Journal of Food Protection, Vol. 82, No. 6, 2019, Pages 1007–1015
https://doi.org/10.4315/0362-028X.JFP-18-456

Research Note

Occurrence of Multiple Mycotoxins in Various Types of Rice and Barley Samples in Thailand

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MS 18-456: Received 27 September 2018/Accepted 30 January 2019/Published Online 22 May 2019

ABSTRACT

The prevalence of mycotoxins is often increased by climatic conditions prevailing in tropical regions. Therefore, consumers in tropical countries such as Thailand have a higher risk of mycotoxin exposure. Existing reports have revealed mycotoxin contamination in rice. This study was conducted to determine the occurrence of multiple mycotoxins in barley and nine types of rice sold in Thailand and to assess consumer health risk. A total of 300 samples collected from various markets in Thailand were analyzed for the presence of 16 mycotoxins using a QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure and a triple quadrupole mass spectrometer equipped with an electrospray ionization source. Of the 300 samples, 124 (41.33%) were contaminated with at least one mycotoxin, and 38.71% of the mycotoxin-positive samples were simultaneously contaminated with more than one toxin. The incidence of mycotoxin contamination differed among the rice and barley samples. Beauvericin, diacetoxyscirpenol, zearalenone, and aflatoxins were the most frequently found mycotoxins. However, the concentrations of regulated mycotoxins were below the regulatory limits. The assessed mycotoxin exposure does not represent a health risk for Thai consumers because the estimated exposure concentrations were lower than the tolerable daily intake values established by the Joint FAO/WHO Expert Committee on Food Additives. However, our findings suggest that continued monitoring of mycotoxin contamination in rice and barley and concomitant risk assessments are warranted.

HIGHLIGHTS

- Contamination with multiple mycotoxins was found in rice and barley.
- BEA, DAS, ZEA, and aflatoxins were the mycotoxins most frequently found in samples.
- The assessed mycotoxin exposure does not represent a health risk for consumers.

Key words: Barley; Liquid chromatography–tandem mass spectrometry; Mycotoxins; QuEChERS; Rice

Mycotoxins are toxic substances produced by certain species of fungi. Contamination of agricultural commodities with mycotoxins during pre- and postharvest processing is a global problem that leads to serious risks to human and animal health. Environmental conditions, especially high humidity and temperature, can favor fungal proliferation, resulting in such contamination *(3)*. Consumers in tropical countries such as Thailand encounter high risk of mycotoxin exposure. Mycotoxin contamination occurs in a wide variety of agricultural products, especially seeds and grains such as rice (*Oryza sativa*), which is one of the most important staple foods for the world's population. In Thailand, rice is the most important food crop and plays an important role in the economic system. Thailand is one of the world's most important rice producers, producing rice for both domestic consumption and export, and is ranked among the top five Asian rice exporters *(8)*. However, rice is relatively susceptible to fungal contamination, which can produce mycotoxins *(17, 24, 25)*. Mycotoxins are very persistent in food and cannot be completely eliminated during food processing *(18)*. Human health risks are usually associated with the direct consumption of food products contaminated with mycotoxins.

To date, several reports of mycotoxin contamination in rice from various countries have been published *(2, 22, 26, 29, 32)*. Some studies have revealed the occurrence of aflatoxin B_1 (AFB_1) in Thailand *(4, 11, 21, 28)*. Considering the health impact of mycotoxin consumption, data on contamination of rice by multiple mycotoxins are required to better estimate the potential problem. The main aim of this study was to evaluate barley and nine types of rice consumed in Thailand for the occurrence of various mycotoxins: AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), citrinin (CTN), nivalenol (NIV), deoxynivalenol (DON), diacetox-

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yscirpenol (DAS), T-2 toxin (T2), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), zearalenone (ZEA), sterigmatocystin (STG), beauvericin (BEA), and alternariol (ATL). These data were then used to assess consumer health risks.

MATERIALS AND METHODS

Toxins and chemicals. The analytical standards of mycotoxins AFB_1, AFB_2, AFG_1, AFG_2, OTA, CTN, NIV, DON, DAS, T2, FB_1, FB_2, ZEA, STG, and BEA were purchased from Romer Labs (Tulln, Austria), and the standard for ATL was from Sigma-Aldrich (Taufkirchen, Germany). Primary secondary amine (PSA) and octadacyl (C18) were purchased from Macherey-Nagel (Düren, Germany). Sodium chloride and magnesium sulfate anhydrous ($MgSO_4$) of analytical grade were purchased from AppliChem GmbH (Darmstadt, Germany). Other reagents and chemicals were of analytical grade.

Sample collection. A total of 300 samples were collected, 30 samples of each type of grain: black sticky rice, brown rice, GABA (γ-aminobutyric acid) rice, Japanese rice, jasmine rice, red rice, riceberry rice, white rice, white sticky rice, and barley. Samples were randomly collected from supermarkets and retail shops in Bangkok, Thailand, April to July 2017. Different brands were selected to obtain a representative sample of products sold in supermarkets in Bangkok. The samples were stored at 4°C until analyzed.

