



Simple chromatographic determination of aflatoxins in Korean fermented soybean products doenjang, ganjang, and gochujang, with comparison of derivatization methods

So Young Woo¹ · Hyun Ee Ok¹ · Sang Yoo Lee¹ · A-Yeong Jeong¹ · Tae Kyun Jeong¹ · Hyang Sook Chun¹

Received: 7 October 2021 / Revised: 23 January 2022 / Accepted: 8 February 2022 / Published online: 28 February 2022
© The Korean Society of Food Science and Technology 2022

Abstract Korean fermented soybean products doenjang, ganjang, and gochujang are vulnerable to contamination with aflatoxigenic fungi in the open fermentation environment. Therefore, simple and effective methods to determine aflatoxins (AFs) in these complex food matrices are needed. High-performance liquid chromatography with fluorescence detection using two derivatization methods for AF determination in three fermented soybean products was optimized and validated. Pre-column derivatization (preCD) of the AF extracts was performed using trifluoroacetic acid, and post-column derivatization (PCD) was performed in a photochemical reactor for enhanced detection. Both derivatization methods resulted in acceptable performances for linearity ($R^2 > 0.999$), recovery (71–118%), and precision ($< 10.6\%$) values. Recovery and precision with preCD and PCD were similar, but the limit of detection was superior with PCD. When these analytical methods were applied to commercially available fermented

soybean products, the AF levels of all commercial products were ranged from not detected to 6.06 $\mu\text{g}/\text{kg}$.

Keywords Aflatoxin · Photochemical reactor for enhanced detection · Doenjang · Ganjang · Gochujang

Introduction

Soybeans form the bulk matrices of various products that undergo fermentation by microorganisms; products like miso, shoyu, dajiang, doubanjiang, chiangyu, and kinema are some of the most important seasonings in Eastern dishes (Kwon et al., 2005). Korean traditional fermented soybean products are called “jang,” meaning “soy fermented paste or sauce” (Shin and Jeong, 2015). These products generally fall into one of three major categories: doenjang (soybean paste), ganjang (soy sauce), and gochujang (red pepper paste). Because the composition, taste, and flavor of these products differ, they are used not only as raw materials but also as seasonings in the manufacture of various foods (Shin, 2011; Shin and Jeong, 2015).

The fungi present in meju (Korean soybean fermentation starter), which consist mainly of *Aspergillus oryzae*, *Mucor* spp., and *Penicillium* spp., are the microorganisms responsible for fermentation, and thus play an important role in decomposing soybeans and other raw materials into nutrient molecules (Hong et al., 2015; Jung et al., 2012; Lee et al., 1993). However, several isolates of *A. oryzae/flavus* groups from meju possess aflatoxin (AF) biosynthetic genes, and consequently AF productions by these isolates have been reported, especially the four major AFB1, AFB2, AFG1, and AFG2 (Jung et al., 2012). Previous studies have determined the AF levels in doenjang,

✉ Hyang Sook Chun
hschun@cau.ac.kr

So Young Woo
mochalatte9@naver.com

Hyun Ee Ok
ohe0904@hanmail.net

Sang Yoo Lee
dm3822@naver.com

A-Yeong Jeong
hahaha784@naver.com

Tae Kyun Jeong
jtk8387@naver.com

¹ School of Food Science and Technology, Chung-Ang University, Anseong 17546, Korea

ganjang, and their raw materials (Bae et al., 2003; Kim et al., 2000; Kim and Kim, 2012; Ok et al., 2007). However, most analytical methods used in these studies did not sufficiently consider the matrix effects of jang samples and had not been validated. Indeed, the main challenges to accurately determining AF levels in jang products are the matrix effects, such as adsorption and interference, in addition to low AF recoveries.

Doenjang, ganjang, and gochujang contain large quantities of salt (7–24%) and high levels of pigments and molecular fermentation byproducts, which, as matrix components, have the potential to interfere with AF analyses (Kim et al., 2000). Doenjang, a brown paste-type matrix, is made entirely of soybean and brine and is rich in flavonoids, vitamins, minerals, proteins, essential amino acids, fatty acids, and phytoestrogens (Shin and Jeong, 2015). Ganjang is a liquid-type matrix with a distinctive dark brown color that is traditionally prepared using the same process as doenjang, with the exception that ganjang is produced by collecting only the supernatant of the fermented soybeans in salt brine (Shin, 2011). Ganjang contains large quantities of melanoidins, which are dark brown pigments, as well as amino acids, peptides, saccharides, organic acids, fatty acids, and alcohol generated during fermentation and maturation (Shin and Jeong, 2015). Gochujang is a red paste-type matrix made with red pepper powder, fermented soybean powder, barley malt powder, glutinous rice flour, and salt (Bae et al., 2003). It is rich in small molecules, including carotenoids, capsaicinoids, flavonoids, saccharides, and fatty acids (Shin and Jeong, 2015). Despite the complexity of these matrix components, there is a lack of common sample preparation and analysis methods for doenjang, ganjang, and gochujang matrices.

