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Original article

Limited survey on aflatoxin contamination in rice

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ABSTRACT

Aflatoxins (AFS) are toxic and carcinogenic fungal metabolites. Aflatoxin B1 is the most toxic and has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). Samples of imported rice were analyzed for their AFS content. Finley ground rice subsamples were extracted with water/methanol (100:150 v/v) followed by purification with Immunoaffinity columns (IAC). AFS purified from extracts were determined with RP-HPLC-FLD using post column electrochemical derivatization with a Kobra Cell. Concentrations of aflatoxin B1 and total AFS in test rice samples were ≤0.123 and ≤2.58 μg/kg, respectively. Tween 80 improved recoveries (86 and 106%) of aflatoxin B1 and aflatoxin G1 from brown rice. Recoveries of Aflatoxin B2 and aflatoxin G2 were substantially reduced (non-detected to 27%) by Tween 80 used in IAC cleanup of brown rice extracts. Visible dense growth of *Aspergillus parasiticus* (food isolate) occurred at 25 °C but higher aflatoxin B1amounts (23.9–39.3 μg/kg) accumulated when the mold grew at 37 °C in rice seeds stored for three weeks. It could be concluded that levels of aflatoxin B1 and total AFS in rice samples were within the permissible amounts of the EU and other international legislations.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food worldwide. Twenty-three *Oryza* species are known while only *Oryza glaberrima* and *Oryza sativa* are widely cultivated (Reiter et al., 2010). Rice is stored for several months or even years as rough rice. Rough rice is dehulled to produce brown rice and the bran layer of brown rice is removed to produce white (Trucksess et al., 2011; Choi et al., 2015). Approximately 75% of world population and 60% of South Asians' food intake consists of rice. Large amounts of rice are consumed per capita per year, especially in Asian countries such as China. White rice is the commonly consumed type, but demand for brown rice is increasing because of its high nutritional value (Choi et al., 2015). *Basmati* rice (*Oryza sativa* Linn.) ranked second, after wheat, in world production of cereals (Lutfullah and Hussain, 2012). *Basmati* is a common white variety belonging to long grain rice and is known to cook dry and fluffy while the medium and short grain rice ones are clumping together during preparation. Japanese prefer consumption of fresh rice while Indians favor stored rice (Reiter et al., 2010). In fact, aging of freshly cultivated rice is a common practice for reducing cohesiveness, increasing volume and producing fine texture of the cooked rice (Butt et al., 2008).

Rice is largely cultivated in subtropical environments which are characteristically warm and humid. After harvesting, it is generally dried and under inappropriate storage conditions, rice is considered as an appropriate substrate for fungal growth (Reiter et al., 2010; Lai et al., 2015). According to FAO, 15% of the rice harvest is lost every year due to inappropriate storage conditions resulting in fungal growth and other deleterious agents (Dors et al., 2009). Rice is often contaminated with mycotoxins such as aflatoxins. The temperatures and moisture conditions prevailing during storage promote aflatoxin production resulting in annual losses of useful food bioresources such as rice and thus affecting the economy of rice producing countries (Súarez-Bonnet et al., 2013; Naseer et al., 2014; Lai et al., 2015). The most important fungal genera producing mycotoxins that are found in food products are *Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium* (Probst and Cotty, 2012; Berthiller et al., 2013).

Contamination with aflatoxins is the main food safety problem for field crops produced in tropical and subtropical climate regions where high temperature and humidity promote growth and proliferation of *Aspergillus* spp. Major food commodities affected are rice,