QuEChERS sample extraction and cleanup. The analysis was performed using a QuEChERS (quick, easy, cheap, effective, rugged, and safe) method modified from those of Arroyo-Manzanares et al. *(1)* and Jettanajit and Nhujak *(11)*. A 1.0-g portion of a rice or barley sample was mixed with 5 mL of Milli-Q water and 5 mL of 10% (v/v) acetic acid in acetonitrile for 2 to 3 min with a vortex mixer. Then 2.0 g of $MgSO_4$, 0.5 g of NaCl, 0.5 g of sodium citrate tribasic dehydrate, and 0.25 g of sodium citrate dibasic sesquihydrate were added, and the mixture was shaken for 1 to 2 min. The acetonitrile fraction was separated by centrifugation at $1,968 \times g$ for 5 min, and 2 mL of the supernatant was transferred into a tube containing 300 mg of $MgSO_4$, 50 mg of C18, and 25 mg of PSA and shaken for 2 to 3 min. This mixture was separated by centrifugation at $1,968 \times g$ for 15 min, then 1 mL of supernatant was completely evaporated to dryness under a nitrogen stream at 40°C on a heating block. The residue was reconstituted with the mobile phase solution, injected onto a 0.22-μm-pore-size syringe filter (Sartorius AG, Goettingen, Germany), and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

LC-MS/MS with electrospray ionization. A high-performance LC-MS/MS system with electrospray ionization was used to determine the presence of 16 mycotoxins in samples of barley and nine types of rice. The LC-MS/MS method was modified from that of Puangkham et al. *(23)*. The chromatographic separation was performed on a C18 column (50 by 4.6 mm, 1.8-μm particle size; ZORBAX Eclipse plus RRHD, Agilent, Santa Clara, CA). The column was maintained at 40°C. The mobile phase consisted of 5 mM ammonium acetate with 0.2% acetic acid in water (mobile phase A) and 0.2% acetic acid in acetonitrile (mobile phase B) with a gradient elution protocol: 0 to 1 min, 10% B; 1 to 5 min, 10 to 95% B; 5 to 10 min, 95% B; and 10 to 12 min, 10% B. The column was reequilibrated for 3 min between injections. The mobile phase solution was filtered through a 0.22-μm-pore-size membrane (Sartorius AG) and ultrasonically degassed prior to application. The flow rate was 0.3 mL/min, and the injection volume was 5 μL. The assay time was 14 min. The triple quadrupole mass spectrometer (6460 triple, Agilent, Waldbronn, Germany) was equipped with an electrospray ionization source run in both positive and negative ion modes under the multiple reaction monitoring mode, with two separate chromatographs analyzed per sample. The ionization source parameters were optimized as follows: capillary voltage, 3,500 V; gas temperature, 320°C; gas flow rate, 11 L/min; nebulizer, 50 lb/in^2. The parameters for the mass spectrometer were optimized as shown in Table 1.

Quantification and method validation. Validation of the LC-MS/MS method for AFB_1, AFB_2, AFG_1, AFG_2, OTA, CTN, NIV, DON, DAS, T2, FB_1, FB_2, ZEA, STG, BEA, and ATL was performed to assess the efficiency of this analytical method by investigating the recovery, repeatability, linear working range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and matrix effects in accordance with the guidelines of Commission Decision 2002/657/EC *(5)*. We checked the suitability of the proposed method for the determination of mycotoxins in nine types of rice and in barley, and linearity, LOD, LOQ, precision, and trueness were determined using representative matrices from each category. The LOD and LOQ of the method were evaluated at signal/noise values of 3:1 and 10:1, respectively. Linear regression analysis was conducted for the 16 mycotoxin standards under the optimized LC-MS/MS conditions. Within-day recovery and precision (repeatability, expressed as percent relative standard deviation [RSD]) were determined by analyzing seven replicates of spiked samples containing the 16 mycotoxins at three quality control concentrations. The interday precision was determined by analyzing quality control samples on 5 days (one batch per day). The matrix-matched calibration curves were prepared in three replicates by spiking the working standard solution into blank samples to yield final concentrations of 1 to 50 μg/kg (1, 2.5, 5, 10, 25, and 50 μg/kg) for AFB_1, AFB_2, AFG_1, AFG_2, OTA, BEA, and STER; 1.5 to 100 μg/kg (1.5, 5, 10, 25, 50, and 100 μg/kg) for FB_1 and FB_2; 2 to 200 μg/kg (2, 5, 10, 50, 100, and 200 μg/kg) for ALT, CIT, and T2; 10 to 500 μg/kg (10, 20, 50, 75, 100, 250, and 500 μg/kg) for DAS, ZEA, and DON; and 100 to 1,000 μg/kg (100, 200, 400, 500, 750, and 1,000 μg/kg) for NIV. The matrix effects were determined by comparing the slope of six concentration calibration curves of the target analyte in postextraction spiked samples with those obtained in solvent alone.

