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Occurrence of aflatoxins and ochratoxin A in rice samples from six provinces in China

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ABSTRACT

In this study, a total of 370 rice samples collected from six provinces in China were surveyed for the presence of aflatoxins (AFs) and ochratoxin A (OTA) using dispersive liquid-liquid microextraction coupled to liquid chromatography with fluorescence detection. The results obtained in this survey showed that 63.5% (235/370) and 4.9% (18/370) of rice samples contained detectable amounts of AFs and OTA, respectively. The average levels of AFB$_1$, total AFs and OTA in positive samples were 0.60, 0.65 and 0.85 µg/kg, respectively. AFB$_1$ and AFB$_2$ were present in samples from all six provinces and could be quantified in 87 and 32 samples, respectively. OTA was present in samples from four provinces and could be quantified in 6 samples. The percentages of rice samples with AFB$_1$ and OTA levels above the EU limit were 1.4% (5/370) and 0.3% (1/370), respectively. The results show that the levels of the mycotoxins in rice are below regulatory limits and the rice commodities are generally safe. It also indicates the need to establish a continuous monitoring program to prevent and manage the occurrence of these contaminants.

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1. Introduction

Rice is one of the most important staple foods in the world and provides 20% of the world’s dietary energy supply. High amounts of rice are consumed per capita each year, especially in Asian countries. According to the Food and Agriculture Organization (FAO, 2009), the worldwide rice production is predicted to be 68501.3 million tons. The main rice-producing countries are China, India, Indonesia, Bangladesh, Myanmar, Thailand and Vietnam (Http://www.fao.org/newsroom/common/ecg/1000820/en/Rmprod0308.pdf). Rice cultivation is usually conducted in subtropical environments, which are characteristically warm and humid. At the same time, rice is generally dried after harvesting. Under inappropriate storage conditions, rice can be an ideal substrate for mycotoxin-producing fungi. A study by Reddy et al. (2008), which established the presence of these major mycotoxins (aflatoxins, fumonisins, ochratoxin A, deoxynivlenol and zearalenone) and the fungi that produce them in rice from several parts of the world, demonstrates that rice supports the growth of fungi and mycotoxin production. Therefore, rice grains can be contaminated with fungi and mycotoxins during cultivation and subsequent handling, if conditions are favorable.

Currently, the presence of mycotoxins, particularly aflatoxins (AFs) and Ochratoxin A (OTA), in rice is a subject of considerable research interest. The occurrence of AFs in rice has also been reported in a number of countries: Bangladesh (Dawlatana et al., 2002), Brazil (Almeida et al., 2012), China (Liu, Gao, & Yu, 2006; Wang, & Liu,
2007), Canada (Bansal et al. 2011), India (Reddy, Reddy, & Muralidharan, 2009), Korea (Park et al., 2005; Ee OK et al., 2014), Pakistan (Majeed, Iqbal, Asi, & Iqbal, 2013), Turkey (Aydin, Aksu, & Gunsen, 2011), Tunisia (Ghali et al. 2008), Vietnam (Nguyen, Tozlovanu, Tran, & Pfohl-Leszkowicz, 2007) and the United States (Abbas & Shier, 2009). Aflatoxins (AFs) are the most important human mycotoxins and are primarily produced by certain *Aspergillus* species, in particular *A. flavus* and *A. parasiticus*. The International Agency for Research on Cancer (IARC) has designated AFB$_1$ as carcinogenic to humans (group 1 carcinogen) and AFM$_1$ as possibly carcinogenic to humans (group 2 carcinogen) (IARC, 1993). To ensure food safety in the European Union, maximum aflatoxin levels were set within the Commission Regulation No. 1881/2006. The MLs for aflatoxin B$_1$ and the limit for total aflatoxins in rice intended for direct consumption are 2 µg/kg and 4 µg/kg, respectively (European Commission, 2006).

OTA is a mycotoxin produced by toxigenic mold species (*Penicillium verrucosum, Aspergillus ochraceus, A. niger and A. carbonarius*) that occur in foods and animal feeds. The IARC has classified OTA in group 2B as a possible carcinogenic compound to humans (IARC, 1993). The maximum residue levels (MRLs) fixed by European regulations for OTA in cereals and cereal products are 5 and 3 µg/kg, respectively (European Commission, 2006). The natural occurrence of OTA has been reported in temperate subtropical and tropical climates for several foods, including rice (Karin, Gunnar, & Karl, 1998). OTA occurrence in rice has been reported in the UK (Scudamore, Patel, & Breeze, 1999), Brazil (Almeida et al., 2012), Canada
(Bansal et al., 2011), Vietnam (Nguyen, Tozlovanu, Tran, & Pfohl-Leszkowicz, 2007), Korea (Park, Choi, Hwang, & Kim, 2005), Morocco (Juan et al., 2008), Malaysia (Samsudin, & Abdullah, 2013), Turkey (Aydin, Aksu, & Gunsen, 2011), Tunisia (Zaied et al., 2009) and Pakistan (Majeed, Iqbal, Asi, & Iqbal, 2013).

