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A study on aflatoxin content in barley flour in India

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ABSTRACT

Barley (*Hordeum vulgare*), is one of the major cereal grain grown in temperate regions. It is believed to be originated from western Asia or Ethiopia. Barley is the fourth largest grain crop globally, after wheat, rice, and maize corn. Barley is generally used in breads, soups, stews, and health products and sometimes as animal fodder also. Aflatoxin is secreted due to fungal contamination with *Aspergillus flavus* or *A. parasiticus* under humid and warm conditions. Aflatoxins can cause liver necrosis, bile duct proliferation, edema or lethargy. There are no reports regarding the level of Aflatoxin content in Barley flour. The objectives of this study was to determine the concentrations of Aflatoxin B_1 in Barley Flour collected from various parts of India and also to assess whether the Barley Flour were safe for human consumption. Out of 27 samples of Barley Flour analysed for Aflatoxin B1 by HPTLC method, it has been observed that all samples were found to be free from Aflatoxin B_1 i.e aflatoxin content was below detection limit (less than 1.6 ppb). The study showed that the Barley Flour samples collected from various regions of India were found to contain aflatoxin less than 1.6 ppb and which is within the permissible limits as specified by Indian Standards, European Standards and FDA standards and are safe for human consumption.

Keywords: Barley Flour, Aflatoxin, HPTLC, Human consumption, safety.

INTRODUCTION

Barley was called as *bere*, in old English which traces back to Proto-Indo-European and "flour" word originated from the Latin word *farina*. The word *barn*, means "barley-house", is also rooted in these words [1]. The common products are barley flakes, and barley flour. Barley flour is also a component of composite flours used for making yeast-raised bread [2]. Barley contains large amount of carbohydrates. Besides that it contains protein, calcium, and phosphorus in moderate amount and Vitamin B in small amount. It has nutlike flavour. Barley is not generally used to produce bread as it contains little gluten, which imparts its elastic property. The presence of aminoacids viz. Lysine, Threonine and valine is high in barley flour compared to wheat flour.[3] Aflatoxins are one of highly toxic secondary metabolites produced by fungal species such as *Aspergillus flavus*, *A.parasiticus* and *A.nomius*[4,5]. These fungi usually infect cereal crops and can lead to serious threats to human and animal health by causing various complications such as hepatotoxicity, teratogenicity and immunotoxicity [6,7, 8]. The major aflatoxins are B_1, B_2, G_1 and G_2 which can poison the body through respiratory, mucous or cutaneous routes resulting in over activation of the inflammatory response [9]. Aflatoxin B_1 is a human carcinogen classified by the International Agency for Research in Cancer (IARC) [10] in Group 1, and clearly genotoxic. Aflatoxins can cause bile duct proliferation, liver necrosis, edema, or lethargy [11]. Aflatoxins are found in cereals such as rice, wheat, barley, oils seeds, spices and nuts [12]. Fungal contamination can occur in field, or during harvest, transport and storage. There are no reports in the literature about any study on the level of aflatoxins in the Barley flour available in India. Therefore, it is important to study the aflatoxin contamination in the Barley flour. The present study was conducted to assess aflatoxin B_1 contamination in market samples of barley flour using HPTLC method with regard to Maximum permissible level in European Union, USFDA and Indian Standards.

MATERIALS AND METHODS

Sampling

For detection and estimation of aflatoxins in Barley flour, samples were collected from different regions of India such as Kochi, Mumbai, Chennai, Jaipur, Bhopal, Amritsar, Guntur. Sampling was carried out in a way that ensured the analytical sample is the representative sample. 27 samples were analysed for Aflatoxin B_1.

Method

The procedure of solvent extraction and subsequent analysis by HPTLC as reported by Ashish *et al*., 2019[13] was followed for analysis of Aflatoxin B_1 in Barley flour.

Calculation

The concentration of Aflatoxin B_1 in µg/kg was calculated as per formula:

$$\mu g / kg = \frac{B \times Y \times S \times V}{Z \times X \times W}$$

Where, B = average Area/Height of Aflatoxin B_1 peaks in test aliquots.
Y = concentration of Aflatoxin B_1 standards, µg/ml
S = µl of Aflatoxin B_1 standards spotted
V = final volume of test solution, µl
Z = average Area/Height of Aflatoxin peaks in standards aliquots.
X = µl test solution spotted.
W = g test portion represented by test solution.

The final results are obtained by taking average of concentration of Aflatoxin after calculation with respect to Height and Area.

RESULTS

Aflatoxin content in Barley Flour

A total of 27 samples were collected from different parts of India. Table 1 showed the level of Aflatoxin content in 27 samples of which all samples are found to be below detection limit (less than 1.6 ppb). The Limit of Detection (LOD) and Limit of Quantification (LOQ) are 0.5 ppb and 1.6 ppb respectively as determined using method validation study.

