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Natural occurrence of aflatoxins and ochratoxin A in *meju* and soybean paste produced in South Korea

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Abstract

In this study, we investigated the occurrence of aflatoxins (AFs) and ochratoxin A (OTA) in *meju* and soybean paste produced in South Korea. Samples were collected from three regions divided on the basis of climate in South Korea. A total of 100 *meju* samples were analyzed over 3 years (2012–2015), and 45 soybean paste samples were analyzed in 2016. Mycotoxins were extracted with an immunoaffinity column method and quantified by high-performance liquid chromatography. AFs were detected in 10 of *meju* (10%) and 11 of soybean paste samples (24.4%) with concentrations of 0.2–48.3 μg/kg and 0.88–16.17 μg/kg, respectively. OTA was detected in 50 of *meju* (50%) and 22 of soybean paste samples (48.9%) with concentrations of 0.1–193.2 μg/kg and 0.88–26.29 μg/kg, respectively. Mycotoxin contamination in *meju* was more common in the central region than in the southern areas. Thus, more mycotoxins were produced in the central region owing to less fungal competition in *meju* during fermentation inside households. We also found that about 91% of AFs and 73% of OTA in *meju* were degraded after the production of soybean paste and soy sauce. Even after degradation of AFs and OTA, the levels of AFB_1 and OTA were 0.5 μg/kg and 7.5 μg/kg in soy sauce and 11.9 μg/kg and 190.4 μg/kg in soybean paste, respectively. Thus, our results suggest the need for constant monitoring of *meju* and soybean paste for AFs and OTA.

Keywords: Aflatoxin, HPLC, *Meju*, Ochratoxin A, Soybean

Introduction

Soybeans and its products are among the major protein sources in East Asia. *Meju* is a fermented soybean food used as a raw material for the preparation of traditional fermented foods such as soy sauce and soybean paste in South Korea [1, 2]. These products have been manufactured for centuries at home by traditional means, wherein the natural microflora, especially storage fungi, participates in the fermentation process. These molds produce the enzymes for conversion of proteins and carbohydrates into amino acids, sugars, organic acids, alcohol, and esters, thereby conferring the characteristic flavor of *meju* [3]. In South Korea, the manufacture of fermented foods such as *meju* using traditional methods, which are preferred over commercial products, is raising concerns, owing to the possibility of contamination with mycotoxins, especially aflatoxins (AFs) and ochratoxin A (OTA).

AFs are a group of toxic metabolites produced by *Aspergillus* species such as *A. flavus*, *A. parasiticus*, *A. pseudotamarii*, and *A. nomius* [4, 5]. In general, *A. flavus* and *A. pseudotamarii* produce AFB_1 and AFB_2, while *A. parasiticus* and *A. nomius* produce AFG_1 and AFG_2. The carcinogenicity, mutagenicity, and teratogenicity of AFB_1 and its metabolites have been well documented. Thus, AFs are classified as Group 1 carcinogens (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [6]. OTA is a toxic secondary metabolite produced by several *Aspergillus* species and *Penicillium verrucosum*, which are found in contaminated agricultural crops [7]. OTA poses a serious threat to public health and causes severe economic losses worldwide because it can be found in animal feed such as corn and stored grains as well as in food products such as flour,

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peas, peanuts, spices, and coffee beans [8]. IARC has categorized OTA as a possible human carcinogen (Group 2B) [9]. Both groups of mycotoxins may contaminate various food commodities, including *meju* [1, 2, 10, 11]. Considering that *meju* is a main ingredient in soybean paste and soy sauce, which are used as seasoning agents and condiments in many Korean foods, the contamination of *meju* with AFs and OTA may affect most Korean foods. The Korean government has set the maximum limits of total AF at 15 μg/kg, AFB_1 at 10 μg/kg, and OTA at 20 μg/kg in *meju*. Several analytical methods have been developed to quantify the two mycotoxins, such as enzyme-linked immunosorbent assay (ELISA) [12], thin-layer chromatography (TLC) [13], and high-performance liquid chromatography (HPLC) [14]. ELISA and TLC methods are less selective than other analytical methods and are prone to interference by sample matrices [15, 16]. HPLC has been employed to determine AFs and OTA at low levels in food [10, 15]. The analysis of AFs and OTA in *meju* is complicated because of the distribution of lipids and proteins in the soy-fermented materials that are co-extracted with AFs and OTA. Therefore, a specific purification method is desirable before HPLC analysis. Highly pure AFs and OTA can be obtained by passing *meju* through an immunoaffinity column (IAC), which uses specific antibody binding. Such an approach would provide an easy and straightforward solution to extract pure mycotoxins and consequently overcome the problems of interference from other compounds present in *meju*. In this regard, the present study aimed to investigate the contamination of AFs and OTA in *meju* and soybean paste samples collected in South Korea. We also observed the transfer rates of mycotoxins to soy sauce and soybean paste produced from the collected *meju* samples, which were severely contaminated with AFs and OTA.

Materials and methods

Sample collection

Meju was randomly collected from traditional markets in South Korea between 2012 and 2015, while soybean paste was obtained in 2016 under the previously described conditions. The sampling areas were chosen by dividing the nation into three climatic zones based on the temperature and precipitation characteristics observed at 60 points by the Korea Meteorological Administration for 25 years [17]. The first region included the Yeongdong region in Gangwon and Gyeonggi provinces (average temperature, 11.7 °C/year; average precipitation, 1286.6 mm/year), while the second region included Chungcheong, Gyeongsangbuk, and Jeollabuk provinces (average temperature, 11.3 °C/year; average precipitation, 1263.4 mm/year). The third region included southern Yeongho-Nam and the coastal areas of Jeollanam and Gyeongsangnam provinces (average temperature, 13 °C/year; average precipitation, 1346.9 mm/year). About 16–18 samples were collected each year. All samples were stored in a freezer (− 20 °C) until analysis.

Standards and reagents

AF and OTA analytical standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at − 18 °C until use. Stock solutions of AF were stored in a mixture of benzene and acetonitrile (ACN) (98:2, v/v) and working standard solutions were prepared by diluting the stock standard solution to 10 μg/mL in 10% ACN. OTA was stored in a mixture of toluene and acetic acid (99:1, v/v). Working standard solutions were prepared by diluting stock standard solutions to 10 μg/mL in a mixture of ACN, methanol (MeOH), and acetic acid (HAC) (99:99:2, v/v). All solvents used for the preparation of the mobile phase were of HPLC grade and obtained from J.T. Baker (Center Valley, PA, USA). Trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich. All solvents were filtered through 0.45 μm membrane filters (Whatman plc, Maidstone, UK). Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA). IACs were supplied by VICAM (Milford, MA, USA).