China has the highest rice planting area and rice production yield in the world. According to the National Bureau of Statistics of China, the planting area and rice production in 2012 were 30137 thousand hectares and 20423.6 million tons, respectively, and rice consumption per person was approximately 67.6 kg/year (http://www.stats.gov.cn/tjsj/ndsj/2013/indexch.htm). In China, rice is grown in almost every province, with the exception of Qinghai province (Peng, Tang, & Zou, 2009). Investigations conducted in certain areas of China have demonstrated the contamination of rice with mycotoxins, and especially with AFs (Li et al. 2014; Liu et al. 2006; Sun et al. 2011; Wang, & Liu, 2007). However, there are few data on the contamination of mycotoxins in rice collected from other areas of China. Northeastern China (including Heilongjiang, Liaoning and Jilin province), which locate between 40°N and 50°N latitude and between 120°N and 130°N longitude, is an important grain production areas. The cultivated area of rice is 4432.8 thousand hectares, and the production of rice is 3211 million tons in 2012. South China (including Guangdong, Guangxi and Hainan province) locate at latitude 40°N-25°31'N and longitude 104°26'N-117°20'N, the cultivated area of rice is 4331.4 thousand hectares, and the production of rice in 2012 is 2424.4 million tons. The main objective of the present study was to investigate the occurrence and contamination level of AFs and
OTA in rice from six provinces (Guangdong, Guangxi, Hainan, Heilongjiang, Liaoning and Jilin) in China. The findings presented here provide further data evaluating the occurrence of AFs and OTA in rice and will be invaluable in creating awareness among local farmers, regulatory agencies and food factories about the risks and health hazards associated with these mycotoxins.

2. Materials and methods

2.1 Sampling

A total of 370 rice samples were selected from farmer stores, granaries and markets in six provinces (South China: Guangdong, Guangxi and Hainan province; Northeast: Heilongjiang, Liaoning and Jilin province) in China between 2009 and 2011. To avoid the sampling error due to highly heterogeneous nature of fungal distribution, each 2 kg composite sample collected one storehouse was a mixture of 10 subsamples (200 g each). The 10 such subsamples were collected from 5 diagonals on each of the upper, middle and lower layers of each storehouse (Liu & Yu, 2006). Each 2 kg sample was transported to the laboratory in one batch within 24 h of collection and stored at 4°C in the refrigerator until analysis. All samples were ground to a homogeneous particle size, and subsamples of 500 g were analyzed for aflatoxins and ochratoxin A.

2.2 Chemicals and reagents

All reagents and chemicals used in this study were of analytical grade or higher. Analytical-grade carbon chloroform (CHCl₃) was obtained from Tianjin Xingyue Chemical Co., China. HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were
supplied by Shanghai ANPEL Scientific Instrument Co., China. Standards for aflatoxin B$_1$ (AFB$_1$), aflatoxin B$_2$ (AFB$_2$) and ochratoxin A (OTA) were purchased from PriboLab (Singapore).

2.3 Simultaneous detection and quantification of AFB$_1$, AFB$_2$ and OTA by DLLME-HPLC

Extraction, purification and HPLC quantification of AFB$_1$, AFB$_2$ and OTA were performed by dispersive liquid-liquid microextraction coupled to high performance liquid chromatography with fluorescence detection (DLLME-HPLC) according to our established method (Lai, Sun, Ruan, Zhang, & Liu, 2014). The limits of detection (LOD) and limits of quantification (LOQ) were 0.009, 0.006, 0.08 µg/kg and 0.03, 0.02, 0.3 µg/kg for AFB$_1$, AFB$_2$ and OTA, respectively. Average recoveries were between 85.2% and 112.0% for three mycotoxins, and precision was lower than 10%.

