



Contamination of White and Brown Rice with Aflatoxin and Evaluation of Strategies for their Decontamination

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ABSTRACT

Rice is a primary food crop for over 50% of the world's population, with approximately 500 million tons of milled rice produced globally each year. Significant food wastage occurs due to poor storage conditions, with an estimated economic loss of US\$1.6 million in the rice industry attributed to aflatoxins (AFs), which also pose serious health risks. Various research efforts are underway to improve storage methods and reduce AF contamination. In this study, white and brown rice samples were analyzed for aflatoxin contamination and treated for detoxification. A total of 50 samples, 30 white rice and 20 brown rice were collected from different areas in and around Lahore, Pakistan. Aflatoxins were quantified using Thin Layer Chromatography. Various detoxification methods, including physical, chemical, and natural approaches, were applied to contaminated rice. Contamination was found in 36% of white rice samples, with 23% exceeding European Union permissible limits. In brown rice, 40% of samples were contaminated, with 20% exceeding permissible levels. The highest aflatoxin concentration ($16.45 \pm 0.06 \mu\text{g/kg}$) was found in brown rice. Detoxification results showed a maximum reduction of 40.09% through cooking, 62.31% with 10% citric acid, and 82.24% with 10% clove oil for physical, chemical, and natural methods, respectively. Among these, essential oils proved to be the most effective for detoxification. The study concludes that aflatoxins are a major cause of rice spoilage, and the use of natural storage methods, such as essential oils, offers a promising solution for reducing contamination.

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Authors' Contribution

RY, SA: Investigation. NZ: Supervision.

Key words

Aflatoxins, Thin layer chromatography, Contamination, Detoxification, Essential oils

INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food all over the world (Trucksess *et al.*, 2011; Choi *et al.*, 2015). It is consumed by around 75% of the global population and 60% of South Asians. In grain crops, rice (*Oryza sativa* Linn.) are second large crop after wheat (Lutfullah and Hussain, 2012). Rice production in Pakistan is approximately 4.9 tons/hectare which makes it world's 11th biggest producer and it contributes 1.3% in gross domestic production of country (Eslami *et al.*, 2015; Suleman *et al.*, 2018; Arshad *et al.*, 2019; Khan *et al.*, 2019). A lot of food waste occurred due to storage incapability as approximately US\$ 1.6 million economical loss is only related to rice industry due to AFs. AFs are a group of nearly

20 structurally similar mycotoxins found in a wide range of foods, such as cereals, spices, nuts, figs, and dried fruit. Though, the highest levels are produced in the food crops that cultivated in the tropical regions around the globe. Because of the worldwide trade of important crops, AFs are at high concern not just for producing countries as well as for consuming ones (Prandini *et al.*, 2009). AFs (AFTs) production is caused by the fungi *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus parasiticus* and *Aspergillus pseudotamarii* (Hassan *et al.*, 2017; Iqbal *et al.*, 2019; Nazir *et al.*, 2019). Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) are the four primary naturally occurring AFs labelled depending on their color variations in Ultraviolet (UV) light and their retention factor (Rf) on Thin Layer chromatography (TLC). AFG2 < AFB2 < AFG1 < AFB1 is the sequence of poisonous of above mentioned AFs from acute to chronic (Tahir *et al.*, 2018).

Rice is mostly grown in subtropical climates that are known for being hot and moist. Rice is usually dried after harvesting, however when stored improperly, it can become an ideal substrate for the mould production (Reiter *et al.*, 2010; Lai *et al.*, 2015). Improper storage conditions lead to the fungal development and other harmful substances

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resulting in the loss of 15% of produced rice each year as per FAO (Dors *et al.*, 2009).

AFs pose a potent threat to human health, either through direct intake of food or through the transmitted AFs and their byproducts in milk and meat (Nordkvist *et al.*, 2009; Reiter *et al.*, 2010; Naseer *et al.*, 2014). AF B1 is the most hazardous of the AFs, having being linked to hepatocellular carcinoma (HCC) along with growth retardation, immunological alteration, and malnutrition (IARC, 2012). Considering AF toxicity, a lot of states have established AF tolerance levels in foods. The maximal tolerated levels for the AFs in white rice are 4 µg/kg for all AFs (AFG1 + AFG2 + AFB1 + AFB2) and 2 µg/kg for AF B1 and in brown rice these are recorded as 10 µg/kg for all AFs and 5 µg/kg for AF B1 as set by the European Unions (EU, 2010).