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oil seeds, nuts, dried fruit, spices, and beans (Reddy et al., 2011; Ruadrew et al., 2013). Aflatoxins (AFS) are a class of mycotoxins produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus*, and rarely by *Aspergillus nominus*. The four major AFS are known as aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). AFB1 and AFB2 are produced by *A. flavus*. The four AFS are produced by *A. parasiticus* (Bennett et al., 2007; Reddy et al., 2011; Ruadrew et al., 2013). AFS chemically correspond to bis-dihydrofurancoumarins. Their melting points are above 250 °C, and they are stable at a pH range of 3 to 10 (Ruadrew et al., 2013; Súarez-Bonnet et al., 2013). The removal of AFS is very difficult due to their stability and thermal resistance in dried products (Lee et al., 2015). In fact, AFS are resistant to food processing and thus they may remain throughout the food chain (Ruadrew et al., 2013). Therefore, AFS are potential threats to human health, either by consumption of direct contaminated food products or by carry over aflatoxins and their metabolites in milk and meat (Nordkvist et al., 2009; Reiter et al., 2010; Naseer et al., 2014). AFB1 is the most toxic and has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). It has been associated with liver cancer and acute hepatitis based on epidemiological studies (El tawila et al., 2013; Ruadrew et al., 2013; Súarez-Bonnet et al., 2013). Most of the AFS are detoxified by liver metabolism and this fact can explain why individuals do not always develop cancer. Conjugation with glutathione (GSH, γ-glutamyl-cysteinyl-glycine) is an important detoxification reaction for AFS in animals (Berthiller et al., 2013). Once AFB1 is ingested, it is metabolized to the active intermediate AFB1-exo-8,9-epoxide through a series of metabolic processes. The detoxification of AFB1-exo-8,9-epoxide is unknown, but might contribute to the fact that human hepatocellular carcinoma (HCC) does not always develop (Súarez-Bonnet et al., 2013). Unfortunately, dietary AFS is a chronic problem in the tropical regions and worldwide due to the increasing global trade and transportation of food across countries. Although rice is not immediately thought of as a high risk commodity, in terms of contamination levels of aflatoxins, there is substantial evidence indicating endemic low mg/kg occurrence of AFB1 contamination in rice (Trucksess et al., 2011; Zhu et al., 2013). AFB1 and other mycotoxins were detected in rice varieties in different countries including USA, UK, Egypt, Pakistan, Malaysia, Philippines, India, Nepal, Iran and China (Tanaka et al., 2007; Rahmani et al., 2011; Lutfullah and Hussain, 2012).

For managing this safety risk, more than hundred countries have established regulations to limit AFS content in rice and other agricultural commodities (Rahmani et al., 2011; Reddy et al., 2011; Trucksess et al., 2011; Majeed et al., 2013). However, maximum tolerance levels differ greatly among countries (Rahmani et al., 2011; Reddy et al., 2011; Zhu et al., 2013). In Asia, for example, rice is considered to be contaminated at >20 μg AFS/kg (Súarez-Bonnet et al., 2013).

The study is a limited survey to analyze rice for aflatoxins (AFB1, AFB2, AFG1 and AFG2). Data were compared to some international permissible limits for those toxins in rice. Effect of a common emulsifier (Tween 80) on extraction and recovery of aflatoxins from rice was evaluated. AFS production in rice grains by *Aspergillus parasiticus* under two different temperatures was also studied.

2. Materials and methods

2.1. Safety precautions

Due to the toxicity and carcinogenicity of AFS, safety considerations were adhered to during experiments by wearing gloves and other protective clothes. Residual AFS in glassware such as tubes and autosampler vials were destroyed by soaking, for 48 h, in a sodium hypochlorite solution (5%) prior to washing. Standard AFS solutions were kept in autosampler amber vials (Agilent Technology, USA) to protect them from light.

2.2. Samples

Rice samples were randomly collected from local retail markets in Al-Ahsa (Eastern Region), Saudi Arabia. They were purchased in their original imported packages (5 and 10 kg) in 2014–2015. Samples were originating from India, Pakistan, USA, Egypt and Australia. Long grain white rice (*Basmati*), American (parboiled), Australian (white medium grain) rice and round grain rice (Egypt) were evaluated for their AFS content. Meanwhile, a locally produced brown rice cultivated at limited scale was also evaluated for AFS. *Basmati* rice has a distinct flavor and is the most common rice variety consumed in India, Pakistan, Saudi Arabia and nearby Gulf states. It is classified, by the Codex Alimentarius, as long grain rice (Reiter et al., 2010). The water content of rice samples was below 10%, determined by drying samples in an oven at 105 °C until constant weights. For AFS analysis, rice subsamples (250 g) were ground in a Waring blender and sieved using kitchen mesh to obtain fine powder.