AFs are natural compounds that strongly fluoresce. Therefore, their analysis by high-performance liquid chromatography (HPLC) often involves fluorescence detection (FLD). However, because reversed-phase eluents quench the fluorescence of AFB1 and AFG1 (Kok, 1994), chemical derivatization is frequently required for their detection. More specifically, either pre-column derivatization (preCD) or post-column derivatization (PCD) can be performed with a suitable fluorophore to improve detectability. In terms of preCD, trifluoroacetic acid (TFA) is extensively used to generate hemiacetals and has been adopted as the standard for AF determination in several countries, including South Korea. However, the disadvantages of preCD include being time-consuming and resulting in limited derivative stability. As an alternative, PCD can be performed by reacting the 8,9-double bonds in AFs with halogens, as was initially achieved in the context of iodination (Shepherd and Gilbert, 1984). However, iodination causes peak broadening and iodine crystallization; thus, post-column UV irradiation methods are now

common. Furthermore, the UV irradiation system, i.e., the photochemical reactor for enhanced detection (PHRED), requires the use of lower amounts of toxic solvent additives (e.g., TFA, bromine) and a shorter preparation time because it provides AF derivatives using only UV light (Walkling et al., 2006). One PCD technique that appears analytically equivalent to iodination and bromination is the photochemical method based on the formation of AFB1 and AFG1 hemiacetals through UV irradiation of the HPLC eluate (Joshua, 1993; Walkling et al., 2006). Although PCD has been widely used owing to its high stability and reproducibility, to the best of our knowledge, there have been few studies applying it to analyses of AFs in jang products. Almost all HPLC-FLD studies carried out to date for AF determination in doenjang, ganjang, and gochujang have been conducted using preCD methods (Cho et al., 2008; Jeong et al., 2019; Oh et al., 2006; Park et al., 2008; 2013).

According to the Korean Food Code published by the Ministry of Food and Drug Safety in Korea (MFDS, 2016), only TFA derivatization has been adopted for HPLC analysis of AFs in food. The Association of Official Analytical Chemists (AOAC) have suggested both preCD and PCD for PHRED as official methods for AF determination in corn, raw peanuts, and peanut butter, while the Food and Drug Administration has adopted the AOAC method for the determination of AFs in several food commodities (AOAC, 2005; FDA, 2013). Therefore, the aims herein were to develop a modified analytical method that can be applied to all three traditional Korean fermented soybean products (i.e., doenjang, ganjang, and gochujang), with their different matrix characteristics, and to compare TFA derivatization with PHRED for the detection of AFs in terms of analytical performance of HPLC-FLD in each matrix.

Materials and methods

Samples

Doenjang, ganjang, and gochujang were selected from the most popular commercially available brands. Samples with a minimum size of 1 kg were purchased from online retailers and local supermarkets. All samples were stored at 4–5 °C prior to use and were equilibrated at 20 °C before analysis.

Materials

An AF standard mixture was purchased from Supelco (Bellefonte, PA, USA) and used as a total AF standard stock solution. The mix contained 2600 ng/mL total AFs

comprising 1000 ng/mL AFB1, 1000 ng/mL AFG1, 300 ng/mL AFB2, and 300 ng/mL AFG2. HPLC-grade methanol (MeOH), LC-grade acetonitrile (ACN), and HPLC-grade water (H₂O) for extractions were purchased from Burdick & Jackson (Muskegon, MI, USA). PEG 8000 and TFA were purchased from Sigma-Aldrich (St. Louis, MO, USA). No. 4 cellulose filters, glass microfiber filters, and 0.22 µm polyvinylidene difluoride (PVDF) syringe filters were obtained from Whatman (Maidstone, UK). An AflaTest WB immunoaffinity column (IAC) was purchased from VICAM (Watertown, MA, USA).

Sample preparation

The sample preparation procedure was optimized based on the AF testing methods for cereals, beans, peanuts, nuts and their processed products, sauces, red pepper powder, curry powder, and spices, as specified by the Korean Food Code (MFDS, 2016). Each sample (25 g) was placed in a 250 mL beaker with MeOH:H₂O (70:30 or 80:20, v/v, 100 mL) containing PEG or NaCl (1 g), then homogenized for 5 min in a high-speed blender (Ultra-Turrax, IKA, Staufen, Germany). The sample was then filtered through No. 4 filter paper (Whatman), and an aliquot (30 mL) of the filtrate was diluted with 120 mL of 1% tween 20 solution (v/v). After filtration through a glass microfiber filter, a portion (20 mL) of the filtrate was passed through the IAC at a flow rate of one drop per second. The IAC was then washed with water (10 mL) and air-dried on the column. The toxins were eluted into an amber screw-cap sample vial with ACN (3 mL), and the eluate was evaporated to dryness in a heated aluminum block at 50 °C under a gentle nitrogen stream. AFs are prone to degradation by light; therefore, all procedures were carried out under reduced lighting with protection from direct UV light.

