



A STUDY ON AFLATOXIN CONTENT IN RICE FLOUR IN INDIA

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Abstract: Rice flour (also rice powder) is a form of flour made from finely milled rice. Rice is the staple food in most of the Asian countries and is scientifically called as *Oryza Sativa*. Rice flour is highly beneficial as it contains large amount of fibre, it is gluten free and helps to maintain healthy liver function. Aflatoxin can be formed as a result of fungal contamination with *Aspergillus flavus* or *A. parasiticus* under warm and humid conditions at both pre and post harvest. Aflatoxins caused neurotoxicity, genotoxicity and mostly lethal in nature. The objectives of this study was to determine the concentrations of Aflatoxin B₁ in Rice Flour collected from various parts of India and also to assess whether the Rice Flour were safe for human consumption. As per FSSAI, the maximum permissible limit for Aflatoxin is 30 ppb. The study showed that the 35 nos. of Rice Flour samples collected from various regions of India were not detected for Aflatoxin B₁ stating that are within the permissible limit and are safe for human consumption.

Index Terms- Rice Flour, Aflatoxin b1, HPTLC

1. INTRODUCTION:

Rice flour is made by grinding whole kernels and or broken rice kernels recovered from the milling process into a powder. Rice flour may be made from either white rice or brown rice. Rice is the main staple food in Southeast and Northeast Asia with highest levels of consumption. Of the world's total rice production, 90% is grown and consumed in Asia [1]. As one of the world's most important staple foods other than wheat, rice represents 27% of the global energy uptake and 20% of the dietary protein in developing countries [2].

It has around 7–10% protein, 75–82% carbohydrates, and 0.7–1% fat [1] and as an important commercial commodity, it is enriched with vitamins and minerals to meet nutrient requirements. It is used for preparation of various traditional home made products like cakes, idli, noodles. It is now employed in production of baby foods, RTE breakfast cereals and snacks[3]. As rice is devoid of gluten, it is non allergic by nature and makes suitable for wheat-intolerant people for those suffering from coeliac disorder. Its non allergenic property also makes rice as one of the first cereals to be used in infant feeding [4].

Aflatoxins are one of highly toxic secondary metabolites produced by fungal species such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. 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. The major aflatoxins are B₁, B₂, G₁ and G₂ which can poison the body through respiratory, mucous or cutaneous routes resulting in overactivation of the inflammatory response. Aflatoxin B₁ is considered to be a human carcinogen (classified by the International Agency for Research in Cancer, IARC 1993) in group 1, and clearly genotoxic. Aflatoxins can acutely cause liver necrosis, bile duct proliferation, edema, or lethargy. Aflatoxins are found in cereals such as rice, wheat, barley, oils seeds, spices and nuts. It has been reported in different varieties of rice. Fungal contamination can occur in field, or during harvest, transport and storage [5]. According to the Food and Agriculture Organization, during

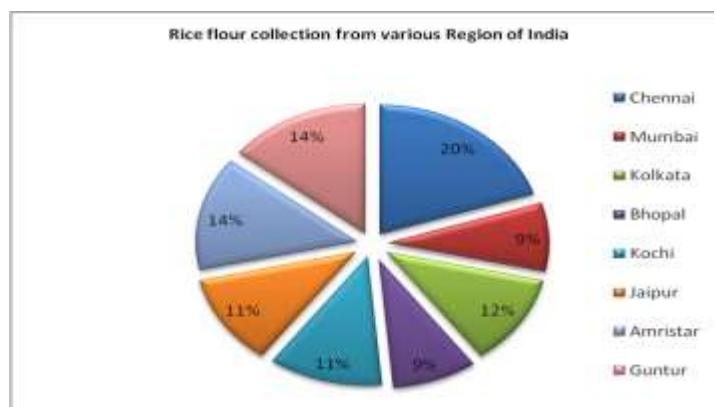
inappropriate storage circumstances, 15% of cultivated rice is thrown away every year because of fungi contamination along with other harmful species [6].

