INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 1 | Jan – Mar - 2019
Journal Home page: www.ijpar.com

Research article *Open Access*

A study on AFLATOXIN content in black scented rice in India

Dr Ashish Mukherjee*, Dr Manvi Sharma, Smt Savita Latkar

Central Agmark Laboratory, North Ambazari Road, Nagpur-440010, Maharashtra, India

***Corresponding Author: Dr Ashish Mukherjee**
Email: cal@nic.in

ABSTRACT

Black rice is a range of rice types of the species oryza sativa L. It is also known as purple rice, some of which are glutinous rice. Varieties include Indonesian black rice and Thai jasmine black rice. Black rice is known as *chak-hao* in Manipur which is an Indian state on the eastern border with Myanmar, where desserts made from black rice are served at major feasts. Black rice is a source of iron, vitamin E, and antioxidants (more than in blueberries). The bran hull (outermost layer) of black rice contains one of the highest levels of anthocyanins found in food, which helps in fighting against heart disease, cancer and associated diseases. The grain has similar amount of fiber to brown rice and, like brown rice, has a mild, nutty taste. It has a deep black color and when cooked, it usually turns purple. Its dark purple color is primarily a product of its anthocyanin content, which is higher by weight than that of other colored grains. It is very useful when making porridge, dessert, traditional Chinese black rice cake, bread, and noodles. Black scented rice also known as Chakhao Amubi is a type of glutinous rice. It is indigenous to northeastern regions of India. It is also called Gold rice as the rice can be exported to countries where there is high demand of sticky rice and fetch foreign exchange.

Rice is largely cultivated in subtropical environments which are characteristically warm and humid. After harvesting, it is generally dried and under inappropriate storage conditions, rice is considered as an appropriate substrate for fungal growth. Rice is often contaminated with mycotoxins such as aflatoxins. The temperatures and moisture conditions prevailing during storage promote aflatoxin production resulting in annual losses of useful food bioresources such as rice and thus affecting the economy of rice producing countries. The aflatoxins are a group of chemically similar toxic fungal metabolites (mycotoxins) produced by certain moulds of the genus *Aspergillus* growing on a number of raw food commodities. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally found in foods. These are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is the most commonly found in food and also the most toxic and classified by the International Agency for Research on Cancer (IARC) as 1st class carcinogen. To control the contamination of Aflatoxin in Black Scented Rice, the Rice to be free from Aflatoxin contamination or contain the permissible limit of same.

The objectives of this study was to determine the concentrations of Aflatoxin B1 in Black scented rice collected from Gauwahti and Shillong i.e North east part of India and also to assess whether the black scented rice were safe for human consumption.
The aflaoxin in Black Scented Rice has been analysed using HPTLC. Out of 87 samples of black scented rice analysed for Aflatoxin, 85 samples were found to be free from Aflatoxin i.e Below detection limit. Only two samples showed aflatoxin content of 21.581 ppb and 22.989 ppb respectively. As per FSSAI, the maximum permissible limit for Aflatoxin is 30 ppb. The study showed that the Black scented rice samples collected from North-Eastern regions of India were safe for human consumption.
Keywords: Black scented Rice; North eastern states; Aflatoxin, HPTLC

INTRODUCTION

Rice, a cereal crop is the major staple food sources for half of the world population. Black scented rice (also known as purple rice) is a range of rice types of the species *Oryza sativa* L., some of which are glutinous rice. Varieties include Indonesian black rice and Thai jasmine black rice. Black rice is known as *chak-hao* in Manipur, an Indian state on the eastern border with Myanmar, where desserts made from black rice are served at major feasts. Black rice is a source of iron, vitamin E, and antioxidants (more than in blueberries). Black rice has a deep black color and usually turns deep purple when cooked. Its dark purple color is primarily due to its anthocyanin content, which is higher by weight than that of other colored grains.