For the study of AFs, different techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and ELISA can be employed (Matabaro *et al.*, 2017). However, Thin layer chromatography is one of the best technique which has potential to detect levels of toxins and can produce good and reliable results (Namir *et al.*, 2019). AFs have been found in rice and rice products reported from various countries such as Iran (Mazaheri, 2009), Pakistan (Majeed *et al.*, 2013; Iqbal *et al.*, 2014), China (Zhu *et al.*, 2013), and Scotland (Ruadrew *et al.*, 2013). The escalating number of studies on AFs prevalence in foodstuff and feed necessitates the development of detoxification processes that not only lowers the AFs concentration to “secure” levels under established limits but also meet the following criteria: simple to utilize, affordable and free of the risk of producing toxic substances or affecting the nutrient profile of the treated item (Méndez-Albores *et al.*, 2004).

This study was aimed to determine the AF concentration in white and brown rice, to compare the incidence levels in both types of rice and was compared to European Union maximum limits. The study also purposed to see appropriate detoxification methods such as physical, chemical and natural for reduction of AF contamination in white and brown rice.

MATERIALS AND METHODS

Collection of samples

A total of 50 samples of white (30) and brown (20) rice were collected from various shops of five areas in and around Lahore that included Gulberg, Iqbal town, Walton, Shahdara and Kasur during August to October 2020. Ten samples were collected from each area; 6 of white rice and 4 of brown rice. One kg of rice sample was taken from a large sized jute sacks from 2-3 different places of jute

bag diagonally. The collected rice samples were then passed through sample divider and obtained 200g of rice from each sample and mixed thoroughly and grinded to fine powder in a grinding mil for experimental analysis (Trucksess, 2005). All the samples were properly labelled as Gulberg (G1-G10), Iqbal town (I1-I10), Walton (W1-W10), Shahdara (S1-S10), and Kasur (K1-K10) and quantified for AFs by TLC.

Extraction of aflatoxin from rice samples

The extraction procedure was performed by taking approximately 50 g of powdered rice samples in a 500 ml conical flask. Then 150 ml chloroform and 25 ml distilled H₂O was added the flask. The flasks containing rice samples were put on shaking on wrist-action shaker for 30 mins and samples were filtered in beakers by filter paper. Approximately 50 ml of filtrate was placed on heavy duty hot plate for evaporation. Dilutions for spotting were obtained in micro-liter. 25 µL spot of the sample solution was put on a TLC plate with micro syringe. 5 or 10 µL standard spots of AFs (B1, B2, G1, and G2) were also placed on the same plate as internal standards. Then the TLC plate was subjected to two TLC tanks containing mobile phases. The TLC plate was developed with anhydrous ether in first TLC tank up to the mark. After the plate was completely developed it was taken out from tank and dried. Then plate was redeveloped in the same direction in the second TLC tank with acetone and chloroform concentration of 1:9. TLC plate was then removed and left for drying (Nisa *et al.*, 2016; Summia *et al.*, 2021).

Identification and quantification of AFs in rice samples

For the identification and quantification of AFs in the test solution spots, the plate was scanned using ultraviolet light scanner. Comparison was made between fluorescing concentrations of specimen spots with standard spots of AFs. In case of fluorescing spot of sample lied between two standard spots, the average value of two standard spots was taken into consideration. Another very important step in the AF analysis was the fluorescing sample spots. This was carried out by spraying evenly the TLC plate with aqueous sulphuric acid (50/50 v/v). After the spraying TLC plate was allowed to dry and then viewed under UV light (365 nm).

Decontamination of contaminated samples

Detoxification of AFs were carried out by physical methods using washing and cooking (Hwang and Lee, 2006; Park and Kim, 2006), by chemical using hydrochloric acid (0.3% HCl) and citric acid (10%) (Zahra *et al.*, 2013) and natural methods using essential oils such as 10% clove oil and cinnamon oil (Anzlovar *et al.*, 2017).