2.3. Preparation of mold inoculum

A loopful of *Aspergillus parasiticus* (isolated from moldy peanuts) maintained in a Potato Dextrose Agar slant (PDA, pH 5.6, Oxoid Ltd., UK) was streaked onto PDA plates followed by incubated at 28 °C for five days. Each plates was washed with ten ml saline (sterile) and spores were harvested by gentle scraping of agar surface with a sterile glass rod. The resulting spore suspension was filtered through layers of cheesecloth to remove any hyphal fragment. The suspension was diluted with sterile double distilled water (DDW) to provide 10^7 spore per ml, verified by surface plating on PDA.

2.4. Spiking experiments

Recovery procedures of AFS from samples were done at two different levels with aflatoxin standard solutions, as previously described (Zhu et al., 2013). Dry aflatoxin standard (Trilogy Analytical Lab., Washington, MO, USA) was dissolved by adding 10 ml acetonitrile, as directed by supplier. The stock solution (total AFS 5 μg/ml) contained 2, 0.5, 2 and 0.5 μg/ml of AFB1, AFB2, AFG1 and AFG2, respectively.

Blank *Basmati* rice analytical units (50 g each) were intentionally contaminated with 63 μl of stock solution corresponding to 2.5 μg AFB1/kg (level 1, 6.25 μg total AFS/kg). Level two was 1.25 μg AFB1/kg (3.125 μg total AFS/kg). Similarly, 50 g of brown rice powder was spiked with same levels of AFS. Beakers (150 ml capacity) containing spiked rice (triplicates per level) were left, in the dark, at ambient temperatures overnight to allow toxin diffusion and solvent evaporation. AFS recoveries from three replicates were determined (Section 2.6).

2.5. Aflatoxin production in rice grains

The method described by Choi et al. (2015) for AFS production in rice was adopted with modifications. Simply, twenty g (triplicates) of sound and healthy white rice (*Basmati*) grains were placed in plastic Petri plates (sterile, Ø 90 mm, 15 mm height) and their moisture was adjusted to 31% with predetermined amount of sterile DDW. Plates were covered and samples were allowed to equilibrate at room temperature for 12 h (in the dark) prior to inoculation with one ml spore suspension (10^7 conidia/20 g) of *Aspergillus parasiticus*. Plates of both treatments and control were

covered and incubated at 25 °C or 37 °C for one to three weeks. Prior to AFS extractions, rice grains were dried at 60 °C for 18 h in a draft oven to facilitate sample grinding by means of a pestle and mortar. Extraction and quantification of AFS produced by *Aspergillus parasiticus* in rice were carried out as described below.

2.6. Aflatoxin extraction and purification

The procedure of Zhu et al. (2013) was used (without Tween 20) for sample extraction and cleaning up. AFLAPREP® columns (IAC, R-Biopharm Rhône Ltd., Glasgow, UK) were used for sample purification and AFS extraction. Briefly, fifty g portions of powdered rice were separately added to a Waring blender. Four g of NaCl and 100 ml DDW was added followed by mixing for 1 min at high speed. NaCl clarified extracts and protect AFS from degradation by UV or alkaline conditions (Prasanna et al., 1975; Súarez-Bonnet et al., 2013). After adding 150 ml methanol, mixture was blended again for 2 min at high speed. Samples were filtered through Whatman no. 1 filter papers. Five ml filtrate (equivalent to 1 g sample) was transferred into a beaker and 15 ml of PBS (pH 7.4) was added. Results were also compared to those when filtrates (spiked brown rice extracts) was diluted with 15 ml of 15% Tween 80 in PBS. IAC were allowed to warm to room temperature prior to conditioning by passing 10 ml of PBS. Diluted filtrates (20 ml) was passed through IAC at approximately 0.6 ml/min by gravity. A slow flow rate is necessary for capturing AFS by their antibodies in IAC, as recommended by the manufacturer. IAC was washed twice, each with 10 ml DDW. Then IAC was dried by passing air (syringe) for 10 s. AFS were eluted from IAC by 0.5 ml methanol then 0.5 ml DDW accompanied by back flushing. Air was passed through the column using a syringe to collect the last few drops. Collected eluates were clarified by a disposable filter membrane (sterile 0.45 μm) and stored in autosampler amber vials (Agilent Technology) at 4 °C prior to injection into HPLC system.