Extraction procedure and IAC clean-up

The levels of AFs in *meju* were detected according to the previously published procedures with some minor modifications [18–20]. Briefly, 20 g of each sample was mixed in a 250 mL Erlenmeyer flask containing 100 mL of ACN and water (60:40, v/v), and the mixture was mechanically shaken by a wrist action shaker (EYELA, Tokyo, Japan) for 1 h. The extract was filtered through Whatman filter paper No. 4, and 10 mL of the filtrate was diluted with 40 mL of phosphate buffered saline (PBS, pH 7.4). After 50 mL of mixture was passed through a glass microfiber filter (Whatman GF/A, UK), 10 mL of the filtrate was passed through an IAC (Aflatest®, VICAM, USA). The IAC was washed with 10 mL distilled water. AFs were then eluted with 2 mL MeOH at a flow of 1–1.5 mL/min. The methanol eluate was evaporated to dryness under a stream of nitrogen at 50 °C, and AFs were reconstituted in 500 μL of 10% ACN-TFA.

The levels of AFs in soybean paste were analyzed according to the previously published procedures with some minor modifications [21]. In brief, 25 g of each sample was extracted with 100 mL of MeOH:water (70:30, v/v) containing 1% sodium chloride (NaCl) using a mechanical shaker for 20 min. The extract was filtered through Whatman No. 1 filter paper, and 10 mL of the filtrate was diluted with 30 mL with deionized water. After vigorously mixed, the mixture was passed through an

Aflatest® IAC at a flow rate of about 3 mL/min (1 drop/s). The IAC was washed with distilled water (10 mL) at the same flow rate until 2–3 mL of air passed through it to remove water. AFs were finally eluted from the column with 3 mL ACN at the same flow rate and it was flushed with air. The eluate was evaporated under a gentle stream of N_2 at 50 °C. The dry residues were re-dissolved and derivatized in 200 µL TFA, allowing them to stand for 15 min. The sample was then diluted with 800 µL of ACN:water (20:80, v/v), filtered through a 0.45 µm syringe filter (13 mm × 0.2 µm, GHP; Pall Corporation, Ann Arbor, MI, USA), and transferred into HPLC vials for auto injection.

We analyzed the levels of OTA in *meju* according to the previously published methods with some minor modifications [20, 22, 23]. Each sample (25 g) was put in a 250 mL Erlenmeyer flask containing 100 mL of ACN:water (60:40, v/v), and the mixture was mechanically shaken with a wrist action shaker (EYELA, JAPAN) for 1 h. The extract was filtered through Whatman No. 4 filter paper, and 5 mL of the filtrate was diluted in 55 mL PBS. After about 55 mL of mixture was passed through a glass microfiber filter (Whatman GF/A, UK), 10 mL of the filtrate was passed through an IAC (OchrTest IAC®, VICAM, USA). The IAC was washed with 10 mL distilled water and OTA was eluted with 2 mL methanol at a flow rate of 1–1.5 mL/min. The methanol eluate was evaporated to dryness under a stream of nitrogen at 50 °C, and the OTA was reconstituted in 500 µL of the mobile phase solution (ACN:MeOH:HAC, 99:99:2, v/v).

The OTA in soybean paste was analyzed according to the annual report of the Korean Food and Drug Administration (KFDA) [24]. Briefly, 25 g of each sample was extracted with 100 mL MeOH:water (70:30, v/v) using a mechanical shaker for 3 min. The extract was filtered with Whatman No. 1 filter paper, and 4 mL of the filtrate was diluted in 40 mL PBS and subjected to vigorous agitation. After the mixture (44 mL) was passed through an OchrTest IAC® at a flow rate of about 3 mL/min (2 drop/s), the column was washed with distilled water (10 mL) at the same flow rate until 2–3 mL of air passed through it to ensure the removal of water. The OTA was eluted with 2 mL MeOH at a flow of 1–1.5 mL/min. The MeOH eluate was evaporated to dryness under a stream of nitrogen at 50 °C, and the OTA was reconstituted in 500 µL of the mobile phase solution (ACN:MeOH:HAC, 99:99:2, v/v).

Assessment of the linearity, precision and sensitivity of the analytical method for determination of levels of AFs and OTA

The linearity of a series of AFs concentrations in the analytical method was assessed by a standard curve using eight levels of AFs (0.01 µg/kg, 0.05 µg/kg, 0.1 µg/kg, 0.5 µg/kg, 1.0 µg/kg, 2.0 µg/kg, 5.0 µg/kg, and 10 µg/kg) for *meju* and AFs (0.005 µg/kg, 0.01 µg/kg, 0.05 µg/kg, 0.1 µg/kg, 0.5 µg/kg, 1.0 µg/kg, 5.0 µg/kg, and 10 µg/kg) for soybean paste, which were dissolved in the mobile phase solution (ACN:MeOH:distilled water = 17:17:66, v/v/v). The linearity of a series of OTA concentrations in the analytical method for *meju* and soybean paste samples was assessed by a standard curve using eight levels of OTA (0.005 µg/kg, 0.01 µg/kg, 0.05 µg/kg, 0.1 µg/kg, 0.5 µg/kg, 1.0 µg/kg, 5.0 µg/kg, and 10 µg/kg) dissolved in the mobile phase solution (ACN:water:HAC = 99:99:2, v/v/v). Each standard solution for AFs or OTA was injected into HPLC-FLD in triplicate. The calibration curve was constructed by plotting the peak areas (y axis) versus AF or OTA concentrations (x axis) in the HPLC analysis. The linearity was determined by linear regression analysis and expressed as coefficient of determination (r^2).

The precision of the analytical method was evaluated by the recovery experiments. The recovery experiments were performed with AF- and OTA-free *meju* and soybean paste samples, which were spiked with either AFs at 1.0 µg/kg and 10.0 µg/kg or OTA at 2.0 µg/kg and 10.0 µg/kg. The experiment was carried out in triplicate, including a double blank. Recovery was calculated by the following equation.

$$\text{Recovery} = \frac{\text{AF or OTA concentration measured from the spiked sample}}{\text{AF or OTA concentration used for spiking the sample}} \times 100$$

The sensitivity of the methods was determined by limit of detection (LOD) and limit of quantification (LOQ) for *meju* and soybean paste samples. These were calculated as a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively, which were determined by using HPLC software (Analyst 1.6 software program).

