Risk Estimation of Hepatocellular Carcinoma due to Exposure to Aflatoxins in Maize from Yogyakarta, Indonesia

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HIGHLIGHTS
- Mean total Aflatoxins (AFs) in raw material, dried maize chips, and fried chips were 46.58, 17.58, as well as 13.24 ppb, respectively.
- The yearly maximum exposure to AFB1 ranged from 85.07 to 92.80 ng/kg body weight per day.
- The estimation of hepatocellular carcinoma (HCC) cases ranged from no case to 43 cases per year.
- Preventive hygienic efforts are needed to reduce AF contamination and risk of HCC in maize consumed in this region.

ABSTRACT

Background: Besides their mutagenic, carcinogenic, and teratogenic effects, Aflatoxins (AFs) also act as the main contributor to hepatocellular carcinoma (HCC) which is known as the most common primary liver cancer worldwide. The main aim of this study was risk estimation of HCC due to exposure to aflatoxins in maize from Yogyakarta, Indonesia.

Methods: As a model, maize samples were taken from Yogyakarta province, Indonesia. The present research was conducted by risk assessment approach which includes hazard identification, hazard characterization, exposure assessment, and risk characterization. The aflatoxin analysis was performed using High Performance Liquid Chromatography method and the secondary data was taken from the province report.

Results: The mean total AF in raw material, dried maize chips, and fried chips were 46.58, 17.58, and 13.24 ppb, respectively. The raw material clearly had higher level of all AF types. The mean of contamination of total AF in raw material (46.58 ppb) exceeded the standard level (20 ppb), while the other products were not more than standard level. The yearly maximum exposure to AFB1 ranged from 85.07 to 92.80 ng/kg body weight per day. The estimation of HCC cases ranged from no case to 43 cases per year.

Conclusion: Preventive hygienic efforts are needed to reduce AF contamination and risk of HCC in maize consumed in this region of Indonesia.

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climate change is the increase in the growth of toxigenic molds in maize. Toxigenic molds are dangerous because they produce hazardous mycotoxins. Toxigenic mold contamination can result in aflatoxin contamination. Therefore, it is necessary to increase the awareness of the government, because aflatoxin contamination in products would not only be detrimental to human health but also would result in huge economic losses (Gnonlonfin et al., 2013; Wu, 2015).

Aflatoxins (AFs) have been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (Liu and Wu, 2010). Besides their mutagenic, carcinogenic, and teratogenic effects, AFs also act as the main contributor to hepatocellular carcinoma (HCC) which is known as the most common primary liver cancer worldwide (Magnussen and Parsi, 2013).

The risk assessment approach has been widely used in an effort to estimate the harmful effects of AFs especially that come from maize products (Adetunji et al., 2017; Kamala et al., 2017; Vaghela and Afshari, 2017). This topic becomes important because the harmful effect of AFs could cause acute and chronic aflatoxicosis. The four main steps of food safety risk assessment are hazard identification, hazard characterization, exposure assessment, and risk characterization (Rahayu et al., 2004; WHO/FAO, 2009). Chemical risk assessment needs to be done in order to evaluate the possibility of health effects emergence after the body is exposed to chemical contaminants such as mycotoxin. Application of risk assessment can be used to estimate the magnitude of the risk that may arise. This strategic step is a prevention effort to food-borne diseases that can affect widely and spread repetitively (Wu and Guclu, 2012).

In Indonesia, the practical methodology of food-borne disease risk assessment was developed by the National Agency for Drug and Food Control. This responsibility is to assure the safety of the products available in the market so the consumers can gain safe and wholesome foods. The standard of maximum level for total AFs in maize as raw materials in Indonesia is 20 ppb (National Standardization Body, 2009). The main aim of this study was risk estimation of HCC due to exposure to aflatoxins in maize from Yogyakarta, Indonesia.

Materials and methods

Hazard identification

In the stage of hazard identification, sampling was done from March to December 2014 in four districts (Bantul, Kulonprogo, Sleman, and Gunung Kidul) of Yogyakarta province, Indonesia. Totally, 19 samples were obtained, consisting of 5 raw maize, 7 dried maize chips, and 7 fried maize chips. Samples of maize were taken from the whole line of the production process until the final products, the maize fried chips product. The AFB1 level contained in cooked material was used in the risk characterization.