2.7. High performance liquid chromatography (RP-HPLC-FLD)

A reversed phased (RP) HPLC procedure was used for AFS determination in samples. The method described by Zhu et al. (2013) was followed with few modifications. A 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump and vacuum degasser (model G1354A), an autosampler (model G1329A, 100 μl total loop volume) and a fluorescence detector (FLD model G1321A) was used. The separation column connected to guard cartridge was a Zorbax Eclipse XDB C18 (5 μm, 250 mm, 4.6 I.D.). It was maintained at 30 °C in a Thermostated column compartment (model G1316A). Post column derivatization was done with a Kobra Cell® (R-Biopharm Rhône Ltd., Glasgow, UK). The isocratic mobile phase was a mixture of water/methanol/acetonitrile (62:22:16, v/v/v) with a flow rate of 1 ml/min. For post column derivatization, 120 mg potassium bromide and 350 μl nitric acid (4 M) was added to one liter of mobile phase. The mobile phase was freshly prepared on the day of analysis, as recommended for the post column derivatization with the Kobra Cell®. Level of detections (LOD) of AFB1, AFB2, AFG1, and AFG2 were established by practical experimentation as being 0.025, 0.015, 0.05, and 0.05 μg/kg, respectively (Zhu et al., 2013).

Filtered samples (20 μl) were injected into system through the autosampler and AFS signals were detected with FLD at excitation of 365 nm and 435 nm emission. The data of three replicates were acquired and analyzed with the Agilent Data Handling Chemstation3 (Agilent Technologies model G1656B). Averaged recoveries of spiked rice (Table 1) were used for obtaining corrected 100% recovery of AFS in samples. The equation reported previously (Trucksess et al., 2011) was used to calculate 100% recovery of AFS from samples:

$$100\%\ \text{recovery} = (\text{measured AFS}/\%\text{recovery}) \times 100.$$

2.8. Statistical analysis

Data are means of triplicates. Student's t-test was applied to determine significance differences between AFS production by *Aspergillus parasiticus* in rice at 25 °C and 37 °C for each storage period. The analysis of variance (one-way ANOVA) was used and significant differences among means were determined by the Duncan's test (SPSS 13, SPSS Inc., Chicago, Il., USA) at a significant level of 0.05. Probabilities <5% were considered statistically significant.

3. Results and discussion

3.1. Aflatoxin recovery

Fig. 1 shows HPLC chromatogram and elution times of AFS standards. Their order is AFG2 (7.38 min), AFG1 (8.79 min), AFB2 (9.87 min) and AFB1 (11.98 min).

Spiked studies are used to correct for the actual concentration of an analyte (e.g. AFS) in samples (Trucksess et al., 2011). Recoveries of AFS form spiked Basmati rice (blank) are shown in Table 1. Total AFS recovery was 75.68% and 86.11% at spiked concentrations of 6.25 and 3.125 μg AFS/kg, respectively. Those percentages were within acceptable values for AFS of the AOAC, Codex Alimentarius and EU Commission. The AOAC guideline for the acceptable recovery at the 10 μg/kg is 70–125% and those of the Codex Alimentarius are 70–110% for 10–100 μg/kg, and 60–120% for 1–10 μg/kg (Trucksess et al., 2008). The EU Commission Regulation (EC No 401/2006) stipulates recovery values for AFS from 50 to 120% at concentration of <1 μg/kg and 70–110% for 1–10 μg/kg (Golge et al., 2016).

From Table 1, at a total AFS of 3.125 μg/kg, recoveries were 88% (AFB1), 83.33% (AFB2), 84% (AFG1) and 89.74% (AFG2). At 6.25 μg/kg AFS, recoveries of each individual toxin did appreciably improved. Recovery percentages (Table 1) were comparable to those reported earlier by Zhu et al. (2013) for spiked white rice analyzed under similar conditions. It was clearly stated that increasing spiked concentrations of AFS from 8 to 30 μg/kg did not result in appreciable increases of recovered AFS from rice varieties (Trucksess et al., 2011; Súarez-Bonnet et al., 2013). In botanical plants, Trucksess et al. (2008) reported that recoveries (68–74%) of AFS and AFB1 had the same trend as their spiked amounts in ginseng increased from 2 to 16, and 1 to 8 μg/kg, respectively. Different % recoveries of AFS from various rice types were reported (Park and Kim, 2006; Ruadrew et al., 2013; Liu et al., 2012; Senyuva and Gilbert, 2010; Súarez-Bonnet et al., 2013).