HPLC analysis

A Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA) was used to detect AF and OTA. Separation was carried out using a Nova-Pack C18 column (4.6 mm × 250 mm, 5 µm; Waters, Milford, MA, USA). The injection volume for the AF or OTA standards and samples was 50 µL. For AF analysis, the mobile phase (ACN:MeOH:distilled water = 17:17:66, v/v/v) was

Table 1 Recovery of AFB_1, AFB_2, AFG_1, AFG_2, and OTA in *meju*

Mycotoxin	Spiked concentration (μg/kg)	Mean recovery (%)[a]	Relative standard deviations (RSD) (%)
AFB_1	1.0	99.8	6.49
	10.0	97.5	3.36
AFB_2	1.0	91.2	7.97
	10.0	90.5	7.31
AFG_1	1.0	92.2	6.98
	10.0	79.7	4.76
AFG_2	1.0	71.2	7.24
	10.0	95.1	2.13
OTA	2.0	75.6	4.26
	10.0	93.3	4.22

[a] Mean recovery indicate the average of triplicates at each level

pumped at a flow rate of 0.5 mL/min, giving a total run time of 20 min. A fluorescence detector was used for the determination of AFs at an excitation wavelength of 360 nm and emission wavelength of 440 nm. For OTA analysis, the mobile phase (ACN:water:HAC = 99:99:2, v/v/v) was pumped at a flow rate of 0.8 mL/min, giving a total run time of 20 min. A fluorescence detector was used for the determination of OTA at an excitation wavelength of 330 nm and emission wavelength of 460 nm.

Statistical analysis

Statistical analyses were performed by Student's *t* test using SigmaStat scientific statistical software (version 1.0, Jandel corporation, San Rafael, CA, USA).

Results and discussion

Assessment of the linearity, precision, and sensitivity of the analytical method to determine levels of AFs and OTA

The precision of the method was evaluated from the recovery rate of AFs and OTA obtained from the samples spiked with known concentrations of AF and OTA standard solutions. The recovery rate was calculated using the equation described in the Materials and Methods. The recovery rates of AFs and OTA in *meju* samples are shown in Table 1, while the typical HPLC chromatograms of AFs and OTA are shown in Fig. 1. The recovery rates of AFs were in the range of 71.2–99.8% at 1 μg/kg and 10 μg/kg of levels spiked with AFs, and the recovery rates of OTA were in the range of 75.6–93.3% at 2 μg/kg and 10 μg/kg of levels fortified with OTA. The recovery rates of AFs and OTA in soybean paste samples are shown in Table 2. The recovery rates of AFs were in the range of 82.8–97.6% at 1 μg/kg and 10 μg/kg of levels spiked with AFs, and the recovery rates of OTA were in the range of 90.5–92.8% at 2 μg/kg and 10 μg/kg of levels fortified with OTA.

The recovery rates of AFs and OTA in all the samples satisfied the permissible limits of the recovery recommended by the Codex or Association of Official Analytical Chemists (AOAC) [25]. The Codex recommends 60–120% of recovery rates in food samples contaminated with 1–10 μg/kg mycotoxins, and the guideline for the recoveries by AOAC is 70–125% in food samples contaminated with 10 μg/kg mycotoxins. In addition, the relative standard deviation (RSD) values of AFs and OTA (2.13–7.97%) were below 15%, which is in accordance with the recommendation for food samples contaminated with 10 μg/kg mycotoxins by AOAC. Thus, we concluded that the analytical method had good recoveries from *meju*.

The sensitivity of the method using HPLC was determined by LOD and LOQ. The LOD was 0.05 μg/kg for AFB_1 and AFB_2, 0.2 μg/kg for AFG_1 and AFG_2, and 0.03 μg/kg for OTA in *meju*, whereas the LOD was 0.01 μg/kg for AFB_1 and AFB_2, 0.05 μg/kg for AFG_1 and AFG_2, and 0.01 μg/kg for OTA in soybean paste samples (Tables 3 and 4). The LOQ was 0.15 μg/kg for AFB_1 and AFB_2, 0.6 μg/kg for AFG_1 and AFG_2, and 0.1 μg/kg for OTA in *meju*, whereas the LOQ was 0.03 μg/kg for AFB_1 and AFB_2, 0.15 μg/kg for AFG_1 and AFG_2, and 0.03 μg/kg for OTA in soybean paste samples. They were as low as those for the detection of trace amounts of AFs and OTA.

The linearity of a series of AF or OTA concentrations in the analytical method was assessed by each standard curve using 8 levels of standard solutions for each toxin. The linearity was determined by linear regression analysis. The curves for AFs or OTA were greater than 0.99 (Tables 3 and 4). Therefore, we concluded that the calibration curves were linear in the range of 0.01–10 μg/kg of AFs for *meju*, in the range of 0.005–10 μg/kg of AFs for soybean paste, and in the range of 0.005–10 μg/kg of OTA for *meju* and soybean paste.

Monitoring the levels of AFs and OTA in *meju* samples

The analytical method validated above was used for the determination of levels of AFs and OTA in 100 *meju* samples collected from local markets in South Korea. Ten out of 100 samples (10%) were contaminated with total AFs at 0.2–41.3 μg/kg. In addition, 9 samples (9%) were contaminated with AFB_1 at 0.6–21.4 μg/kg (Table 5). The mean concentration was 1.45 μg/kg and 0.86 μg/kg for AFB_1 and total AFs, respectively. The levels of AFB_1 and total AF in three *meju* samples analyzed (3%) exceeded the regulatory limits for AFB_1 (10 μg/kg) and total AFs (15 μg/kg) set by the KFDA. In general, soybeans are believed to be a poor substrate for AF production [26]. Therefore, it is highly likely that AF production occurs during the fermentation process for making *meju*. Several

