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Total Aflatoxin Contamination Level in Flour in Mongolia and Aflatoxin Intake

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Objectives: This study aimed to assess the total aflatoxin contamination level in flour, flour storage conditions, and the aflatoxin intake in Mongolia. **Methods:** A total of 71 samples of flour were randomly collected from the survey area in Ulaanbaatar, the capital city of Mongolia, from March to December 2015. An ELISA test was used for detection the total aflatoxins B_1 + B_2. The relative humidity and temperatures of the storage areas were measured at the time of sampling the flour. The personal daily intake of aflatoxin was estimated. **Results:** The survey found that 43.7% of all analyzed flour samples had detectable aflatoxin at 0.04 μg kg^{-1} and 0.05 μg kg^{-1}. The mean relative humidity and temperatures of the storage areas were higher than the recommended level. The aflatoxin intakes were 0.1271 μg kg^{-1} body weight day^{-1} and 0.3615 μg kg^{-1} body weight day^{-1} as calculated from the mean daily flour intake and the 95th percentile intake, respectively. **Conclusion:** The potential health hazard associated with aflatoxin in flour was not determined as a serious threat in Mongolia, but the storage conditions of flour may encourage the growth of fungi-producing aflatoxins. Thus, a national strategy for the elimination of aflatoxin in foods is needed in Mongolia.

Keywords: Fungi, Mycotoxins, *Aspergillus flavus,* Aflatoxins, Carcinogens

Introduction

Mycotoxins are toxic substances produced as secondary metabolites by fungi. According to the Food and Agricultural Organization (FAO) of the United Nations, up to 25% of the world's food crops have been estimated to be significantly contaminated with mycotoxins [1]. Aflatoxins are a type of mycotoxin produced by the *Aspergillus* species of fungi, such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* [2, 3]. Aflatoxins are classified into subtypes, which are B_1, B_2, G_1 and G_2 [4]. Aflatoxins B_1 and B_2 are produced by both *Aspergillus flavus* and *Aspergillus*

parasiticus and aflatoxins G_1 and G_2 are produced exclusively by *Aspergillus parasiticus* [5]. Aflatoxin B_1 is a toxic and potent carcinogen which has been correlated to liver cancer in many animals [6], and it has been classified as a group I carcinogen by the International Agency of Research on Cancer [7]. The growth of *Aspergillus flavus* and *Aspergillus parasiticus* and their aflatoxin-producing activity are favored by high humidity (>85%) and high temperature (>25°C) in cereals during their storage period [8]. Some researchers suggested that the synthesis of mycotoxins is highest when the humidity is above 13% and when the temperature is between 24 °C and 37 °C in foods [9].

There are three types of food contamination with mycotoxins. Primary pollution of agricultural products occurs in the field during vegetation, after harvesting and during storage time. Secondary pollution can occur during processing in poor sanitary conditions. The third type of pollution can occur as mycotoxins exert residual effects as a result of feeding animals by fodder containing mycotoxins [10]. During the post-harvest stage, proliferation of aflatoxin can be exacerbated in susceptible commodities under storage conditions such as hot and humid storage environment [11]. Aflatoxins frequently contaminate food, especially cereals and cereal products [9, 12]. Wheat flour is a powder made from the grinding of wheat and all its products can be contaminated by molds at all phases of the production chain [13]. Researchers have found high levels of mycotoxin-producing fungi in grain and flour during storage time and at the mechanical cleaning point in flour manufacture [14].

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Aflatoxin can cause both acute and chronic toxicity. High-level aflatoxin exposure produces an acute hepatic necrosis, cirrhosis and failure of liver function [15]. Chronic toxicity is more important from a food safety standpoint. Ingestion of low levels over long periods has been implicated in primary liver cancer [15 -17]. Chronic aflatoxicosis in humans as well as primary liver cancer have been reported in many Asian countries. In a recent study in China, Li et al. found that the levels of aflatoxins B_1, B_2, and G_1 were significantly higher in corn from the area of high human hepatocellular carcinoma incidence and the average daily intake of the aflatoxin B_1 from the high-risk area was 184.1 μg [15]. Jager et al. estimated the degree of exposure to aflatoxins based on the consumption of foodstuffs contaminated with aflatoxins and on the average concentration level of aflatoxins [18].

