Assessment of health risks from aflatoxins in rice commercialised in Riyadh, Kingdom of Saudi Arabia

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RESEARCH ARTICLE

Abstract

This study aimed to assess the occurrence and health risk of aflatoxins (AF) in rice imported to Riyadh, Kingdom of Saudi Arabia (KSA). Rice samples (n=41) including long grain white rice (LGW), short grain white rice (SGW) and long grain yellow rice (LGY) were analysed using high performance liquid chromatography with fluorescence detection. Accordingly, the hepatocellular carcinoma (HCC) risk for the total population was estimated with regard to individuals positive for the hepatitis B virus surface antigen (HBsAg+) and HBsAg-negative individuals. The results of this study indicated that 68% of the samples were contaminated with an average AF concentration of 1.45±1.57 µg/kg. The SGW had the highest value of contamination (mean=2.34 µg/kg) followed by the LGY (1.36 µg/kg) and the LGW (0.59 µg/kg). The AF levels in all analysed samples were found to be below the prescribed limit of the KSA regulation (20 µg/kg), and 1 sample (2.4%) had total AF level higher than European Commission limit (4 µg/kg). However, estimation of the chronic effects revealed that the HCC risk was found 0.04 for HBsAg- and 1.04 for HBsAg+ per 10^5 individuals per year. The margin of exposure was found to be 72 for total AF. The results indicate that consumption of AF contaminated rice imported into Riyadh (KSA) may have public health consequences including risk of HCC, although levels are below the prescribed limit in the kingdom of Saudi Arabia as well as in European Union. This is the first study to investigate the occurrence of AF, and the related health risks, in rice imported to KSA.

Keywords: probable daily intake, hepatocellular carcinoma, high performance liquid chromatography, legal limit

1. Introduction

Aflatoxins (AF) are hepato-carcinogenic toxins produced by some toxigenic strains of Aspergillus species in food commodities and foodstuffs, such as peanuts, nuts, maize, dried fruits, vegetable oils and rice. Aspergillus flavus only produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) whereas Aspergillus parasiticus may also produce aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Elzupir et al., 2010, 2011; Osman et al., 1999; Tanaka et al., 2007; Tchana et al., 2010). The most potent hepato-carcinogenic compound is AFB_1, followed by AFG_1, AFB_2 and AFG_2. AFB_1 is classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogen (IARC, 1993; Leong et al., 2012). The Global Environmental Monitoring System of the World Health Organization reported that rice (Oryza sativa or Oryza glaberrima) is the major diet constituent for half of the world and the second consumable cereal food worldwide after wheat (WHO, 2003). Rice constitutes 27% of the global diet and 20% of dietary protein intake in developing countries (Ok et al., 2014; Sales and Yoshizawa, 2005). According to the Food and Agriculture Organization of the United Nations (FAO, 2012), there are 156 million cultivated hectares of rice, producing 721 million metric tons globally in 2011. Nevertheless, several reports have shown that rice can be contaminated with AF (Almeida et al., 2012; Iqbal et al., 2012; Makun et al., 2011; Nguyen et al., 2007; Park et al., 2004; Ruadrew et al., 2013; Sangare-Tigori et al., 2006). Further, the literature indicates a positive relationship between daily intake of rice that is
contaminated with aflatoxin and hepatocellular carcinoma (HCC) incidence in some rice-consuming countries in Asia, despite the average contamination levels being within the permitted limit. This relationship is explained by the chronic exposure to AF (Elzupir et al., 2015). Therefore, it could conceivably be hypothesised that the AF contaminated rice in rice-consuming countries is very important, regardless of the concentration. Recently, there has been a growing body of literature that recognises the importance of assessing the health risk of AF exposure (Elzupir and Alamer, 2014; Liu and Wu, 2010).

Riyadh, a capital of the Kingdom of Saudi Arabia (KSA), is one of the largest cities in the world and is densely populated with a population of 4.2 million in 2017 according to the General Authority for Statistics, KSA. Generally, rice is the main meal for its citizens. It is imported from several countries around the world, such as India, Pakistan, USA and Thailand. The value of the imports in 2014 is about 1.4 million MT of rice according to the Saudi Customs (2014). The most commonly consumed species are long grain yellow (LGY) and long grain white (LGW) as both of them used in Kabsa (the main dish for lunch and sometimes dinner), however short grain white rice (SGW) has little uses. In reviewing the literature, no data was found regarding AF levels in imported rice. Therefore, the HCC risk related to this exposure is unknown. The current study set out to determine the occurrence and health effects of AF in rice imported to the KSA, with a particular focus on the city of Riyadh as a case study.

