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Comparative study of aflatoxins in brown rice samples of local and import quality

[1*]Nisa, A., [2]Zahra, N. and [3]Yasha, N. B.

[1]*Pakistan Council of Scientific and Industrial Research,* [2]*Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan*

[3]*Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan*

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Abstract

Aflatoxins are harmful and most carcinogenic substances known which may affect rice quality to greater extent. Aflatoxin concentrations in local and import quality Brown Rice samples were analysed for determination of quality difference with respect to the limits set by European Commission i.e.10 ppb. A total of 50 samples of local and import quality brown rice were obtained from market for analysis using Thin Layer Chromatography. 92% local brown rice samples were contaminated, 56% were contaminated above permissible limits; whereas, 36% were below permissible limits. In import quality only 48% of samples were contaminated where 44% were below and only 4% were above permissible limits. The results of aflatoxin concentration analysis revealed that import quality of rice is quite improved as compared to local quality rice. Local quality brown rice samples can be unsafe and dangerous for human health due to its lethal effects.

Introduction

Aflatoxins are harmful secondary metabolites which are produced by fungi belonging to the species *Aspergillus parasiticus* and *Aspergillus flavus* (Binder *et al*., 2007). These chemical poisons cause the contamination of world's crops and bring about economic losses and many serious health hazards (Williams *et al*., 2004).

Aflatoxins are carcinogenic and hepatotoxic, their consumption is strictly monitored by different food and health authorities including European Commission and Food and Drug Administration through various pre-harvest and post-harvest methods. These authorities have set several range limits for the presence of aflatoxins in different foodstuffs beyond which food becomes unsafe for consumption (Van Egmond and Jonker, 2004).

About 18 types of various aflatoxins are discovered uptill now and a few renowned ones include G_1, G_2, B_1, B_2, M_1, and M_2 type aflatoxins but the most harmful type of aflatoxin is the type B1 aflatoxin (Krishnamurthy and Shashikala, 2006). Stored food is highly subjected to the fungal invasion which poses great threat to global food safety and security by reducing the nutritional value and increasing health hazards through food spoilage (Set and Erkman, 2010). Food Safety and Food Security are now found to be much associated with public health and problems because various mycotoxins have caused outbreak of diseases and many deaths in the past.

The presence of aflatoxins can be determined through various processes such as HPLC, TLC, ELISA, and Fluorescence Detector (Trucksess *et al*., 1989). Aflatoxins are more frequent in the developing countries due to poor harvesting, drying, and storage facilities (WHO, 2005). Of many different types of crops, rice is one of the major polished cereal grains with different varieties depending on the environmental conditions, seed types, harvest operations and optimum time for sowing (Pittet, 1998).

Rice is widely grown and consumed throughout Asia as a major food source. There are many countries such as Thailand, India, Pakistan and Uruguay which cultivates and supplies import quality rice to the other countries in the world (FAO, 2004). Pakistan being an agro based developing country exports rice too; but it still needs to establish safety regulations to bring the local quality rice to the same level as that of the import quality of rice. It is reported that many foodstuffs including corn, figs, walnuts, dried fruits and cereals contain harmful aflatoxins in them (Juan *et al*., 2008).

In Iran around 83% of import quality rice samples were reported to be contaminated with aflatoxins with average amount of 2.09 μgKg^{-1} (Mazaheri, 2009).

*Corresponding author.
Email: *nisaalim64@yahoo.com*

Like many other crops, rice also contains aflatoxins and might become unfit for human consumption so it must be prevented from contamination (Patel *et al.*, 1996). Many favorable environmental factors such as high temperature, high humidity and moisture, frequent rainfalls, and poor soil conditions also play an important role in aflatoxin contamination of crops and this is the reason why aflatoxins are somewhat found in the local quality rice samples in Pakistan (Shamma *et al*., 2012). The standard regulatory range limits for the presence of aflatoxins in rice samples set by European Commission is around 2 μgKg^{-1} for AFB_1 type Aflatoxins; whereas, the aflatoxin range acceptable as in whole is around 4 μgKg^{-1} (EC, 2006).