The literature surveys in the study of Aflatoxin B₁ for the rice flour is limited. Hence a Study the aflatoxin contamination in the rice flour was carried out.

MATERIALS AND METHODS:

2.1 Extraction of aflatoxins from Rice Flour

For detection and estimation of aflatoxins in Rice flour, 35 samples were collected from different parts of India. The analytical procedure of solvent extraction and subsequent analysis were done using HPTLC. 20 g homogenised each sample were accurately weighed in 500 ml Conical flask. The samples were mixed in asolution containing 1 g NaCl, 50 ml Hexane and 125 ml Methanol: Water (55:45) and allowed to stand for 30 minutes with intermittent shaking. The mixture was filtered and solution was taken in separating funnel. Hexane layer was discarded and Collect Methanol: Water layer. 25 ml of this layer was taken in another separating funnel, and 25 ml of Chloroform was added and shake. After layer being separated, discarded the aqueous layer, and Chloroform layer collected. The chloroform layer evaporated to dryness on water bath. The residue was dissolved again with 2.5 ml of chloroform and stored in darkness for quantitative analysis through HPTLC.



2.2. Quantitative estimation of aflatoxins

Quantitative estimation of aflatoxin was done by High performance thin layer chromatography (HPTLC). The analytical equipment for HPTLC (CAMAG Linomat 5) with CAMAG TLC Scanner-3/081123 and operated with winCATs software.

Pre-coated TLC sheets silica gel Merck 60 F₂₅₄ 10x10 cm were used in this analysis. Apply band with CAMAG Linomat, distance from lower edge of sheet 12 mm and distance from left edge 12 mm. Spotted 10 µl volume samples extract with band length 5 mm. The standard Aflatoxin B₁ were applied also applied. The spotted TLC **sheets are placed in the** development chamber filled with chloroform-acetone (9:1) upto a depth of about 8 mm. The solvent migrates up to 70 mm. Then plate is air dried and mounted on Scanner Tray and fixed with the magnets and scanned under UV light at 366 nm.

2.4 Calculation:

The concentration of Aflatoxin B₁ in µg/kg were calculated as follows:

$$\text{Aflatoxin B}_1 \mu\text{g/kg} = \frac{B \times Y \times S \times V}{Z \times X \times W}$$

Where, B = average Area/Height of Aflatoxin B₁ peaks in test aliquots.

Y = concentration of Aflatoxin B₁ standards, µg/ml

S = µl of Aflatoxin B₁ 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 have been obtained by taking average of concentration of Aflatoxin after calculation with respect to Height and Area.

3 RESULTS AND DISCUSSION:

A total of 35 samples were collected from different parts of India with a distribution on 20% from Chennai, 9% from Mumbai, 12% from Kolkata, 11% from Kochi, 11% from Jaipur, 14% from Amristar, 14 % from Guntur were analysed for the contamination of Aflatoxin B₁.

Table 1. Level of Aflatoxin content in ppb in Rice flour samples obtained from different parts of India

S No	Region	Sample Code	Aflatoxin in ppb
1	Chennai	CALT-194	BDL
2	Chennai	CALT-195	BDL
3	Chennai	CALT-196	BDL
4	Chennai	CALT-197	BDL
5	Chennai	CALT-70	BDL
6	Chennai	CALT-71	BDL
7	Chennai	CALT-72	BDL
8	Mumbai	CALT-210	BDL
9	Mumbai	CALT-211	BDL
10	Mumbai	CALT-213	BDL
11	Kolkata	CALT-234	BDL
12	Kolkata	CALT-235	BDL
13	Kolkata	CALT-236	BDL
14	Kolkata	CALT-237	BDL
15	Bhopal	CALT-250	BDL
16	Bhopal	CALT-105	BDL
17	Bhopal	CALT-106	BDL
18	Kochi	CALT-30	BDL
19	Kochi	CALT-31	BDL
20	Kochi	CALT-32	BDL
21	Kochi	CALT-33	BDL
22	Jaipur	CALT-78	BDL
23	Jaipur	CALT-80	BDL
24	Jaipur	CALT-81	BDL
25	Jaipur	CALT-82	BDL
26	Amristar	CALT-113	BDL
27	Amristar	CALT-114	BDL
28	Amristar	CALT-115	BDL
29	Amristar	CALT-116	BDL
30	Amristar	CALT-117	BDL
31	Guntur	CALT-131	BDL
32	Guntur	CALT-132	BDL
33	Guntur	CALT-133	BDL
34	Guntur	CALT-134	BDL
35	Guntur	CALT-135	BDL