RESULTS

In this study, 50 rice samples, including 30 white rice and 20 brown rice samples, were analyzed for aflatoxins (AFs). Among the 30 white rice samples, 11 (36%) were found to be contaminated, with 7 samples (23%) within the permissible range and 4 samples (13%) exceeding the permissible range (Table I).

Of the 20 brown rice samples, 8 (40%) were contaminated, with 4 samples (20%) within the permissible range and 4 samples (20%) exceeding it. A larger proportion of brown rice samples (40%) were found to be contaminated compared to white rice samples (36%) (Table II).

Detoxification of highly contaminated samples was performed using methods such as washing, cooking, acids, and oils. Physical methods were the least effective, reducing AFs by 24.31% to 40.09%. Chemical treatments achieved a reduction of 53.73% to 62.31%. However, the most effective results were observed with natural methods using plant oils, where clove and cinnamon oils reduced AFs by 73.88% to 82.24% (Table II).

DISCUSSION

Rice is a primarily essential food crop in several states. Rice is mainly cultivated in these countries during the rainy season. During this season, sun drying of rice, as is done by majority of the farmers, sometimes may not be enough to keep the moisture level of grains low enough to avoid fungal growth. As a result, rice grains having moisture level greater than the required threshold (>14%) may enter the storage

system. Change in color of the grain or hull, quality loss, loss in vitality, and toxin contamination are all detrimental outcomes of such fungal invasion (Reddy *et al.*, 2009).

Table I. Aflatoxins contamination of white rice and brown rice samples collected from different sites of Lahore permissible range according to EU (2010) is 4 µg/kg for white rice and 10 µg/kg for brown rice.

S. No.		Sample ID	Mean ± SD
White rice samples			
1	Gulberg	G3	1.89 ±0.02
2	Iqbal Town	I4	1.74 ±0.01
3	Walton	W2	2.42 ± 0.03
4		W3	1.85 ± 0.07
5		W5	7.44 ± 0.2
6	Shahdara	S1	8.73 ± 0.02
7		S4	2.41 ± 0.03
8		S6	1.67 ± 0.04
9	Kasur	K3	4.60 ± 0.04
10		K5	1.54 ± 0.03
11		K6	5.45 ±0.1
Brown rice samples			
1	Gulberg	G1	13.21 ± 0.03
2		G3	6.83 ± 0.1
3	Walton	W2	4.14 ±0.02
4		W4	3.92 ± 0.05
5	Kasur	K4	1.35 ± 0.02
6	Iqbal Town	I2	16.45 ± 0.06
7	Shahdara	S1	15.72 ± 0.01
		S4	11.06 ± 0.04

Table II. Detoxification of contaminated white rice samples S1 and brown rice sample I2 by different physical, chemical, and natural methods.

Sample	Initial AF contaminations (µg/kg)	Methods	Type	AF concentration after decontamination process	Reduction in contamination (%)
Shahdara SI (white rice)					
	8.73 ± 0.02	Physical	Washing with water	6.45 ± 0.03	26.11
			Cooking	5.23 ± 0.1	40.09
			0.3% HCl	3.97 ± 0.07	54.52
		Chemical	10% Citric acid	3.29 ± 0.12	62.31
		Natural	10% Clove oil	1.55 ± 0.2	82.24
			10% Cinnamon oil	2.28 ± 0.1	73.88
Iqbal Town I2 (Brown Rice)					
	16.45 ± 0.06	Physical	Washing with water	12.45 ± 0.02	24.31
			Cooking	10.03 ± 0.05	39.02
		Chemical	0.3% HCl	7.61 ± 0.3	53.73
			10% Citric acid	6.58 ± 0.1	60
		Natural	10% Clove oil	2.97 ± 0.04	81.94
			10% Cinnamon oil	4.84 ± 0.15	70.57

38% of all samples were found tainted with further differentiation of 36% from white rice and 40% from the brown rice in the present study. A research study that was carried out in Pakistan revealed the prevalence of higher AFs contamination of 70% in the rice samples and the mean contaminated levels were 4.9 µg/kg that surpassed the secure level established by European Union 4 µg/kg. AF B1, AF B2, AF G1, and AF G2 were present in levels up to 18, 10.4, 8.4, and 12 µg/kg, accordingly (Ali *et al.*, 2011).