Instrumentation and analytical procedures

TFA derivatization

Each dried residue was derivatized by first adding TFA (200 µL) and allowing the mixture to stand for 15 min under protection from direct UV light. Subsequently, the sample was diluted with ACN:H₂O (20:80, v/v, 800 µL), and the derivatized sample was vortexed for 30 s prior to filtering through a 0.22 µm membrane filter into an HPLC vial for auto-injection. A reversed-phase Synergi Hydro-*RP* column (150 mm × 4.6 mm, 4 µm; Phenomenex, Torrance, CA, USA) was used to separate the TFA-derivatized samples at 35 °C with the mobile phase (ACN:H₂O = 25:75, v/v) eluted at a flow rate of 0.7 mL/min. The injection volume was 10 µL for both the sample and standard solutions, and the average retention times

(RTs) of AFG1, AFB1, AFG2, and AFB2 were 6.0, 7.5, 14.2, and 20.2 min, respectively.

Post-column photochemical derivatization (PHRED) cell

Dried eluate was redissolved in MeOH:H₂O (1:1, v/v, 1 mL) and filtered through a 0.22 µm PVDF syringe filter for auto-injection. The PCD reactor (Aura Industries, NY, USA) was equipped with a UV lamp ($\lambda = 254$ nm) and a 0.74 mL knitted reactor coil (15 m × 0.25 mm). The reactor was inserted between the chromatographic column and the FLD. A Symmetry C18 reversed-phase column (150 mm × 4.6 mm, 3.5 µm; Waters, Ireland) was used for separation at 35 °C. The mobile phase was ACN:H₂O (0.1% acetic acid, v/v):MeOH (14:59:27, v/v/v), the flow rate was 1.0 mL/min, and the sample injection volume was 50 µL. Under these conditions, the average RTs for AFG2, AFG1, AFB2, and AFB1 were 9.5, 10.6, 11.8, and 14.0 min, respectively.

HPLC apparatus

All chromatographic separations were performed with an Agilent 1260 Infinity Quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) consisting of pumps, an autosampler, a column oven, and an FLD. The AFs were monitored at an excitation wavelength of 360 nm and emission wavelength of 450 nm for all analyses.

Method validation

The method was validated according to IUPAC guidelines (Thompson et al., 2002). Matrix-matched calibration curves were obtained for the four mycotoxins diluted using five final concentration points prepared by spiking standard from 0.2 to 10 µg/kg for AFB1/AFG1 and 0.06 to 3 µg/kg for AFB2/AFG2. Ten replications were performed for each calibration point. Linearity of the method was assessed from the matrix-matched calibration curves, and the slope, intercept, and coefficient of determination (R²) were calculated. The limit of detection (LOD) was calculated by the “b + 3 σ ” method, where the standard deviation (σ) from 10 injections of a blank sample (b) was calculated. The LOQ was defined as “b + 10 σ .” The accuracy was represented by recovery experiments according to the following equation: analyzed concentration of spiked samples calculated from matrix-matched standard/spiking concentration × 100 (%). Precision was expressed as the relative standard deviation of repeatability (RSD_r) of ten independent experiments for each spiking level. The recovery and RSD_r values for each of the derivatization methods were determined using the three Korean fermented products after spiking with three different level: AFB1 and AFG1 at

1, 2, or 5 µg/kg, and with AFB2 and AFG2 at 0.3, 0.6, or 1.5 µg/kg.

Results and discussion

Selection of the optimal extraction solvent

The optimal extraction solvent was selected after evaluating the AF recoveries from each solvent system using the preCD method (Table 1). The inclusion of an appropriate amount of water in the extraction solvent can help the organic solvent penetrate the sample matrix (Alshannaq and Yu, 2017). Except for AFG1, the highest AF recoveries were obtained when MeOH:H₂O (70:30, v/v) containing PEG 8000 was used for extraction. Among the AF isomers, AFB1 and AFB2 showed low recoveries, in close agreement with the results reported by Diaz et al. (2012). They reported that AFB isomers have one lactone ring, while AFG isomers have two lactone rings that can be cleaved and potentially bind to the matrix. Among AFG isomers, when MeOH:H₂O (70:30, v/v) containing PEG 8000 was used for extraction, the recovery of AFG1 in the three matrices was slightly lowered, but AFG2, which had the lowest recovery, was markedly increased. Taken together,

the overall AF recoveries from the traditional Korean fermented soybean products were the highest with MeOH:H₂O (70:30, v/v) containing PEG 8000 rather than NaCl specified in the Korean Food Code. Although AFs are soluble in most organic solvents, they are poorly soluble in water. It was therefore considered that adding NaCl to the samples breaks down the hydrogen bonds present in the AFs, thereby improving the distribution coefficient between the organic solvent and water for the extraction process (Cranwell et al., 2017). Nonetheless, a slight decrease in extraction efficiency was observed at higher NaCl concentrations, likely because of the increase in viscosity of the aqueous phase, which reduced mass transfer of the analyte from the aqueous to the organic phase (Diaz et al., 2012). It can be assumed that the addition of NaCl was ineffective because the doenjang, ganjang, and gochujang samples were already high in salt. Instead, the use of a nonionic surfactant such as PEG 8000 appeared to improve recovery by increasing the analyte solubility or the degree of dispersion in high-protein matrices (Pourhossein and Alizadeh, 2018). The optimal extraction solvent for AFs was MeOH:H₂O (70:30, v/v) containing PEG 8000 in doenjang matrix, and method validation was performed based on this extraction solvent system.