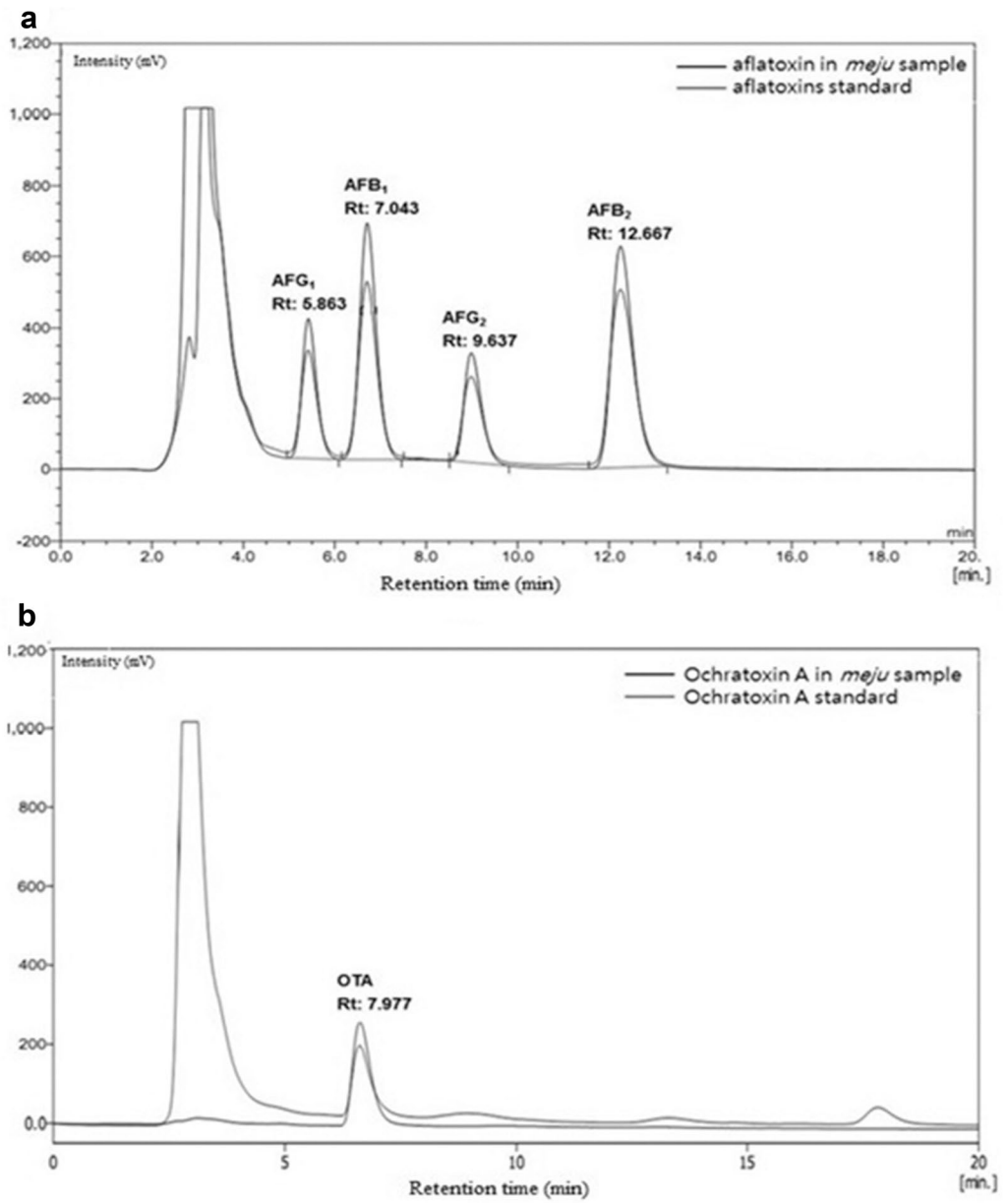

Fig. 1 HPLC chromatograms of **a** standard solution containing 10 μg/L of AFs and a *meju* sample naturally contaminated with 9 μg/kg of AFs and **b** standard solution containing 10 μg/L of OTA and a *meju* sample naturally contaminated with 8.7 μg/kg of OTA

studies have reported on the levels of AFs in soybean and soybean products. One study showed AF contamination in 41.7% of *meju* samples, and the mean levels were lower (6.9 μg/kg) than the legal limits [18]. Despite the detection of contamination, the authors concluded that the presence of AFs in *meju* sold in South Korea was not a serious threat to human health. In addition, in Pakistan, Lutfullah and collaborators found AFB_1 contamination in 15% of soybean samples, with an average concentration of 6.4 μg/kg [16]. The levels of AFs in a traditional Korean fermented soybean food was similar to those of AF in soybean products marketed in other countries.

Fifty samples of 100 *meju* samples (50%) were contaminated with 0.1–193.2 μg/kg of OTA, which were analyzed with HPLC (Table 5). The average concentration of OTA was 10.24 μg/kg. The levels of OTA in nine *meju* samples analyzed (9%) exceeded the legal limits for OTA set by the KFDA (20 μg/kg). In particular, six *meju* samples exceeded the permissible limits by 5–10 times. Information about levels of OTA in soybean fermented food such

Table 2 Recovery of AFB_1, AFB_2, AFG_1, AFG_2, and OTA in soybean paste

Mycotoxin	Spiked concentration (μg/kg)	Mean recovery (%)[a]	Relative standard deviations (RSD) (%)
AFB_1	1.0	97.6	1.65
	10.0	94.1	3.33
AFB_2	1.0	91.3	2.95
	10.0	87.3	4.68
AFG_1	1.0	88.2	7.00
	10.0	83.1	2.00
AFG_2	1.0	82.8	4.67
	10.0	83.0	4.73
OTA	2.0	92.8	4.51
	10.0	90.5	8.24

[a] Mean recovery indicate the average of triplicates at each level

as *meju* is scarce. Therefore, greater attention is required for assessing and monitoring OTA contamination in *meju*.

We also investigated the co-occurrence of AFs and OTA in the same *meju* samples. The co-occurrence and co-contaminated levels were relatively lower than those in samples contaminated with only one of the toxins (Fig. 2). These results suggest that the same *meju* products are not likely to be co-infected with different fungi, which produce either AFs or OTA; even if the same products are co-infected, those fungi are not likely to produce toxins due to fungal competition.

The occurrence of AFs and OTA in *meju* was investigated on the basis of climate.

Samples from the first region (Yeongdong region in Gangwon and Gyeonggi provinces; average temperature, 11.7 °C/year; average precipitation, 1286.6 mm/year) showed higher mycotoxin contamination levels than those from the southern region (Jeollanam and Gyeongsangnam provinces; average temperature, 13 °C/year; average precipitation, 1346.9 mm/year) ($p < 0.05$) (Table 6). Also, samples from the second region (Chungcheongbuk, Chungcheongnam, Gyeongsangbuk, and Jeollabuk provinces; average temperature, 11.3 °C/year; average precipitation, 1263.4 mm/year) showed higher mycotoxin contamination levels than those from the third region (Jeollanam and Gyeongsangnam provinces) ($p < 0.05$) (Table 6). This result might be due to differences in the average winter temperatures in South Korea. Some parts of South Korea have very low temperatures in winter, which are unsuitable for the fermentation of *meju*. The average temperature in winter is below zero in the first and second regions (−6.0 to 0.7 °C), while it is above zero in the southern region (2.5 to 3.9 °C) [17]. As a result, in the southern region, *meju* can be exposed to different

Table 3 LOD and LOQ of AFs and OTA by HPLC analysis in *meju*

Mycotoxin	LOD[a] (μg/kg)	LOQ[b] (μg/kg)	Linear equation[c]	R^2 [d]	Range (μg/kg)
AFB_1	0.05	0.15	$Y = 2441.0X + 7.8015$	0.9974	0.01–10
AFB_2	0.05	0.15	$Y = 2900.1X - 7.1994$	0.9974	0.01–10
AFG_1	0.2	0.6	$Y = 1293.4X + 1.7867$	0.9988	0.01–10
AFG_2	0.2	0.6	$Y = 1173.8X + 2.0294$	0.9976	0.01–10
OTA	0.03	0.1	$Y = 3935.1X + 324.6316$	0.9996	0.005–10