3.2. Aflatoxin production in rice

In this study, visible growth of *A. parasiticus* in rice grain samples was more dense at 25 °C than that at 37 °C. Rice extracts of those grown at 25 °C had more deeper color. In this regard, growth temperatures of aspergilli and other toxigenic fungi vary, where a minimum from 10 to 12.8 °C, a maximum between 43 and 48.8 °C and an optimum near 33.8 °C were stated (Reiter et al., 2010). Table 2 presents production profile of AFS in rice grain samples. None of the four structural forms of AFS was detected in controls (non-inoculated rice). *A. parasiticus* formed only AFB1 and AFB2 at 25 °C. AFG1 and AFG2 were not detected at 25 °C throughout the storage period (Table 2). As stated by previous researchers, it is possible that some enzyme systems and genes may play a role on the relative accumulation of the different AFS forms under various conditions (Sorensen et al., 1967; Lin et al., 1980; Breckenridge and Arseculeratne, 1985; Schmidt-Heydt et al.,

Table 1
Recovery (%) of aflatoxins (AFS) from *Basmati* rice samples spiked with two different concentrations.

Aflatoxin concentration (μg/kg)							
	Level 1			Level 2			
Aflatoxin	Added	Determined	Recovery (%)	Added	Determined	Recovery (%)	Mean (%)
AFB1	2.5	1.91	76.4	1.25	1.1	88	82.2
AFB2	0.625	0.52	83.2	0.312	0.26	83.33	83.27
AFG1	2.5	1.82	72.8	1.25	1.05	84	78.4
AFG2	0.625	0.49	78.4	0.312	0.28	89.74	84.07
Total (AFS)	6.25	4.73	75.68	3.125	2.69	86.11	80.89

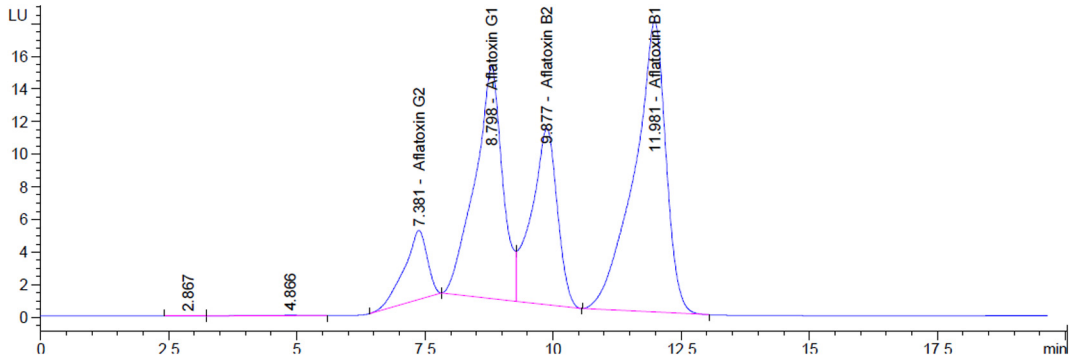

Fig. 1. HPLC chromatogram of aflatoxin (AFS) standard solution containing 2 AFB1, 0.5 AFB2, 2 AFG1 and 0.5 μg/ml AFG2 (5 μg/ml total AFS).

Table 2
Aflatoxin production in rice samples by *Aspergillus parasiticus* at two different temperatures for three weeks.

Temperature (°C)	Period (week)	Concentration (μg/kg)[a]				
		AFB1	AFB2	AFG1	AFG2	Total (AFS)
25	1	4.32	0.26	–	–	4.58
	2	2.16	0.18	–	–	2.34
	3	0.72	0.16	–	–	0.88
37*	1	36.30	10.35	12.96	42.53	102.15
	2	23.85	7.46	6.78	26.91	65.00
	3	39.31	9.87	25.37	10.11	84.66

–, not detected.
[a] Corrected for 100% recovery.
* $P < 0.05$, (Student's *t*-test).

