

Simultaneous determination of 17 regulated and non-regulated *Fusarium* mycotoxins co-occurring in foodstuffs by UPLC-MS/MS with solid-phase extraction

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ABSTRACT

Fusarium species produce numerous mycotoxins known to co-occur in food. While some of these mycotoxins (e.g., deoxynivalenol, fumonisins) are regulated in several countries, others are non-regulated (e.g., nivalenol, beauvericin). In this study, UPLC-MS/MS with solid-phase extraction cleanup was used to determine 17 *Fusarium* mycotoxins (FTs) simultaneously. The method showed excellent performance in terms of linearity ($R^2 > 0.99$), LOD ($<1.2 \mu\text{g/kg}$), LOQ ($<3.6 \mu\text{g/kg}$), accuracy (70.0–116.3 %), repeatability (<15.7 %), reproducibility (<25.3 %), and expanded uncertainty (<41.7 %). The validated method was successfully applied to 198 marketed food samples collected in South Korea. Of the tested samples, 79 % were contaminated with at least one FT. Job's tears showed the highest prevalence of 14 FTs, and sorghum had the highest total FTs level (3.03 mg/kg). The results suggest that this method can be used for the simultaneous analysis of 17 FTs in food samples, which would serve as crucial information for risk management.

1. Introduction

Mycotoxins are one of the hazards that threaten food safety. Globalization of the food market coupled with climate change makes it increasingly important to monitor and track mycotoxins through the food supply chain. A single food product can be contaminated with several types of mycotoxins simultaneously (Borutova et al., 2012; Siri-Anusornsak et al., 2022). Of particular importance is the need to assess the combined risk from co-exposure to mixtures of mycotoxins with similar modes of action (Zhao et al., 2021), including the possibility of synergisms between mycotoxins (Ficheux et al., 2012). Despite this, the number of analytical approaches that determine multiple mycotoxins within one analytical run is still relatively low (Steiner et al., 2021).

Fusarium species are common and widespread in nature and one of the most dominant mycotoxin-producing fungi on field crops (Topi et al., 2020). *Fusarium* mycotoxins (FTs) are secondary metabolites produced by *Fusarium* spp. They commonly occur in cereal grains and

their processed foods and feeds and can cause hepatotoxicity, immunotoxicity, reproductive toxicity, and nephrotoxicity (Kabak et al., 2006). Therefore, due to food safety concerns, the monitoring of FTs has become pertinent, and there is growing concern about the potential health risks presented by co-occurring FTs in foods.

Most countries regulate the maximum permitted levels of FTs in food and feed, mainly for the T-2 toxin (T-2), HT-2 toxin (HT-2), deoxynivalenol (DON), zearalenone (ZEN), and fumonisin B toxins (FBs). However, the regulatory limits for FTs vary among food commodities and countries. The EC and South Korea (MFDS) have set maximum levels of 200–1,750 and 200–2,000 $\mu\text{g/kg}$ for DON, 20–200 and 20–200 $\mu\text{g/kg}$ for ZEN, and 200–2,000 and 1,000–4,000 $\mu\text{g/kg}$ for the sum of FB1 and FB2, respectively. The United States Food and Drug Administration (FDA) has issued advisory and guidance levels for DON and total FBs (sum of FB1, FB2, and FB3) in wheat products of $< 1,000$ and 2,000–4,000 $\mu\text{g/kg}$, respectively (European Commission [EC], 2006, 2014; Food and Drug Administration [FDA], 2023; Ministry of Food and

Abbreviations: 3ADON, 3-acetyl deoxynivalenol; 15ADON, 15-acetyl deoxynivalenol; ACN, acetonitrile; BEA, beauvericin; DON, deoxynivalenol; EN, enniatin; ESI, electrospray ionization; FB, fumonisin B toxin; FT, *Fusarium* mycotoxin; HPLC, high-performance liquid chromatography; HT-2, HT-2 toxin; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; ME, matrix effect; MeOH, methanol; NIV, nivalenol; QqQ, triple quadrupole; QuEChERS, dilute-and-shoot™ or “quick, easy, cheap, effective, rugged, and safe”; SD, standard deviation; SPE, solid-phase extraction; T-2, T-2 toxin; UPLC, ultra-high-performance liquid chromatography; ZEN, zearalenone.

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Drug Safety [MFDS], 2016).

Fusarium spp. can produce several mycotoxins simultaneously: *F. graminearum* is an important producer of type B trichothecenes, such as DON, nivalenol (NIV), and acetylated DON, but it is also capable of producing ZEN. Additionally, some multi-mycotoxin-producing *Fusarium* spp. produce not only regulated mycotoxins but also beauvericin (BEA) and enniatins (ENs), so-called “emerging mycotoxins”, which are not regulated because of their low prevalence and limited toxicological data (Siri-Anusornsak et al., 2022). For instance, *F. oxysporum* can produce FB1, FB2, and BEA, *F. sporotrichioides* is known to produce T-2, DON, NIV, ZEN, and ENs, and *F. poae* can produce T-2, HT-2, NIV, BEA, and ENs (Haidukowski et al., 2022; Kotowicz et al., 2014). BEA and ENs co-occur naturally with regulated FTs, and their synergistic toxicity with regulated FTs, such as T-2, DON, and ZEN, has been reported (Ficheux et al., 2012; Klarić et al., 2008; Siri-Anusornsak et al., 2022). Therefore, an accurate and reliable analytical method for the simultaneous quantitative analysis of multiple mycotoxins in complex food matrices is necessary to obtain realistic exposure estimations with a low level of uncertainty.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is suitable in this context because of its sensitivity to mycotoxins without fluorophore structures, such as trichothecenes (Lattanzio et al., 2009). However, matrix effects (MEs) can lead to inaccurate quantification, necessitating an appropriate sample cleanup method. Several recent studies on the simultaneous determination of regulated and emerging FTs adopted “dilute-and-shoot” or “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) as the cleanup method (González-Jartín et al., 2021; Krausová et al., 2022; Leite et al., 2023). Although these are straightforward techniques, they present some disadvantages, such as high ME and low sensitivity. Solid-phase extraction (SPE) is the most frequently applied cleanup technique to mitigate the ME. In addition to the analytical challenges mentioned above, it is rather difficult to develop a method for the simultaneous analysis of FTs because of their chemical diversity and varying concentrations in foods and feeds (Jestoi et al., 2009). Some studies performed two separate sample preparations on account of the polarity and solubility differences among the analytes, or FBs were excluded from the analytes because of their water-soluble properties (Habler & Rychlik, 2016; Njumbe Ediage et al., 2011).

Therefore, we hypothesized that LC-MS/MS using SPE cleanup with enhanced retention of polar mycotoxins would enable the accurate and reliable simultaneous quantitative determination of FTs in food matrices. In this study, an LC-MS/MS-based analytical method with SPE cleanup was developed and validated for simultaneous determination of regulated FTs (T-2, HT-2, DON, 3-acetyl deoxynivalenol [3ADON], 15-acetyl deoxynivalenol [15ADON], ZEN, FB1, FB2, and FB3) and non-regulated emerging FTs (NIV, BEA, ENA, ENA1, ENA2, ENB, ENB1, and ENB4). The validated method was then successfully applied to 198 marketed food samples collected in South Korea to investigate the natural co-occurrence of 17 FTs.

2. Materials and methods

2.1. Sample collection

A total of 198 commercial food products distributed in South Korea were purchased from online retailers, supermarkets, and local markets in 2021–2022 to analyze the co-occurrence of FTs. The collected samples were classified into the following categories: cereal grains (rice, brown rice, barley, oats, Job's tears, wheat flours, sorghum, foxtail millet, and maize), processed grain products (*Sunsik* [dry grain food] and breakfast cereals), cereal formula baby food, edible oils (corn oil, soybean oil, grapeseed oil, sesame oil, and perilla oil), and eggs (chicken eggs and quail eggs). Collected samples were homogenized and stored at -20°C . Samples were removed from frozen storage and equilibrated at room temperature before analysis.

2.2. Chemicals and reagents

Certified mycotoxin standards were purchased as follows: T-2 (99.9 % purity), HT-2 (99.6 %), NIV (99.3 %), DON (99.4 %), 3ADON (99.3 %), 15ADON (99.4 %), FB1 (99.6 %), FB2 (98.5 %), and FB3 (99.1 %) from Romer Labs (Tulln, Austria); ZEN solution (99.8 %) from Supelco (St. Louis, MO, USA). Several ENs all from *Gnomonia errabunda*, including ENA (99.3 %), ENA1 (97.1 %), ENB (96.4 %), and ENB1 (98.5 %), were purchased along with BEA (98.0 %) from Sigma-Aldrich (St. Louis, MO, USA). The reference standards of ENA2 and ENB4 were purified and supplied by Prof. Yunying Xie (Chinese Academy of Medical Sciences & Peking Union Medical College) (Li et al., 2020). Working standard mixtures were prepared as follows: ZEN and trichothecenes (T-2, HT-2, NIV, DON, 3ADON, and 15ADON) were diluted in acetonitrile (ACN) at 1 mg/mL, FBs were diluted in 50 % ACN (v/v) at 1 mg/mL, and BEA and ENs were prepared in ACN at 0.2 mg/mL. High-performance liquid chromatography (HPLC)-grade water, ACN, and methanol (MeOH) from Honeywell Burdick & Jackson (Muskegon, MI, USA), and acetic acid (glacial $\geq 99.7\%$) from J.T. Baker (Philipsburg, NJ, USA) were used for sample preparation. LC/MS-grade ammonium acetate was obtained from Merck (Darmstadt, Germany).