Table 1: Level of Aflatoxin content in ppb in Barley flour samples collected and obtained from different parts of India

S No	Location	Sample Code	Aflatoxin B_1 in ppb
1	Kochi	CALT-36	BDL
2	Kochi	CALT-37	BDL
3	Kochi	CALT-38	BDL
4	Mumbai	CALT-63	BDL
5	Mumbai	CALT-64	BDL
6	Chennai	CALT-76	BDL
7	Jaipur	CALT-94	BDL
8	Jaipur	CALT-95	BDL
9	Jaipur	CALT-96	BDL
10	Jaipur	CALT-97	BDL
11	Jaipur	CALT-98	BDL
12	Jaipur	CALT-99	BDL
13	Bhopal	CALT-107	BDL
14	Bhopal	CALT-108	BDL
15	Amritsar	CALT-122	BDL
16	Amritsar	CALT-123	BDL
17	Amritsar	CALT-124	BDL
18	Amritsar	CALT-125	BDL
19	Guntur	CALT-129	BDL
20	Guntur	CALT-130	BDL
21	Mumbai	CALT-215	BDL
22	Mumbai	CALT-216	BDL
23	Kolkata	CALT-238	BDL
24	Kolkata	CALT-239	BDL
25	Kolkata	CALT-240	BDL
26	Kolkata	CALT-241	BDL
27	Bhopal	CALT-251	BDL

BDL: - Below Detection Limit, <1.6 ppb

DISCUSSION

The study showed that aflatoxin content was below detection limit (less than 1.6 ppb) in 27 samples analysed collected from different parts of India. In a similar study, Haubruge et al (2003) [14] also reported that aflatoxin was detected in less than 4% of barley samples at a low level (< 0.05 mg/kg) using competitive direct enzyme linked immunosorbent assay. In another study, 125 barley kernels samples were collected from Spanish grain stores from 2008 to 2010 and analysed using optimized method involving accelerated solvent extraction, clean up immunoaffinity column, liquid chromatographic separation, post column derivatization with iodine and fluorescent detection. Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2 and Ochratoxin were detected in 12.4%, 2.9%, 4.8%, 2.9% and 20% of the samples. The maximum mycotoxin levels were found to be 0.61 ng/g for Aflatoxin B_1, 0.06 ng/g for Aflatoxin B_2, 0.26 ng/g for Aflatoxin G_1 , 0.05 ng/g for Aflatoxin G_2 and 2.0 ng/g Ochratoxin A as reported by Mateo *et al* 2011 [15]. However, Jedidi *et al* (2017) [16] reported that Aflatoxin was not detected in Barley and wheat samples collected in Tunisia which is similar in the present study. Ramesh *et al.* (2013) [17] carry out the survey for detection of Aflatoxin B_1 contamination in Bengal Gram, Bajra, Maize, Jowar and grain flour procured locally from Chennai, Tamil Nadu, India. It has been reported that 100% of grain flour samples were found to be contaminated with mean concentration of 60.41 ppb which is above the maximum permissible limit (MPL) in European Union, USFDA and Indian standards. Similarly, Feizy and Ahmadi (2020) [18] reported that Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2 was found in 19.59%, 9.28%, 4.12% and 1.03% of barley samples purchased from wholesalers in Mashhad city, Iran with mean values of 3.53, 1.73, 1.46 and 0.29 ng/g respectively analysed by HPTLC. Beheshti and Asadi (2014) [19] also reported that Aflatoxin B_1 was detected in 5 out of 60 barley samples collected from Iran with a mean value of 0.48 ng/g and Aflatoxin B_2, G_1, G_2 were not detected. The total aflatoxin should not be more than 15 µg/kg and Aflatoxin B_1 should not be more than 10 µg/kg as per FSSAI i.e Indian Standards. The European Committee Regulations (ECR) establish the maximum acceptable level of AFB_1 in cereals, peanuts and dried fruits either for direct human consumption, or as an ingredient in foods as 4 ppb for total aflatoxins (AFB_1, AFG_1, AFB_2 and AFG_2) and 2 ppb for AFB_1 alone. FDA Specifies maximum limit of Total Aflatoxin as 20 ppb. In the present study, Aflatoxin B_1 was found to be below detection limit i.e less than 1.6 ppb in barley flour samples collected from different regions of India which is within the permissible limits as specified by Indian Standards, European Standards and FDA standards.

CONCLUSION

In the present study, all samples were found to contain Aflatoxin B_1 below detection limit i.e less than 1.6 ppb as determined during the method validation study. It indicates that the good manufacturing practices and storage conditions were implicated that lead to safe food for human consumption. It complies the FSSAI Standard, EU Standards and USFDA Standards.

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