2. Materials and methods

Sampling

The sample collection was designed to represent the different types of rice consumed in Riyadh in KSA. Accordingly, 41 samples (1-5 kg) were randomly collected from different retail shops and supermarkets in October and November 2015, and were kept at -20 °C until the time of analysis. The crops of the collected samples were produced in 2012-2014, and imported from different countries, including: India (10 samples), USA (9 samples), Thailand (5 samples), Pakistan (4 samples), Australia (4 samples), Italy (4 samples), Spain (2 samples), Egypt (2 samples) and Greece (1 sample). Samples included LGW, LGY and SGW.

Extraction procedure

AFs in rice samples were extracted using the BF method (the AOAC official method 970.45) as described previously (Elzupir et al., 2009) with some modification. In brief, the samples were subsampled to about 100 g using mixing and quartering techniques. The subsamples were grinded and 25 g was placed in a 250 ml volumetric flask. Then, 3 g sodium chloride and 100 ml of extraction solvent (methanol:water, 80:20, v/v) were added to the flask and shaken for 1 h. Subsequently, 50 ml of the filtrated extract and 30 ml hexane were added to a separation funnel, which was gently shaken by hand for approximately 30 s (three times), and then left to settle. The extract was collected into a new volumetric flask, and the hexane was discarded. Thereafter, 25 ml of water was added to the extract after it was cleaned up twice with 25 ml chloroform by the separation funnel. The collected chloroform extract was concentrated under a vacuumed rotary evaporator at 60 °C. The residue was transferred to a small vial, followed by 3-5 washes of the rotary flask with about 1 ml chloroform. The chloroform solvent was removed with a hotplate at 60 °C under a fume hood.

Derivatisation

For derivatisation, 150 µl trifluoroacetic acid was added to the dry film, shaken for seconds and left for 30 minutes. Then, 850 µl of an acetonitrile solution (acetonitrile: water 1:9 v/v) was added to the film for high performance liquid chromatography (HPLC) analysis.

Separation and quantification

Separation of the AF was performed by HPLC with Flexar fluorescence detector, Flexar LC pump (N291-0401), FlexarLC solvent manager, 3-channel vacuum degasser (N260-0581) and Flexar LC autosampler equipped with a 100-μl loop (Perkin Elmer, San Francisco, CA, USA). The chromatographic column used was a reverse phase Brownlee validated C18 (150×4.6 mm i.d. and 5 μm particle size; Perkin Elmer). Excitation and emission wavelengths were set at 360 and 440 nm, respectively, by using the super high sensitivity mode in the fluorescence detector. The isocratic mobile phase was water:methanol:acetonitrile (60:20:20, v/v/v) set to run at a flow rate of 1 ml/min. The injection volume was 20 µl.

For quantification, standard concentrations of AFB$_1$, AFG$_1$, AFB$_2$ and AFG$_2$ at 0.98, 0.88, 0.31, and 0.32 µg/ml, respectively, were obtained from Supelco (Bellevfote, PA, USA). Standard concentrations of AF used for calibration were prepared after derivatisation to be: 0.0198, 0.0594, 0.0999 and 0.198 ng per 20 µl for AFG$_1$: 0.0176, 0.0528, 0.088 and 0.176 ng per 20 µl for AFB$_1$: 0.0064, 0.0192, 0.032 and 0.064 ng per 20 µl for AFG$_2$: and 0.0062, 0.0186, 0.031 and 0.062 ng per 20 µl for AFB$_2$. The correlation coefficient (linear regression) was >0.99 for each AF standard. The limit of detection (LOD) of each AF was determined based on the signal to noise ratio of 3:1 and was equal to 0.032, 0.020, 0.016 and 0.008 µg/kg for AFG$_1$, AFB$_1$, AFG$_2$, and AFB$_2$, respectively. As that, the limit of quantification (LOQ) was equal to 0.106, 0.066, 0.053 and 0.026 µg/kg (Figure 1), and the recovery rate for different concentrations was found to be 87±3%, 96±15%, 87±8%, 85±5% for the corresponding AFs.
The daily intake of AF in rice and the associated cancer risk were calculated as previously described (Elzupir and Alamer, 2014; Liu and Wu, 2010). The average consumption of rice was reported to be 144 g per person per day in 2016 as documented by the United States Department of Agriculture (USDA, 2016), and the average body weight per person was 60 kg. A prevalence percentage of hepatitis B virus (HBV) of 8.3% (Al-Wayli, 2009) was used to assess the chronic risk for a total population of 30.7 million in 2016, according to the General Authority for Statistics, KSA. The

### Assessment of chronic exposure and cancer risk

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HCC risk was estimated to be $0.01/10^5$/year/ng AFB$_1$/kg body weight for those HBV negative and $0.3/10^5$/year/ng AFB$_1$/kg body weight for those HBV positive (WHO, 1999).