Table 1 Accuracy and precision for analyses of AFs in doenjang after TFA derivatization of the extracts, using different extraction procedures (n = 3)

Sample	Analyte	Spiking level (µg/kg)	70% MeOH and 1 g NaCl ^a		80% MeOH and 1 g NaCl		70% MeOH and 1 g PEG 8000	
			Accuracy (%) ^b	Precision (%) ^c	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Doenjang	AFB1	2.0	69.7	2.6	75.3	9.6	82.6	2.1
	AFB2	0.6	71.7	5.7	70.1	3.2	83.9	2.8
	AFG1	2.0	95.9	6.7	87.3	2.0	86.5	4.0
	AFG2	0.6	47.3	7.8	53.2	7.8	87.8	5.2
Ganjang	AFB1	2.0	73.1	1.2	79.4	1.1	85.6	3.3
	AFB2	0.6	76.1	6.0	76.0	3.0	81.5	3.8
	AFG1	2.0	94.7	3.6	96.4	1.4	78.4	5.8
	AFG2	0.6	50.8	22.3	59.6	1.7	69.5	7.6
Gochujang	AFB1	2.0	67.1	1.8	74.0	2.0	84.8	5.2
	AFB2	0.6	69.0	8.0	71.5	1.8	80.0	5.5
	AFG1	2.0	82.3	4.4	92.1	2.3	83.7	12.5
	AFG2	0.6	39.9	12.2	56.5	7.2	74.1	6.3

^aKorean Food Code method

^bAccuracy: % recovery

^cPrecision: Relative standard deviation of repeatability (RSDr)

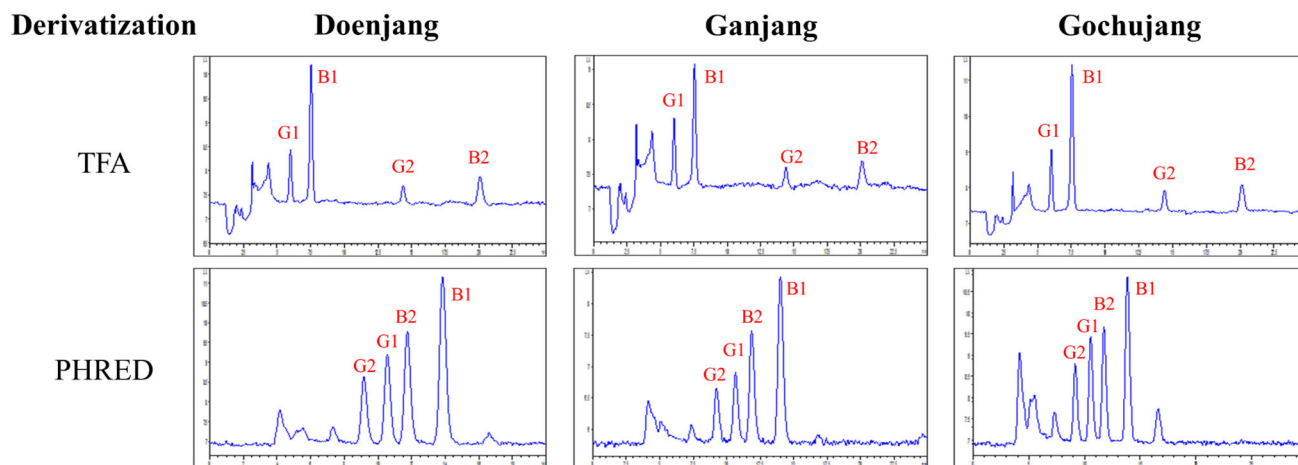


Fig. 1 Chromatograms of AFs-spiked doenjang, ganjang, and gochujang following TFA and PHRED cell derivatization (2 $\mu\text{g}/\text{kg}$ for AFB1 and AFG1; 0.6 $\mu\text{g}/\text{kg}$ for AFB2 and AFG2)

Table 2 Linearity and sensitivity of mycotoxin analyses using optimal HPLC conditions (n = 10)

Derivatization method	Toxins	Calibration range ($\mu\text{g}/\text{kg}$)	Slope	Intercept	R^2	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
TFA	AFB1	0.2–10	30.92	− 6.10	0.9992	0.04	0.11
	AFB2	0.06–3	32.49	− 0.87	0.9995	0.03	0.08
	AFG1	0.2–10	8.71	− 1.11	0.9992	0.11	0.32
	AFG2	0.06–3	14.54	− 0.12	0.9993	0.06	0.18
PHRED	AFB1	0.2–10	53.78	0.32	0.9999	0.04	0.13
	AFB2	0.06–3	100.47	− 0.05	1.0000	0.01	0.04
	AFG1	0.2–10	33.17	− 0.35	1.0000	0.05	0.17
	AFG2	0.06–3	82.43	− 0.31	0.9999	0.01	0.04