2.3. Ultra-high-performance liquid chromatography (UPLC) electrospray ionization (ESI)-triple quadrupole (QqQ)-MS equipment conditions

The UPLC-MS/MS system consisted of an Agilent Infinity 1290 series and an Agilent 6470B QqQ system equipped with an Agilent Jet Stream ESI source (Santa Clara, CA, USA). Data analysis and quantification were performed with the Agilent MassHunter Acquisition software (version 10.0). Chromatographic separation was carried out on a Waters HSS C18 column (2.1×100 mm, $1.8\ \mu\text{m}$; Milford, MA, USA) at 40°C . The injection volume was $5\ \mu\text{L}$. Gradient conditions were established to achieve complete separation of 17 FTs, with emphasis on the separation of isomers with identical mass-to-charge ratios (e.g., 3ADON and 15ADON, ENA and ENA2, and ENB1 and ENB4). Elution was achieved in 25 min using the following gradient of solvent A (5 mM ammonium acetate in water containing 0.5 % acetic acid, v/v) and solvent B (5 mM ammonium acetate in MeOH) with a flow rate of 0.2 mL/min: 0–2 min, 80 % A, 20 % B; 2–6 min, 24 % B; 6–9.5 min, 30 % B; 9.5–12.5 min, 60 %; 12.5–14 min, 70 % B; 14–17 min, 75 % B; 17–17.1 min, 95 % B; 17.1–20 min, 95 % B; 20–20.5 min, 20 % B; 20.5–25 min, 20 % B. The MS parameters were as follows: gas flow, 9 L/min; gas temperature, 250°C ; nebulizer, 30 psi; sheath gas temperature, 350°C ; sheath gas flow, 12 L/min; capillary voltage, $-2,000\ \text{V}$ in the ESI-negative mode and $+3,500\ \text{V}$ in the ESI-positive mode; and ultrapure nitrogen ($>99.99\%$) was used as the collision gas. The optimized multiple reaction monitoring (MRM) conditions of the analytes are shown in Table 1.

2.4. Sample preparation

The 17 FTs from food matrices were optimized in the following cleanup steps: 4 g of sample was extracted with 16 mL of MeOH:water:acetic acid (79:20:1, v/v/v) by shaking (60 min, 270 rpm), followed by centrifugation ($3,100 \times g$, 7 min). The supernatant was diluted 16-fold with water. The polymer-based hydrophilic–lipophilic-balanced (HLB) SPE cartridge (200 mg, $6\ \text{cm}^3$; Waters) was conditioned with 6 mL of MeOH and equilibrated with 6 mL of water. A reservoir was attached to the SPE cartridge, and 32 mL of diluted supernatant was loaded into the reservoir. The cartridge was washed with 3 mL of water and dried thoroughly under vacuum for 3 min. Next, 6 mL of MeOH was applied to the SPE to elute toxins. The eluate was evaporated to dryness under a nitrogen stream at 45°C . The residue was reconstituted with 0.5 mL of MeOH, followed by 0.5 mL of water. The reconstituted solvent was filtered through a syringe filter ($0.2\ \mu\text{m}$ polytetrafluoroethylene, 13 mm) and injected into the LC-MS/MS apparatus.

Table 1
Optimized LC-MS/MS parameters.

Analyte*	Molar mass (g/mol)	Precursor ion (m/z)	Molecular ion	Fragmentor (V)	Product ion (m/z)	Collision energy (V)	Retention time (min)
T-2	466.5	484.3	[M + NH ₄] ⁺	110	<u>305.1</u> ** 185.1	10 20	16.3
HT-2	424.5	442.2	[M + NH ₄] ⁺	110	<u>263.1</u> 215.1	10 10	15.6
NIV	312.3	371.0	[M + CH ₃ COO] [−]	90	<u>281.1</u> 311.1	12 10	2.2
DON	296.3	355.1	[M + CH ₃ COO] [−]	90	<u>295.1</u> 265.1	8 10	3.7
3ADON	338.4	397.1	[M + CH ₃ COO] [−]	90	<u>337.1</u> 307.1	10 15	11.2
15ADON	338.4	339.1	[M + H] ⁺	90	<u>321.1</u> 264.1	10 10	11.6
ZEN	318.4	317.7	[M−H] [−]	190	<u>175.0</u> 130.8	25 33	17.0
FB1	721.8	722.4	[M + H] ⁺	180	<u>352.3</u> 334.3	45 45	15.6
FB2	705.8	706.5	[M + H] ⁺	180	<u>336.3</u> 318.3	45 45	17.6
FB3	705.8	706.5	[M + H] ⁺	180	<u>336.3</u> 318.3	45 45	16.4
BEA	783.9	784.3	[M + H] ⁺	240	<u>134.0</u> 244.1	64 50	19.5
ENA	681.9	682.4	[M + H] ⁺	220	<u>210.1</u> 228.1	24 26	19.9
ENA1	667.9	668.4	[M + H] ⁺	220	<u>210.1</u> 228.1	26 28	19.7
ENA2	681.9	682.4	[M + H] ⁺	220	<u>210.1</u> 228.1	28 28	19.7
ENB	639.8	640.4	[M + H] ⁺	220	<u>196.1</u> 214.1	24 26	19.4
ENB1	653.8	654.4	[M + H] ⁺	220	<u>196.1</u> 210.0	28 24	19.6
ENB4	653.8	654.4	[M + H] ⁺	220	<u>196.1</u> 214.1	24 26	19.4

*3ADON: 3-acetyl deoxynivalenol; 15ADON: 15-acetyl deoxynivalenol; BEA: beauvericin; DON: deoxynivalenol; ENA: enniatin A; ENA1: enniatin A1; ENA2: enniatin A2; ENB: enniatin B; ENB1: enniatin B1; ENB4: enniatin B4; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; HT-2: HT-2 toxin; NIV: nivalenol; T-2: T-2 toxin; ZEN: zearalenone.

**indicates the quantifier ion.

2.5. Method validation

Method validation was performed based on SANTE/11312/2021 (EC, 2021). The performance of the method was evaluated by the Association of Official Analytical Chemists (AOAC) Guidelines for Standard Method Performance Requirements (SMPRs) for non-regulated FTs (AOAC International, 2016) and by EC No. 519/2014 for regulated FTs (EC, 2014). The matrix-matched calibration curves covering 1 to 200 µg/kg were prepared by spiking FTs to each blank matrix. The linearity was evaluated by the coefficient of determination (R^2) of six-point matrix-matched calibration curves constructed by plotting the peak area (signal intensity) against the analyte concentration. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the slope of the matrix-matched calibration curve (S) and the standard deviation (SD) of the peak area from the lowest concentration in the matrix-matched calibration curve. LOD and LOQ were calculated by the following equations: $LOD = 3.3 \times SD / S$; $LOQ = 10 \times SD / S$. The ME was calculated using the following equation to evaluate the signal suppression or enhancement: $\text{slope of the matrix-matched calibration curve} / \text{slope of the solvent-based calibration curve} \times 100 (\%)$.

The accuracy was evaluated by recovery experiments according to the following formula: $\text{analyzed concentration of spiked samples} / \text{calculated from matrix-matched standard} / \text{spiking concentration} \times 100 (\%)$. Precision was calculated as the relative SD of the replicated recovery experiments. The measurement uncertainty was quantified based on the EURACHEM/CITAC Guide CG 4 (Ellison & Williams, 2012). Uncertainty sources were identified using the Fishbone diagram. Sample weight, reference material (purity of standard and preparation of

working standard), linear calibration curve interpolation, instrumental factors, matrix (recovery), and final volume were considered sources of uncertainty. Each uncertainty factor was calculated according to type A (SD of the series of the measured values) and type B (calibration certificate of the balance and pipette) uncertainties. The combined standard uncertainty ($u_c(y)$) was calculated as the positive square root of the total variance obtained by combining all the uncertainty components based on Equation (1). The expanded measurement of uncertainty ($U(y)$) was calculated using a standard coverage factor (k) of two for an approximate level of confidence of 95 % based on Equation (2).

$$u_c^2(y) = \sum_{i=1}^N \left(\frac{\partial y}{\partial x_i} \right)^2 u^2(x_i) \quad (1)$$

$$U(y) = k_{uc}(y) \quad (2)$$

3. Results and discussion

3.1. Method validation

Method validation was conducted on rice, baby food, and corn oil matrices because FTs are known to have the highest degree of contamination in grains, such as corn, wheat, and their products (Gallardo et al., 2023; Han et al., 2019). The validation matrices were selected based on EC No. 519/2014 (EC, 2014). Rice, a high starch and low water and fat contents matrix, was a representative matrix of cereal grain and products thereof. Furthermore, rice is the most consumed cereal grain in South Korea. The corn oil matrix was selected to represent commodities

with high oil content and very low water content. Among edible oils, it is reported that corn oil showed the highest contamination level of FTs (European Food Safety Authority Panel on Contaminants in the Food Chain [CONTAM], 2011; European Food Safety Authority [EFSA], 2014).