[a] Limit of detection

[b] Limit of quantification

[c] X = AFs or OTA concentration (μg/kg), Y = intensity

[d] Coefficient of determination

Table 4 LOD and LOQ of AFs and OTA by HPLC analysis in soybean paste

Mycotoxin	LOD (μg/kg)[a]	LOQ (μg/kg)[b]	Linear equation[c]	R^2 [d]	Range (μg/kg)
AFB_1	0.01	0.03	$y = 2.5259x + 0.4332$	0.9978	0.005–10
AFB_2	0.01	0.03	$y = 0.6885x + 0.1409$	0.9981	0.005–10
AFG_1	0.05	0.15	$y = 1.4595x + 0.2503$	0.9978	0.005–10
AFG_2	0.05	0.15	$y = 1.0506x - 0.0995$	0.9926	0.005–10
OTA	0.01	0.03	$y = 0.6885x + 0.1409$	0.9971	0.005–10

[a] Limit of detection

[b] Limit of quantification

[c] X = AFs or OTA concentration (μg/kg), Y = intensity

[d] Coefficient of determination

Table 5 Natural occurrence of AFs and OTA in *meju*

Mycotoxin	Positive samples/total samples (percentage)	Mean (μg/kg)	Range (μg/kg)	Number of positive samples in the range				
				ND	0.1–1.0 (μg/kg)	1.1–10.0 (μg/kg)	10.1–100.0 (μg/kg)	100.1–200.0 (μg/kg)
AFB_1	9/100 (9.0%)	0.86 ± 63.51	0.6–21.4	91	1	5	3	0
AFB2	3/100 (3.0%)	0.38 ± 2.59	1.4–19.9	97	0	1	2	0
AFG_1	5/100 (5.0%)	0.20 ± 1.86	0.1–18.6	95	4	0	1	0
AFG_2	1/100 (1.0%)	0.01 ± 0.11	1.1	99	0	1	0	0
AFs	10/100 (10%)	1.45 ± 6.60	0.2–41.3	90	2	5	3	0
OTA	50/100 (50.0%)	10.31 ± 33.49	0.1–193.2	50	27	12	5	6

ND none detectable

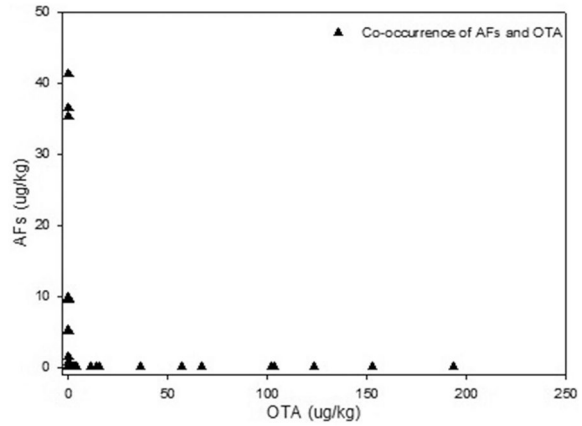

Fig. 2 Co-occurrence of AFs in OTA positive *meju* samples

fungi in the outside air, causing competition among many different fungi and resulting in the inhibition of mycotoxin production [3, 27]. In contrast, in the first and second regions, *meju* is dried inside households. Thus, only a few specific fungi such as mycotoxin-producing fungi can contaminate *meju*. In this case, less competition between different fungi could increase the probability of mycotoxin contamination in *meju*. A previous study showed no AFs in samples from northern Korea [28]. Our data demonstrate that mycotoxin contamination is a function of climatic conditions to a certain extent.

Monitoring the levels of AFs and OTA in soybean paste samples

In total, 45 soybean paste samples were analyzed for AFs using HPLC. Eleven out of 45 samples (24.4%) were

Table 6 Occurrence of AFs and OTA in *meju* from three areas

Region		Number of positive samples/total number of samples							
		Range						Mean	
		AFB_1 (μg/kg)	AFB_2 (μg/kg)	AFG_1 (μg/kg)	AFG_2 (μg/kg)	Total AFs (μg/kg)	OTA (μg/kg)	Total AFs (mean, μg/kg)	Total OTA (mean, μg/kg)
Area I	Seoul	1/3 (0.6)	0/3 (< 0.0)	1/3 (0.1)	0/3 (< 0.0)	0/3 (< 0.0)	2/3 (57–115.5)	4/31 (0.8)	15/31 (24.6)
	Gyeonggi	1/17 (4.9)	0/17 (< 0.0)	1/17 (0.2)	0/17 (< 0.0)	1/17 (5.1)	6/17 (0.3–152.4)		
	Gangwon	3/11 (5.5–9.7)	1/11 (1.4)	1/11 (0.2)	0/11 (< 0.0)	3/11 (5.7–9.8)	7/11 (0.1–193.2)		
Area II	Chungcheong-buk	0/7 (< 0.0)	0/7 (< 0.0)	0/7 (< 0.0)	0/7 (< 0.0)	0/7 (< 0.0)	3/7 (0.7–3.0)	4/35 (3.2)	18/35 (6.8)
	Chungcheong-nam	1/7 (19.6)	1/7 (16.9)	0/7 (< 0.0)	0/7 (< 0.0)	1/7 (36.5)	5/7 (0.1–67.0)		
	Gyungsangbuk	1/12 (21.4)	1/12 (19.9)	0/12 (< 0.0)	0/12 (< 0.0)	1/12 (41.3)	6/12 (0.6–36.0)		
	Jeollabuk	1/9 (15.6)	0/9 (< 0.0)	2/9 (0.2–18.6)	1/9 (1.1)	2/9 (0.2–35.3)	4/9 (0.2–102.0)		
Area III	Jeollanam	1/17 (4.5)	0/17 (< 0.0)	1/17 (0.6)	0/17 (< 0.0)	1/17 (5.2)	12/17 (0.1–3.7)	2/31 (0.2)	18/34 (0.7)
	Gyungsang-nam	1/17 (1.5)	0/17 (< 0.0)	0/17 (< 0.0)	0/17 (< 0.0)	1/17 (1.5)	6/17 (0.1–11.1)		

Table 7 Natural occurrence of AFs and OTA in soybean paste

Mycotoxin	Positive samples/total samples (percentage)	Mean (μg/kg)	Range (μg/kg)	Number of positive samples in the range				
				ND	0.1–1.0 (μg/kg)	1.1–10.0 (μg/kg)	10.1–20.0 (μg/kg)	20.1–50.0 (μg/kg)
AFB_1	4/45 (8.9%)	0.59 ± 2.52	1.06–15.25	41	0	3	1	0
AFB_2	5/45 (11.1%)	0.34 ± 1.29	0.92–7.80	40	1	4	0	0
AFG_1	1/45 (2.2%)	0.12 ± 0.78	5.24	44	0	1	0	0
AFG_2	2/45 (4.4%)	0.05 ± 0.25	0.88–1.44	43	0	2	0	0
AFs	11/45 (24.4%)	1.09 ± 2.93	0.88–16.17	34	1	9	1	0
OTA	22/45 (48.9%)	4.43 ± 7.25	0.88–26.29	23	1	14	3	4