Effects of the derivatization method

Selectivity

The HPLC chromatograms of doenjang, ganjang, and gochujang spiked with AFB1, AFG1, AFB2, and AFG2, and derivatized using two methods, are shown in Fig. 1. The low noise was attributed to the selective IAC cleanup step, wherein the AFs were isolated and concentrated. The use of an IAC efficiently removed endogenous matrix components that could cause interference, and the RTs of the remaining matrix components were clearly different from those of the AFs. All AFs were detected without any overlapping peaks, and the chromatograms for three matrices were similar.

Linearity

For each of the derivatization methods, the linear calibration range for AFB1 and AFG1 was 0.2–10 $\mu\text{g}/\text{kg}$, and the linear range for AFB2 and AFG2 was 0.06–3.0 $\mu\text{g}/\text{kg}$. The

coefficients of determination (R^2) of the matrix-matched calibration curve with the corresponding spiking level for each method ranged from 0.9992 to 0.9995 for preCD and 0.9999 to 1.0000 for PCD. The calibration sensitivity is indicated by the slope of the calibration curve at the concentration of interest; the steeper the slope, the higher the sensitivity. The slope and intercept of the calibration curves for the extracts obtained using PCD were higher than those obtained from the extracts subjected to preCD (Table 2).

LOD and LOQ

LOD and LOQ values for three matrices were determined for each derivatization method (Table 2). For the preCD method, the LOD and LOQ were 0.04 and 0.11 $\mu\text{g}/\text{kg}$, respectively, for AFB1; 0.03 and 0.08 $\mu\text{g}/\text{kg}$, respectively, for AFB2; 0.11 and 0.32 $\mu\text{g}/\text{kg}$, respectively, for AFG1; and 0.06 and 0.18 $\mu\text{g}/\text{kg}$, respectively, for AFG2. For the PCD method, the LOD and LOQ were 0.04 and 0.13 $\mu\text{g}/\text{kg}$, respectively, for AFB1; 0.01 and 0.04 $\mu\text{g}/\text{kg}$,

respectively, for AFB₂; 0.05 and 0.17 $\mu\text{g}/\text{kg}$, respectively, for AFG₁; and 0.01 and 0.04 $\mu\text{g}/\text{kg}$, respectively, for AFG₂. PHRED resulted in lower LODs than TFA because PCD gave highly reproducible results when the same calibrators were used (Trucksess et al., 2006). It should be noted that the LOD and LOQ values herein are lower than those obtained with other preCD methods for determining AFs present in doenjang, ganjang, and gochujang matrices (Jeong et al., 2019; Ok et al., 2007; Park et al., 2008).

Recovery and RSD

The recovery and precision values of the AF measurements after preCD and PCD for the doenjang, ganjang, and gochujang extracts at the indicated concentrations are shown in Fig. 2. For the doenjang matrix, recovery and precision from the TFA-derivatized samples ranged from 76.3% to 107.8% and 1.5% to 10.6%, respectively. For the PHRED-derivatized samples, recovery and precision ranged from 70.6% to 105.1% and 1.6% to 8.4%, respectively. The results from both derivatization methods meet the EU regulatory performance criteria (EC, 2006); regulation (EC) No. 401/2006 requires AF recoveries of 70–110% and 50–120% for concentrations of 1–10 and < 1 $\mu\text{g}/\text{kg}$, respectively. The relative standard deviation of reproducibility (RSD_R) values recommended by the EU are derived from the Horwitz equation, and RSD_R is defined as 0.66 times the RSD_R . The regulatory RSD_R values for AFB₁ and AFG₁ at 1, 2, and 5 $\mu\text{g}/\text{kg}$ are 29.9%, 26.9%, and 23.4%, respectively, and those for AFB₂ and AFG₂ at 0.3, 0.6, and 1.5 $\mu\text{g}/\text{kg}$ are 35.8%, 32.3%, and 28.1%, respectively.

The recovery and precision obtained for the ganjang extracts using TFA derivatization ranged from 78.3% to 118.1% and 3.8% to 9.1%, respectively. TFA recoveries tended to decrease as spiking level of AFs increases. This observation is considered to be related to the fact that the stability of TFA derivatives is affected by various factors such as light, pH, and solvent (Kok, 1994), but additional research is needed to clarify this. Nevertheless, TFA recovery at all levels and matrices tested satisfied EU regulatory performance criteria (EC 401/2006). For the PHRED-derivatized samples, recovery and precision ranged from 93.6% to 105.1% and 0.8% to 9.5%, respectively. Both methods also yielded good recoveries and precisions that meet the EU regulatory performance criteria. For AF derivatization with TFA, recoveries from the spiked gochujang samples ranged from 75.5% to 109.5%, and precision ranged from 1.9% to 7.2%. These results conform to the values recommended by the EU. In the case of the PHRED-derivatized extracts, recovery and precision ranged from 93.9% to 106.4% and 1.7% to 7.0%, respectively. PreCD with agents such as TFA generally involves a