In most countries, including South Korea (EC, 2006; MFDS, 2016), regulatory limits for mycotoxins in infant foods are lower than those in foods for adults. In addition, baby food has a complex heat-processed matrix containing high protein, high carbohydrate, vitamins, and minerals. For these reasons, baby food was selected as a matrix for method validation.

3.1.1. Selectivity, linearity, LOD, LOQ, and ME

Seventeen FTs were successfully separated in 25 min on a C18 column under optimized gradient conditions (Fig. 1). Chromatographic separation of isomers with identical precursor and product ions (3ADON and 15ADON; ENA and ENA2; ENB1 and ENB4) was achieved with good selectivity. The linearity (R^2) of the matrix-matched external calibration curve of six points was in the range of 5–100 $\mu\text{g/kg}$ for ZEN, 5–200 $\mu\text{g/kg}$ for trichothecenes and FBs, and 1–40 $\mu\text{g/kg}$ for BEA and ENs. Good linearity of the calibration curves was obtained for all FTs in all validated matrices ($R^2 > 0.99$). The LOD and LOQ values are listed in Table 2. The LODs ranged from 0.03 to 1.20 and 0.01 to 0.42 $\mu\text{g/kg}$ for regulated and non-regulated toxins, respectively. The LOQs ranged from 0.08 to 3.63 and 0.02 to 1.26 $\mu\text{g/kg}$ for regulated and non-regulated toxins, respectively. These values are much lower than those reported in previous studies that simultaneously determined FTs in various matrices: 0.1–70.0 $\mu\text{g/kg}$ in starch (Habler & Rychlik, 2016), 0.40–92.4 $\mu\text{g/kg}$ in infant stool (Krausová et al., 2022), 0.2–21.1 $\mu\text{g/kg}$ in maize (Leite et al., 2023), and 0.1–33 $\mu\text{g/kg}$ in maize, cassava, and peanut cake (Njumbe Ediage et al., 2011). The MEs of FTs in rice, baby food, and corn oil were 81.2–117.5 %, 80.2–115.4 %, and 83.6–119.2 %, respectively. An ME between 80 and 120 % is generally considered an acceptable signal suppression or enhancement (Dong et al., 2019), and the MEs of all FTs in three validated matrices were within this range.

3.1.2. Accuracy, precision, and measurement uncertainty

For accuracy and precision evaluation, the blank samples of rice, baby food, and corn oil were fortified with 5, 10, and 20 $\mu\text{g/kg}$ of BEA and ENs and 25, 50, and 100 $\mu\text{g/kg}$ of other mycotoxins based on their reported occurrence levels. The accuracy, precision (repeatability, RSD_r), and U(y) ($k = 2$) are shown in Table 3; all values were rounded to the first decimal place. The performance of the method was evaluated according to EC No. 591/2014 (EC, 2014) for regulated toxins and AOAC International (2016) specifications for non-regulated toxins.

The accuracies of regulated and non-regulated FTs were in the range of 88.6–109.2 % and 70.0–99.2 % for rice, 74.4–116.3 % and 72.7–108.3 % for baby food, and 72.4–105.8 % and 72.1–99.9 % for corn oil, respectively. The precision values for rice, baby food, and corn oil matrices were 0.6–9.3 % and 0.3 %–15.7 %, 0.3–14.2 % and 0.3–10.7 %, and 0.2–11.8 % and 0.2–14.1 % for regulated and non-regulated FTs, respectively. The reproducibility of the method was validated in baby food and corn oil matrices. Three different laboratories participated in the validation, and the results are summarized in Table S1. The reproducibility values (RSD_R) for baby food and corn oil matrices were in the range of 4.3–18.5 % and 4.4–21.7 % for regulated FTs, and 1.9–25.3 % and 2.1–25.3 % for non-regulated FTs, respectively.

The measurement uncertainty calculation is an essential parameter for the metrological evaluation of the results. The U(y) values for the regulated and non-regulated FTs were under 38.3 % and 41.7 %, respectively. The contributions of each uncertainty factor to $u_c(y)$ are presented in Fig. S1. The standard materials of ENA2 and ENB4 were confirmed by MS/MS and nuclear magnetic resonance (NMR), but purity information was not provided, so the relative uncertainty of the reference material for ENA2 and ENB4 was calculated by the preparation of working standards. The relative uncertainty from calibration curves ($U_{\text{calibration}}$) and recovery (U_{matrix}) were the most significant factors in all three matrices, contributing about 30 % to $u_c(y)$.

All the obtained accuracy, repeatability, and reproducibility results satisfied the requirements of EC No. 591/2014 (EC, 2014) and AOAC International (2016). Furthermore, the U(y) values of all analytes in three matrices were acceptable according to the report presented by the

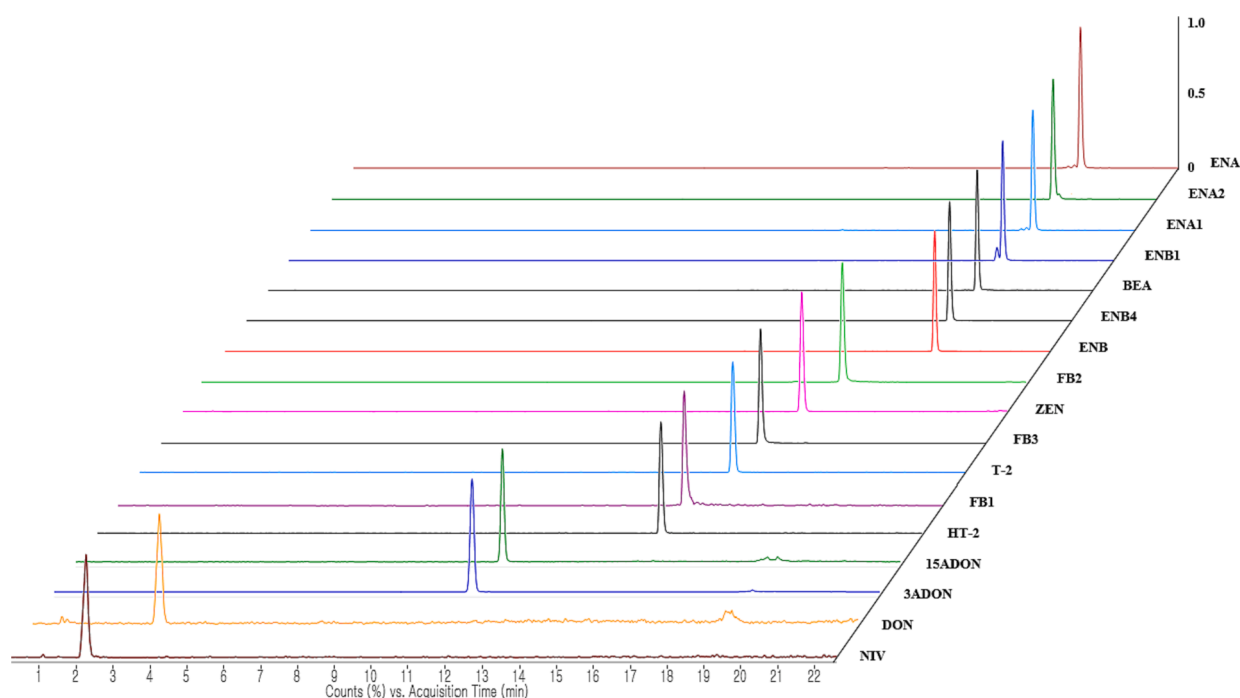


Fig. 1. Chromatograms of 17 *Fusarium* mycotoxins (10 $\mu\text{g/kg}$ for regulated and 2 $\mu\text{g/kg}$ for non-regulated toxins). 3ADON: 3-acetyl deoxynivalenol; 15ADON: 15-acetyl deoxynivalenol; BEA: beauvericin; DON: deoxynivalenol; ENA: enniatin A; ENA1: enniatin A1; ENA2: enniatin A2; ENB: enniatin B; ENB1: enniatin B1; ENB4: enniatin B4; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; HT-2: HT-2 toxin; NIV: nivalenol; T-2: T-2 toxin; ZEN: zearalenone.

Table 2
Linearity, LOD, LOQ, and ME of *Fusarium* mycotoxins in validated matrices.