Such a risk assessment survey on aflatoxin level in foods and dietary exposure to total aflatoxin from food consumption has never been conducted in Mongolia. Thus, we aimed to carry out a monitoring survey on the aflatoxin contamination level in flour and to assess aflatoxin intake from flour based on data from Ulaanbaatar. Our specific aims were: (1) to determine the contamination level of the total aflatoxins (B_1 + B_2) in flour samples, (2) to study the environmental storage conditions of flour in food markets, (3) to study the flour consumption in individuals, (4) to establish the total aflatoxin intake in individuals from flour consumption.

Materials and Methods

2. Sampling design

2.1 Primary sampling design

This cross-sectional survey was conducted in seven districts of Ulaanbaatar, the capital city of Mongolia, from March to December 2015. A total of 27 high-risk markets including hypermarkets, supermarkets, food department stores and bazaars were randomly selected in this survey.

2.2 Secondary sampling design

A total of 71 samples of various flour types were randomly collected from the hypermarkets, supermarkets, food department stores and bazaars. All sampling design was performed according to the Commission Regulation (EC) 401/2006 [19]. Sampling was carried out in a way that ensured the analytical sample truly represented the lot of the consignment under investigation. The lot weights of the consignments of flour were between 0.05 and 500 tons in each field in our survey. Here, the numbers of samples taken for each lot was between 3 and 5. The aggregate sample weight ranged from 1.0 to 10.0 kg.

All samples were packed into polyethylene bags and taken to the National Reference Laboratory, General Agency for Specialized Inspection, Ulaanbaatar, Mongolia and kept in the refrigerator until analysis. All samples were analyzed within 10 to 60 days after collection.

3. Assessment of environmental storage conditions of flour in trade markets

To determine the effect of environmental factors in aflatoxin

formation, characteristics of flour storage conditions including temperature and relative humidity were measured and recorded during sampling in each survey field. A digital thermometer and humidity meter (DT-625, CEM, China) was used for the measurements.

4. Laboratory methods

4.1 Sample preparation

An enzyme-linked immunosorbent assay (ELISA) test was used for the quantitative analysis of the total aflatoxin detection according to the Mongolian National Standards (MNS:6044:2009) and the Official Methods of Analysis (AOAC International) method with certain modifications [20, 21].

To 2.0 g of the ground flour sample was added 10 mL of a methanol:distilled water solution (70:30, v/v) and was mixed for 10 minutes at room temperature (20 to 25 ºC). The extract was subsequently centrifuged at room temperature for 10 minutes. The extract was filtered through filter paper and 100 µL of the filtrate was added to 600 µL of phosphate-buffered saline (R-Biopharm, Germany) and centrifuged. Then 50.0 µL of enzyme conjugate (R-Biopharm) and 50.0 µL anti-aflatoxin antibodies (R-Biopharm) were added to a 50.0-µL extract aliquot and incubated at room temperature for 30 minutes, then washed twice with distilled water. After washing, 50.0 µL of substrate solution and 50.0 µL of chromogen solution (Red Chromogen Pro, R-Biopharm) were added to the extract and mixed, incubated at room temperature for 30 minutes, and then the stop solution was added to it (Stop Reagent, R-Biopharm). The extract was applied to the immunoaffinity column and allowed to flow at a rate of 1 mL per minute. The eluate was collected in a vial, filtered and placed in the ELISA reader.

4.2 Sample analysis

Total aflatoxin (B_1 + B_2) was measured at 450 nm in the Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, USA). The instrument was calibrated with a single aflatoxin standard solution RIDACREEN Aflatoxin Total (R-Biopharm). The limit of detection (LOD) and quantification (LOQ) were calculated for the method of analysis as 3 times and 10 times the noise, respectively. The LOD was 0.04 µg kg^{-1}. The LOQ was 1.75 µg kg^{-1}. Test implementation time was 45 minutes.

5. Food frequency questionnaire

In order to assess the total aflatoxin intake from daily flour consumption, information from 97 individuals who participated in this survey was collected through a food frequency questionnaire. Portion sizes of flour were chosen by the participants according to the usual measures of cups and tablespoons in order to describe their daily flour intake.