2010; Liu et al., 2012). All four AFS types were produced at 37 °C (Table 2). During the three week period, amounts of AFB1 were significantly ($P < 0.05$) higher at 37 °C (23.85–39.31 μg/kg) than at 25 °C (0.72–4.32 μg/kg). Naseer et al. (2014) reported more AFS accumulation occurred above 25 °C. In contrast, a previous finding stated maximum AFB1 production occurred in rice at 25 °C to below 35 °C (Sorensen et al., 1967). Strains of *A. flavus* and *A. parasiticus* accumulated varied amounts (<0.1–1214 μg/g) of AFB1 in powdered rice stored at 25 °C for ten days (Wei and Jong, 1986). It was indicated that AFB1 biosynthesis in *A. parasiticus* was optimal at 37 °C in YES agar media (Schmidt-Heydt et al., 2010). No correlation existed between AFS production in grains and in liquid fermentation media. *A. flavus* producing AFB1on viable maize (*in vivo*) frequently failed to produce (*in vitro*) detectable AFB1 in synthetic media (Probst and Cotty, 2012). It is noteworthy to mention that AFS were not secreted by *Aspergillus* species in rice and other grains having moisture content <18%. Besides, maximal AFS production was at 28–31% moisture (Chang and Markakis, 1981; Naseer et al., 2014). Rice is harvested at moisture content between 16% and 28%, depending on the harvest technique. Those conditions, therefore, could promote growth and AFS production by contaminating *Aspergillus* species (Reiter et al., 2010).

3.3. Effect of Tween 80 on aflatoxins

For better recovery of AFS from pigmented food (brown rice or black sesame), Tween 20 (polyoxyethylenesorbitan monolaurate) was added to PBS for IAC purification. Washing the IAC with PBS containing 15% Tween 20 reduced non-specific binding to the matrix by antibodies and thus improved AFS recoveries to >70% (Trucksess et al., 2011; Liu et al., 2012; Zhu et al., 2013).

In the present study, a closely related emulsifier (Polyoxyethylenesorbitan monooleate, Tween 80) was used in IAC purification of spiked brown rice. Tween 20 and 80 are nonionic surfactants, which are soluble in water and other solvents. Table 3 summarizes recovery of the different structural forms of AFS with or without Tween 80. Tween 80 increased % recoveries of AFB1 from 65.6 to 85.6% (2.5 μg/kg spiked level), and from 81.6 to 106.4% (1.25 μg/kg spiked level, Table 3). Lower spiked amounts of AFB1 and Tween 80 treatment resulted in better % recoveries of the toxin (Table 3). In fact, improving recoveries of AFB1 by Tween 80 from grains such as rice has not been published.

Among AFS, AFB1 is the most toxic and has been classified as a Group I carcinogen by IARC (El tawila et al., 2013; Ruadrew et al., 2013; Súarez-Bonnet et al., 2013). Tween 80 reduced significantly ($P < 0.05$) recovery of AFG2 and AFB2 (Table 3). It was possible that

Table 3
Effect of Tween 80 on recovery (%) of aflatoxins spiked in brown rice at two different concentrations.

Aflatoxin	Spiked amount (μg/kg)	No Tween 80		With Tween 80	
		Concentration (μg/kg)*	Recovery (%)	Concentration (μg/kg)	Recovery (%)
AFB1	2.5	1.64b	65.60	2.14a	85.6
AFB2	0.625	0.56a	89.60	0.16b	25.6
AFG1	2.5	2.19b	87.60	2.30a	92
AFG2	0.625	0.65	104.00	–	–
AFS (Total)	6.25	5.04a	80.64	4.6b	73.6
AFB1	1.25	1.02b	81.60	1.33a	106.40
AFB2	0.312	0.34a	108.97	0.085b	27.24
AFG1	1.25	1.26a	100.80	1.31a	104.8
AFG2	0.312	0.36a	115.38	0.071b	22.76
AFS (Total)	3.125	2.99a	95.68	2.81b	89.92

–, not detected.
* Mean concentrations within a row with different letters are significantly different ($P < 0.05$).

Tween 80 did not reduce nonspecific binding of those two types (AFB2 and AFG2) to IAC matrix, and thus most of the toxins could not be eluted during the purification step. While lower concentrations improved recovery, higher levels (>15% Tween 20) resulted in considerable losses of AFS (Liu et al., 2012).