Health risk evaluation. The type of mycotoxin exposure through rice consumption and data on the distribution of exposure concentrations within the studied population were combined with the average concentration for each contaminant under investigation. The mean and 97.5 percentile exposure (p97.5) values were evaluated, and a risk assessment was performed for average and high exposure. To compare dietary exposure concentrations with toxicological reference concentrations, which are expressed per kilogram of body weight, an average body weight of 60 kg was used *(31)*. The estimate dietary exposure (EDI; μg/kg of body weight per day) of the chemical residues were calculated with equation 1:

$$\mathrm{EDI} = \sum \mathrm{RL}_i \times \frac{A_i}{\mathrm{BW}} \quad (1)$$

where RL_i is the mycotoxin residue in the rice or barley (μg/kg), A_i is the rice or barley consumption rate (g per person per day), and BW is body weight (kg). Risk assessment is calculated by comparing the concentrations of residues detected with the provisional maximum tolerable daily intake (PMTDI). In this

TABLE 1. *MS/MS parameters for the determination of 16 mycotoxins*

Analyte	Precursor ion (m/z)	Product ions (m/z)[a]	Mean ion ratio[b]	Collision energy (eV)	Fragmentor (V)	Retention time (min)	Polarity
AFB_1	313.07	285.1	0.75	21	150	6.32	Positive
		241.0		35	150		
AFB_2	315.09	287.1	0.79	25	160	6.18	Positive
		259.0		29	160		
AFG_1	329.07	311.0	0.91	25	160	6.16	Positive
		243.0		43	160		
AFG_2	331.08	313.0	0.86	25	180	6.02	Positive
		245.0		29	180		
OTA	404.00	192.9	0.68	48	130	6.79	Positive
		102.1		80	130		
ZEA	319.16	283.0	0.83	5	80	7.24	Positive
		187.0		17	80		
BEA	801.40	784.3	0.52	13	160	9.13	Positive
		244.1		35	160		
FB_1	722.40	352.5	0.89	40	160	5.59	Positive
		334.4		45	160		
FB_2	706.30	336.2	0.75	35	200	5.99	Positive
		318.3		40	200		
T2	489.40	387.3	0.88	20	170	7.00	Positive
		245.2		26	170		
DAS	384.20	307.1	0.59	5	60	6.28	Positive
		199.0		13	60		
STER	325.10	309.9	0.68	25	160	7.46	Positive
		281.0		35	160		
ALT	259.10	187.9	0.81	25	240	6.08	Positive
		160.1		33	240		
CIT	251.10	233.1	0.21	3	120	5.79	Positive
		205.1		20	120		
DON	355.10	265.1	0.58	4	90	3.71	Negative
		59.1		10	90		
NIV	371.10	281.0	0.84	4	80	2.26	Negative
		59.1		10	80		

[a] Bottom number of each couplet is the qualitative product ion.
[b] Ratio of peak area of quantifier to qualifier transition.

TABLE 2. *Performance characteristics of the proposed method*

	White rice types			Colored rice types			Barley		
Analyte	LOD (μg/kg)	LOQ (μg/kg)	R^2	LOD (μg/kg)	LOQ (μg/kg)	R^2	LOD (μg/kg)	LOQ (μg/kg)	R^2
AFB_1	0.28	0.92	0.9953	0.31	1.0	0.9974	0.25	0.85	0.9987
AFB_2	0.21	0.71	0.9972	0.27	0.86	0.9979	0.21	0.63	0.9985
AFG_1	0.14	0.47	0.9981	0.20	0.67	0.9985	0.16	0.52	0.9991
AFG_2	0.17	0.52	0.9955	0.22	0.74	0.9962	0.17	0.55	0.9967
OTA	0.20	0.65	0.9991	0.20	0.69	0.9989	0.19	0.62	0.9991
ZEA	6.05	20.15	0.9967	10.6	35.2	0.9955	7.73	25.5	0.9973
BEA	0.20	0.68	0.9995	0.26	0.88	0.9989	0.15	0.51	0.9967
FB_1	0.31	1.04	0.9994	0.47	1.55	0.9992	0.38	1.25	0.9959
FB_2	0.44	1.48	0.9985	0.66	2.20	0.9987	0.53	1.77	0.9963
T2	0.76	2.53	0.9966	1.35	4.60	0.9936	0.98	3.26	0.9971
DAS	1.87	6.22	0.9985	2.63	8.75	0.9972	2.05	6.84	0.9989
STER	0.16	0.53	0.9978	0.22	0.75	0.9934	0.19	0.62	0.9959
DON	7.53	25.11	0.9956	10.3	34.5	0.9932	8.47	28.2	0.9948
NIV	26.80	89.34	0.9943	28.2	92.8	0.9935	25.6	85.3	0.9944
ALT	0.55	1.85	0.9989	0.68	2.30	0.9991	0.56	1.88	0.9981
CIT	0.64	2.14	0.9986	0.82	2.65	0.9985	0.67	2.23	0.9976

TABLE 3. *Accuracy and precision in white rice types, colored rice types, and barley*[a]