ND none detectable

Table 8 Occurrence of AFs and OTA of soybean paste from three areas

Region		Number of positive samples/total number of samples							
		Range						Mean	
		AFB_1 (μg/kg)	AFB_2 (μg/kg)	AFG_1 (μg/kg)	AFG_2 (μg/kg)	Total AFs (μg/kg)	OTA (μg/kg)	Total AFs (mean, μg/kg)	Total OTA (mean, μg/kg)
Area I	Seoul	2/5 (7.3–15.3)	3/5 (0.9–7.8)	0/5 (< 0.0)	0/5 (< 0.0)	4/5 (1.1–16.2)	3/5 (6.9–22.9)	7/15 (2.58)	9/15 (4.04)
	Gyeonggi	1/8 (1.1)	2/8 (1.8–3.4)	0/8 (< 0.0)	0/8 (< 0.0)	3/8 (1.1–3.4)	5/8 (1.6–6.2)		
	Gangwon	0/2 (< 0.0)	0/2 (< 0.0)	0/2 (< 0.0)	0/2 (< 0.0)	0/2 (< 0.0)	1/2 (0.88)		
Area II	Chungcheongbuk	0/3 (< 0.0)	0/3 (< 0.0)	0/3 (< 0.0)	0/3 (< 0.0)	0/3 (< 0.0)	2/3 (1.6–1.7)	1/15 (0.8)	13/15 (9.3)
	Chungcheongnam	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	4/4 (2.5–24.1)		
	Gyungsangbuk	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	1/4 (1.44)	1/4 (< 1.44)	3/4 (2.8–26.3)		
	Jeollabuk	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	0/4 (< 0.0)	4/4 (3.2–14.6)		
Area III	Jeollanam	1/8 (2.8)	0/8 (< 0.0)	1/8 (5.2)	0/8 (< 0.0)	2/8 (2.8–5.2)	0/8 (< 0.0)	3/15 (0.59)	0/15 (0.0)
	Gyungsangnam	0/7 (< 0.0)	0/7 (< 0.0)	0/7 (< 0.0)	1/7 (0.9)	1/7 (0.9)	0/8 (< 0.0)		

contaminated with total AFs at levels of 0.88–16.17 μg/kg (Table 7). Furthermore, four samples (8.9%) were contaminated with AFB_1 at 1.06–15.25 μg/kg. The mean concentrations of AFB_1 and total AFs were 0.59 μg/kg and 1.09 μg/kg, respectively (Table 7). The levels of AFB_1 and total AF in one sample analyzed exceeded the regulatory limits for AFB_1 (10 μg/kg) and total AF (15 μg/kg) set by the KFDA. In a previous study, 5 out of 11 samples analyzed (45.5%) were within the permissible levels, ranging 0.04–2.46 μg/kg [21]. These data indicate that continuous management of AF risk is necessary during soybean paste manufacturing. In addition, 45 soybean paste samples were analyzed for OTA levels using HPLC. Twenty-two samples (48.9%) were contaminated with OTA at 0.88–26.29 μg/kg (Table 7); the mean concentration was 4.43 μg/kg. These results are similar to the average contamination (7.1 μg/kg) of soybean paste described by park et al. [24]. The levels of OTA in five soybean paste samples analyzed (11.1%) exceeded the permissible limits for OTA in *meju* set by the KFDA (20 μg/kg) when the limit for OTA in *meju* was used for comparison as the legal limits for OTA in soybean paste are not yet established in South Korea. Thus, the establishment of legal limits for OTA in soybean paste and risk management are required for the soybean paste industry.

In addition, the occurrence of AFs and OTA in soybean paste was investigated on the basis of local climate. Unlike the severe contamination of *meju*, the contamination level was low in soybean paste (Table 8). There were no regional differences in the levels of AFs and OTA in the samples.

Reduction in the levels of AFs and OTA in *meju* samples contaminated with high amounts of the toxins during soy sauce and soybean paste production

Soy sauce and soybean paste are produced after the ripening of fermented *meju* (Fig. 3). To investigate the levels of AFs and OTA in soy sauce and soybean paste after the

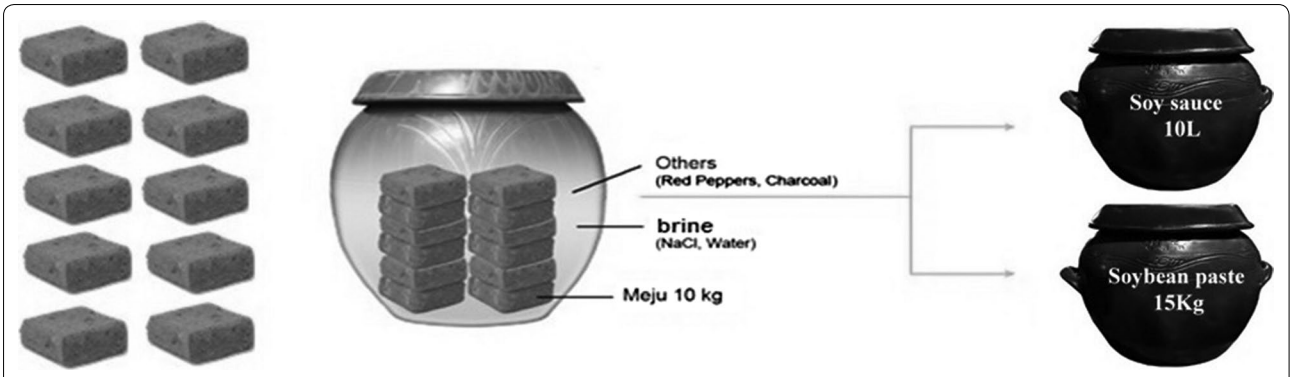

Fig. 3 General scheme for the production of soy sauce and soybean paste from *meju*

Table 9 Levels of AFs and OTA in *meju*, soy sauce, and soybean paste collected from the same household