It is suitable for preparation of porridge, dessert, traditional Chinese black rice cake, bread, and noodles. The Chakhao Amubi is one type of sticky black rice that is indigenous to Manipur. 'Chakho' means delicious while 'Ambui' means black. In Manipur, it is generally served in special occasions and festive events. Chahaomubi may be considered as black gold of Manipur because of its scope for earning foreign exchange. The demand for good quality chahaomubi has been increasing in international market because of its organic in character. The bran hull (outermost layer) of black rice contains one of the highest levels of anthocyanins found in food. It plays a role in preventing plaque building in artery walls. It is also helpful in lowering cholesterol levels in body. Antioxidants like anthocyanins in Black scented rice helps in fighting against heart disease, cancer and associated diseases.

The grain has a similar amount of fibre to brown rice and, like brown rice, has a mild, nutty taste.

The aromatic black glutinous rice of Manipur has been characterized with 34 morphological characters. Medhabati et al [1]. has developed a novel rice hybrid of "Chakhao Amubi and Basmati 370" to improve yield and provide better grain quality rice having high Nutraceutical properties using anther culture development techniques of homozygous breeding lines of double haploid. The improved varieties can be exported to South east and East Asian Countries where there is a huge demand of aromatic glutinous rice.

Rice bran, the outer layer of rice grain is a rich source of gamma-oryzonol and vitamin E including tocopherols and tocotrienols [2]. These nutritional compounds play an important part in preventing oxidative damage in foods and have a wide range of application in biological activities. The rice bran from red and purple rice has a higher concentration of phenolic and flavonoid concentrations than the lighter rice bran colour [3]. Bran also reduces the serum cholesterol levels in blood that help in lower of bad low density lipoprotein (LDL) and increase good high density lipoprotein (HDL) levels of cardiovascular health. Food and Beverage industry could explore the potential of black rice bran to boost human health and provide supplementary nutrients to the human body as synthetic compounds have side effects [4].

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus (A. flavus, A. parasiticus* and *A. nomius)* [5-7]. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally found in foods. These are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is the most commonly found in food and also the most toxic and classified by the International Agency for

Research on Cancer (IARC) as 1st class carcinogen [8-10]. Aflatoxin producing fungi may contaminate cereals such as rice, wheat, barley, oils seeds etc. if grown, stored and/or processed under conditions which favour fungal growth. Hot, humid climates and any pest pressures resulting in bruising or cuts on the commodity will favour the growth of the Aflatoxin producing fungi, either in the field or in storage. Growth of these fungi on certain foods and feeds may result in Aflatoxin production which results in illness or death in humans and animals and thus is an important public health concern [11-14]. It has been reported in different varieties of rice [15]. Prolonged storage and/or contamination during storage or transport have also been associated with higher Aflatoxin levels.

There are no reports available in the literature about any study on the level of aflatoxins in the black scented rice available in India. The Blak Scented Rice, which is being consumed by human being, to be free from Aflatoxin contamination or contain the permissible limit of same. Therefore, it is important to study the aflatoxin contamination in the black scented rice. In the present study, aflatoxin B1 content was determined in black scented rice collected from North eastern regions of India and also to assess whether the Black Scented Rice is safe for human consumption.

MATERIALS AND METHODS

Total 87 Black Scented Rice samples were investigated for Aflatoxin B_1 levels. The samples were collected from North Eastern part of India i.e. Guwahati and Shillong.

Extraction of aflatoxins from Dates

For detection and estimation of aflatoxins from Dates, samples collected from different parts of India, the analytical procedure of solvent extraction and subsequent analysis by HPTLC was employed. About 20 g. dried finely crushed sample accurately weighed in 500 ml. Conical flask containing mixture of 1 gm NaCl, 50 ml Hexane and 125 ml Methanol: Water (55:45) and allowed to stand for 30 minutes with intermittently shaken Thereafter, the mixture was filtered through Whattman filter paper and solution has been taken in separating funnel. Discard Hexane layer. Wash again with Hexane, if require. Collected Methanol: Water layer. 25 ml of this layer taken in separating funnel, and added 25 ml of Chloroform 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 with 2.5 ml of chloroform and stored in darkness for quantitative analysis.

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 171005, CAMAG Visualizer 171113 and operated with winCATs software.