Various varieties of rice, comprising parboiled, white, brown, paddy, and broken, were documented to have AFs (Iqbal *et al.*, 2012) and results of white and brown rice samples were comparable to our existing findings. Paddy rice were majorly infected (64%), while brown rice were least effected (33%) followed by white rice (42%). The study also claimed dietary rice as a main source of AFs in human beings. Asghar *et al.* (2014) documented AFB1 and AFB2 prevalence in 95.4% and 7.6% in Pakistani brown rice, respectively that as very high as compared to our findings for the brown rice that was 40%. Whereas AFG1 and AFG2 were not found in any sample and that was very similar to current study. Overall AFs occurred between 1.07 and 27.27 µg/kg.

Different physical, chemical and biological methods were also adopted to reduce AF levels in rice. Park *et al.* (2005) concluded that the reduction of AFB1 in rice after washing with water ranged from 20 to 24%, with an average of 22% which support our results.

In different studies conducted in Korea, cooking positively decreased total AFs 34% and 31-36% in originally contaminated refined rice (Park *et al.*, 2005; Park and Kim, 2006). Both the studies were in line with our work. The impact of steaming at 160 °C by ordinary cooker on AF deposits in the cooked rice had been documented previously by others (Park *et al.*, 2005).

Zahra *et al.* (2013) documented 55% decrease of AFB1 in rice by 0.3% HCl which is in agreement with the results of our experiment. As HCl is a strong acid so its mild concentration is used in experiment because high concentration can change the organoleptic properties of food.

Citric acid (C₆H₈O₇) was utilized for the conservation measures. It has particular, distinctive, corrosive and tart flavors, incorporated into different kinds of beverages and edibles. Lemon juice comprises of 5 % citric acid. Reaction with the diluted citric acid degraded AF B1 in rice. In present study tainted samples were immersed in lemon juice for decontamination. The outcomes of that experiment revealed that citric acid was 63.59% to 90% effectual, against AFB1 in the various types screened (Safara *et al.*, 2010; Karlovsky *et al.*, 2016).

Few essential oils and compounds in plant extracts are taken up as generally recognized as safe (GRAS). Different amounts (0, 2, 4, 6, 8, and 10% (w/v)) of *Syzygium aromaticum* pure diluted extracts were screened for their potential resistive impact on *Aspergillus* growth and AF formation in 50 g of rice. The outcomes revealed that *Syzygium aromaticum* at 10% (w/v) and restricted the *Aspergillus* growth and also lowered the AF formation rate in rice (Thanaboripat *et al.*, 1997). Reddy *et al.* (2009) noticed that mold growth and AF development were totally restricted after employing of diluted extracts from clove on healthy kernels of a rice cultivator for five days. Eugenol (C₁₀H₁₂O₂) is a phenolic substance obtained chiefly from buds and leaves of *Syzygium aromaticum* and from *Cinnamomum zeylanicum*, comprising the most important and active compound of clove oil (85 to 95%) along with methyleugenol and iso-eugenol (Souza *et al.*, 2005). The most effectual compounds of cinnamon essential oil because of their biological activities were suggested to be cinnamaldehyde and eugenol (Siddiqua *et al.*, 2015).

Physical and chemical decontamination strategies may have certain restrictions like loss of minerals, ineffectiveness and variations in the organoleptic properties of food. But, the application of natural methods (plant oils) is a secure way of detoxification of AF contamination in rice samples. This study provided updated knowledge regarding AFs contamination in different areas and results provided a good contribution to food authorities and they can form and imply regulations to control this.

CONCLUSION

It was concluded that the contamination percentages of aflatoxins (AFs) differed between brown and white rice samples, with higher concentrations detected in brown rice. Rice samples collected from various locations exhibited varying levels of AF contamination, with the highest levels found in samples from Walton and Shahdara, likely due to improper storage conditions. While physical and chemical detoxification methods were moderately effective, natural methods proved more effective for long-term storage. This study provides updated insights into AF contamination across different rice types and regions, contributing valuable information for food authorities regarding the implementation of safety regulations.

DECLARATIONS

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Statement of conflict of interest

The authors have declared no conflict of interest.

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