3.4. Aflatoxins in rice

Rice samples were analyzed for their AFS content using RP-HPLC-FD. Up until now, RP- HPLC using C18 columns is the most commonly used method for AFS determinations in rice and other food products (Reiter et al., 2010; Reddy et al., 2011; Khayoon et al., 2012; Zhu et al., 2013; Golge et al., 2016). The concentrations (corrected for 100% recovery) of each individual aflatoxin in rice samples are shown in Table 4. With the exception of parboiled rice, at least one AFS type was detected in rice extracts. AFB1 contamination ranged from 0.014 to 0.123 μg/kg. Meanwhile, total AFS values were 0.052–2.58 μg/kg (Table 4). Published data on AFS in rice in Saudi Arabia are lacking.

In Pakistan, rice (*Basmati*) contained higher levels of both AFB1 and AFS. Their levels were 4.9–8.8, and 8.9–12.5 μg/kg, respectively (Iqbal et al., 2014). Rice (white, *Basmati* and parboiled) from Spain, Mexico, Pakistan, USA and other sources exceeded levels of AFB1 and AFS tolerated in cereals in the European Community (Súarez-Bonnet et al., 2013). In Austria and West Scotland, AFB1 levels were ≤10 μg/kg in rice varieties originating from India, Pakistan, Italy, Egypt and other places (Ruadrew et al., 2013; Reiter et al., 2010). Interestingly, it was reported that conjugated or masked AFS may not be detected by common protocols used for detection of their original forms (Berthiller et al., 2013; Ruadrew et al., 2013).

In Asian countries, rice consumption is estimated to be 110 g per day per person, which corresponds to about 40.2 kg per capita per a year. However, in the European countries, such as Austria the annual consumption of rice is only 3.9 kg per capita (Mazaheri, 2009; Reiter et al., 2010). Despite rice being a low-risk commodity, EU regulations controlling AFS levels in rice and other cereals impose limits of 2.0 μg/kg and 4.0 μg/kg for AFB1 and total AFS, respectively. Other limits for AFS ranging from 5 μg/kg in Russia to 10 μg/kg in China and Japan. In the USA, total AFS in rice is 20 μg/kg. The limit in Brazil and India is 30 μg/kg (Súarez-Bonnet et al., 2013; Ruadrew et al., 2013; Zhu et al., 2013). Korea Republic has set limits of 15 ng/g and 10 ng/g for total AFS and AFB1, respectively, in grains (Choi et al., 2015). In Saudi Arabia, the Gulf standard (No. 841/1997) and Saudi standard (No. 1151/1998) specified the maximum limits of AFS in foods and feeds of 20 μg/kg for nuts, cereals and other food products, except dairy products (AFM1 0.2 μg/kg) and animal feeds (10 μg/kg), and no limit was set for AFB1 (El tawila et al., 2013).

As seen in Table 4, AFB1 and total AFS were within the acceptable levels stipulated by the EU regulations for AFB1 and AFS in rice. It should be taken into consideration that no exposure to any level of AFS could be regarded as safe (Ruadrew et al., 2013).

One of the Australian rice sample had AFG1 (Table 4). In this regard, it was reported that AFB1 is considered to be a precursor of AFG1 and thus explaining the relative accumulation of both toxins (Lin et al., 1980; Breckenridge and Arseculeratne, 1985). Interestingly, it was reported that cross reactivity by AFG1 to the AFB1 antibodies occurred at higher percentages in immunoultrafilteration cleanup of AFS (Reiter et al., 2009). The order of toxicity is AFB1 > AFB2 > AFG1 > AFG2. The terminal furan moiety of AFB1 is the critical point for determining the degree of biological activity of this group of fungal toxins (Quinto et al., 2009).

Fig. 2 shows the chromatogram of *Basmati* rice samples (an outlier) naturally contaminated with AFG1, AFG2 and AFB1. Their concentrations were 5.12, 1.42 and 0.03 μg/kg, respectively. From safety point of view, AFB1 is the most toxic and classified as a Group I carcinogen (Wang et al., 2016). Molecular biology information and epidemiological studies predicted the LD_{50} of AFB1 for humans to be five mg/kg (Ruadrew et al., 2013). According to the European Commission, as low as one ng/kg/person/day of AFB1

Table 4
Aflatoxin contents of different rice samples.

