ± 0.0116	-	-	-	0.0069 ± 0.0010	-	-
18	Cat food	-	-	-	-	-	-	-	-	-	-	-	-	-
19	Cat food	-	-	-	-	-	-	0.1643 ± 0.0055	-	-	-	0.0076 ± 0.0001	-	-
20	Cat food	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	-	-	-
23	Formula animal food for dogs	-	-	-	0.0447 ± 0.0069	-	-	-	-	-	-	-	-	-
24	Formula animal food for dogs	-	-	-	-	-	-	-	-	-	-	0.0056 ± 0.0006	-	-
25	Feedstuffs for pigs	-	-	-	0.0527 ± 0.0035	0.0418 ± 0.0040	-	-	-	-	-	-	-	-
26	Feedstuffs for chickens	-	-	-	0.2931 ± 0.0199	0.4962 ± 0.0495	0.1634 ± 0.0226	-	-	-	-	0.0370 ± 0.0016	-	-
27	Feedstuffs for chickens	-	-	-	-	0.1064 ± 0.0092	0.0337 ± 0.0061	-	-	-	-	-	-	-
28	Maize gluten	-	-	-	-	0.0427 ± 0.0030	-	-	0.0236 ± 0.0031	0.0168 ± 0.0016	-	-	-	-
29	Feedstuffs for pigs	-	-	-	0.0614 ± 0.0042	0.2474 ± 0.0129	0.0928 ± 0.0142	-	-	-	-	-	-	-
30	Sodium chloride	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Feedstuffs for chickens	-	-	-	0.1411 ± 0.0054	0.0931 ± 0.0179	0.0181 ± 0.0032	-	-	-	-	-	-	-
32	Stall food for animals	-	-	-	2.3115 ± 0.3352	4.2907 ± 0.1852	2.0989 ± 0.0751	-	-	-	-	2.0785 ± 0.1932	-	-
33	Stall food for animals	-	-	-	4.8073 ± 0.1580	18.0943 ± 0.8066	5.3277 ± 0.0954	-	-	-	-	4.5617 ± 0.3116	-	-

**Table 5** (continued)

No	Type of feedstuffs	Contents (average ± standard deviation, mg/kg)												
		AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	DON	FMB <sub>1</sub>	FMB <sub>2</sub>	OTA	T-2 toxin	HT-2 toxin	ZON	α-ZEL	β-ZEL
34	Cakes of soy and cereal residue for animal consumption					0.0791 ± 0.0016	-	-				-		
35	Mazie gluten feed					3.4782 ± 0.0523	8.0416 ± 0.6234	2.4445 ± 0.1270				1.4160 ± 0.0394		
36	Mazie gluten					0.1177 ± 0.0106	1.2025 ± 0.0610	1.6055 ± 0.0691				0.6674 ± 0.0281		
37	Cakes of soy and cereal residue for animal consumption					-	-	-				-		
38	Cotton seed					-	-	-				0.0513 ± 0.0099		
39	Cakes of soy and cereal residue for animal consumption					0.8027 ± 0.0359	1.2528 ± 0.0635	0.4420 ± 0.0224				0.1420 ± 0.0171		

AFB<sub>1</sub>, Aflatoxin B<sub>1</sub>; AFB<sub>2</sub>, aflatoxin B<sub>2</sub>; AFG<sub>1</sub>, aflatoxin G<sub>1</sub>; AFG<sub>2</sub>, aflatoxin G<sub>2</sub>; DON, deoxynivalenol; FMB<sub>1</sub>, fumonisin B<sub>1</sub>; FMB<sub>2</sub>, fumonisin B<sub>2</sub>; OTA, ochratoxin A; ZON, Zearalenone; α-ZEL, α-Zearalenol; β-ZEL, β-Zearalenol

1000 µg/kg [32]. Mycotoxin contamination varies by country. Further, weather condition, such as temperature and humidity, may be key factors for the fungal production of mycotoxins [33, 34].

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-021-00602-9>.

**Additional file 1: Figure S1.** Diagram of the employed sample preparation for the analysis of mycotoxins. **Figure S2.** Matrix effects of bean and maize as shown as signal suppression and enhancement. **Figure S3.** Average recovery ( $n = 5$ ) of mycotoxins obtained from QuEChRES sample preparation (a: Bean, B: Maize). **Table S1.** Concentrate ranges of each mycotoxin for linearity. **Table S2.** Matrix-matched calibration curve preparation. **Table S3.** Peak intensity (Area) with final dilution. **Table S4.** Recovery by two types of absorbents after extraction towards mycotoxins. **Table S5.** Calibration ranges, MLOD and MLOQ of the optimized analytical method. **Table S6.** Determination coefficient ( $R^2$ ) for the targeted mycotoxins in two validated raw feed material-based matrices such as bean and maize

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## Authors' contributions

SC, SL, and JM designed the experiments and wrote the manuscript. HJ, MP, HH, and HJ performed all the experiments. HJ, SL, and JM performed data analysis. All authors read and approved the final manuscript.

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## Availability of data and materials

Yes.

## Declarations

## Competing interests

The authors declare that they have no competing interests.

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