BDL :- Below Detection Limit

The food safety and standard Authority of India had established health based limits for contaminant residues through Food safety and standards Act, 2006 and Food Safety and standards Regulations 2011 as tolerance limit of 30µg/Kg for aflatoxin for all foods meant for human consumption. The EU established maximum levels of AFB₁ and total AFs (2 and 4 µg/kg, respectively) in rice for human consumption and also implemented maximum levels of AFB₁ and total AFs (5 and 10 µg/kg, respectively) in rice before ingestion [7].

In the present study, no samples were found to be contaminated with aflatoxin B₁. From the available literatures, it was observed that the Aflatoxin B₁ contamination in Lebanon was exceed than EU limits. The range of aflatoxin B₁ in rice samples collected from Lebanon were ranged between 0.06 to 2.08 µg/kg;

Turkey, from “not detected” to 1.86 µg/kg in rice samples obtained from five provinces in the eastern side of the country and from China and ranged from 0.1 to 1.4 µg/kg; in Saudi Arabia in packed basmati, white, parboiled, and brown rice contamination ranged from 0.014 to 0.123 µg/kg [8].

4 CONCLUSION:

In the present study for contamination of aflatoxin in rice flour with HPTLC, 35 samples collected from various region of india are having aflatoxin below detection limit ie it is free from contamination and safe for human consumption. The rice flour samples satisfies the FSSAI standards and EU standards

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6. REFERENCES:

1. Steiger, G., et al., Fortification of Rice: Technologies and Nutrients. *Annals of the New York Academy of Sciences*, 2014, pp. 29–39, doi:10.1111/NYAS.12418.
2. Al-Zoreky, N. S., and F. A. Saleh. 2019. Limited survey on aflatoxin contamination in rice. *Saudi J. Biol. Sci.* 26:225–231. <https://doi.org/10.1016/j.sjbs.2017.05.010>.
3. Rice Quality: A Guide to Rice Properties and Analysis (2011), Kshirod R Bhattacharya, Agricultural Sciences, p 307, DOI:10.1533/9780857092793.
4. 4. Brown Rice, Annamalai Manickavasagan, Chandini Santhakumar, N. Venkatachalapathy, p-204, Springer, ISBN 978-3-319-59011-0(E book)
5. 5. Dr.Ashish Mukherjee, Dr.Manvi Sharma, Smt.Savitha Latkar (2019), A study on Aflatoxin content in rolled oats available I domestic market of India, *International Journal of Pharmacy and Analytical Research*, Vol.8,Issue3, 266-271.
6. Food and Agriculture Organization (FAO). 2018. Rice Market Monitor, vol. XXI. Available at: <http://www.fao.org/3/I9243EN/i9243en.pdf>.
7. Ali, N. 2019. Aflatoxins in rice: worldwide occurrence and public health perspectives. *Toxicol. Rep.* 6:1188–1197. <https://doi.org/10.1016/j.toxrep.2019.11.007>
8. Hussein F. Hassan, Rita Kordahi, Hani Dimassi, Andre El Khoury, Rouaa Daou, Nisreen Alwan, Samar Merhi, Joyce Haddad, and Layal Karam (2022), Aflatoxin B1 in Rice: Effects of Storage Duration, Grain Type and Size, Production Site, and Season, *Journal of Food Protection*, Vol. 85, No. 6, Pages 938–944 <https://doi.org/10.4315/JFP-21-434>