Analyte*	Rice						Baby food						Corn oil					
	Calibration curve			LOD** (µg/kg)	LOQ*** (µg/kg)	ME**** (%)	Calibration curve			LOD (µg/kg)	LOQ (µg/kg)	ME (%)	Calibration curve			LOD (µg/kg)	LOQ (µg/kg)	ME (%)
	Slope	Intercept	R ²				Slope	Intercept	R ²				Slope	Intercept	R ²			
T-2	591.9	-533.7	1.000	0.03	0.08	117.5	346.5	-397.5	0.999	0.10	0.29	112.9 %	614.6	-339.7	0.999	0.20	0.59	103.6
HT-2	157.4	-47.5	1.000	0.04	0.13	99.0	114.2	-95.1	0.999	0.09	0.27	81.3 %	161.9	-18.8	0.999	0.09	0.29	101.8
NIV	50.9	-18.2	1.000	0.42	1.26	115.4	17.2	-17.9	0.999	0.40	1.21	115.4 %	48.0	-5.5	1.000	0.35	1.05	117.9
DON	117.2	-116.3	0.999	0.14	0.43	87.4	47.4	403.6	0.997	1.20	3.63	80.2 %	123.1	-99.6	0.999	0.26	0.78	97.1
3ADON	152.5	31.1	1.000	0.15	0.45	113.3	152.2	73.6	1.000	0.10	0.31	113.1 %	160.5	205.4	0.999	0.32	0.96	119.2
15ADON	34.3	-50.1	0.999	0.10	0.29	98.9	34.5	-38.0	0.999	0.61	1.86	89.9 %	34.9	18.5	1.000	0.75	2.27	101.2
ZEN	26.7	24.6	0.999	0.07	0.22	81.2	26.6	41.3	0.999	0.07	0.22	80.8 %	115.7	40.9	0.999	0.19	0.57	111.7
FB1	38.0	-45.2	0.999	0.28	0.84	90.2	37.0	-45.0	0.999	0.76	2.31	87.9 %	37.7	3.0	1.000	0.66	2.00	89.5
FB2	66.5	-71.9	0.999	0.16	0.48	85.8	64.0	-68.0	0.999	0.65	1.98	82.6 %	64.8	-12.3	1.000	0.31	0.93	83.6
FB3	66.7	-100.8	0.999	0.26	0.79	116.0	56.5	-71.7	0.999	0.53	1.60	98.3 %	65.5	-72.7	0.999	0.46	1.38	113.9
BEA	5,183.7	673.5	1.000	0.01	0.03	101.5	5,657.6	968.8	1.000	0.08	0.23	110.7 %	6,979.3	1,157.9	1.000	0.01	0.04	100.0
ENA	6,952.6	696.0	1.000	0.04	0.11	93.9	6,599.3	1,651.2	1.000	0.06	0.19	89.1 %	7,549.2	1,786.0	1.000	0.07	0.23	101.9
ENA1	5,913.1	1,034.5	1.000	0.04	0.11	93.4	5,693.2	1,207.7	1.000	0.08	0.25	89.9 %	6,406.8	1,718.0	1.000	0.04	0.13	101.2
ENA2	6,826.2	306.7	1.000	0.03	0.08	93.9	6,371.0	117.6	1.000	0.08	0.23	87.6 %	8,256.6	479.4	1.000	0.04	0.12	113.6
ENB	5,214.4	-204.1	1.000	0.02	0.07	109.5	4,582.1	191.2	1.000	0.07	0.22	96.2 %	5,057.0	233.9	1.000	0.01	0.02	106.2
ENB1	3,709.8	-278.4	1.000	0.02	0.07	109.8	3,249.6	-139.4	1.000	0.01	0.04	96.2 %	3,603.4	164.1	1.000	0.06	0.19	106.6
ENB4	5,234.4	185.8	1.000	0.07	0.22	103.7	5,451.3	662.5	1.000	0.03	0.09	108.0 %	6,546.0	1,149.4	1.000	0.04	0.12	117.4

* 3ADON: 3-acetyl deoxynivalenol; 15ADON: 15-acetyl deoxynivalenol; BEA: beauvericin; DON: deoxynivalenol; ENA: enniatin A; ENA1: enniatin A1; ENA2: enniatin A2; ENB: enniatin B; ENB1: enniatin B1; ENB4: enniatin B4; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; HT-2: HT-2 toxin; NIV: nivalenol; T-2: T-2 toxin; ZEN: zearalenone.
** LOD: limit of detection;
*** LOQ: limit of quantification.
**** ME: matrix effect.

EC (2004): the U(y) was < 44 % if the concentration of the analyte was < 100 µg/kg (EC, 2004). Hence, the validated method was applied to investigate the occurrence of FTs in marketed foods in South Korea.

3.2. Natural occurrence of FTs in marketed foods

The natural occurrence of FTs was investigated in 198 marketed cereal grains, processed grain products, cereal formula baby foods, edible oils, and eggs distributed in South Korea. Of all tested foodstuffs, 79 % (157/198) were contaminated with at least one FT. The overall frequency (No. of positive samples / Total no. of tested samples × 100 (%)), overall mean, mean of the positive samples, and detected range are presented in Table 4.

Regulated FTs were detected in 66 % (131/198) of tested foodstuffs, with a range of 0.10 to 2,447.17 µg/kg, which is under regulatory limits (MFDS, 2016). T-2 and HT-2 were detected in 19 % (37/198) and 8 % (16/198) of the total foodstuffs at levels ranging from 1.25 to 4.21 and 0.51 to 2.89 µg/kg, with mean values of 0.45 and 0.15 µg/kg, respectively. T-2 occurred most frequently in perilla oils (8/10) but was detected at the highest concentration in Job's tears (4.21 µg/kg). HT-2 was detected most frequently and at the highest concentration in Job's tears (6/9, 2.89 µg/kg). Among all foodstuffs, Job's tears presented the top three highest contamination levels for the sum of T-2 and HT-2, albeit the concentrations were low, ranging from 6.12 to 6.35 µg/kg. It was reported that T-2 and HT-2 frequently occurred in oats at relatively high concentrations (Kiš et al., 2021; van der Fels-Klerx, 2010). In this study of various foodstuffs distributed in South Korea, oats and Job's tears were the major sources of T-2/HT-2 contamination, but the detected concentration range in oats (1.89–2.48 µg/kg) was much lower than reported in oats in the Czech Republic (32–165 µg/kg), Austria (56–645 µg/kg), and Croatia (25.8–31.9 µg/kg) (Mahato et al., 2022).

Of all foodstuffs examined, 45 % (90/198) were contaminated with at least one DON or its acetylated forms. DON (44 %, 87/198) was the most frequently detected type B trichothecene, followed by 15ADON (9 %, 17/198) and 3ADON (7 %, 14/198). The highest concentration of DON was found in foxtail millet (387.90 µg/kg), whereas the highest concentrations of its acetylated derivatives were found in Job's tears (14.41 µg/kg for 3ADON and 146.25 µg/kg for 15ADON). The foodstuffs with the highest and second-highest concentrations for the sum of DON, 3ADON, and 15ADON were Foxtail millet (387.90 µg/kg) and Job's tears (379.03 µg/kg), respectively. Among 42 edible oils, 92 % (39/42) were devoid of contamination with DON and its acetylated forms, and only three samples of perilla oil (3/10) were contaminated with DON or 3ADON or both FTs.

ZEN was detected in 31 % (61/198) of all analyzed foodstuffs at concentrations ranging from 0.10 to 61.19 µg/kg, with a mean value of 3.01 µg/kg. Sorghum had the highest concentration of ZEN (61.19 µg/kg) among all foodstuffs and ranked third after Job's tears (89 %, 8/9) and corn oil (83 %, 5/6) for frequency of ZEN, with an 80 % (8/10) detection rate. The mean occurrence level was similar to that reported in grains for human consumption in Europe (2.7 µg/kg, n = 2,190) (CONTAM, 2011).

For the FBs, at least one FB was found in 34 % (68/198) of the foodstuffs. FB2 (33 %) was detected at a higher frequency than FB1 (26 %), and FB3 showed the lowest frequency (21 %) among the FBs. Mean occurrence levels of 49.37, 7.50, and 6.50 µg/kg for FB1, FB2, and FB3, respectively, were found considering all tested foodstuffs. Among all foodstuffs, sorghum had the highest concentrations for each of the three FBs, with 2,447.17 µg/kg for FB1, 216.02 µg/kg for FB2, and 280.44 µg/kg for FB3. In addition, FB1 and FB2 were found in all investigated samples of Job's tears (9/9) and sorghum (10/10). By contrast, no FBs were detected in barley and sesame oil samples. Job's tears and sorghum were major sources of the contamination of regulated FTs in marketed foodstuffs (Fig. S2).

Non-regulated FTs occurred in 68 % (134/198) of all tested foodstuffs, similar to the regulated FTs. NIV, a type B trichothecene, was

Table 3
Accuracy, precision, and measurement uncertainty of the optimized method.