The body weight of each individual was recorded to calculate the probably daily intake (PDI) value by the following expression: PDI = (mean concentration of aflatoxins x mean consumption of food type)/(each individual body weight). The PDI was calculated based only on aflatoxin exposure from flour and no other foodstuffs. PDI is expressed as µg kg^{-1} of body weight day^{-1}. The degree of exposure to aflatoxin was calculated based on the amount of flour likely to be contaminated with aflatoxin and on the average concentration level of aflatoxin, a method previously used by other researchers [18].

6. Statistical analysis

SPSS version 21 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Frequency statistic analysis was used to calculate the mean levels of the aflatoxins in flour samples. A one-way ANOVA test was used to compare the mean levels of aflatoxins and the mean values of the storage relative humidity and temperatures of flour samples. The data of the daily intake of flour and the PDI of aflatoxin from flour consumption were analyzed using bootstrapping.

7. Ethical considerations

The survey methodology was discussed by the Ethical Committee under the auspices of the Ministry of Health on December 1, 2014 and was granted approval. Written and oral informed consent was obtained from the heads and quality managers of the hypermarkets, supermarkets, food department stores, bazaars and all individual participants randomly selected to participate in the survey.

Results

1. Total aflatoxin contamination level in flour

Total aflatoxin (B_1 + B_2) was determined in the 71 collected flour samples. Frequency statistics analysis showed that 43.7% of all analyzed flour samples had positive detection of total aflatoxin

Table 1. Prevalence of total aflatoxin ($B_1 + B_2$) in flour

Sample type	Number of analyzed samples	Number of samples positive for total aflatoxin	Percentage of samples positive for total aflatoxin	Mean concentration (μg kg^{-1}) of total aflatoxin in all analyzed samples[a]	Mean concentration (μg kg^{-1}) of total aflatoxin in positive samples
Flour "Ulaanbaatar"	5	3	60.0	0.03	0.05
Flour "Altantaria"	6	4	66.7	0.03	0.04
Flour "Millhouse"	12	4	33.3	0.01	0.04
Flour "Khyrakh"	5	2	40.0	0.02	0.05
Flour "Aliaca"	6	4	66.7	0.03	0.05
Flour "Tsesna"	14	9	64.3	0.03	0.05
Rye	10	5	50.0	0.02	0.04
Barley	13	0	0	0	0
Total	71	31	43.7	0.02	0.046

[a]Total aflatoxin concentration values below the LOD were replaced by zero

and the concentrations ranged from 0.04 μg kg^{-1} to 0.05 μg kg^{-1}. By the descriptive statistics analysis, the prevalence of the flour samples containing aflatoxin ranged from 33.3% to 66.7% in analyzed samples of the "Millhouse", "Khyrakh", rye, "Ulaanbaatar", "Tsesna", "Aliaca" and "Altantaria" samples (Table 1). Barley had no detectable aflatoxin. The prevalence of samples with the total detectable aflatoxin was significantly lower in "Millhouse" flour samples than the other flour samples ($p < 0.02$). The prevalence of flour samples with detectable total aflatoxin was higher in samples of flour "Aliaca", "Altantaria" and "Tsesna" ($p < 0.01$).

The mean concentrations of the total aflatoxin were 0.05 μg kg^{-1} in positive flour samples "Ulaanbaatar", "Khyrakh", "Aliaca", "Tsesna", while positive samples of rye, "Altantaria", and "Millhouse" had 0.04 μg kg^{-1}. A one-way ANOVA with Tukey's honest significant difference test showed that there were no significant differences between the mean concentrations of total aflatoxin in positive flour samples ($F = 1.020$, $p = >0.05$).

2. Assessment of environmental storage conditions of flour in trade markets

Frequency statistics analysis showed that the mean values of the storage relative humidity ranged from 24.4 to 58.0% in flour in trade markets and those parameters were higher than the recommended storage humidity (<13%). Furthermore, the mean values of the storage temperatures ranged from 16.3 to 33.0 °C at the time of sampling and these parameters were higher than the recommended storage temperature (<24°C) in the flour "Millhouse", rye and barley.

The mean values of the storage time of flours ranged from 1.6 to 10.6 months. A one-way ANOVA test showed that the mean values of the storage duration were significantly longer for the flour "Millhouse", "Aliaca" and rye than other types of flour ($F = 3.082$, $p < 0.001$). Statistically significant associations were not found between the prevalence of positive samples and the environmental storage conditions of flour.