Recently, Joint FAO/WHO Expert Committee on Food Additives (JEFCA) and European Food Safety Authority (EFSA) recommended the use of the margin of exposure (MOE) to determine the risk of substances which are both genotoxic and carcinogenic. The MOE is known as the ratio between the no-observed-adverse-effect level or the benchmark dose lower confidence limit that causes a 10% increase in tumour formation in the fisher rat (BMLD$_{10}$), and the estimated exposure dose or concentration (WHO, 2009). The BMLD$_{10}$ of developing liver cancer is 250 ng/kg body weight (bw)/day, as estimated for AFB$_1$ (Benford et al., 2010a).

$$\text{MOE} = \frac{\text{BMLD}_{10}}{\text{Total intake}}$$

### 3. Results and discussion

The results showed that the prevalence of AF in imported rice is up to 68% with a contamination range between 0.07-7.09 µg/kg and an average concentration of 1.45±1.57 µg/kg. AFG$_1$ had the highest value of contamination, with a concentration range 2.13-3.22 µg/kg, followed by AFB$_2$ (0.07-3.98 µg/kg), AFG$_2$ (0.13-1.79 µg/kg), and AFB$_1$ (0.08-0.2 µg/kg) (Table 1). Table 2 shows the AF distribution with particular attention to rice variety. The LGW showed the lowest value of contamination with a concentration range of (0.07-2.53 µg/kg) followed by LGY (0.19-3.86 µg/kg) and SGW (2.13-7.09 µg/kg). The concentration of AFG$_1$ was not expected as obtained, because AFB$_1$ is usually the most common in nature. This may be due to special chemical treatments for AFB$_1$ prior to introduction the rice on the market, or another factor/factors.

In this study, the results were below the legal limit prescribed by the KSA regulation of 20 µg/kg (GSO, 1997), and only 2.4% had AF level higher than the EU legislation of 4 µg/kg. That is not unexpected as the ability of rice to be contaminated with AF is less than that of other crops such as oil seeds. A possible explanation for this might be differences in the crop substrate, pH value, presence of competitive organisms and/or moisture content. Although these grains are kept at room temperature (24-27 °C), they are kept in a relatively dry environment. This low relative humidity can limit the secretion of aflatoxin. Aflatoxin is known to be excreted at a temperature between 15-30 °C and relatively high humidity (Diener and Davis, 1967). Further, the obtained average concentration is less than that mentioned in the literature reviews of Ivory Coast, Nigeria, Brazil, Vietnam, Pakistan, Korea and the UK for AF in rice samples (Almeida et al., 2012; Iqbal et al., 2012; Makun et al., 2011; Nguyen et al., 2007; Park et al., 2004; Ruadrew et al., 2013; Sangare-Tigori et al., 2006), and is in agreement with those obtained in Canada, Austria, Malaysia, Iran and the Philippines (Bansal et al., 2011; Mazaheri, 2009; Reiter et al., 2010; Reddy et al., 2011; Sales and Yoshizawa, 2005).

Regardless of AF levels and if they are within the permissible limit, AF can still pose a health risk. The calculated HCC potency revealed that the risk per 10$^5$ individuals per year was found to be 0.04 for HBsAg- and 1.04 for HBsAg+. The risk for the total population is 9.8 for HBsAg- and 26.65 for HBsAg+. AFG$_1$ was found to have high concentrations relative to AFB$_1$, which was detected at low concentrations. The order of potency of total AF in rice with a particular

### Table 1. Aflatoxin levels (µg/kg), probable daily intake (PDI) (ng/kg body weight/day), hepatocellular carcinoma (HCC) cases risk, and the margin of exposure (MOE) attributed to different type of aflatoxin in rice imported to Riyadh, Kingdom of Saudi Arabia.$^1$

<table>
<thead>
<tr>
<th>Statistic</th>
<th>AFG$_1$</th>
<th>AFB$_1$</th>
<th>AFG$_2$</th>
<th>AFB$_2$</th>
<th>Total aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of detected samples</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>% of contamination</td>
<td>24</td>
<td>15</td>
<td>39</td>
<td>41</td>
<td>68</td>
</tr>
<tr>
<td>Minimum value</td>
<td>2.13</td>
<td>0.08</td>
<td>0.13</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Maximum value</td>
<td>3.22</td>
<td>0.20</td>
<td>1.79</td>
<td>3.98</td>
<td>7.09</td>
</tr>
<tr>
<td>Average value</td>
<td>2.55</td>
<td>0.16</td>
<td>0.42</td>
<td>0.43</td>
<td>1.45</td>
</tr>
<tr>
<td>Median value</td>
<td>2.42</td>
<td>0.17</td>
<td>0.30</td>
<td>0.18</td>
<td>0.65</td>
</tr>
<tr>
<td>STD</td>
<td>0.41</td>
<td>0.05</td>
<td>0.40</td>
<td>0.93</td>
<td>1.57</td>
</tr>
<tr>
<td>PDI</td>
<td>6.12</td>
<td>0.38</td>
<td>1.01</td>
<td>1.03</td>
<td>3.48</td>
</tr>
<tr>
<td>HCC cases for HBsAg+/$10^5$ individuals</td>
<td>1.84</td>
<td>0.11</td>
<td>0.30</td>
<td>0.31</td>
<td>1.04</td>
</tr>
<tr>
<td>HCC cases for HBsAg-/$10^5$ individuals</td>
<td>0.06</td>
<td>0.004</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>MOE (BMLD$_{10}$ = 250 ng/kg bw/d)</td>
<td>41</td>
<td>658</td>
<td>248</td>
<td>242</td>
<td>72</td>
</tr>
</tbody>
</table>