Method of Spotting and Development of TLC plate

Pre-coated TLC sheets silica gel Merck 60 F_{254} 10x10 cm was taken.

Sample application

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.

Standards application

Apply side by side, 3.0, 6.0 and 10.0 µl standard Aflatoxin B_1 (Concentration 0.5µl/ml).

Chromatography

The development chamber should be filled up () with chloroform-acetone (9:1) upto a depth of about 8 mm and insert the sheet, The solvent migrates up to 70 mm. Then plate is air dried.

Scanning of TLC

Mounted air dried plate on Scanner Tray and fixed with the magnets. Scanned plate in TLC scanner, under UV light at 366 nm.

CALCULATION

The concentration of Aflatoxin B_1 in µg/kg has been calculated as follows:

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

Where, B = average Aea/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 = gm 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.

RESULTS AND DISCUSSION

A total of 87 samples were collected from north eastern part of India i.e. Gauwahti and Shillong.

Table 1 showed the level of Aflatoxin content in 87 samples. The Aflatoxin has not been detected in 85 samples. Only two samples with code number BSR-7 and BSR-19 showed aflatoxin content of 21.581 ppb and 22.989 ppb respectively.

Table 1. Level of Aflatoxin content in ppb in Black scented rice samples obtained from North eastern regions of India

S No	Sample Code	Aflatoxin in ppb	S No	Sample Code	Aflatoxin in ppb	S No	Sample Code	Aflatoxin in ppb
1	BSR-1	ND	30	BSR-30	ND	59	BSR-59	ND
2	BSR-2	ND	31	BSR-31	ND	60	BSR-60	ND
3	BSR-3	ND	32	BSR-32	ND	61	BSR-61	ND
4	BSR-4	ND	33	BSR-33	ND	62	BSR-62	ND
5	BSR-5	ND	34	BSR-34	ND	63	BSR-63	ND
6	BSR-6	ND	35	BSR-35	ND	64	BSR-64	ND
7	BSR-7	21.581	36	BSR-36	ND	65	BSR-65	ND
8	BSR-8	ND	37	BSR-37	ND	66	BSR-66	ND
9	BSR-9	ND	38	BSR-38	ND	67	BSR-67	ND
10	BSR-10	ND	39	BSR-39	ND	68	BSR-68	ND
11	BSR-11	ND	40	BSR-40	ND	69	BSR-69	ND
12	BSR-12	ND	41	BSR-41	ND	70	BSR-70	ND
13	BSR-13	ND	42	BSR-42	ND	71	BSR-71	ND
14	BSR-14	ND	43	BSR-43	ND	72	BSR-72	ND
15	BSR-15	ND	44	BSR-44	ND	73	BSR-73	ND
16	BSR-16	ND	45	BSR-45	ND	74	BSR-74	ND
17	BSR-17	ND	46	BSR-46	ND	75	BSR-75	ND
18	BSR-18	ND	47	BSR-47	ND	76	BSR-76	ND
19	BSR-19	22.989	48	BSR-48	ND	77	BSR-77	ND
20	BSR-20	ND	49	BSR-49	ND	78	BSR-78	ND
21	BSR-21	ND	50	BSR-50	ND	79	BSR-79	ND
22	BSR-22	ND	51	BSR-51	ND	80	BSR-80	ND
23	BSR-23	ND	52	BSR-52	ND	81	BSR-81	ND
24	BSR-24	ND	53	BSR-53	ND	82	BSR-82	ND
25	BSR-25	ND	54	BSR-54	ND	83	BSR-83	ND
26	BSR-26	ND	55	BSR-55	ND	84	BSR-84	ND
27	BSR-27	ND	56	BSR-56	ND	85	BSR-91	ND
28	BSR-28	ND	57	BSR-57	ND	86	BSR-92	ND
29	BSR-29	ND	58	BSR-58	ND	87	BSR-93	ND

ND- Not detected and may be taken as “0”.