Briefly, 20 g of ground rice samples were extracted in 80 mL of a 79/20/1 (v/v/v) mixture of MeCN/H$_2$O/CH$_3$COOH and followed by filtration. 1 mL of filtrate was mixed with 200 µL CHCl$_3$ and then rapidly injected into a 15 mL glass centrifuge tube containing 5 mL 2% NaCl in water (pH 3.0). The ternary component system was mixed and centrifuged, and the sedimented CHCl$_3$ phase was quantized and evaporated to dryness. A derivative of AFB$_1$ and AFB$_2$ was prepared by adding 200 µL n-hexane and 100 µL trifluoroacetic acid and analyzed by HPLC-FLD. The gradient elution was used with mobile phase of MeCN, MeOH and 1% H$_3$PO$_4$. The detection wavelengths of AFB$_1$ and AFB$_2$ were 360 nm (excitation wavelength) and 440 nm (emission wavelength) from start to 15 min and were then changed to 333 nm.
(excitation) and 460 nm (emission) for OTA from 15 to 32 min. The retention times were 5.9, 14.1 and 23.7 min for \( \text{AFB}_1 \), \( \text{AFB}_2 \) and OTA, respectively.

### 2.4 Statistical analysis

The concentrations of AFs and OTA were statistically analyzed and given as mean ± standard deviation (SD) using SPSS software (IBM, PASW Statistics 18.0, USA). One-way analysis of variance (ANOVA) was applied to the data from the different sample sources to determine the significance of the results and regression/correlation analysis was used to determine \( R^2 \).

### 3. Results and discussion

The incidence and contamination of \( \text{AFB}_1 \), \( \text{AFB}_2 \), total AFs and OTA in rice samples collected from six provinces in China are shown in Table 1 and 2. As can be observed in Table 1, 235 out of 370 rice samples had detectable levels of AFs and \( \text{AFB}_1 \) contamination, with average levels of 0.65 \( \mu \text{g/kg} \) and 0.60 \( \mu \text{g/kg} \) in positive samples, respectively. It should be noted, however, that \( \text{AFB}_1 \) and \( \text{AFB}_2 \) were present in samples from all six Chinese provinces. Five rice samples (3 samples from Guangxi and 2 samples from Guangdong) exceeded the maximum levels of \( \text{AFB}_1 \) for rice as set by the European Union (European Commission, 2006). The maximum contamination of \( \text{AFB}_1 \) was a concentration of 20.0 \( \mu \text{g/kg} \) in one rice sample from Guangxi province.

Results of this study indicated that there were difference in the contamination of AFs and \( \text{AFB}_1 \) in rice samples collected from different locations. The average content
of AFs and AFB$_1$ in rice samples from south China (Guangdong, Guangxi and Hainan province) was higher than that in the three northeast provinces (Heilongjiang, Liaoning and Jilin). In Liaoning province, 96.7% (29/30) of rice samples were contaminated with AFB$_1$ with no sample exceeding the LOQ (0.03 µg/kg). It is well known that contamination of toxicogenic fungi in cereal grains is influence by various environmental factors. For the different geographical location, the climate in south China is significant different from that of northeastern China. In northeast China, it is very hot and wet in summer but very cold and dry in winter. However, it is very hot and wet in all year in south China. The climatic conditions in south China may favor for fungal growth and result in the high contamination levels of AFs in rice. Some previous studies in China have different results with that in this investigation. Wang and Liu (2007) reported that 27.38% of rice samples, which collected from eight regions (Chongqing, Fujian, Guangdong, Guangxi, Hubei, Jiangsu, Shanghai and Zhejiang) in China, were contaminated with AFs, with an average contaminant content of 0.79 µg/kg. Li et al. (2014) reported that the incidence of AFs was 14.5% in rice, corn and oil products collected from Yangtze Delta region of China, with the levels of 6.9 µg/kg in positive samples. Liu, Gao, & Yu (2006) found that 90.3% of analyzed rice samples in Liaoning province was contaminated with AFs, the average contents in whole grain rice and brown rice were 3.87 µg/kg and 0.88 µg/kg, respectively. Sun et al. (2011) reported that all of twenty-nine rice samples collected from Shandong, Jiangsu and Guangxi province were measured for AFB$_1$ with the median levels of 0.57 µg/kg. The occurrence of mycotoxins in rice might be related to
the storage condition, geographical/ climatological location, rice type and agricultural practice.