	White rice types									Colored rice types									Barley								
	Recovery (%) ($n = 7$)			Intraday precision (%RSD) ($n = 7$)			Interday precision (%RSD) ($n = 18$)			Recovery (%) ($n = 7$)			Intraday precision (%RSD) ($n = 7$)			Interday precision (%RSD) ($n = 18$)			Recovery (%) ($n = 7$)			Intraday precision (%RSD) ($n = 7$)			Interday precision (%RSD) ($n = 18$)		
Mycotoxin	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
AFB_1	88.5	95.3	97.7	5.1	2.6	3.7	3.9	3.4	2.6	80.3	83.8	85.6	7.9	5.3	4.7	8.4	9.1	6.7	83.3	86.8	89.5	5.7	5.5	4.4	6.3	5.9	5.2
AFB_2	87.6	95.9	95.7	2.5	3.1	1.9	2.1	1.4	1.4	81.2	86.7	87.9	6.9	4.8	3.1	7.8	7.9	7.6	86.4	90.1	88.6	4.5	4.9	4.6	5.9	5.4	4.9
AFG_1	90.7	97.7	101	4.3	2.7	2.1	2.7	2.7	2.1	83.6	87.6	90.5	6.3	5.5	4.6	8.2	8.7	9.1	85.2	92.5	93.4	4.4	5.2	5.0	6.1	5.8	7.4
AFG_2	85.7	93.5	94.3	3.4	2.7	1.7	1.8	2.2	1.3	80.7	83.5	88.6	7.4	4.7	3.5	8.5	9.3	7.2	88.1	85.7	90.3	5.9	6.1	4.7	7.5	7.1	7.7
OTA	92.9	94.1	99.5	2.6	2.1	3.2	2.4	1.9	2.5	84.5	87.7	88.3	5.8	6.1	4.7	7.4	8.5	7.2	92.8	90.4	91.7	4.3	3.8	2.9	4.6	4.2	5.6
ZEA	89.2	90.6	90.5	3.3	4.6	3.4	3.4	2.8	2.2	78.4	80.2	83.4	8.7	8.3	6.2	9.1	9.3	8.4	83.4	88.3	87.9	6.4	6.7	7.5	8.8	7.5	9.3
BEA	97.7	99.4	103	3.5	2.3	1.8	2.8	1.7	2.2	91.1	95.2	93.7	4.6	3.9	4.2	6.5	5.8	4.9	89.7	91.8	93.4	4.1	5.3	3.6	4.5	5.0	4.8
FB_1	93.5	94.8	93.4	2.1	1.6	1.9	2.3	3.8	2.6	82.3	83.1	87.6	5.5	5.8	4.7	8.6	7.7	8.1	79.5	83.7	85.6	4.9	4.5	5.1	6.5	5.9	5.4
FB_2	90.6	92.5	94.2	2.3	1.8	2.1	2.4	2.0	2.5	81.6	80.8	85.2	6.2	5.4	4.8	7.8	8.3	8.6	81.4	86.6	80.2	5.1	5.3	4.8	7.4	6.6	7.9
T2	92.0	93.2	102	4.2	3.3	4.2	3.0	2.4	3.7	85.3	84.4	87.9	5.7	4.9	3.9	9.7	9.6	7.3	81.3	84.8	88.6	3.8	4.4	3.9	4.2	3.2	5.1
DAS	95.4	96.6	98.6	2.7	2.7	2.0	2.5	1.7	1.8	87.7	89.6	86.8	4.5	4.7	4.2	6.9	5.7	5.5	85.7	87.9	90.4	4.2	3.9	4.5	4.3	4.5	3.8
STER	92.3	95.7	98.6	3.4	2.9	2.2	4.3	1.5	2.3	82.6	83.3	88.1	5.1	3.9	3.6	6.7	5.9	4.6	88.3	90.8	92.7	4.3	4.0	4.6	4.7	5.5	6.4
DON	81.6	88.1	89.4	6.3	5.7	4.9	5.6	4.9	6.4	73.6	72.2	78.6	8.3	6.5	5.7	9.1	7.7	7.2	78.2	82.6	80.3	7.8	6.5	7.9	7.5	8.1	7.6
NIV	79.5	83.8	82.1	7.7	5.4	4.9	4.4	5.4	3.2	75.2	78.3	79.8	8.5	7.6	6.1	9.8	8.1	7.0	74.5	79.7	83.6	6.9	7.1	8.3	8.1	8.5	7.7
ALT	89.0	91.1	95.4	3.0	2.2	2.7	2.6	1.9	2.2	83.2	85.9	86.2	4.2	3.8	3.5	6.7	6.0	5.9	86.8	89.4	92.4	4.6	4.8	3.9	5.3	6.2	6.5
CIT	90.2	92.2	93.7	3.5	1.7	2.3	2.4	2.1	1.7	82.5	85.7	80.4	4.7	4.5	3.6	6.6	7.2	5.5	85.3	88.8	90.6	4.7	3.2	2.8	5.7	5.1	3.2