Rice (no. samples)	Origin	Concentration (μg/kg)[a]				
		AFB1	AFB2	AFG1	AFG2	AFS (Total)
Basmati (19)	India	0.049	0.0107	<LOD	<LOD	0.059
Basmati (17)	Pakistan	0.025	0.012	1.973	0.5662	2.577
Parboiled (13)	USA	<LOD	<LOD	<LOD	<LOD	<LOD
White medium (13)	Australia	<LOD	<LOD	0.052	<LOD	0.052
White round (13)	Egypt	0.123	0.0152	<LOD	<LOD	0.135
Brown (13)	Saudi Arabia	0.014	0.019	0.037	0.043	0.116

LOD, below detection limit.
[a] Corrected for 100% recovery.

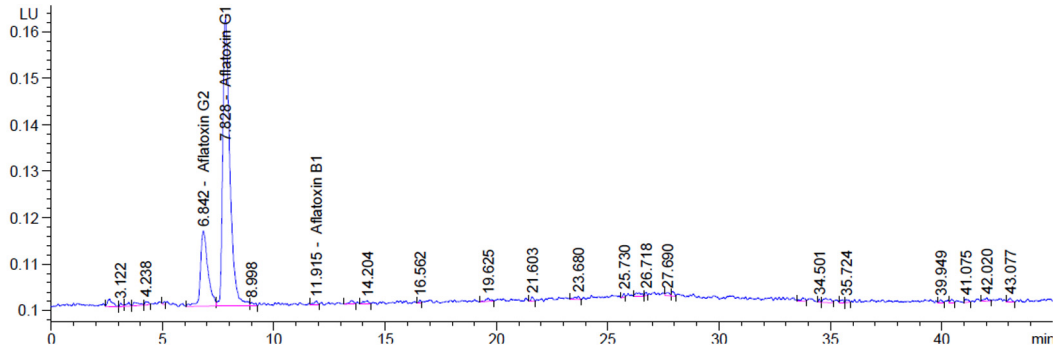

Fig. 2. HPLC chromatogram of *Basmati* rice naturally contaminated with 0.03 μg/kg AFB1, 5.12 μg/kg AFG1, and 1.42 μg/kg AFG2.

could be enough to contribute to a high risk for liver cancer (Súarez-Bonnet et al., 2013).

AFS contamination of rice and other field crops has been the main food safety concern in tropical and subtropical climates where high temperature and humidity promote growth of mycotoxigenic fungi such as *Aspergillus* species. In fact, rice is harvested at moisture content between 16% and 37%, which favor fungal growth (Reiter et al., 2010; Sarker et al., 2015). In Japan, rice is stored in warehouses at below 15 °C and 70–75% relative humidity. Those conditions prevented postharvest contamination of rice with mycotoxins (Tanaka et al., 2007). The moisture level of <9% in rice is recommended for a storage period of more than one year in labeled plastic bags (Súarez-Bonnet et al., 2013). Storage of rice with less than 10% moisture in rodent proof rooms would control AFS production (Naseer et al., 2014).

To reduce their risk, physical (*e.g.*, gamma radiation), chemical (*e.g.*, hydrogen peroxide), or biological treatments have been used as detoxification methods of AFS from contaminated food and feed (Súarez-Bonnet et al., 2013; Naseer et al., 2014; Lee et al., 2015). Plants' essential oils and phenolic constituents offered inhibition activities against some mycotoxin producing molds (Nakahara et al., 2003; Tajkarimi et al., 2010; Naseer et al., 2014).

AFS loss during food processing may occur because toxins could be washed away, bound to food matrices or transformed to unknown decomposed derivatives (Park and Kim, 2006). Pressure cooking of rice reduced AFS by 60 to 88% while 37% reduction resulted from ordinary rice cooking (Park and Kim, 2006; Lee et al., 2015).

4. Conclusion

AFS content of various rice tested were in compliance with EU and other international standards for AFS. Endemic low levels exposure to AFS could constitute a risk factor for humans. Treating rice extracts and probably other food with emulsifiers such as Tween 80 may result in partial or complete loss of AFB2 and AFG2.

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