The incidence of AFs was 63.5% in this investigation which was similar that reported by other countries. But, the contamination level of AFs in our study was lower than these reports. Reddy et al. (2009) reported that 70% of paddy rice samples and 64.1% of milled rice samples contained up to 308 µg/kg AFB₁ (in paddy rice samples) in India. Villa and Markaki (2009) reported a very high incidence of AFs (56%), with levels between 0.05 and 4.3 µg/kg in rice samples analyzed from Athens. The LOD and LOQ were 0.40 and 1.06 µg/kg for this study, respectively. Mazaheri (2009) documented that 83% of rice samples collected from Iran contained detectable AFs and AFB₁, with mean concentrations of 2.09 and 1.89 µg/kg, respectively. Majeed et al. (2013) reported that 56% of rice samples from Pakistan were found to be contaminated with AFs, with average levels of 8.23 and 19.52 µg/kg for AFB₁ and total AF, respectively. Makun et al. (2011) found that AFs were detected in all twenty-one rice samples collected from Nigeria, at total AF concentrations of 28-372 µg/kg.

Ochratoxin A has received increased attention owing to its toxic effects on human health and high incidence in a wide range of food commodities. In this study, 4.9% (18/370) of rice samples had detectable levels of OTA contamination, with average levels of 0.85 µg/kg in positive samples and 4.8% (3/62), 8.5% (5/59), 9.5% (9/138) and 1.5% (1/67) of analyzed samples from Heilongjiang, Jilin, Guangdong and Guangxi province have found to be detected with OTA, respectively (Table 2).
Ochratoxin A was not detected in any of the analyzed rice samples collected from Liaoning and Hainan provinces. Out of the 18 rice samples in which OTA was detectable, six samples had OTA concentrations greater than the LOQ (0.3 µg/kg); one was from Heilongjiang, two were from Jilin, and three were from Guangdong province. However, only one rice sample from Guangdong province contained an ochratoxin A level higher than the maximum tolerable limit of 3.0 µg/kg set by European Union Commission regulation (European Commission Regulation, 2006).

The occurrence and contamination levels of OTA in this report are low compared with another study in China (Li et al., 2014). Li et al. (2014) showed that 14.5% of rice samples in the Yangtze Delta region of China were contaminated with OTA at an average level of 3.5 µg/kg. Similarly, the occurrence and contamination levels OTA in rice are low than that reported in the other countries. Juan et al. (2008) documented that 26% of rice samples from Morocco were contaminated with OTA, with an average level of 3.5 µg/kg. Zaied et al. (2009) reported that 28% of all analyzed rice samples from Tunisia contained OTA at an average level of 44 µg/kg. In Pakistan, 34 out of 68 rice samples were found to be contaminated with OTA at an average level of 12.94 µg/kg (Majeed et al., 2013). Samsudin and Abdullah (2013) reported that OTA was present in all 50 analyzed red rice samples from Malaysia, with concentrations ranging from 0.23-2.48 µg/kg.

In this study, co-occurrence of AFs and OTA was detected in 4.6% (17/370) of all analyzed rice samples. The AFs/OTA contamination was observed in 6.5% (9/138), 1.5% (1/67), 4.8% (3/62) and 6.8% (4/59) of rice samples from Guangdong, Guangxi,
Heilongjiang and Jilin provinces, respectively. This co-occurrence has been observed in Vietnam rice samples (Nguyen, Tozlovanu, Tran, & Pfohl-Leszkowicz, 2007).

Rice, along with wheat and corn, is an important food crop worldwide and is a major food in several countries, especially in China. Rice is not spared from natural AFs and OTA contamination. Mold spores may infect rice crops in the field and during harvest. Additionally, rice is an aquatic plant and is usually harvested at very high moisture levels (35-50%). Therefore, mycotoxin-producing molds could also contaminate the grain and produce mycotoxins during storage if environmental conditions are favorable. Some studies have found that the contamination of rice by mycotoxins could be a result of inappropriate storage and climate conditions (Reddy et al., 2009; Saleemullah et al., 2006). So, it is necessary for local farmers and food factories to implement strict measures and to improve storage conditions. However, according to the present findings, the contamination levels of AFs (with a mean of 0.65 µg/kg) in rice collected from China are very low compared with previous reports (Mazaheri, 2009; Majeed, Iqbal, Asi, & Iqbal, 2013; Makun et al. 2011; Reddy, Reddy, & Muralidharan, 2009) and with the regulated safe consumption levels established in most countries. In addition, the incidence level and contamination level of OTA in rice samples was also low in our study. It can be concluded that rice produced in China is generally safe for consumption and for national and international trading without restriction.