Analyte*	Fortification level (µg/kg)	Rice								Baby food								Corn oil							
		Intra-day (n = 9)		Inter-day (n = 9)		Result ± Uncertainty (µg/kg)		Uncertainty** / Result (%)		Intra-day (n = 9)		Inter-day (n = 9)		Result ± Uncertainty (µg/kg)		Uncertainty / Result (%)		Intra-day (n = 9)		Inter-day (n = 9)		Result ± Uncertainty (µg/kg)		Uncertainty / Result (%)	
		Recovery (%)	RSD %	Recovery (%)	RSD %					Recovery (%)	RSD %	Recovery (%)	RSD %					Recovery (%)	RSD %	Recovery (%)	RSD %				
T-2	25	106.1	1.5	105.5	2.0	26.3 ± 3.1	12.5			106.1	2.2	102.4	2.5	26.0 ± 4.1	16.3			104.2	1.1	99.3	3.4	25.4 ± 3.2	12.8		
	50	102.9	0.9	103.4	1.0	51.7 ± 3.7	7.5			99.5	0.8	99.3	2.7	49.8 ± 4.2	8.4			102.6	0.2	100.8	1.6	50.8 ± 3.9	7.9		
	100	101.2	0.9	101.2	0.8	101.4 ± 5.9	5.9			100.6	2.6	97.5	0.3	99.3 ± 6.9	6.9			101.3	1.7	99.7	0.9	100.7 ± 6.4	6.4		
HT-2	25	102.8	1.9	104.6	0.6	25.9 ± 2.2	8.8			105.1	3.3	99.8	1.7	25.6 ± 3.6	14.3			105.2	1.5	100.3	3.5	25.6 ± 3.2	12.6		
	50	104.2	1.9	102.6	1.6	51.6 ± 3.9	7.8			100.7	2.7	98.5	0.6	50.0 ± 4.1	8.2			101.6	1.7	101.1	1.1	50.7 ± 3.8	7.5		
	100	101.7	0.6	101.8	0.6	101.6 ± 5.8	5.8			97.9	3.5	94.4	0.6	96.5 ± 7.7	7.7			102.7	0.9	101.8	0.3	102.4 ± 6.6	6.6		
NIV	25	90.6	6.4	87.8	5.9	22.6 ± 10.4	41.7			88.6	5.6	86.3	5.4	21.7 ± 5.6	22.5			84.7	7.6	86.7	9.9	21.8 ± 4.6	18.6		
	50	78.1	6.9	74.1	2.0	38.3 ± 19.5	39.0			86.0	4.5	80.5	10.7	40.9 ± 10.9	21.8			74.2	6.7	72.1	7.1	36.5 ± 18.5	36.9		
	100	70.0	2.0	72.5	5.2	71.2 ± 38.6	38.6			81.1	3.5	72.7	8.8	76.3 ± 28.3	28.3			76.9	3.5	72.5	1.9	74.9 ± 35.7	35.7		
DON	25	106.2	1.6	109.2	3.6	27.0 ± 4.2	17.0			108.9	5.5	116.3	4.9	28.2 ± 4.4	17.5			104.0	1.0	105.8	2.2	26.3 ± 3.9	15.4		
	50	101.9	1.8	95.2	6.9	48.9 ± 4.6	9.2			104.1	4.8	108.1	7.6	53.8 ± 5.4	10.9			99.1	2.3	97.6	4.8	49.0 ± 4.5	9.0		
	100	98.7	2.1	96.0	6.6	96.6 ± 7.1	7.1			95.1	4.2	102.3	11.4	100.3 ± 10.4	10.4			99.1	0.7	101.2	2.4	100.3 ± 6.7	6.7		
3ADON	25	102.7	6.8	107.3	2.8	26.0 ± 2.8	11.1			102.9	3.2	93.7	6.6	24.5 ± 2.6	10.3			102.0	1.6	99.1	3.3	25.0 ± 2.8	11.0		
	50	104.9	2.8	101.5	3.4	51.4 ± 4.3	8.6			103.0	0.8	99.6	2.5	50.6 ± 3.7	7.3			102.5	0.2	100.4	2.0	50.6 ± 3.8	7.7		
	100	101.1	2.6	101.9	3.0	101.0 ± 6.5	6.5			102.0	1.7	100.4	2.1	101.4 ± 6.4	6.4			103.2	1.2	104.4	1.0	103.8 ± 7.2	7.2		
15ADON	25	100.1	5.4	95.0	3.1	24.4 ± 5.3	21.2			98.4	4.7	95.4	6.0	24.0 ± 4.1	16.3			97.4	3.2	102.5	4.6	24.9 ± 2.8	11.0		
	50	99.0	2.7	95.5	2.4	48.6 ± 5.6	11.2			94.4	2.0	95.3	5.5	47.3 ± 5.9	11.8			99.6	2.8	98.4	3.6	49.8 ± 4.2	8.5		
	100	96.1	3.0	92.8	4.7	93.8 ± 9.3	9.3			98.3	1.5	95.5	3.1	96.8 ± 8.1	8.1			97.8	2.2	100.3	4.1	99.4 ± 8.2	8.2		
ZEN	25	99.0	3.9	98.1	6.5	24.4 ± 5.7	22.9			100.9	3.2	89.9	12.1	23.5 ± 4.2	17.0			74.9	3.2	75.0	4.6	18.8 ± 9.5	38.1		
	50	104.5	4.4	101.6	4.5	51.8 ± 6.8	13.5			102.5	2.8	90.9	14.2	47.4 ± 5.0	10.0			80.8	1.1	77.0	6.3	39.2 ± 14.3	28.7		
	100	104.5	1.1	101.3	4.0	102.3 ± 9.1	9.1			100.7	2.1	87.1	11.2	93.0 ± 7.0	7.0			82.1	1.0	79.5	3.6	80.6 ± 26.0	26.0		
FB1	25	102.0	2.6	101.8	3.8	25.3 ± 3.1	12.6			98.9	3.5	89.9	10.1	23.3 ± 4.3	17.0			83.8	9.8	87.3	3.6	21.3 ± 4.5	17.8		
	50	100.8	3.4	98.7	3.0	49.8 ± 4.3	8.5			90.6	3.5	85.1	2.3	44.0 ± 8.2	16.4			80.6	4.2	84.6	8.2	41.8 ± 10.0	20.0		
	100	98.8	1.5	94.0	6.1	95.6 ± 6.6	6.6			88.4	3.0	82.4	3.1	85.4 ± 17.7	17.7			82.5	1.0	80.6	2.6	81.3 ± 25.5	25.5		
FB2	25	100.3	2.7	93.7	6.5	24.1 ± 3.2	12.8			88.2	1.5	82.4	7.6	21.2 ± 5.7	22.6			74.8	8.9	77.5	3.6	19.1 ± 7.7	30.9		
	50	95.7	3.1	90.1	5.9	46.1 ± 5.1	10.2			79.7	3.1	75.2	3.1	38.8 ± 15.7	31.4			72.8	7.6	76.4	11.8	38.0 ± 17.6	35.3		
	100	88.6	8.4	88.8	7.2	87.2 ± 19.7	19.7			79.3	3.9	74.4	1.6	77.0 ± 31.9	31.9			75.3	3.2	72.5	5.8	73.8 ± 38.3	38.3		
FB3	25	101.8	1.2	97.3	5.2	24.7 ± 3.8	15.2			93.5	4.7	87.9	9.6	22.3 ± 4.6	18.4			78.5	5.3	77.8	1.7	19.5 ± 8.6	34.2		
	50	96.9	4.7	91.7	9.3	46.6 ± 5.5	11.0			87.5	5.1	78.2	6.2	41.3 ± 10.1	20.1			73.4	5.7	78.4	9.9	38.5 ± 14.8	29.5		
	100	90.9	4.4	90.8	4.2	90.0 ± 14.4	14.4			84.4	2.9	76.9	5.3	80.4 ± 23.0	23.0			73.4	5.7	72.4	7.5	73.1 ± 37.8	37.8		
BEA	5	93.4	13.7	74.7	4.8	4.3 ± 5.8	23.0			95.5	4.4	92.4	3.5	4.7 ± 2.8	11.2			85.6	2.5	88.5	4.8	4.4 ± 5.5	21.9		
	10	82.4	14.4	75.3	2.7	8.0 ± 16.4	32.8			100.4	2.9	98.8	4.4	9.9 ± 3.7	7.5			84.2	1.3	80.1	3.8	8.2 ± 11.4	22.7		
	20	88.5	15.7	74.8	4.6	16.7 ± 26.2	26.2			97.5	5.0	88.2	5.5	18.6 ± 9.2	9.2			87.5	1.3	81.0	12.1	16.8 ± 17.6	17.6		
ENA	5	81.9	4.6	83.4	3.3	4.1 ± 1.5	29.2			99.3	2.3	101.3	3.5	5.0 ± 0.5	9.3			84.1	0.8	98.1	11.9	4.6 ± 1.2	24.9		
	10	83.1	1.5	83.4	1.3	8.3 ± 2.5	24.8			103.5	1.2	106.7	3.7	10.6 ± 0.8	7.6			83.6	2.3	91.1	9.3	8.9 ± 2.4	24.2		
	20	80.6	3.7	81.6	2.6	16.1 ± 5.8	28.9			105.7	3.6	101.2	2.7	20.8 ± 1.9	9.3			88.5	0.2	89.1	9.6	17.8 ± 3.3	16.3		
ENA1	5	82.0	3.1	82.9	3.8	4.1 ± 1.4	28.6			100.9	2.7	103.5	3.3	5.1 ± 0.5	9.6			83.3	1.0	95.7	12.1	4.5 ± 1.3	26.5		
	10	86.2	2.2	85.6	2.3	8.6 ± 2.0	20.2			103.9	1.3	106.3	3.3	10.6 ± 0.9	8.7			82.4	1.6	91.3	9.6	8.8 ± 2.6	26.2		
	20	84.3	3.8	83.1	5.2	16.6 ± 4.6	23.0			105.4	2.8	103.0	2.2	20.9 ± 1.9	9.6			87.3	1.1	88.9	8.1	17.7 ± 3.7	18.4		
ENA2	5	75.4	5.8	78.2	2.3	3.8 ± 1.7	34.1			97.7	3.8	99.5	2.3	4.9 ± 0.5	10.5			82.2	1.3	97.0	14.1	4.6 ± 1.4	27.7		
	10	79.5	2.6	79.7	2.2	7.9 ± 2.9	29.2			99.7	2.3	104.6	5.7	10.3 ± 0.7	6.8			79.7	2.0	85.7	8.5	8.4 ± 3.0	30.4		
	20	78.8	1.8	76.8	4.5	15.5 ± 6.3	31.7			99.0	4.6	98.0	5.3	19.9 ± 1.6	7.8			86.1	0.6	85.5	8.7	17.1 ± 3.9	19.5		
ENB	5	95.5	5.6	95.4	5.6	4.7 ± 0.6	12.6			105.9	1.0	103.4	1.3	5.2 ± 0.7	13.1			95.4	1.4	99.5	3.5	4.9 ± 0.6	11.5		
	10	99.2	3.1	95.0	4.0	9.7 ± 0.8	7.6			106.1	1.0	103.5	2.6	10.5 ± 1.1	10.8			92.3	3.3	91.0	0.6	9.2 ± 1.3	12.9		
	20	97.0	2.7	94.6	5.0	19.0 ± 1.5	7.6			106.3	2.1	102.5	1.4	20.9 ± 2.1	10.6			95.0	1.5	89.9	10.9	18.4 ± 1.9	9.4		
ENB1	5	95.6	5.5	95.1	6.0	4.7 ± 0.6	11.4			106.1	3.0	103.2	1.7	5.2 ± 0.7	13.7			95.5	0.4	99.9	3.8	4.9 ± 0.6	11.2		
	10	98.2	3.7	94.5	5.0	9.6 ± 0.8	8.1			108.3	1.2	105.1	4.1	10.7 ± 1.2	12.4			91.8	2.3	92.2	1.2	9.2 ± 1.3	12.9		
	20	95.3	3.1	93.7	4.6	18.7 ± 1.9	9.3			105.3	1.2	103.5	0.5	20.9 ± 2.0	9.8			95.2	1.4	91.4	12.9	18.6 ± 1.8	9.0		
ENB4	5	87.8	9.5	90.3	7.1	4.4 ± 1.1	21.8			103.1	0.3	104.7	1.5	5.2 ± 0.5	10.5			88.3	1.2	97.9	9.3	4.7 ± 1.0	19.6		
	10	95.5	2.6	89.9	7.0	9.2 ± 0.9	8.9			106.4	0.8	105.2													