AFs analysis

AFs analysis was performed using High Performance Liquid Chromatography (HPLC) method according to AOAC (2012). Each sample which consisted of 25 g of maize was crushed with a grinder. Then, the powdered samples were added by 5 g of NaCl and 125 ml of methanol: water (7:3 v/v) and blended in two min at high speed. This fluid was filtered with preloaded filter paper and its filtrate was drawn as much as 15 ml. After that, the filtrate was eluted with 30 ml of H2O and filtered using filter paper 24 cm (VICAM, USA). The extract was eluted again using glass microfiber paper <30 min. Next, 15 ml of the filtrate was put in the IAC column containing a monoclonal antibody specific to AFB1, AFB2, AFG1, and AFG2 to be purified. The column was washed with 20 ml of deionized water. Next, AFs was eluted from the column with one ml of methanol, and then the fraction was filtered using a membrane of 0.2 μm. The filtrate was collected in vials, stored at -18 to -20 °C for next analysis.

AFs concentration analysis phase began with the making of standard solutions series at various AFs levels. The standard solution stock of AFB1, AFB2, AFG1, and AFG2 was made by each concentration of 500, 125, 250, and 125 ng/ml. Each standard solution was injected into HPLC (by 3 repetitions) to obtain several peak areas. The standard curve was made by plotting the peak area to the concentration of AFB1, AFB2, AFG1, and AFG2. AFs concentration was calculated by following the formula:

\[ \text{AF concentration (ppb)} = \frac{\text{(concentration (ng/ml) on standard curve= methanol solution volume)}}{\text{1 mg sample}} \]

Hazard characterization

In AFs hazard characterization, toxic effects and its predefined threshold were reviewed (Adetunji et al., 2017). The identified hazards were hazards posed by the AFs to its consumer health. Data used was secondary data from the libraries of the FAO (2006) and JECFA (2007). The hazard characterization of AFs in food can be seen in Table 1.

Exposure assessment

The exposure from the consumption of AFs-contaminated maize products was obtained from primary
and secondary data. Primary data consisted of the level of hazard in the raw material and handling/storage condition. The data were used to track changes in the overall level of the food production chain. These data were obtained from interviews with managers of maize storage facilities. The secondary data, i.e. consumption patterns of the target population, were used to assess the exposure of hazard during a specific time period. The exposure assessment in this study was based only on AFB$_1$ exposure due to its properties as potent liver carcinogen and its very high potential to cause HCC in individuals (Wu et al., 2013). In addition, the other AFs concentrations were too small compared to AFB$_1$. Exposure assessment was calculated based on AFs concentration in maize and maize consumption rate in the area divided by the body weight of the average population. In this study, the assumption of public consumption was associated with the total production of maize divided by the population. The average-population body weight was assumed 60 kg. According to FAO/WHO (2009), the formula used in the calculation of the amount of exposure was as follows:

$$
\text{Exposure (ng/kg bw per day)} = \frac{\text{Concentration (ug/kg or pg)} \times \text{consumption (kg/person per day)} \times 1000}{\text{the average body weight of population (kg)}}
$$

Data on maize production and consumption from the local government in Yogyakarta can be seen in Table 2. The prediction of average maize consumption per person was quite high and it was around 220-240 g per person per day.

### Table 1: Characteristics of aflatoxins hazards

<table>
<thead>
<tr>
<th>Aflatoxicosis type</th>
<th>Risks for humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Anorexia due to impaired absorption and metabolism of nutrients</td>
</tr>
<tr>
<td></td>
<td>• Lethargy, vomiting, and stomachache</td>
</tr>
<tr>
<td></td>
<td>• Decrease in immunity causing inhibited growth on children</td>
</tr>
<tr>
<td></td>
<td>• Risk of hepatitis B</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Causing HCC or liver cancer. For patients with hepatitis, liver cancer risk can be increased up to more than 30 times</td>
</tr>
<tr>
<td></td>
<td>• Causing lung cancer</td>
</tr>
<tr>
<td></td>
<td>• Teratogenic, toxic to the fetus</td>
</tr>
<tr>
<td></td>
<td>• Cancer potential from specific epidemiological data of aflatoxin B1 (JECFA, 2007)</td>
</tr>
<tr>
<td></td>
<td>• HBsAg$: 0.3 cancer/year per 100000 population per ng AFB1/kg body weight per day (hepatitis B carrier)</td>
</tr>
<tr>
<td></td>
<td>• HBsAg$: 0.01 cancer/year per 100000 population per ng AFB1/kg body weight per day (non-hepatitis B carrier)</td>
</tr>
</tbody>
</table>