$^1$ AFB$_1$ = aflatoxin B$_1$; AFB$_2$ = aflatoxin B$_2$; AFG$_1$ = aflatoxin G$_1$; AFG$_2$ = aflatoxin G$_2$; total aflatoxin = AFB$_1$ + AFB$_2$ + AFG$_1$ + AFG$_2$; HBsAg = hepatitis B virus surface antigen; STD = standard deviation.
focus on their variety is SGW, LGY, and LGW (Table 1 and Table 2).

The range of the MOE gives a good an indication of the level of concern. The larger the MOE, the smaller the potential risk and vice versa. However, it cannot be used to quantify risk (Benford et al., 2010b). In 2005, the EFSA suggested 10,000 or more as the limit of safe exposure (EFSA, 2005); however, along with WHO and the International Life Sciences Institute, European Branch, the EFSA recommended adopting specific MOE values. Based on the study by Wogan et al. (1974), the MOE of AFB1 was estimated to range from 100 to 600 (Benford et al., 2010a). In the present investigation a MOE of 868 was found for AFB1, which indicated safe exposure. However, the MOE of 72 for the total AF level is far below the accepted limit of 600. These results support the hypothesis that a high consumption of rice can be an important source of chronic exposure to aflatoxins.

Table 2. Probable daily intake (PDI) (ng/kg body weight/day), hepatocellular carcinoma (HCC) cases risk, and the margin of exposure (MOE) of total aflatoxin (µg/kg) in imported rice samples in Riyadh, Kingdom of Saudi Arabia, with a particular focus to rice variety.1,2

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Long grain-yellow rice</th>
<th>Short grain-white rice</th>
<th>Long grain-white rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>13</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Number of detected samples</td>
<td>10</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>% of contamination3</td>
<td>24</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>% of contamination4</td>
<td>77</td>
<td>42</td>
<td>80</td>
</tr>
<tr>
<td>Minimum value</td>
<td>0.19</td>
<td>2.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Maximum value</td>
<td>3.86</td>
<td>7.09</td>
<td>2.53</td>
</tr>
<tr>
<td>Average value</td>
<td>1.36</td>
<td>3.34</td>
<td>0.59</td>
</tr>
<tr>
<td>Median value</td>
<td>0.77</td>
<td>2.34</td>
<td>0.49</td>
</tr>
<tr>
<td>STD</td>
<td>1.22</td>
<td>2.13</td>
<td>0.67</td>
</tr>
<tr>
<td>PDI</td>
<td>3.26</td>
<td>8.02</td>
<td>1.42</td>
</tr>
<tr>
<td>HCC cases for HBsAg+/105 individuals</td>
<td>0.03</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>HCC cases for HBsAg+&lt;105 individuals</td>
<td>0.98</td>
<td>2.40</td>
<td>0.42</td>
</tr>
<tr>
<td>MOE (BMDL50 = 250 ng/kg bw/d)</td>
<td>77</td>
<td>31</td>
<td>177</td>
</tr>
</tbody>
</table>

1 HBsAg = hepatitis B virus surface antigen; STD = standard deviation.
2 Only one long grain-brown rice sample is not included: in this sample AFB1 and AFG1 were detected with combined concentration of 0.096 µg/kg.
3 This with regard to all collected rice samples.
4 This regarding to rice variety (according to a shape and/or colour).

4. Conclusions

To conclude, we investigated AF in imported rice in Riyadh, KSA. The AF levels were found to be less than the legal limit of 20 µg/kg. However, the quantification of HCC risks and the MOE has conveyed awareness to handlers and specialists. This study suggests the importance of reconsidering the legal limit of AF in rice in the KSA as well as enacting specific legislation. According to our best knowledge, this is the first study regarding AF occurrence in imported rice in KSA and related health risk assessment. We recommend further studies on aflatoxin in rice and other commodities, such as peanuts and nuts, and to what extent they affect the health of citizens, especially in coastal cities such as Dammam and Jeddah, because the temperature and humidity in these areas is relatively high.

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