Table 4
Occurrence of *Fusarium* mycotoxins* in marketed foods distributed in South Korea.

Commodity	Parameters	Occurrence (µg/kg)																
		T-2	HT-2	NIV	DON	3ADON	15ADON	ZEN	FB1	FB2	FB3	BEA	ENA	ENA1	ENA2	ENB	ENB1	ENB4
Rice (n = 20)	Incidence	10%	5%	30%	35%	-**	-	20%	5%	15%	-	25%	-	-	65%	5%	-	
	Total mean (Positive mean)	0.26 (2.61)	0.08 (1.66)	2.73 (9.10)	1.91 (5.46)	-	-	0.57 (2.85)	0.32 (6.48)	0.49 (3.26)	-	0.08 (0.33)	-	-	0.07 (0.11)	0.01 (0.18)	-	
	Range	1.95–3.27	1.66	4.69–24.65	3.27–11.18	-	-	0.63–6.46	6.48	2.76–3.85	-	0.11–0.54	-	-	0.09–0.19	0.18	-	
Brown rice (n = 20)	Incidence	10%	10%	85%	70%	-	15%	75%	25%	20%	-	85%	-	-	60%	10%	-	
	Total mean (Positive mean)	0.25 (2.47)	0.15 (1.46)	7.37 (8.67)	4.87 (6.95)	-	3.11 (20.72)	6.78 (9.04)	1.23 (4.92)	0.57 (2.87)	-	10.34 (12.17)	-	-	0.06 (0.10)	0.02 (0.16)	-	
	Range	2.41–2.53	1.09–1.84	2.52–30.00	2.99–11.61	-	15.80–26.06	0.48–32.95	3.53–7.16	2.52–3.15	-	0.14–150.03	-	-	0.08–0.17	0.16	-	
Barley (n = 10)	Incidence	-	-	100%	100%	-	-	-	-	-	-	-	50%	50%	100%	100%	100%	
	Total mean (Positive mean)	-	-	18.59 (18.59)	16.8 (16.8)	-	-	-	-	-	-	-	0.71 (1.41)	0.08 (0.17)	9.60 (9.60)	0.49 (0.49)	3.14 (3.14)	
	Range	-	-	5.00–58.34	6.44–66.18	-	-	-	-	-	-	-	0.85–2.49	0.08–0.40	0.83–25.13	0.16–0.95	0.08–7.95	
Oats (n = 10)	Incidence	70%	20%	40%	100%	-	-	10%	-	10%	-	40%	-	10%	-	90%	80%	
	Total mean (Positive mean)	1.50 (2.15)	0.44 (2.19)	11.83 (29.59)	11.41 (11.41)	-	-	0.01 (0.10)	-	0.28 (2.82)	-	0.16 (0.41)	-	0.04 (0.39)	-	3.18 (3.18)	0.21 (0.23)	0.50 (0.63)
	Range	1.89–2.48	2.11–2.27	22.88–34.44	8.64–14.38	-	-	0.10	-	2.82	-	0.08–1.21	-	0.39	-	0.21–9.90	0.16–0.48	0.08–1.78
Job's tears (n = 9)	Incidence	78%	67%	100%	100%	67%	78%	89%	100%	100%	89%	100%	-	-	100%	56%	67%	
	Total mean (Positive mean)	2.43 (3.12)	1.21 (1.82)	70.27 (70.27)	96.58 (96.58)	3.87 (5.81)	48.09 (61.83)	19.48 (21.91)	67.20 (67.20)	22.06 (22.06)	9.13 (10.27)	26.61 (26.61)	-	-	1.35 (1.35)	0.11 (0.20)	0.11 (0.17)	
	Range	2.18–4.21	0.97–2.89	16.28–195.90	14.62–262.84	2.15–14.41	15.80–146.25	3.02–58.84	8.79–148.46	3.66–71.72	3.77–18.83	0.61–92.29	-	-	0.13–2.47	0.19–0.21	0.08–0.23	
Wheat flour (n = 10)	Incidence	-	-	20%	40%	-	-	10%	-	-	-	-	10%	-	90%	30%	20%	
	Total mean (Positive mean)	-	-	16.23 (81.15)	9.76 (24.39)	-	-	0.50 (5.04)	-	-	-	-	0.04 (0.40)	-	1.25 (1.39)	0.08 (0.28)	0.33 (1.65)	
	Range	-	-	37.62–124.68	5.31–65.48	-	-	5.04	-	-	-	-	0.40	-	0.09–8.86	0.17–0.44	0.58–2.71	
Sorghum (n = 10)	Incidence	10%	10%	70%	80%	60%	60%	80%	100%	100%	90%	70%	-	-	50%	-	-	
	Total mean (Positive mean)	0.34 (3.40)	0.20 (2.02)	4.96 (7.09)	32.55 (40.68)	3.09 (5.16)	35.47 (59.12)	16.15 (20.19)	636.76 (636.76)	58.86 (58.86)	76.82 (85.36)	6.02 (8.59)	-	-	0.8 (1.60)	-	-	
	Range	3.40	2.02	0.82–17.95	8.78–101.70	2.60–7.28	29.46–93.58	2.17–61.19	13.24–2447.17	6.34–216.02	17.11–280.44	0.13–42.79	-	-	0.53–3.18	-	-	
Foxtail millet (n = 10)	Incidence	-	-	80%	90%	-	-	50%	80%	80%	80%	70%	-	-	60%	10%	20%	
	Total mean (Positive mean)	-	-	38.40 (48.00)	56.26 (62.51)	-	-	4.01 (8.01)	113.22 (141.52)	24.14 (30.18)	15.82 (19.78)	5.00 (7.14)	-	-	1.41 (2.35)	0.01 (0.07)	0.04 (0.18)	
	Range	-	-	10.04–90.14	3.69–387.90	-	-	4.74–11.05	19.66–571.18	3.98–133.36	5.49–73.01	0.10–34.52	-	-	0.12–10.20	0.07	0.06–0.30	
Maize (n = 7)	Incidence	-	29%	43%	86%	-	14%	57%	86%	86%	29%	-	-	-	-	-	-	
	Total mean (Positive mean)	-	0.31 (1.08)	0.72 (1.67)	39.67 (46.29)	-	12.32 (86.25)	3.33 (5.83)	192.58 (224.68)	43.65 (50.92)	31.43 (36.66)	0.42 (1.49)	-	-	-	-	-	
	Range	-	0.51–1.66	0.72–2.48	13.43–123.15	-	86.25	2.54–12.68	75.83–499.58	14.15–98.06	11.67–75.20	0.12–2.85	-	-	-	-	-	
Baby food (n = 10)	Incidence	-	10%	10%	10%	10%	-	-	-	30%	10%	20%	-	10%	40%	60%	10%	
	Total mean (Positive mean)	-	0.28 (2.81)	1.28 (12.83)	0.39 (3.93)	0.48 (4.83)	-	-	-	1.11 (3.70)	0.35 (3.46)	0.10 (0.49)	-	0.01 (0.09)	0.45 (1.13)	0.07 (0.12)	0.02 (0.24)	
	Range	-	2.81	12.83	3.93	4.83	-	-	-	3.37–3.93	3.46	0.31–0.66	-	0.09	0.22–3.03	0.10–0.16	0.24	
<i>Sunsik</i> (n = 10)	Incidence	30%	-	80%	60%	-	-	50%	60%	70%	30%	100%	-	70%	50%	100%	100%	90%
	Total mean (Positive mean)	0.85 (2.83)	-	34.38 (42.98)	11.91 (19.85)	-	-	0.96 (1.91)	24.71 (41.18)	8.19 (11.70)	2.97 (9.89)	7.84 (7.84)	-	0.83 (1.19)	0.14 (0.28)	12.58 (12.58)	0.50 (0.50)	2.99 (3.32)
	Range	2.63–3.22	-	13.18–94.00	4.06–56.41	-	-	0.43–4.86	6.97–92.21	4.43–25.17	8.02–11.59	0.64–21.22	-	0.22–3.42	0.09–0.60	0.32–40.27	0.10–1.43	0.54–10.00