### Table 2: Maize production and consumption in Yogyakarta province, Indonesia

<table>
<thead>
<tr>
<th>Year</th>
<th>Total maize production (tons)*</th>
<th>Total population (persons)*</th>
<th>The average prediction of maize consumption per person per day (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>312236</td>
<td>3637116</td>
<td>0.24</td>
</tr>
<tr>
<td>2015</td>
<td>299084</td>
<td>3679176</td>
<td>0.22</td>
</tr>
<tr>
<td>2016</td>
<td>310257</td>
<td>3720912</td>
<td>0.23</td>
</tr>
<tr>
<td>2017</td>
<td>311764</td>
<td>3762167</td>
<td>0.23</td>
</tr>
</tbody>
</table>

References: * District Agricultural Agency (2017a,b); * Central Statistic Agency (2019)
Risk characterization

As the final stage of risk assessment, risk characterization is obtained based on the results of qualitative and quantitative calculations including the uncertainty of opportunities that occur and their severity. The results obtained at the risk characterization stage in the form of risk estimates for the healthy and vulnerable population. This study used level of exposure to cause a minimal effect. It means the Appropriate Level of Protection (ALOP) was set below the dose that may significantly endanger the health of people. Standards used were based on the “worst-case” exposure scenario to keep risks under ALOP (Hahn et al., 2010). Risk characterization was calculated based on the concentration of AFB1 in maize and maize consumption by people in the district where the samples were taken. The assumptions used were all maize productions were used for food and consumed by the public in this area. According to JECFA (2007), HCC potential can be calculated by as follows:

\[ \text{HCC} = \frac{\text{AFB1 concentration (ppb) \times body weight (kg) \times days per year}}{100000 \text{ population per ng AFB1/kg body weight per day}} \]

The calculation of risk, in particular, the estimated occurrence of HCC, was obtained by multiplying the value of the exposure of AFB1 to the potential of HCC cancer and to the population (Liu and Wu, 2010). The proportion of hepatitis B carrier was calculated based on the assumption of the prevalence of hepatitis B. Based on the basic health research 2013, the prevalence of hepatitis in Yogyakarta was 0.9% (Ministry of Health of Indonesia, 2013). It was assumed all hepatitis in Yogyakarta were hepatitis B.

Results

The mean total AFs in raw material, dried maize chips, and fried chips were 46.58, 17.58, and 13.24 ppb, respectively. The raw material clearly had higher level of all AFs types. The mean of contamination of total AFs in raw material (46.58 ppb) exceeded the standard level (20 ppb), while the other products were not more than standard level (Table 3).

The exposure assessment calculation results can be seen in Table 4. The yearly maximum exposure to AFB1, based on the highest AFB1 concentration, ranged from 85.07 to 92.80 ng/kg body weight per day. Based on the exposure of AFB1, the estimates of HCC were calculated (Table 5). The estimation of HCC cases ranged from no case to 43 cases per year in 2014-2017. The highest number of estimations happened in 2014 in which the total maize production was the highest over the examined years. The estimated number of HCC cases did not change (maximum at ±9%) in the studied years.

| Year | Lowest aflatoxin concentration (ppb) | Highest aflatoxin concentration (ppb) | Maximum exposure to aflatoxin B1 (ng/kg body weight per day) *
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>ND</td>
<td>23.20</td>
<td>88.93</td>
</tr>
<tr>
<td>2015</td>
<td>ND</td>
<td>23.20</td>
<td>85.07</td>
</tr>
<tr>
<td>2016</td>
<td>ND</td>
<td>23.20</td>
<td>88.93</td>
</tr>
<tr>
<td>2017</td>
<td>ND</td>
<td>23.20</td>
<td>88.93</td>
</tr>
</tbody>
</table>