In the present study, only two out of 87 black scented rice samples (2.3%) were found to contain aflatoxin B_1. It has been reported that 184 out of 413 rice samples (44.8%) were contaminated by aflatoxin in a study carried out by Iqbal et al., [15]. The average concentration of aflatoxins in paddy rice, parboiled rice, brown rice, white rice and broken rice were 16.35 ± 1.67 µg/kg, 14.20 ± 2.04 µg/kg, 9.85 ± 1.25 µg/kg, 7.10 ± 1.39 µg/kg and 8.50 ± 1.71 µg/kg respectively. In the present study, two samples showed aflatoxin content of 21.581 ppb and 22.989 i.e about 23 ppb or 23µg/kg respectively. The higher concentration is related to the fact that aflatoxin accumulation in the outer layers of the grains. FSSAI has given maximum limit for aflatoxin as 30 ppb in any food products. Hence, all the samples were found to be within the maximum permissible limits. However, different countries have different maximum permissible limits. European Union (commission regulation No 165/2010) set maximum levels of aflatoxin B1 (5 µg/kg) and total aflatoxins (10 µg/kg) in rice subjected to sorting or other physical treatment before human consumption and EU has regulated aflatoxin levels of B1 (2 µg/kg) and total aflatoxins (4 µg/kg) in rice intended for human consumption (Commission regulation No 1881/2006). In the present study, two samples have shown higher concentration of aflatoxin B_1 than maximum limit as prescribed by EU legislation. The presence of aflatoxins in rice has been documented in several countries. High incidence of aflatoxin contamination up to 96 µg/kg was reported in rice by Abdullah et al., [16] in Malaysia. In United Arab Emirates, aflatoxin level ranging from 1.2 to 16.5 µg/kg [17] while contamination level of aflatoxin B1 in rice ranged from 0.025 µg/kg to 11 µg/kg in Phillipines [18]. The percent positive for aflatoxin in Columbian rice samples were 10% and mean concentrations was 7.1 µg/kg [19]. Post harvest treatments to remove aflatoxins such as alkalization, ammonization and heat can be used before consumption to reduce the aflatoxin content. Techniques to eliminate aflatoxin may be either physical or chemical methods. Removing mold damaged kernels has been observed to reduce aflatoxins by 40 to 80% (Park, 2002). Aflatoxins generally decompose at temperatures of 237 to 306°C. The decrease in aflatoxin content depends upon time and temperature combination. Alkaline cooking and steeping of corn reduces aflatoxin by 52% [20]

The percent positive in the present study for black scented rice was very less as compared to previous studies in rice and the concentration of aflatoxin was found to be within the maximum permissible limits as prescribed by FSSAI. Hence, Black scented rice samples analysed from North Eastern regions of India may not produce health risk for human consumption, if other sources of contaminated food are not taken.

CONCLUSION

In present study, the Black Scented Rice samples are collected from North Eastern parts of countries mentioned in Table-1, and the content of Aflatoxin has been determined using HPTLC. In India, a tolerance limit of 30 µg/kg has been prescribed under the Food Safety and Standards(Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption. Screening of 87 samples of black scented rice revealed that except 2 samples all the black scented rice samples were found free from Aflatoxin B_1 content, and the said 2 samples contain Aflatoxin within the permissible limit as specified by Indian legislation. In view of this, it has been concluded that consumption of black scented rice may not produce any health risk for human consumption in respect of Aflatoxin contamination.

Acknowledgments

We would like to express our sincere gratitude to Shri P.K. Swain, Joint Secretary-cum- Agricultural Marketing Adviser to the Govt. of India and all the Staffs of Central Agmark Laboratory and Regional Agmark Labratories have been source of constant inspiration to us. We would like to express our gratitude to Regional Office, Gauwahi and Sub-office Shillong to collect and provide the samples of black scented rice for analysis. The views expressed in the manuscript are that of authors and not binding on the Government of India.