Mycotoxin	*Meju* (μg/kg)	Soy sauce (μg/kg)	Soybean paste (μg/kg)
AFB_1	620.0 ± 6.49	0.49 ± 0.02	13.29 ± 0.70
AFB_2	9.7 ± 1.99	0.48 ± 0.01	13.20 ± 0.60
AFG_1	1.2 ± 0.32	0.49 ± 0.01	13.30 ± 0.61
AFG_2	0	0.48 ± 0.02	13.25 ± 0.50
OTA	1738.31 ± 25.69	7.53 ± 0.32	190.47 ± 4.11

ripening of *meju*, we collected *meju* samples, which were contaminated with high amounts of AFs (620.0 μg/kg) and OTA (1738.3 μg/kg), soy sauce samples, and soybean paste samples in the same household in 2016, which were prepared from the *meju*. Both soy sauce and soybean paste were collected 8 months after *meju* sampling. In this study, we assumed that soy sauce and soybean paste from *meju* were produced as shown in Fig. 3. Briefly, 10 kg of *meju* and 30 L of brine (about 20% NaCl) were used for the production of soy sauce and soybean paste. After the ripening of *meju* in the brine, the mashed *meju* was filtered, and the paste was separated from the liquid. Finally, 25 L of liquid (soy sauce) and 15 kg of soybean paste were obtained without any loss [29]. When soybean paste and soy sauce are made in the traditional way, fermented *meju* is matured for several months in brine. AFs and OTA production would not occur during the ripening of fermented *meju* because the fungus cannot grow and produce AFs under > 14% salt concentration (22%). Therefore, mycotoxins levels are expected to decline or stay at a certain value [30]. Table 9 shows that the levels of AFB_1 and OTA were 0.5 μg/kg and 7.5 μg/kg in soy sauce and 11.9 μg/kg and 190.4 μg/kg in soybean paste, respectively. It was presumed that 12.5 μg of AFB_1 and 1882.5 μg of OTA were present in 25 L soy sauce and that 178.5 μg of AFB_1 and 2790 μg OTA were present in 15 kg soybean paste (Fig. 4). These results suggest that considerable amounts of AFB_1 (97%) and OTA (73%) were degraded during the production of soy sauce and soybean paste from *meju*. AFB_1 and OTA were almost completely degraded. There are various reasons why the mycotoxins were degraded. Various microorganisms are known to inhibit mycotoxin production. Cho and co-workers reported the degradation of OTA using *A. tubingensis* isolated from *meju* [31]. In their study, OTA degradation was about 95% after 14 days. Kim and collaborators studied the effect of mixed culture conditions in a liquid medium and soybean mash and reported that *A. niger* reduced AF formation, but other microorganisms did not interfere with AF production [10]. Petchkongkaew and colleagues reported the detoxification of AFs and OTA by using *Bacillus licheniformis* isolated from fresh *Thuanao* (a fermented soybean product) collected from north Thailand (74% decrease in AFB_1 and 92.5% decrease in OTA) [32]. However, in our study we cannot rule out the possibility that mycotoxins in the final products might be adsorbed onto charcoal during the soybean paste and soy sauce preparation even though charcoal addition to home-made soy sauce had negligible effects on AFB_1 degradation (about 5% AFB_1 degradation) in the soy sauce in one study described by Park et al. [27].

Both AF and OTA contamination in *meju* can cause public health hazards even at low levels. Our data showed that OTA contamination in *meju* was more frequent than AF contamination. Therefore, although greater management of OTA is necessary than of AFs in *meju*, regular monitoring and control of both mycotoxins in *meju* and soybean paste are crucial for the fermented food industry in South Korea.

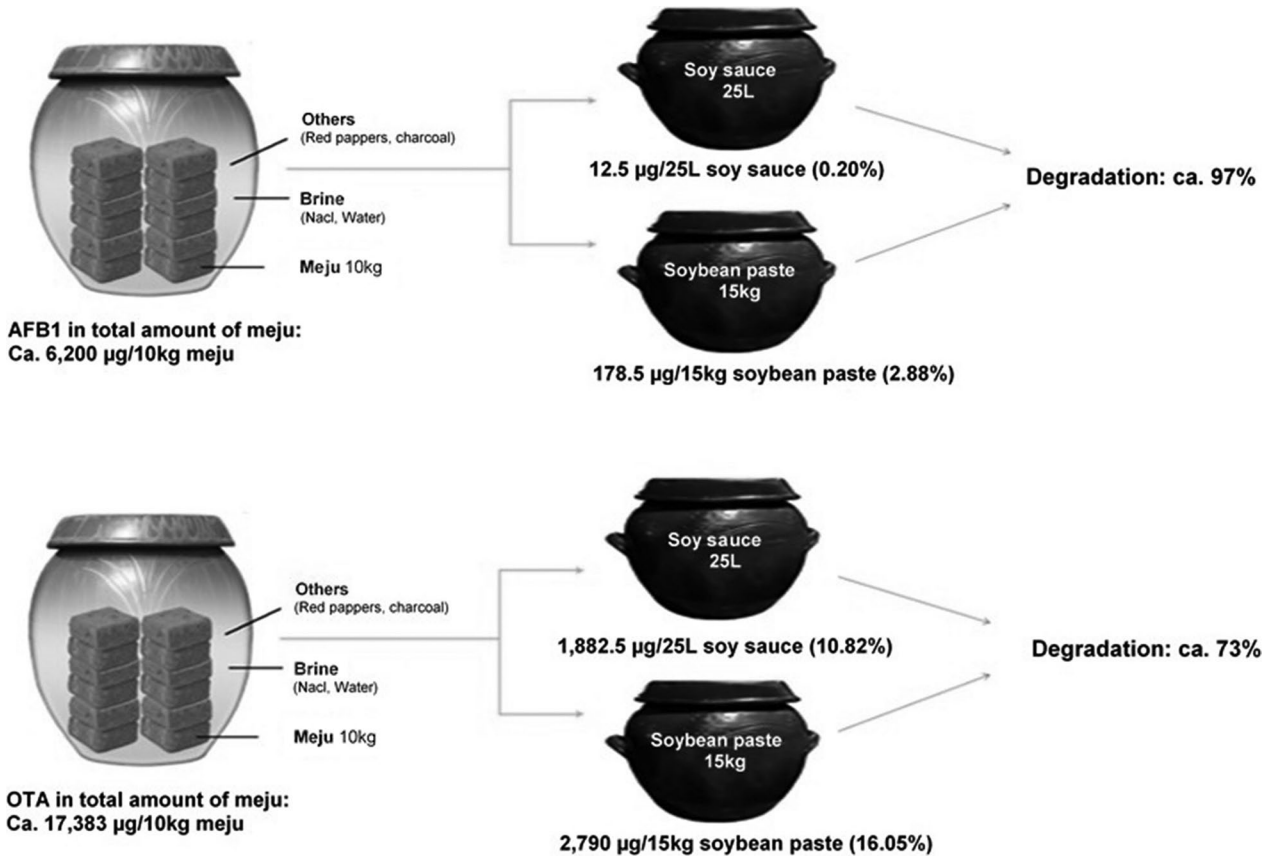

Fig. 4 Putative fate of AFB_1 and OTA during the production of soy sauce and soybean paste from *meju* assuming that all *meju* samples contained the same levels of toxins

Abbreviations

AFs: aflatoxins; OTA: ochtatoxin A; AFB_1: aflatoxin B_1; AFB_2: aflatoxin B_2; AFG_1: aflatoxin G_1; AFG_2: aflatoxin G2; *A.*: *Aspergillus*; IARC: International Agency for Research on Cancer; ELISA: enzyme-linked immunosorbent assay; TLC: thin-layer chromatography; HPLC: high-performance liquid chromatography; IAC: immunoaffinity column; ACN: acetonitrile; MeOH: methanol; HAC: acetic acid; TFA: trifluoroacetic acid; PBS: phosphate buffered saline; NaCl: sodium chloride; LOD: limit of detection; LOQ: limit of quantification; S/N: signal-to-noise ratio; AOAC: Association of Official Analytical Chemists; KFDA: Korea Food and Drug Administration; RSD: relative standard deviation.