[a] Spiking levels. L1 for AFB_1, AFB_2, AFG_1, AFG_2, OTA, BEA, and STER: 1 μg/kg; for FB_1, FB_2, and ALT: 2 μg/kg; for T2 and CIT: 5 μg/kg; for DAS: 10 μg/kg; for ZEA and DON: 50 μg/kg; for NIV: 100 μg/kg. L2 for AFB_1, AFB_2, AFG_1, AFG_2, OTA, BEA, and STER: 10 μg/kg; for FB_1, FB_2, and ALT: 20 μg/kg; for T2 and CIT: 50 μg/kg; for DAS: 100 μg/kg; for ZEA and DON: 250 μg/kg; for NIV: 500 μg/kg. L3 for AFB_1, AFB_2, AFG_1, AFG_2, OTA, BEA, and STER: 40 μg/kg; for FB_1, FB_2, and ALT: 100 μg/kg; for T2 and CIT: 200 μg/kg; for DAS: 250 μg/kg; for ZEA and DON: 500 μg/kg; for NIV: 1,000 μg/kg.

TABLE 4. *Matrix effects for each analyte in difference rice sample types and barley*

	Matrix effect (%)									
Mycotoxin	White rice	Jasmine rice	Japanese rice	White sticky rice	Brown rice	GABA brown rice	Black sticky rice	Red rice	Riceberry rice	Barley
AFB_1	87.27	86.72	86.41	83.35	79.32	81.37	78.16	80.15	78.84	86.48
AFB_2	85.33	84.53	85.62	82.49	77.15	79.94	75.32	79.54	75.32	85.63
AFG_1	83.79	84.65	85.35	84.11	80.26	80.65	79.47	78.36	78.52	88.32
AFG_2	90.41	92.14	91.18	89.65	83.65	84.68	84.88	83.84	80.36	87.15
OTA	92.77	95.43	91.54	89.32	106.37	103.52	98.95	96.38	107.73	102.36
ZEA	68.32	70.46	72.36	71.64	62.95	65.71	64.32	63.11	64.12	70.17
BEA	94.54	98.59	95.15	90.52	111.32	107.58	109.68	107.22	101.35	101.44
FB_1	91.71	93.45	93.16	90.36	78.65	76.23	73.42	79.33	72.59	101.48
FB_2	84.23	84.18	82.47	81.51	73.77	75.85	72.53	76.16	72.65	106.38
T2	73.36	77.36	78.54	74.82	70.48	72.67	72.27	74.58	73.79	80.14
DAS	84.52	85.61	88.63	85.67	79.85	75.23	80.69	78.63	78.37	85.36
STER	86.78	88.79	87.49	82.36	82.63	80.85	81.37	82.37	80.16	87.83
DON	73.37	72.14	70.32	71.59	69.17	68.64	66.34	68.15	68.63	74.47
NIV	79.48	78.37	76.45	75.48	71.43	72.33	70.75	70.66	70.74	77.95
ALT	89.56	90.44	90.59	88.23	83.72	80.79	81.46	80.35	79.45	86.34
CIT	87.45	90.65	88.47	85.62	81.44	83.47	82.97	81.74	83.75	87.78

study, the hazard quotient (HQ) was used to indicate the long-term risk assessment. HQ can be calculated with equation 2:

$$\text{HQ} = \frac{\text{EDI}}{\text{PMTDI}} \times 100\% \quad (2)$$

When the value of HQ is >100%, the rice or barley involved should be considered a risk to consumers; when the value is <100%, the rice or barley is acceptable or low risk.

Because of the genotoxic and carcinogenic effects of aflatoxins, no TDI can be set. Therefore, the risk assessment calculation was performed separately by determination of the population at risk (cases of cancer per year per 100,000 persons) for primary liver cancer *(12)*. The hepatitis B antigen (HBsAg)–positive prevalence rate was assumed to be 5% for the Thai population:

$$\text{Population risk} = \text{exposure} \times \text{average potency}$$

$$\begin{aligned}\text{Average potency} &= (0.3 \times \text{HBsAg}^+ \text{ rate}) \\ &\quad + (0.01 \times \text{HBsAg}^- \text{ rate}) \\ &= (0.3 \times 0.05) + (0.01 \times 0.95) \\ &= 0.0245 \text{ cases/year/}100,000 \text{ persons}\end{aligned}$$

RESULTS

Optimization of LC-MS/MS. The optimization procedure was modified from that of Puangkham et al. *(23)*. In both ionization modes, the highest sensitivity for all 16 mycotoxins was achieved using 5 mM ammonium acetate with 0.2% acetic acid in water (A: positive ion mode) and 5 mM ammonium acetate in 9:1 water-methanol (A: negative ion mode) with a mobile phase of 0.2% acetic acid in acetonitrile (positive ion mode) or methanol (negative ion mode). The MS parameters are shown in Table 1.