REFERENCES

[1]. Medhabati K., Rajiv, D.K., Henary, Ch., Dikash, Th., Sunitibala H. Androgenic callus induction of the Indica Rice Hybrid of chakhao Amubi and Basmati 370. International Research Journal of Biological Sciences. 3(4), 2014, 73-78

[2]. Godber, J.S., Wells, J.H. Rice Bran: as a viable source of high value chemicals. Louisiana Agriculture 37(2), 1994, 13-17

[3]. Min, B., Gu L., McClung A.M., Bergman, C.J, Chen, M Free and bound total phenolics, procyanidin and anthocyanins profiles and their antioxidant capacities in whole grain rice (*Oryza Sativa* L) of different bran colors. Food Chemistry 133, 2012, 715-722.

[4]. Das, K.R., Medhabati, K., Nongalleima, K and Devi, H.S The potential of Dark Purple Scented Rice-From Staple food to Nutraceutical. Current World Environment 9(3), 2014, 867-876

[5]. Tajik H, Rohani SMR, Moradi M, Detection of aflatoxin M1 in raw and commercial pasteurized milk in Urmia, Iran, Pakistan J Biol Sci 10(22), 2007, 4103-07.

[6]. Özdemir M, Determination of aflatoxin M1 levels in goat milk consumed in Kilis province, Vet J Ankara Univ 54, 2007, 99-103.

[7]. Baydar T, Erkekoglu P, Sipahi H, Sahin G, Aflatoxin B_1, M1 and ochratoxin A levels in infant formulae and baby foods marketed in Ankara, Turkey, J Food Drug Analy 15(1), 2007, 89-92.

[8]. Yapar K, Elmah M, Kart A, Yaman H, Aflatoxin M_1 levels in different type of cheese products produced in Turkey, Med Wet 64(1), 2008, 53-55.

[9]. Nuryono N, Agus A, Wedhastri S, Maryudani YB, Sigit Setyabudi FMC, Böhm J, Razzazi-Fazeli E, A limited survey of aflatoxin M1 in milk from Indonesia by ELISA, Food Control 20, 2009, 721-24.

[10]. IARC (International Agency for Research on Cancer) Aflatoxins, Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxin*s*, IARC Monographs on the Evaluation of Carcinogenic Risk to Humans 1993, 245-395.

[11]. Aydin A, Erkan ME, Baskaya R, Ciftcioglu G, Determination of aflatoxin B1 levels in powdered red pepper, Food Control 18, 2007, 1015-18.

[12]. Baydar T, Engin AB, Girgin G, Aydin S, Sahin G, Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey, Ann Agric Environ Med 12 (2), 2005, 193-7.

[13]. Giray B, Girgin G, Engin AB, Aydin S, Sahin G, Aflatoxin levels in wheat samples consumed in some regions of Turkey, Food Control 18, 2007, 23-29.

[14]. Reddy, K.R.N An overview of Mycotoxin contamination in foods and its implications for human health. Toxin Rev. Doi:10.3109/15569541003598553, 29, 2010, 3-26.

[15]. Iqbal, S.Z., Muhammad, R A., Arino A., Akram N and Zuber M Aflatoxin contamination in different fractions of rice from Pakistan and estimation of dietary intakes. Mycotoxin Res. 28, 2012, 175-180 DOI 10.1007/s12550-012-0131-1.

[16]. Abdullah N., Nawawi A and Othman I Survey of fungal counts and national occurrence of aflatoxin in Malaysian starch based foods. Mycopathotogia 143, 1998, 53-58.

[17]. Osman NA, Abdelgadir AM, Moss MO and Bener A Aflatoxin contamination in rice in the United Arab Emrirates. Mycotoxin Res 15, 1999, 39-44.

[18]. Sales A, Yoshizawa T Updated profile of aflatoxin and Aspergillus section Flavi contamination in rice and its by products from the Phillipines. Food Addit. Contam. 22, 2005, 429-436

[19]. Diaz G T, Perilla NS and Rojas Y Occurrence of aflatoxins in selected Columbian foods. Mycotoxin Res 17, 2001, 15-20

[20]. Torres, P., Guzman-Ortiz, M., and Ramirez-Wong, B Revising the role of pH and thermal treatments in aflatoxin content reduction during the tortilla and deep frying processes. J.Agric Food Chem. 49, 2001, 2825-2829. Doi:10.1021/jf0007030.