Many different methods and chemicals are now being used to detoxify aflatoxins from foods such as the usage of 0.3% of NaOCl, 5% $Ca(OH)_2$, and 0.5% HCl to remove around 80% of aflatoxins from white rice (Nisa *et al*., 2013). Crops which are usually contaminated with mycotoxins should be treated with post-harvest treatment methods and proper storage conditions must be ensured in order to prevent harmful maladies like liver cancer (Andrade, 2013). Since Asians are more dependent on rice as a food source so the quality of rice must be improved in all the types of rice crops either import or local quality.

It is easier to carry out pre-harvest methods rather post-harvest methods which are usually expensive and difficult to carry out (Janos *et al*., 2010). Aflatoxins must be removed from food and feed sources through various methods such as detoxification on larger scale so that to prevent great economic loss and illnesses in human beings and animals (Nisa *et al*., 2012). Aflatoxins were analyzed in different samples of Brown Rice which were first prepared through a specific scheme (Begum *et al*., 1985). Aflatoxins present in the Brown Rice samples were then further detected through the method explicated (Romer, 1975). The standard technique was compared with the toxic extracts of aflatoxins for the estimation purpose (AOAC, 2005). In this Study brown rice samples of local and import quality were compared for the presence of Aflatoxins.

Materials and Methods

Collection of samples

Before commencing with the research work, different Brown Rice samples of both local and import quality were collected from various shops and mega stores and used in the laboratory for quantitative analysis.

Sampling of brown rice specimen

Aflatoxins are non-uniformly present in various commodities such as Brown Rice. The contaminated grains of Brown Rice are found to have pockets of quite high concentration of aflatoxin because of varied distribution of aflatoxins in them. A suitable plan for sampling was designed to acquire samples with vivid results.

All Brown Rice samples were obtained from the large jute bags stored in the basements. About 500g of the brown rice samples were collected to obtain the most representative portion of grains. The jute bags were cut diagonally from 2-3 sides and the sample probe was used to fill the plastic bags. After passing the brown rice samples of 500 g through the sample divider, the amount of brown rice was reduced to 200 g each. This step was carried out to obtain greater homogeneity of different portions of rice sample contaminated with aflatoxins. Brown rice samples were then properly mixed and grinded to get a fine powder form for better experimental analysis.

Extraction of brown rice samples

Today, different extraction and analytical methods are used for different types of commodities because of their varied chemical composition. There isn't a common method which can be applied for the brown rice samples; henceforth, chloroform method for the extraction was selected as a suitable method to extract aflatoxins.

The type of aflatoxins which were extracted and analyzed from the brown rice samples included AFB_1, AFB_2, AFG_1, and AFG_2. The extraction method was carried out by taking about 50 g of grinded brown rice samples in a 500 ml Erlenmeyer flask. About 25 ml of water and 150 ml of chloroform was poured into the flask. The Erlenmeyer flask was then shaken for 30 minutes with the help of wrist-action shaker and the brown rice sample was then filtered through Whatman filter paper. About 50 ml of the eluate was put on hot plate for evaporation.

The dilutions were obtained in micro-liter for the spotting purpose. With the help of a micro syringe, a spot of 25 µL of the test solution was then applied on a Thin Layer Chromatographic Plate. Standard spots of 5 µL and 10 µL of aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2 were also spotted on that plate to work as internal standards. The TLC plate was placed in a TLC tank containing anhydrous ether until the solvent travelled half way up. After the TLC plate was finely developed it was taken out and dried.

The redevelopment of plate was then done in the similar direction but with solution of acetone and chloroform to a ratio of 1:9. After the removal of TLC plate from the TLC tank the spot of test solution was properly observed under Ultra-Violet Light to look for the presence or the absence of aflatoxins. New concentration for the spotting purpose was prepared when the first plate showed the necessity of new concentration of the test solution.

Detection and estimation of brown rice samples

The Fluorescing intensities of brown rice sample spots were examined and compared with the standard spots of aflatoxins. If the fluorescing spot of brown rice sample was present between the standard aflatoxin spots then the value which was most likely to be considered was the average value of the two standard spots.