Method validation and quality assurance. The LC-MS/MS method used in this study had an adequate linearity range and adequate intra- and interday precision and accuracy for quantification of the 16 mycotoxins. Linearity of the calibration curves for groups of white rice, groups of colored rice, and barley matrices, expressed as the determination coefficients (R^2), gave values that were all ≥0.9932. The LOD and LOQ for mycotoxin detection in rice and barley were 0.14 to 26.80 and 0.47 to 89.34 μg/kg, respectively (Table 2). The mean recovery and intra- and interday precision (%RSD) of the spiked rice and barley samples are shown in Table 3. The percentage of extraction recovery of FB_1 reported in this study was higher than that reported by others *(15)*. The differences in recovery rate may be associated with the smaller sample size used in our study or the use of alumina-neutral as a sorbent for the clean-up step in the previous study, which causes significant losses of acidic analytes. Considering matrix effects, we found that all compounds were subjected to ion suppression in white rice matrices. Both matrix enhancement and suppression were observed for target mycotoxin analytes in colored rice matrices and barley (Table 4). All compounds were subjected to ion suppression.

Occurrence of mycotoxins in rice and barley. The method described here was applied to a monitoring survey of 16 mycotoxins in barley and nine kinds of rice (black sticky rice, brown rice, GABA rice, Japanese rice, jasmine rice, red rice, riceberry rice, white rice, and white sticky rice). The summary of investigated compounds found in each variety is shown in Table 5. Overall, 124 (41.33%) of the total 300 samples were contaminated with at least one mycotoxin, and 38.71% of the mycotoxin-positive samples were simultaneously contaminated with more than one toxin. The incidence of mycotoxin contamination differed among the types of samples. BEA, DAS, ZEA, and aflatoxins were the mycotoxins most frequently found in all contaminated samples. AFB_1 was quantifiable in 20% (6 of 30) of black sticky rice samples, 10% (3 of 30) of riceberry rice samples, and 6.67% (2 of 30) of GABA brown rice samples; and AFG_1 was quantifiable in 3.33% (1

TABLE 5. *Occurrence of mycotoxins in the analyzed rice and barley samples*

Mycotoxin	Occurrence[a]	Type of sample									
		White rice	Jasmine rice	Japanese rice	White sticky rice	Brown rice	GABA brown rice	Black sticky rice	Red rice	Riceberry rice	Barley
AFB_1	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.67)	6 (20)	0 (0)	3 (10)	0 (0)
	Mean concn (μg/kg)						1.38	1.44		1.48	
	LB–UB estimate (μg/kg)	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0.1–0.4	0.3–0.5	0–0.3	0.2–0.4	0–0.3
AFB_2	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2
AFG_1	No. (%) positive samples	0 (0)	1 (3.33)	0 (0)	1 (3.33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)		1.41		1.78						
	LB–UB estimate (μg/kg)	0–0.1	0.1–0.2	0–0.1	0.1–0.2	0–0.1	0–0.1	0–0.1	0–0.1	0–0.1	0–0.1
AFG_2	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	00 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2
OTA	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2	0–0.2
ZEA	No. (%) positive samples	3 (10)	1 (3.33)	1 (3.33)	11 (36.67)	0 (0)	4 (13.33)	4 (13.33)	0 (0)	14 (46.67)	0 (0)
	Mean concn (μg/kg)	60.91	60.42	58.55	92.79		67.94	54.13		71.94	
	LB–UB estimate (μg/kg)	8.1–13.4	2.0–7.9	1.9–7.8	34.0–37.9	0–6.1	9.1–14.3	7.2–12.5	0–6.1	33.6–36.8	0–6.1
BEA	No. (%) positive samples	6 (20)	5 (16.67)	7 (23.33)	5 (16.67)	15 (50.0)	18 (60.0)	20 (66.67)	14 (46.67)	15 (50.0)	6 (20)
	Mean concn (μg/kg)	1.49	1.21	1.27	1.55	2.45	1.73	1.85	2.03	2.40	2.65
	LB–UB estimate (μg/kg)	0.3–0.5	0.3–0.5	0.2–0.4	0.3–0.4	1.2–1.3	1.0–1.1	1.2–1.3	0.9–1.1	1.2–1.3	0.5–0.7
FB_1	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3	0–0.3
FB_2	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4	0–0.4
T2	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8	0–0.8
DAS	No. (%) positive samples	1 (3.33)	0 (0)	3 (10)	0 (0)	0 (0)	2 (6.67)	4 (13.33)	3 (10)	5 (16.67)	0 (0)
	Mean concn (μg/kg)	4.78		7.25			11.61	11.47	6.77	7.74	
	LB–UB estimate (μg/kg)	0.2–2.0	0–1.9	0.7–2.4	0–1.9	0–1.9	0.8–2.5	1.5–3.2	0.7–2.4	1.2–1.3	0–1.9
STER	No. (%) positive samples	0 (0)	1 (3.33)	0 (0)	0 (0)	0 (0)	0 (0)	7 (23.33)	0 (0)	1 (3.33)	0 (0)
	Mean concn (μg/kg)		3.51					3.76		5.25	
	LB–UB estimate (μg/kg)	0–0.2	0.1–0.3	0–0.2	0–0.2	0–0.2	0–0.2	0.9–1.0	0–0.2	0.2–0.3	0–0.2
DON	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5	0–7.5