3. Estimation of flour consumption

The food frequency questionnaire was administered to 97 subjects, of which 86 were women (88.7%) and 11 were men (11.3%) with ages ranging from 17 to 61 years old. A total of 16 subjects did not consume flour, leaving 81 subjects. Bootstrap statistics showed that the low intake (mean) and high intake (95^{th} percentile) of flour were 170 and 500 g day^{-1} for females, respectively and 217 and 300 g day^{-1} in males, respectively (Table 2).

4. Assessment of aflatoxin intake

The PDI of the aflatoxins from daily low intake (mean) and high intake (95^{th} percentile) of flour were 0.1253 μg kg^{-1} body weight day^{-1} and 0.3686 μg kg^{-1} body weight day^{-1} in studied females, respectively, and 0.1364 μg kg^{-1} body weight day^{-1} and 0.1885 body weight μg kg^{-1} in studied males, respectively (Table 3). The mean of body weight was 62.4 kg in females and 73.2 kg in males.

Table 2. Flour consumption in individuals

Number of all individuals who consumed flour	Percent of individuals who consumed flour	Flour consumption (g day^{-1})			
		Female (n = 71)		Male (n = 10)	
		Low intake[a]	High intake[b]	Low intake[a]	High intake[b]
81	83.5	170	500	217	300

[a]Mean [b]95th percentile

Table 3. Probable daily intake (µg kg $^{-1}$ body weight day $^{-1}$) of aflatoxins from flour

Female (n = 71)		Male (n = 10)		Total (n = 81)	
Low intake[a]	High intake[b]	Low intake[a]	High intake[b]	Low intake[a]	High intake[b]
0.1253	0.3686	0.1364	0.1885	0.1271	0.3615

[a]Mean [b]95th percentile

Discussion

This is the first survey to assess the total aflatoxin (B_1 + B_2) concentrations in flour in Mongolia and the total aflatoxin intake through flour consumption in Mongolian people. Also, the survey findings provided new information on the environmental storage conditions of flour in Mongolia. In this survey, the prevalence of flour samples with detectable total aflatoxin in flour was 43.7%. The result of a study Tamil Nadu, India showed that the prevalence of flour samples contaminated with aflatoxin B_1 was 68.2% in analyzed samples of flour [9]. Furthermore, the results of a study of Golestan Province, Northeast Iran showed that the prevalence of the total aflatoxin G_2 + G_1 + B_2 + B_1 in wheat flour in winter and summer were about 99% and 70%, respectively [25]. In Malaysia, 22.85% of flour samples were contaminated with aflatoxin, of which aflatoxin B_1, B_2, G_1, G_2 were detected in 1.2%, 4.8%, 3.6%, 13.25% of samples, respectively [26]. Prevalence varies by country since a different number of aflatoxins were measured and different methods were used for their determination, therefore direct comparisons cannot be made between them.

In our survey, the total aflatoxin concentration levels in positive samples ranged from 0.04 µg kg^{-1} to 0.05 µg kg^{-1}. The limits for aflatoxins may be controlled as the total aflatoxins referring to the sum of aflatoxin B_1 + B_2 + G_1 + G_2 or B_1+ B_2 [23]. With regards to the total aflatoxins, the European Union Limit for food other than for infants is 4 µg kg^{-1} for cereals and products derived from cereals, including processed cereal products [23]. The concentrations found in our study were below this limit, although the concentrations might have been higher had aflatoxin G_1 and G_2 been included in our analyses.

The total aflatoxin concentration in positive flour samples from our study was 0.046 µg kg^{-1} overall, which is lower than the aflatoxin contamination levels in flour in other countries. For example, a study in Iran found the mean concentration of the total aflatoxins in flour samples to be 0.99 µg kg^{-1} and 0.82 µg kg^{-1} in winter and summer, respectively [25]. In another study, total aflatoxin and aflatoxin B_1 levels in wheat flour samples ranged between 1.3-7.1 µg kg^{-1} and 1.36-1.78 µg kg^{-1}, respectively [27]. Zinedine et al. reported the mean level of total aflatoxin and aflatoxin B_1 in wheat flour as 0.07 µg kg^{-1} and 0.07 µg kg^{-1}, respectively [28]. Thus, the finding of our survey indicated that the level of the total aflatoxin in flour in Mongolia was low, but could have been higher had aflatoxin G_1 and G_2 been included in our analyses.