Breakfast cereals (n = 10)	Incidence	30%	10%	20%	-	-	-	10%	40%	40%	30%	70%	-	10%	10%	60%	40%	10%
	Total mean (Positive mean)	0.86 (2.88)	0.23 (2.35)	1.74 (8.71)	-	-	-	0.02 (0.20)	4.13 (10.32)	1.69 (4.22)	1.31 (4.35)	0.43 (0.61)	-	0.08 (0.79)	0.01 (0.14)	1.98 (3.30)	0.08 (0.19)	0.34 (3.39)
	Range	2.70–3.23	2.35	8.42–9.00	-	-	-	0.20	7.62–13.78	3.46–5.43	3.88–5.26	0.09–2.22	-	0.79	0.14	0.08–16.00	0.10–0.43	3.39
Corn oil (n = 6)	Incidence	-	-	-	-	-	-	83%	-	17%	17%	-	-	-	-	-	-	-
	Total mean (Positive mean)	-	-	-	-	-	-	3.09 (3.71)	-	0.13 (0.78)	0.43 (2.58)	-	-	-	-	-	-	-
	Range	-	-	-	-	-	-	0.23–9.41	-	0.78	2.58	-	-	-	-	-	-	-
Soybean oil (n = 6)	Incidence	-	-	-	-	-	-	17%	17%	17%	17%	-	-	-	-	-	-	-
	Total mean (Positive mean)	-	-	-	-	-	-	0.06 (0.37)	0.49 (1.47)	0.55 (3.29)	-	-	-	-	-	-	-	-
	Range	-	-	-	-	-	-	0.37	0.72–2.23	3.29	-	-	-	-	-	-	-	-
Grapeseed oil (n = 10)	Incidence	30%	-	-	-	-	-	20%	10%	70%	20%	-	-	-	10%	-	-	-
	Total mean (Positive mean)	0.44 (1.47)	-	-	-	-	-	0.74 (3.70)	0.09 (0.90)	1.15 (1.65)	0.67 (3.36)	-	-	-	0.04 (0.38)	-	-	-
	Range	1.25–1.82	-	-	-	-	-	2.84–4.56	0.90	0.97–3.34	2.71–4.02	-	-	-	0.38	-	-	-
Sesame oil (n = 10)	Incidence	10%	-	-	-	-	-	-	-	-	-	60%	-	-	40%	-	-	-
	Total mean (Positive mean)	0.15 (1.51)	-	-	-	-	-	-	-	-	-	0.33 (0.54)	-	-	0.10 (0.26)	-	-	-
	Range	1.51	-	-	-	-	-	-	-	-	-	0.08–1.07	-	-	0.11–0.52	-	-	-
Perilla oil (n = 10)	Incidence	80%	-	-	30%	10%	-	20%	10%	10%	-	100%	-	30%	30%	100%	10%	70%
	Total mean (Positive mean)	1.50 (1.87)	-	-	2.51 (8.36)	0.13 (1.27)	-	0.75 (3.74)	0.27 (2.71)	0.22 (2.20)	-	18.74 (18.74)	-	0.17 (0.58)	0.03 (0.10)	2.45 (2.45)	0.01 (0.09)	0.43 (0.61)
	Range	1.38–2.58	-	-	3.73–15.95	1.27	-	0.75–6.72	2.71	2.20	-	0.14–143.90	-	0.25–1.200	0.05–0.18	0.92–5.26	0.09	0.11–1.43

* 3ADON: 3-acetyl deoxynivalenol; 15ADON: 15-acetyl deoxynivalenol; BEA: beauvericin; DON: deoxynivalenol; ENA: enniatin A; ENA1: enniatin A1; ENA2: enniatin A2; ENB: enniatin B; ENB1: enniatin B1; ENB4: enniatin B4; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; HT-2: HT-2 toxin; NIV: nivalenol; T-2: T-2 toxin; ZEN: zearalenone; Regulated *Fusarium* mycotoxins included T-2, HT-2, DON, 3ADON, 15ADON, ZEN, FB1, FB2, and FB3; and non-regulated *Fusarium* mycotoxins included NIV, BEA, ENA, ENA1, ENA2, ENB, ENB1, and ENB4.

** -: not detected (<LOD, where LOD is the limit of detection).

detected in 39 % (77/198) of all tested foodstuffs. The top three foodstuffs contaminated with NIV were Job's tears, wheat flour, and *Sunsik*. The detection rates of NIV and DON in the most consumed cereal grains in South Korea (rice, brown rice, and wheat flour) were similar, but the mean contamination level of NIV was higher than that of DON. This result was in agreement with a previous report that *Fusarium* spp. isolated from cereal grains in South Korea were mostly of the NIV chemotype (Lee et al., 2020).

BEA was detected in 43 % (86/198) of the analyzed samples at 0.08–150.03 µg/kg, with a mean value of 4.23 µg/kg. The highest

concentration of BEA was 150.03 µg/kg, which was observed in brown rice. All tested samples of Job's tears, *Sunsik*, and perilla oil were contaminated with BEA. At least one EN was detected in 56 % (111/198) of the analyzed foodstuffs. ENA was not detected in any of the tested samples. The highest level of total ENs in the foodstuffs was observed in *Sunsik* (55.73 µg/kg), followed by barley (34.25 µg/kg). All tested barley, oats, *Sunsik*, and perilla oil samples were contaminated with ENs. Non-regulated FTs were found most frequently at high levels in *Sunsik* and perilla oil, considering all analyzed foodstuffs. The occurrence levels of BEA and ENs in this study were much lower than those reported in

foodstuffs in Europe (infant/toddler food: 72.0 µg/kg for BEA, <1,236 µg/kg for ENs), Japan (wheat flours and corn grits: <633 µg/kg for ENs), and China (maize, wheat, and their products: 0.04–1,006.56 µg/kg for BEA, and 0.02–61.77 µg/kg for ENs) (CONTAM, 2014; Han et al., 2019; Yoshinari et al., 2016).

Mycotoxins in naturally contaminated feeds fed to laying hens can be carried over into eggs, and T-2, DON, ZEN, and its metabolites have been identified in eggs (Tangni et al., 2009). In addition, lipophilic molecules BEA and ENs have been observed to bioaccumulate in egg yolk (Jestoi et al., 2009). However, in the current study, no FTs were detected in either chicken or quail eggs.