4. Conclusions
In this work, the occurrence of AFs and OTA in rice collected from six provinces in China was determined, and 63.5% and 4.9% of analyzed rice samples were found to be contaminated with AFs and OTA at average concentrations of 0.65 and 0.85 µg/kg, respectively. AFB$_1$ was present in samples from all six provinces and could be quantified in 87 samples. OTA was detected in samples from four provinces and could be quantified in 6 samples. Of the total number of analyzed samples, 1.4 % (5) and 0.3% (1) were found to contain AFB$_1$ and OTA levels above the maximum limits set by EU regulations in cereal (2 and 3 µg/kg, respectively). Further investigations into the occurrence of AFs and OTA in rice are needed to more thoroughly assess the situation in China.

**Acknowledgements**

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**References**


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**Table 1**

Occurrence and levels of aflatoxin B$_1$, B$_2$ and total aflatoxins (µg/kg) in rice samples

<table>
<thead>
<tr>
<th>Rice origin</th>
<th>Samples (n)</th>
<th>Positive samples (n)</th>
<th>AFs Content (µg/kg)</th>
<th>Mean ± SD (µg/kg)</th>
<th>Positive samples(n)</th>
<th>AFB$_1$ Content (µg/kg)</th>
<th>Mean ± SD (µg/kg)</th>
<th>Positive samples(n)</th>
<th>AFB$_2$ Content (µg/kg)</th>
<th>Mean ± SD (µg/kg)</th>
<th>Positive samples(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heilongjiang</td>
<td>62</td>
<td>43</td>
<td>0.033-0.17</td>
<td>0.062±0.042</td>
<td>43</td>
<td>0.033±0.14</td>
<td>0.058±0.034</td>
<td>9</td>
<td>0.022</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Liaoning</td>
<td>30</td>
<td>29</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>29</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>2</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td></td>
</tr>
<tr>
<td>Jilin</td>
<td>59</td>
<td>23</td>
<td>0.030-0.98</td>
<td>0.12±0.25</td>
<td>23</td>
<td>0.030±0.90</td>
<td>0.11±0.23</td>
<td>9</td>
<td>0.086</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>Guangdong</td>
<td>138</td>
<td>73</td>
<td>0.19-4.1</td>
<td>0.44±0.90</td>
<td>73</td>
<td>0.030-3.7</td>
<td>0.41±0.81</td>
<td>18</td>
<td>0.020-0.47</td>
<td>0.11±0.15</td>
<td></td>
</tr>
<tr>
<td>Guangxi</td>
<td>67</td>
<td>54</td>
<td>0.032-2.1</td>
<td>1.3±3.7</td>
<td>54</td>
<td>0.032-20</td>
<td>1.2±3.4</td>
<td>25</td>
<td>0.029-1.6</td>
<td>0.19±0.36</td>
<td></td>
</tr>
<tr>
<td>Hainan</td>
<td>14</td>
<td>13</td>
<td>0.032-0.71</td>
<td>0.23±0.32</td>
<td>13</td>
<td>0.032-0.66</td>
<td>0.21±0.30</td>
<td>2</td>
<td>0.051</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>370</td>
<td>235</td>
<td>0.030-21.0</td>
<td>0.65±2.3</td>
<td>235</td>
<td>0.030-20.0</td>
<td>0.60±2.1</td>
<td>65</td>
<td>0.02-1.6</td>
<td>0.15±0.28</td>
<td></td>
</tr>
</tbody>
</table>

* Positive mean samples > LOD.
### Tabel 2

Occurrence and level of OTA in analyzed samples of rice

<table>
<thead>
<tr>
<th>Rice origin</th>
<th>Samples (n)</th>
<th>Positive samples (n)</th>
<th>Range of OTA (µg/kg)</th>
<th>Mean ± SD (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heilongjiang</td>
<td>62</td>
<td>3</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>Liaoning</td>
<td>30</td>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Jilin</td>
<td>59</td>
<td>5</td>
<td>0.30-0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Guangdong</td>
<td>138</td>
<td>9</td>
<td>0.33-3.2</td>
<td>1.3±1.6</td>
</tr>
<tr>
<td>Guangxi</td>
<td>67</td>
<td>1</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Hainan</td>
<td>14</td>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Total</td>
<td>370</td>
<td>18</td>
<td>0.30-3.2</td>
<td>0.85±1.2</td>
</tr>
</tbody>
</table>

* Positive mean samples > LOD.

n.d.=not detected.
**Highlights:**

1. The study focused on the occurrence of AFs and OTA in rice in six provinces in China.

2. 63.5% and 4.9% samples contained detectable amounts of AFs and OTA, respectively.

3. 1.4% and 0.3% rice samples have found above the EU limits for AFB$_1$ and OTA, respectively.

4. It is necessary to implement strict measures and to improve good storage conditions.