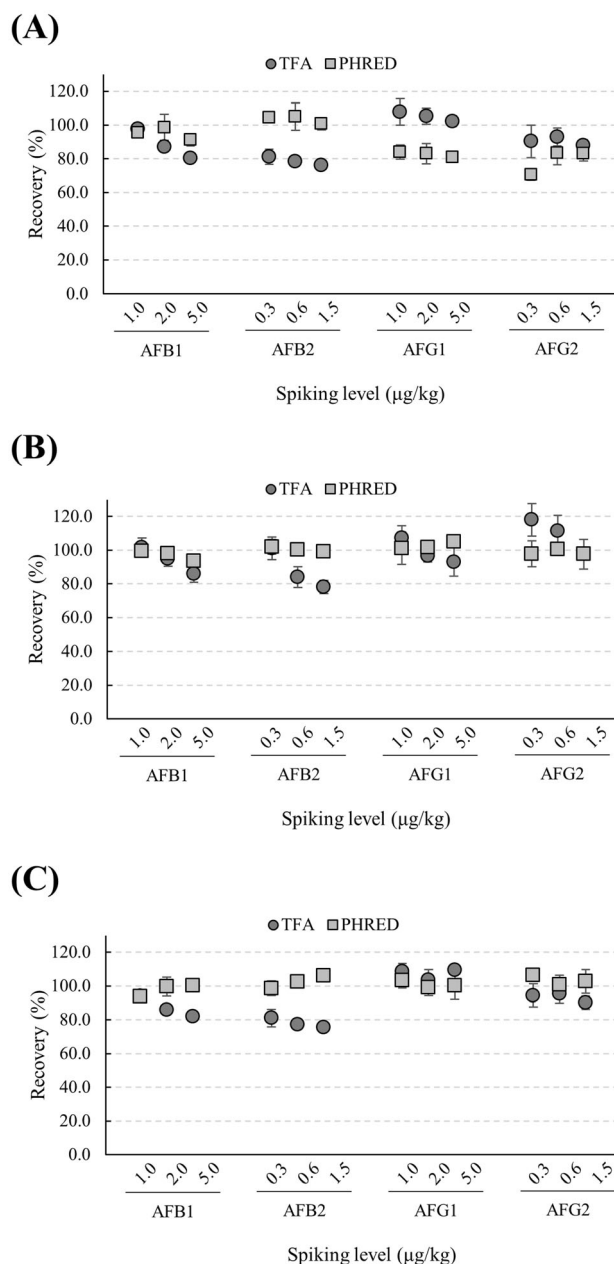


Fig. 2 Accuracy and precision values for AF determination in doenjang (A), ganjang (B), and gochujang (C) after TFA and PHRED derivatizations ($n = 10$)

complex, time-consuming concentration procedure that cannot be performed online. PCD has obvious advantages, including its ease, and is thus used more often than preCD in many countries (Ok et al., 2016). However, few studies have directly compared the analysis efficiencies of the preCD and PCD methods. Trucksess et al. (2006) determined the AFs present in ginseng using the preCD (TFA) and PCD (PHRED) derivatization methods, which were compared based on recovery and precision values. More specifically, recovery was highest for preCD with TFA,

Table 3 Determination of AFs present in commercial doenjang, ganjang, and gochujang samples with different derivatization methods

Sample (No. of samples)	Toxins	No. of positive samples		Mean ($\mu\text{g}/\text{kg}$)		Range ($\mu\text{g}/\text{kg}$)	
		TFA	PHRED	TFA	PHRED	TFA	PHRED
<i>Doenjang</i> (n = 22)	AFB1	7	7	0.58	0.58	0.24–6.06	0.18–6.06
	AFB2	2	5	0.04	0.05	0.33–0.50	0.04–0.50
	AFG1	1	3	0.02	0.09	0.53	0.52–0.82
	AFG2	0	2	–*	0.02	–	0.13–0.31
<i>Ganjang</i> (n = 15)	AFB1	0	3	–	0.05	–	0.16–0.41
	AFB2	0	2	–	0.01	–	0.08–0.14
	AFG1	0	1	–	0.02	–	0.3
	AFG2	0	0	–	–	–	–
<i>Gochujang</i> (n = 15)	AFB1	5	5	0.06	0.03	0.09–0.20	0.10–0.12
	AFB2	–	–	–	–	–	–
	AFG1	–	–	–	–	–	–
	AFG2	–	–	–	–	–	–

*Not detected (< LOD)

whereas precision was superior with PCD, consistent with our findings. Another study determined the AFs present in corn using preCD and PCD methods, and the LOD, LOQ, recovery, and precision were compared as validation parameters (Ok et al., 2016). Their results showed that PCD afforded superior calibration sensitivity, LOD, and LOQ, also consistent with our study. Our findings therefore suggest that in addition to TFA as the standard method for AF analysis in South Korea, PHRED can be used to determine the AFs present in jang products.