TABLE 5. *Continued*

Mycotoxin	Occurrence[a]	Type of sample									
		White rice	Jasmine rice	Japanese rice	White sticky rice	Brown rice	GABA brown rice	Black sticky rice	Red rice	Riceberry rice	Barley
NIV	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–26.8	0–26.8	0–26.8	0–26.8	0–26.8	0–26.8	0–26.8	0–26.8	0–26.8	0–6.8
ALT	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6
CIT	No. (%) positive samples	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean concn (μg/kg)										
	LB–UB estimate (μg/kg)	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6	0–0.6

[a] Number of positive samples from a total of 30 samples of each type of rice or barley. Mean concentrations were calculated from the positive samples. LB, lower bound estimate calculated as the mean concentration for all samples; the undetected values and the unquantified values were replaced by zero and the LOD, respectively. UB, upper bound estimate calculated as the mean concentration for all samples; the undetected values and the unquantified values were replaced by the LOD and the LOQ, respectively.

of 30) of jasmine rice and white sticky rice samples. ZEA was detectable in 46.67% (14 of 30) of riceberry rice samples, 36.67% (11 of 30) of white sticky rice samples, 13.33% (4 of 30) of GABA brown rice and black sticky rice samples, 10% (3 of 30) of white rice samples, and 3.33% (1 of 30) of jasmine rice and Japanese rice samples. BEA was found in 66.67% (20 of 30) of black sticky rice samples, 60% (18 of 30) of GABA brown rice samples, 50% (15 of 30) of brown rice and riceberry rice samples, 46.67% (14 of 30) of red rice samples, 23% (7 of 30) of Japanese rice samples, 20% (6 of 30) of white rice and barley samples, and 16% (5 of 30) of jasmine rice and white sticky rice samples. DAS was found in 16.67% (5 of 30) of riceberry samples, 13.33% (4 of 30) of black sticky rice samples, 10% (3 of 30) of Japanese rice and red rice samples, 6.67% (2 of 30) of GABA brown rice samples, and 3.3% (1 of 30) of white rice samples. STER was detectable in 23.33% (7 of 30) of black sticky rice samples and 3.3% (1 of 30) of jasmine rice and riceberry rice samples. The mean concentrations and lower and upper estimates mycotoxin exposure from these grains are shown in Table 5.

The EDI of each residue of ZEA, DAS, STER, and BEA in rice and barley samples was calculated by multiplying either the mean or p97.5 of the consumption rate. The mean consumption rates for white rice, colored rice, and sticky rice were 255.66, 118.04, and 75.22 g per person per day, respectively, and the p97.5 values of the consumption rate were 720, 180, and 444 g per person per day, respectively *(9, 19)*. The PMTDI value for ZEA was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for humans at 0.5 μg/kg of body weight *(13)*. The HQ_{mean} and $HQ_{97.5}$ values for ZEA, BEA, DAS, and STER in contaminated rice were all $<100\%$. The EDI and HQ values for the contaminated rice and barley samples are shown in Table 6.

DISCUSSION

In our evaluation of mycotoxin contamination in rice and barley, the types of mycotoxins present were consistent with those reported in previous publications *(14, 27)*. However, the mean concentration of AFB_1 was lower than those previously reported. AFB_1 has been analyzed in white rice, brown rice, basmati rice, whole grain rice, long grain rice, short grain rice, and puffed rice from Australia. Iqbal et al. *(10)* found that AFB_1 was quantifiable in 41% (14 of 34) and 54% (15 of 28) of white rice and brown rice samples, respectively. Nguyen et al. *(20)* reported that AFB_1 was detectable in 51% of positive samples from Vietnam, with a mean concentration of 3.31 μg/kg. Toteja et al. *(30)* reported high concentrations of aflatoxins (60 to 600 μg/kg) in parboiled rice samples. In our study, AFB_1 was quantifiable in GABA brown rice, black sticky rice, and riceberry rice but not in white rice and barley samples. This difference may associated with the milling and polishing process, which may reduce the AFB_1 concentration in white rice and barley. Our results are similar to those of Lim et al. *(16),* who reported low AFB_1 concentrations and no evidence of AFB_2, AFG_2, FB_1, FB_2, DON, T2, and OTA in rice samples collected from Thailand. In our study, ZEA was quantifiable

TABLE 6. *Estimated dietary exposure and hazard quotient (HQ) values for mycotoxins through the consumption of rice and barley in Thailand*