3.3. Co-occurrence and correlation between naturally occurring FTs

The number of co-occurring FTs in marketed foodstuffs (Fig. 2A) ranged from 0 to 14, and 67 % (132/198) of all samples contained at least two FTs. About 4 % (7/198) of all samples were contaminated with more than 12 FTs (Table S2). The four samples detected with 14 FTs (T-2 + HT-2 + NIV + DON + 3ADON + 15ADON + ZEN + FBs (FB1, FB2, FB3) + BEA + ENs (ENB, ENB1, ENB4) were all Job's tears samples. Three samples were contaminated with 12 FTs, which were found in the following combinations: T-2 + NIV + DON + ZEN + FB1 + FB2 + FB3 + BEA + ENA1 + ENB + ENB1 + ENB4 for two *Sunsik* samples, and T-2 + HT-2 + NIV + DON + 3ADON + 15ADON + ZEN + FB1 + FB2 + FB3 + BEA + ENB for one sorghum sample. The most prevalent FT combination found in the foodstuffs (6/198, 3 %) was NIV + DON + ENB + ENB1 + ENB4.

The paired FT combinations are described in Fig. 2B. The most common paired combinations were NIV + DON (33 % of all tested samples), DON + ENB (33 %), and BEA + ENB (33 %). Those found in over 20 % of all samples were as follows: NIV + ENB (29 %), DON + BEA (27 %), NIV + BEA (27 %), ENB + ENB1 (25 %), FB1 + FB2 (25 %), DON + FB2 (23 %), DON + ZEN (23 %), ENB + ENB4 (23 %), NIV + ZEN (23 %), ZEN + BEA (23 %), DON + FB1 (21 %), FB1 + BEA (21 %), FB2 + BEA (21 %), FB2 + FB3 (21 %), and FB1 + FB3 (20 %). Reisinger et al.

(2019) reported that over 50 % of maize silage samples (n = 158) were contaminated with DON + FBs, DON + NIV, or DON + ZEN, and Zhao et al. (2021) found that 59 % of wheat samples (200/338) were contaminated with BEA + DON, BEA + NIV, or BEA + ZEN. Both of these frequencies were lower than the highest incidence of co-occurring FTs found among the foodstuffs in the current study. BEA, a non-regulated FT, showed a high co-occurrence with regulated FTs, such as DON, ZEN, and FBs, rather than other non-regulated ones. NIV, DON, ZEN, and BEA often occurred in the presence of other mycotoxins. In particular, all 77 samples contaminated with NIV were co-contaminated with at least one other FT.

The correlations between individual FTs were investigated (Fig. S3). There were strong positive correlations within both the FBs group and the ENs group ($0.90 \leq \text{Spearman's rank correlation coefficients } [r_s] \leq 0.99$ and $0.81 \leq r_s \leq 0.96$, respectively), and a significant positive correlation between DON and all other FTs except for ENs ($0.16 \leq r_s \leq 0.55$, $p < 0.05$). There was no correlation between BEA and ENs. The ENs group only showed a significant positive correlation with NIV (except with other ENs). It seems that the foodstuffs tended to be contaminated by *Fusarium* species capable of simultaneously producing ENs together with NIV and/or DON, such as *F. poae*, *F. avenaceum*, and *F. sporotrichioides*. These results indicated a similar trend to those reported by Gallardo et al. (2023). From the results obtained in the current study, BEA showed a significant positive correlation with regulated FTs, such as T-2, DON, ZEN, and FBs, rather than other non-regulated ones. The combinations of DON + BEA, DON + FBs, DON + ZEN, FBs + BEA, NIV + DON, NIV + ZEN, and ZEN + BEA showed both a high co-occurrence rate (over 20 % of all samples) and a significant positive correlation, which can cause synergistic toxicity effects (Alassane-Kpembé et al., 2015; Fichoux et al., 2012; Klarić et al., 2008).

4. Conclusions

In this study, a method for the simultaneous determination of 17 FTs by LC-MS/MS with SPE cleanup was successfully validated in three

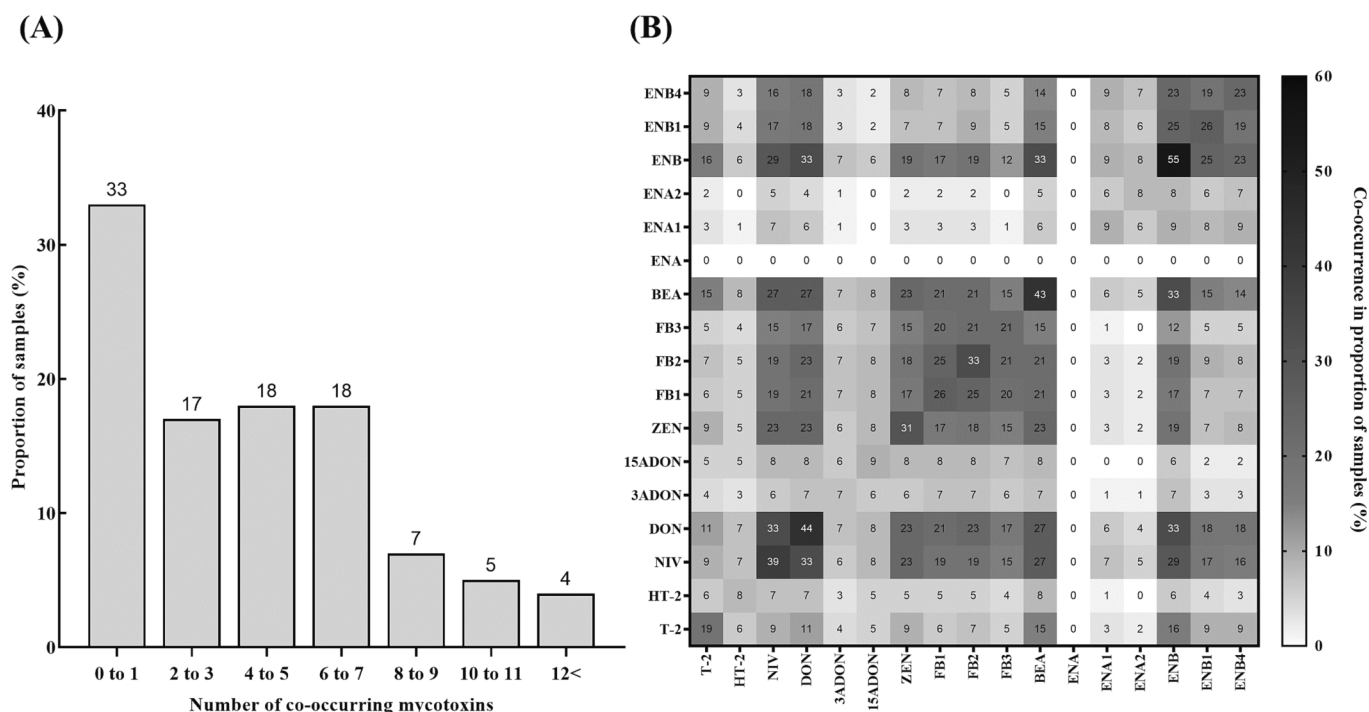


Fig. 2. Co-occurrence of *Fusarium* mycotoxins in marketed foods distributed in South Korea. (A) Number of co-occurring mycotoxins in tested samples, (B) co-occurrence heatmap indicating the prevalence of mycotoxin combinations. 3ADON: 3-acetyl deoxynivalenol; 15ADON: 15-acetyl deoxynivalenol; BEA: beauvericin; DON: deoxynivalenol; ENA: enniatin A; ENA1: enniatin A1; ENA2: enniatin A2; ENB: enniatin B; ENB1: enniatin B1; ENB4: enniatin B4; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; HT-2: HT-2 toxin; NIV: nivalenol; T-2: T-2 toxin; ZEN: zearalenone.

different food matrices. The accuracy, precision (repeatability and reproducibility), and measurement uncertainty satisfied the requirements of EC guidelines for regulated FTs and the AOAC guidelines for non-regulated FTs. To the best of the authors' knowledge, this study is the first attempt to simultaneously determine up to 17 FTs with SPE cleanup. The validated method was applied to investigate the natural co-occurrence of FTs in 198 commercial foods distributed in South Korea. Job's tears, sorghum, and maize were susceptible to contamination by regulated FTs. *Sunsik*, perilla oil, and brown rice were major foods contaminated with non-regulated FTs. Approximately half of the tested samples (99/198) were co-contaminated with at least four FTs. Among the foodstuffs, the Job's tears samples were contaminated with the highest number of co-occurring FTs, followed by the *Sunsik* and sorghum samples. In addition, positive correlations were observed between individual FTs, and these co-contaminations may cause synergistic toxicity. Our results recommend that continuous monitoring for mixtures of FTs should be conducted for risk management.

CRedit authorship contribution statement

So Young Woo: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft. **Sang Yoo Lee:** Formal analysis, Methodology, Validation. **Su Been Park:** Formal analysis, Software, Visualization. **Hyang Sook Chun:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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