Determination of AFs in commercial doenjang, ganjang, and gochujang after preCD and PCD

To evaluate natural AF contamination in the three different product types, 46 samples were purchased from online retailers and local markets. The occurrence of AFs in commercial doenjang, ganjang and gochujang with different derivatization methods was shown in Table 3. As indicated, AFB1 was detected in 7 of the 22 doenjang samples. These AFB1 contamination levels (0.18–6.06) in commercial doenjang are similar or slightly higher than the previously reported ranges of 1.0–6.0 $\mu\text{g}/\text{kg}$ (Bae et al., 2003), 0.04–2.15 $\mu\text{g}/\text{kg}$ (Park et al., 2008), and 0.11–0.96 $\mu\text{g}/\text{kg}$ (Woo et al., 2019). In the case of ganjang, no AFs were detected in any of the 15 samples subjected to TFA derivatization, but AFs were detected in three of the samples subjected to PHRED derivatization (0.16–0.41 $\mu\text{g}/\text{kg}$ AFB1, 0.08–0.14 $\mu\text{g}/\text{kg}$ AFB2, and 0.3 $\mu\text{g}/\text{kg}$ AFG1), which was attributed to this method's higher sensitivity. Though 5 of the 15 gochujang samples were found to be contaminated with an AF, the contamination level was extremely low (0.09–0.20 $\mu\text{g}/\text{kg}$ AFB1). The contamination levels determined by our analyses of ganjang and

gochujang are similar to those previously reported (Jeong et al., 2019; Ok et al., 2007; Park et al., 2004). Among the three soybean paste products, AFs had the highest contamination level and positive rate in doenjang.

Herein, a simple chromatographic method for the determination of AFs in doenjang, ganjang, and gochujang was established, and the preCD and PCD methods were compared. Extraction of AFs from the three jang matrices using a MeOH:H₂O (70:30, v/v) solution containing PEG 8000 was more effective than using a MeOH:H₂O (70:30, v/v) or MeOH:H₂O (80:20, v/v) solution containing NaCl. Both preCD using a TFA agent and PCD using the PHRED system resulted in analytical performance that meets the EU regulatory performance criteria. The procedures based on TFA or PHRED described herein can therefore be equivalently implemented for routine AF analysis of both Korean jang products and various types of fermented soybean products (e.g., Japanese miso and shoyu; Chinese dajiang, doubanjiang, and chiangyu; and Nepalese kinema). Commercial doenjang, ganjang, and gochujang samples were also analyzed to demonstrate the applicability of the described method. More specifically, the AF contamination levels in the actual samples were < 0.41 $\mu\text{g}/\text{kg}$ for AFB1 and < 0.82 $\mu\text{g}/\text{kg}$ for the total AFs, which were significantly lower than current regulatory limits. Although all analyzed samples were well managed under the regulatory limits, a more extensive number of samples should be monitored in future research.

Funding This research was supported by the grant 21162MFDS028 from the Ministry of Food and Drug Safety in 2022 and the Chung-Ang University Graduate Research Scholarship (Academic scholarship for College of Biotechnology and Natural Resources) in 2021.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

- Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environmental Research and Public Health*. 14: 632 (2017)
- AOAC. Official Method 49.2.18A. Association of Official Analytical Chemists, Maryland, USA (2005)
- Bae SI, Kwak BY, Park YK, Kim YH, Shon DH. Survey of aflatoxin B1 in domestic doenjang and kojuchang determined by enzyme linked-immunosorbent assay. *Journal of Food Hygiene and Safety*. 18: 95-100 (2003)
- Cho SH, Lee CH, Jang MR, Son YW, Lee SM, Choi IS, Kim SH, Kim DB. Aflatoxins contamination in spices and processed spice products commercialized in Korea. *Food Chemistry*. 107: 1283-1288 (2008)
- Cranwell PB, Harwood LM, Moody CJ. *Experimental Organic Chemistry*. 3rd ed. John Wiley & Sons, New Jersey, USA (2017)
- Diaz GJ, Cepeda SM, Martos PA. Stability of aflatoxins in solution. *Journal of AOAC International*. 95: 1084-1088 (2012)
- EC. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union*. 70: 12-34 (2006)
- FDA. Food and Drug Administration Office of Regulatory Affairs ORA Laboratory Manual Volume IV Section 7 (2013). Available from: <https://www.fda.gov/science-research/field-science-and-laboratories/field-science-laboratory-manual>. Accessed Aug. 24, 2021.
- Hong SB, Kim DH, Samson RA. *Aspergillus* associated with meju, a fermented soybean starting material for traditional soy sauce and soybean paste in Korea. *Mycobiology*. 43: 218-224 (2015)
- Jeong SE, Chung SH, Hong SY. Natural occurrence of aflatoxins and ochratoxin A in meju and soybean paste produced in South Korea. *Applied Biological Chemistry*. 62: 65 (2019)
- Joshua H. Determination of aflatoxins by reversed-phase high-performance liquid chromatography with post-column in-line photochemical derivatization and fluorescence detection. *Journal of Chromatography A*. 654: 247-254 (1993)
- Jung YJ, Chung SH, Lee HK, Chun HS, Hong SB. Isolation and identification of fungi from a meju contaminated with aflatoxins. *Journal of Microbiology and Biotechnology*. 22: 1740-1748 (2012)
- Kim JG, Roh WS. Changes of aflatoxins during the ripening and storage of Korean soy sauce and soybean paste and the characteristics of the changes-Part 2. *Korean Public Health Research*. 1: 13-21(2000)
- Kim M, Kim YS. Detection of foodborne pathogens and analysis of aflatoxin levels in home-made doenjang samples. *Preventive Nutrition and Food Science*. 17: 172 (2012)
- Kim JG, Lee YW, Bullerman LB. Changes of aflatoxins during the ripening and storage of Korean soy sauce and soybean paste and the characteristics of the changes-Part 3. *Korean Public Health Research*. 1: 21-28 (2008)
- Kok WT. Derivatization reactions for the determination of aflatoxins by liquid chromatography with fluorescence detection. *Journal of Chromatography B: Biomedical Sciences and Applications*. 659: 127-137 (1994)
- Kwon TW. *Utilization History of Soybean*. Korea University Press, Seoul, South Korea, pp. 3-12 (2005)
- Lee SS, Park KH, Choi KJ, Won S. A Study on hyphomycetous fungi found on maejus, a raw material of Korean traditional soy sources. *The Korean Journal of Mycology*. 21: 247-272 (1993)
- MFDS. *Korean Food Code*. Ministry of Food and Drug Safety, Seoul, South Korea (2016)
- Oh KS, Suh JH, Park SS, Sho YS, Choi WJ, An YS, Lee JO, Woo GJ. Advances in the analysis of total aflatoxins in foods. *Journal of Food Hygiene and Safety*. 21: 76-81 (2006)
- Ok HE, Jung H, Lee SE, Peak O, Chun HS. Three liquid chromatographic methods for the analysis of aflatoxins in for different corn (*Zea mays*) matrices. *Journal of Food Composition and Analysis*. 54: 20-26 (2016)
- Ok HE, Kim HJ, Shim WB, Lee H, Bae DH, Chung DH, Chun HS. Natural occurrence of aflatoxin B1 in marketed foods and risk estimates of dietary exposure in Koreans. *Journal of food protection*. 70: 2824-2828 (2007)
- Park JW, Kim EK, Kim YB. Estimation of the daily exposure of Koreans to aflatoxin B1 through food consumption. *Food additives and contaminants*. 21: 70-75 (2004)
- Park JW, Yoo MS, Kuk JH, Ji Y, Lee JH. Simultaneous determination and monitoring of aflatoxin and ochratoxin A in food. *Journal of Food Hygiene and Safety*. 28: 75-82 (2013)
- Park MJ, Yoon MH, Hong HG, Joe TS, Lee IS, Park JH, Ko HU. A survey of the presence of aflatoxins in food. *Journal of Food Hygiene and Safety*. 23: 108-112 (2008)
- Pourhossein A, Alizadeh K. Salt-assisted liquid-liquid extraction followed by high performance liquid chromatography for determination of carvedilol in human plasma. *Journal of Reports in Pharmaceutical Sciences*. 7: 79-87 (2018)
- Sadeghi R, Jahani F. Salting-in and salting-out of water-soluble polymers in aqueous salt solutions. *The Journal of Physical Chemistry B*. 116: 5234-5241 (2012)
- Shepherd MJ, Gilbert J. An investigation of HPLC post-column iodination conditions for the enhancement of aflatoxin B1 fluorescence. *Food Additives & Contaminants*. 1: 325-335 (1984)
- Shin DH. Utilization of soybean as food stuffs in Korea. pp. 81-110. In: *Soybean and Nutrition*. Hany El-Shemy, Rijeka, Croatia (2011)
- Shin DH, Jeong D. Korean traditional fermented soybean products: Jang. *Journal of Ethnic Foods*. 2: 2-7 (2015)
- Thompson M, Ellison SL, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). *Pure and Applied Chemistry*. 74: 835-855 (2002)
- Trucksess M, Weaver C, Oles C, D'Ovidio K, Rader J. Determination of aflatoxins and ochratoxin A in ginseng and other botanical roots by immunoaffinity column cleanup and liquid chromatography with fluorescence detection. *Journal of AOAC International*. 89: 624-630 (2006)
- Waltking AE, Wilson D, Dunn E. Liquid chromatographic analysis of aflatoxin using post-column photochemical derivatization: collaborative study. *Journal of AOAC International*. 89: 678-692 (2006)
- Woo SY, Ryu SY, Tian F, Lee SY, Park SB, Chun HS. Simultaneous determination of twenty mycotoxins in the Korean soybean paste doenjang by LC-MS/MS with immunoaffinity cleanup. *Toxins*. 11: 594 (2019)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.