*Bodyweight assumption was 60 kg per person; ND: Not Detected
Discussion

AFB₁, AFB₂, and AFG₁ were detected in some raw material, dried maize chips, and fried maize chips samples in the current survey. Although, some samples were AFs-negative, the level of AFB₁ in some raw material samples was quite high and exceeded the standard of maximum level. The high contamination of AFs in maize in Yogyakarta, Indonesia is probably linked to temperature and humidity of this region which are favorable for growth of AFs-producing moulds. The average temperature and humidity in the sampling area is approximately 23-32 °C and 56-94%. In addition, it has been proved that there is a positive correlation between Aspergillus flavus and AFB₁ level in maize, because this AFs-producing mould has enough capability to survive and grow in maize. Temperature and humidity are critical factors that can affect the growth of A. flavus to produce AFs. It is known that the optimum conditions for the formation of AFs by the moulds occur at 30 °C and 90% humidity (Pratiwi et al., 2015).

The mean level of AFB₁ (41 ppb) in raw maize in Yogyakarta, Indonesia was quite similar to that of found by Ahsan et al. (2010) in maize grains of Punjab, Pakistan (45 ppb). Another survey in Serbia revealed that AFs was present in 137 out of 200 (68.5%) of maize samples ranging from 1.01 to 86.1 ppb with the mean level of 36.3 ppb (Kos et al., 2013). A higher level of AFB₁ was found in maize obtained from Eastern Croatia, when its mean value reached to 81 ppb with the maximum value of 2072 ppb found in. The contamination might have arisen due to extremely hot and dry weather from May to September during the maize growth and harvesting period Eastern Croatia (Pleadin et al., 2014).

The high AFs contamination in maize leads to higher exposure of AFs to humans. The AFB₁ exposure in Yogyakarta province on this study was estimated as 89-93 ng/kg body weight per day which was much higher than those of Serbia, Croatia, and Greece that only had 0.44-5.59 ng total AFs/kg body weight per day (Udovicki et al., 2019). The study of estimated AFs exposure for infants aged between 6 and 12 months in Tanzania also showed low concentration as 0.017 ng/kg body weight per day (Kamala et al., 2017). Nevertheless, much higher AFB₁ exposure was reported in Nigeria, when its national exposure reached 1976.1 and 4742.7 ng/kg body weight per day for adult and children, respectively (Adetunji et al., 2017).

Several studies confirmed that AFB₁ exposure can increase the risk of HCC (Chu et al., 2017; Kitaya et al., 2010; Lizárraga-Paulín et al., 2011; Wang et al., 2001). The current research showed that there was a direct relationship between consumption rate of maize and estimated risk of HCC. These estimated risk in Yogyakarta ranged from no case to 43 cases per year for around 3.7 million people. Nevertheless, the proportion of estimated risk of HCC related to maize consumption rate in Indonesia was quite higher than the mean estimated cases of HCC in the other Asian countries (Vaghela and Afshari, 2017) such as China (4545 cases for 1371.2 million people), Philippines (854 cases for 100.7 million people), Republic of Korea (14 cases for 50.6 million people), and Malaysia (13 cases for 30.3 million people).

Based on the risk characteristics that have been described, it is known that the presence of AFs in maize and its processed products does not come along processing but from post-harvest handling. This statement is supported by the fact that maize-processed products have lower AFs levels compared to raw material, although there is no process that can reduce levels of AFs. In addition, AFs contamination may be derived from the initial harvest process which was not handled properly. According to Kusumaningrum et al. (2010), there was no correlation between A. flavus and AFs levels in the processed product. Therefore, it is necessary to control the presence of AFs from the first collector or dealer, where the highest contamination is found.

Conclusion

Preventive hygienic efforts are needed to reduce AFs contamination and risk of HCC in maize consumed in Yogyakarta, Indonesia. It is proposed to do more studies in future in order to acquire detailed up-to-date data about the other possible chemical hazards in foods affecting incidence of HCC.

Author contributions

W.P.R. designed the study; D.H., W.B., and S.A. conducted the experimental work; D.H., N.I., as well as W.A. analyzed the data; W.P.R. and W.A. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

All the authors stated that none of them had conflict of interest in this study.

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