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Authors' contributions

SEJ, SHC, and SYH conceived and designed the experiments. SEJ performed the experiments. SEJ, SHC, and SYH analyzed the data. SEJ and SYH wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Competing interests

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References

1. Kim DH, Lee KH, Yook HS, Kim JH, Shin MG, Byun MW (2000) Quality characteristics of gamma irradiated-grain shape improved meju. Korean J Food Sci Technol 32:640–645
2. Lee KH, Kim ND, Yoo JY (1997) Survey on the manufacturing process of traditional meju for and of kanjang (Korean soy sauce). J Korean Soc Food Sci Nutr 26:390–396
3. Kim JS, Kim JG, Kim WJ (2004) Changes in isoflavone and oligosaccharides of soybeans during germination. Korean J Food Sci Technol 36:294–298
4. Abdin M, Ahmad MM, Javed S (2010) Advances in molecular detection of *Aspergillus*: an update. Arch Microbiol 192:409–425
5. Reddy K, Salleh B, Saad B, Abbas H, Abel C, Shier W (2010) An overview of mycotoxin contamination in foods and its implications for human health. J Toxicol Toxin Rev 29:3–26
6. IARC (2002) Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr Eval Carcinog 82:1–556
7. Abrunhosa L, Venâncio A (2007) Isolation and purification of an enzyme hydrolyzing ochratoxin A from *Aspergillus niger*. Biotechnol Lett 29:1909–1914
8. Wang Y, Wang L, Liu F, Wang Q, Selvaraj J, Xing F et al (2016) Ochratoxin A producing fungi, biosynthetic pathway and regulatory mechanisms. Toxicol Toxin Rev 8:83

9. Lee KR, Yang SM, Cho SM, Kim M, Hong S-Y, Chung SH (2017) Aflatoxin B1 detoxification by *Aspergillus oryzae* from meju, a traditional Korean fermented soybean starter. J Biol Biotechnol 27:57–66
10. Kim EJ, Chung Y, Kwon SP (1976) Effect of various fungi on the aflatoxin productivity in the culture of *Aspergillus flavus*. J Prev Med Public Health 9:77–86
11. Kim Y-H, Hwangbo J-S, Lee S-R (1977) Detection of aflatoxins in some Korean foodstuffs. Korean J Food Sci Technol 9:73–80
12. Park K-Y, Lee E-S, Moon S-H, Cheigh H-S (1989) Effects of browning products and charcoal on the degradation of aflatoxin B, in Korean soy sauce (kanjang) and its model system. Bull Environ Contam Toxicol 21:419–424
13. Scott P, Lawrence J, Van Walbeek W (1970) Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl Microbiol 20:839–842
14. Leitner A, Zöllner P, Paolillo A, Stroka J, Papadopoulou-Bouraoui A, Jaborek S et al (2002) Comparison of methods for the determination of ochratoxin A in wine. Anal Chim Acta 453:33–41
15. Dallsta C, Galaverna G, Dossena A, Marchelli R (2004) Reversed-phase liquid chromatographic method for the determination of ochratoxin A in wine. J Chromatogr A 1024:275–279
16. Lutfullah G, Hussain A (2012) Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. Food Control 23:32–36
17. Park C-Y, Choi Y-E, Moon J-Y, Yun W-T (2009) Classification of climate zones in South Korea considering both air temperature and rainfall. J Geo Soc Korea 44:1–16
18. Kim E, Shon D, Yoo J, Ryu D, Lee C, Kim Y (2001) Natural occurrence of aflatoxins in Korean meju. Food Addit Contam 18:151–156
19. Sripathomswat N, Thasnakorn P (1981) Survey of aflatoxin-producing fungi in certain fermented foods and beverages in Thailand. Mycopathologia 73:83–88
20. Wei R, Chang SC, Lee S (1980) High pressure liquid chromatographic determination of aflatoxins in soy sauce and fermented soybean paste. J Assoc Off Anal Chem 63:1269–1274
21. Park M-J, Yoo M-H, Hong H-G, Joe T-s, Lee I-s, Park J-H et al (2008) A survey of the presence of aflatoxins in food. J. Food Hyg Saf 23:108–112
22. Blesa J, Soriano J, Moltó J, Mañes J (2004) Absence ochratoxin A in soy sauce. Int J Food Microbiol 97:221–225
23. Brera C, Grossi S, De Santis B, Miraglia M (2003) Automated HPLC method for the determination of ochratoxin A in wine samples. J Liq Chromatogr Related Technol 26:119–133
24. Park S-K, Kwon K-S, Kim M-H, Jeong S-Y, Jang G-H, Nam T-H et al (2004) Survey of ochratoxin A in cereal-based Korean traditional foods by HPLC. Korean J Food Sci Technol 36:158–161
25. Horwitz W (1975) Official methods of analysis, pp edn. Association of Official Analytical Chemists, Washington, DC
26. Chang Y, Beng C, Ponnapalam J (1966) Mouldy soybeansas potential source of aflatoxin contamination. Far East Med J 2:298–300
27. Park K-Y, Lee K-B, Bullerman LB (1988) Aflatoxin production by *Aspergillus parasiticus* and its stability during the manufacture of Korean soy paste (doenjang) and soy sauce (kanjang) by traditional method. J Food Prot 51:938–944
28. Kim Y-K, Roh J-K (1985) Detection of aflatoxins in soybean food by HPLC. Korean J Food Sci Technol 17:295–303
29. Park K-Y, Hwang K-M, Jung K-O, Lee K-B (2002) Studies on the standardization of doenjang (Korean soybean paste): 1. Standardization of manufacturing method of doenjang by literatures. J Korean Soc Food Sci Nutr 31:343–350
30. Nagarajan V, Bhat R, Tulpule P (1973) Aflatoxin production in some varieties of soybeans (*Glycine max* L.). Experientia 29:1302–1303
31. Cho SM, Jeong SE, Lee KR, Sudhani HP, Kim M, Hong S-Y et al (2016) Biodegradation of ochratoxin a by *Aspergillus tubingensis* isolated from meju. J Microbiol Biotechnol 26:1687–1695
32. Petchkongkaew A, Taillandier P, Gasaluck P, Lebrihi A (2008) Isolation of Bacillus spp. from Thai fermented soybean (Thua-nao): screening for aflatoxin B1 and ochratoxin A detoxification. J Appl Microbiol 104:1495–1502

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