Mycotoxin	Mycotoxin-positive sample	Estimated dietary exposure (μg/kg of BW/day)				EU maximum regulatory limit (μg/kg)	TDI or PMTDI (μg/kg of BW/day)	HQ_{mean} (%)	$HQ_{p97.5}$ (%)
		EDI_{mean}		$EDI_{p97.5}$					
		LB estimate[a]	UB estimate[b]	LB estimate	UB estimate				
ZEA	White rice	0.03	0.05	0.09	0.15	100	0.5	1.68–10.52	17.99–29.61
	Jasmine rice	0.01	0.03	0.02	0.09			1.58–6.18	4.46–17.42
	Japanese rice	0.01	0.05	0.02	0.12			1.66–6.13	4.32–17.28
	White sticky rice	0.04	0.04	0.23	0.26			7.87–8.76	46.48–51.72
	GABA rice	0.02	0.03	0.05	0.08			3.29–5.19	10.03–15.84
	Black sticky rice	0.01	0.01	0.05	0.09			1.67–2.88	9.86–17.02
	Riceberry rice	0.06	0.07	0.19	0.20			12.19–13.36	37.18–40.76
BEA	White rice	0.001	0.002	0.003	0.01	NA[c]	0.1 (TDI)[d]	1.17–1.80	3.29–5.06
	Jasmine rice	0.001	0.001	0.002	0.004			0.79–1.42	2.24–4.01
	Japanese rice	0.001	0.002	0.003	0.005			1.16–1.77	3.28–4.98
	White sticky rice	0.0003	0.0005	0.002	0.003			0.30–0.49	1.77–2.90
	Brown rice	0.002	0.002	0.007	0.007			2.22–2.40	6.78–7.34
	GABA rice	0.002	0.002	0.006	0.006			1.89–2.03	5.75–6.20
	Black sticky rice	0.001	0.001	0.01	0.018			1.41–1.49	8.34–8.80
	Red rice	0.002	0.002	0.005	0.006			1.72–1.91	5.25–5.84
	Riceberry rice	0.002	0.002	0.007	0.007			2.17–2.36	6.64–7.20
	Barley	0.001	0.001	0.004	0.004			0.90–1.20	2.76–6.65
DAS	White rice	0.001	0.01	0.002	0.028	NA	2	0.03–0.39	0.09–1.09
	Japanese rice	0.003	0.01	0.01	0.03			0.14–0.47	0.40–1.33
	GABA rice	0.001	0.005	0.004	0.01			0.07–0.23	0.21–0.70
	Black sticky rice	0.002	0.004	0.01	0.02			0.09–0.18	0.52–1.07
	Red rice	0.001	0.004	0.004	0.01			0.06–0.21	0.19–0.65
	Riceberry rice	0.002	0.005	0.007	0.016			0.12–0.26	0.36–0.79
STER	Jasmine rice	0.001	0.001	0.001	0.003	NA	0.1 (TDI)[d]	0.46–1.07	1.30–3.01
	Black sticky rice	0.001	0.001	0.006	0.007			1.01–1.56	5.99–6.83
	Riceberry rice	0.0003	0.001	0.001	0.002			0.32–0.60	0.97–1.82

[a] LB, lower bound estimate calculated as the mean concentration for all samples; the undetected values and the unquantified values were replaced by zero and the LOD, respectively.
[b] UB, upper bound estimate calculated as the mean concentration for all samples; the undetected values and the unquantified values were replaced by the LOD and the LOQ, respectively.
[c] NA, not available.
[d] Tolerable daily intake (TDI) with reference to TDI of HT-2 toxin of 0.1 μg/kg of BW/day.

in all types of samples except brown rice, red rice, and barley. The prevalence of mycotoxin contamination differed among sample types. BEA, DAS, ZEA, and AFB_1 were found most frequently in all contaminated samples. However, the concentrations of regulated mycotoxins were below the established limits. The European Commission *(7)* has established its regulatory concentration for AFB_1 and total aflatoxins in cereals at 2 and 4 μg/kg, respectively. The maximum limit for ZEA in rice was set at 100 μg/kg *(6)*.

In this study, the EDI and the HQ were used to assess the health risk from rice and barley for adult Thai consumers. The EDIs of AFB_1, AFG_1, ZEA, BEA, DAS, and STER were below the PMTDI values established by JECFA. The risk assessment (HQ) value for ZEA residue was <100% in rice, which indicates no obvious risk to consumers in Thailand. For AFB_1, the risk assessment calculation was performed separately by determination of the population risk. AFB_1 contamination in GABA brown rice, black sticky rice, and riceberry rice was 1.38, 1.44, and 1.48 μg/kg, respectively, resulted in an estimated exposure of 4.14, 4.32, and 4.44 ng/kg of body weight per day. Therefore, the estimated cancer risk to the Thai population through consumption of GABA brown rice, black sticky rice, and riceberry rice was 0.101, 0.105, and 0.109 cases of liver cancer per 100,000 persons per nanogram of AFB_1 per kilogram of body weight per day. Thus, only one liver cancer case per one million persons would be due to consumption of AFB_1 in rice. However, study of emerging mycotoxins in rice, such as BEA and STER, are warranted.

In summary, in this study the LC-MS/MS method was adequate for quantification of 16 mycotoxins in samples of rice and barley. AFB_1 and ZEA concentrations in the analyzed samples were lower than the maximum regulatory limits set by the European Commission. These results also suggest that the risk of harmful mycotoxin exposure via the consumption of barley and these nine kinds of rice (black sticky rice, brown rice, GABA rice, Japanese rice, jasmine rice, red rice, riceberry rice, white rice, and white sticky rice) is very low for Thai consumers. Further studies with a larger sample size are needed to confirm these data because

the samples may not be statistically representative of all types of rice.

ACKNOWLEDGMENTS

This work was partially supported by the Center for Advanced Studies for Agriculture and Food (Institute for Advanced Studies, Kasetsart University) under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

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