Many researchers suggest that long-term human exposure to low level of aflatoxin increases the risk of liver cancer. This is a particularly serious problem in areas where hepatitis B is prevalent. Aflatoxins and hepatitis B have been seen to have synergistic effects on the development of liver cancer. Kuiper-Goodman suggested that the provisional maximum tolerable daily intake for aflatoxin (PMTDI) cannot be greater than 1.0

μg kg^{-1} body weight day^{-1} for adults and children without hepatitis B virus, and 0.4 μg kg^{-1} body weight day^{-1} for adults carrying the hepatitis B virus, respectively [24]. Then by the result of our survey, the PDI of the total aflatoxins from daily high consumption of flour was 0.3615 μg kg^{-1} body weight day^{-1} in all studied subjects, which is close to the provisional maximum tolerable daily intake for aflatoxin in adults with hepatitis B virus.

Higher and lower PDI values than those found in the current study have been reported in surveys worldwide. Among much lower aflatoxin intakes are the data reported in France by Leblanc et al. where values of PDI were 0.117 ng kg^{-1} body weight day^{-1} (0.000117 μg kg^{-1} body weight day^{-1}) and 0.345 ng kg^{-1} body weight day^{-1} (0.000345 μg kg^{-1} body weight day^{-1}) for mean and high adult consumers, respectively [29]. Other lower PDI values of 0.15 ng kg^{-1} body weight day^{-1} (0.00015 μg kg^{-1} body weight day^{-1}) and 0.26 ng kg^{-1} body weight day^{-1} (0.00026 μg kg^{-1} body weight day^{-1}) were reported in Australia and the United States of America, respectively [30]. Also, the recent evaluation conducted in Spain found a PDI of 0.072 ±0.167 ng kg^{-1} body weight day^{-1} (0.000072 ±0.000167 μg kg^{-1} body weight day^{-1}) for adult males and 0.077 ± 0.208 ng kg^{-1} body weight day^{-1} (0.000077 ±0.000208 μg kg^{-1} body weight day^{-1}) for adult females [31]. The highest PDI of aflatoxins found in the literature was in China with values ranging up to 91 ng kg^{-1} body weight day^{-1} (0.091 μg kg^{-1} body weight day^{-1}) [30]. Thus, the finding of our survey (0.3615 μg kg^{-1} body weight day^{-1}) indicated that the total aflatoxin intake through flour consumption was higher than the PDI of aflatoxins in all the other studied countries.

In our survey, flour was stored at the temperature of 16.3 to 33.0 °C and storage air humidity was 24.4 to 58.0% in trade markets. Those parameters do not meet standard requirements for the storage of grain in optimum conditions. *Aspergillus* species can grow at the temperatures between 12 and 39 °C [32, 33]. Furthermore, the results of another study indicated that storage at a temperature of 5 °C decreased the population and types of molds on wheat flour [13]. Our study also found that the mean values of the storage time of flours ranged from 1.6 to 10.6 months. Al-Defiery and Merjan detected *Aspergillus flavus* in wheat flour samples after 3 months of storage at 10.7% humidity [13]. Therefore, the environmental storage conditions of flour meet the favorable condition of growth of fungi-producing aflatoxins in Mongolia. Further study and a national strategy for the elimination of aflatoxin contamination in food are needed in Mongolia.

This study has several limitations. First, flour for sale in rural Mongolia was not included in this survey. Second, the study population was limited for the food frequency questionnaire and most individuals who participated to this survey were female because they work in trade service. Third, the ELISA method has a higher LOQ than other methods, so using an alternative method would have offered more confidence in quantification of aflatoxin in the flour samples. Fourth, this study was conducted only in one season, so differences throughout the year could affect the storage conditions. Fifth, other sources of aflatoxin exposure were not measured. It is recommended to do future study in Mongolia in additional seasons, with additional samples types, with improved methods of quantification, and including aflatoxin G_1 and G_2 as well as B_1 and B_2.

Conflict of Interest

The authors state no conflict of interest.

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