Confirmation of the results

One of the most important steps when it comes to the analysis of aflatoxins includes the fluorescing of sample spots. This step was done by homogenously spraying aq. sulphuric acid (50/50 v/v) on the TLC plate. The TLC plate was then dried and examined under the Ultra-Violet Light of 365 nm.

Calculations for the presence of aflatoxins

The concentration of aflatoxins in brown rice samples in µg/kg was determined by using following formula

$$\text{Aflatoxins in } \mu g/kg = \frac{S \times Y \times V}{Z \times W}$$

Where;

S= the volume of aflatoxin standard in µL with intensity equivalent to Z (the µL of sample)
Y= the concentration of aflatoxin standards µg/mL
V= the volume of solvents in µL required for the dilution of the final extract
Z= the volume of sample extract in µL required to give the fluorescence intensity compared to aflatoxin standard S in µL
W= the weight of original sample in the final extract (g)

Results and Discussion

The extent of contamination of total aflatoxins in the local Brown Rice Samples (AFB_1, AFB_2, AFG_1, and AFG_2) was of great incidence. Of all 25 local Brown Rice quality samples, 23 were found to be contaminated with aflatoxins. The European Commission has set the limit of 10 ppb (parts per billion) for the presence of aflatoxins in rice samples (EC, 2010). Among these contaminated local quality brown rice samples about 14 of them were contaminated beyond the permissible limits and 9 were contaminated below the permissible limits set by the EC.

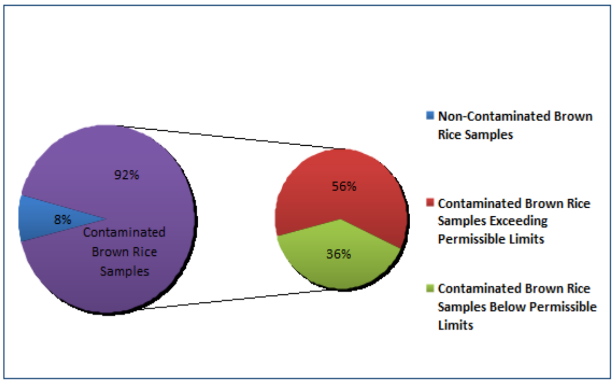

Figure 1. Percentage analysis of local brown rice

The analysis of Local Quality Brown Rice Samples demonstrated that 92% of the total samples were contaminated with aflatoxins; whereas, only 8% had no aflatoxins in them. The incidence of the prevalence of aflatoxins was quite high in the local quality rice samples. The reason behind the presence of abundant aflatoxins in local brown rice samples is because of the absence of food regulation authority in Pakistan. With the help of food regulation authority certain limitations can be imposed on the supply of aflatoxin contaminated commodities like brown rice. Out of contaminated samples 56% had aflatoxins beyond the permissible limits whereas 36% had the aflatoxins below the permissible limits (Figure 1).

Four types of aflatoxins were analyzed including aflatoxin type B_1, B_2, G_1 and G_2 (Lai *et al.*, 2015). The most abundant type of aflatoxin with high concentration was aflatoxin B_1 whereas a few samples had aflatoxin B_2. Aflatoxin type G_2 was totally absent in all the samples. The final results of aflatoxin concentration were concluded on the basis of the sum of all the aflatoxins present in each of the samples of brown rice. Since the extent of aflatoxins in the samples is quite high, there must be preventive measures against these harmful toxic substances to avoid health disorders in Pakistan.

The Import quality brown rice samples demonstrated different results than the local quality brown rice samples. The incidence of aflatoxins present in the import quality rice samples was quite lower as compared to that of the local quality brown rice samples. Total 25 samples of import quality were analyzed from which only 12 of them were contaminated with aflatoxins whereas the remaining were not. Just one sample was found to have aflatoxins beyond the defined limits. Remaining 11 samples had very low aflatoxin contamination.

The common thing among both the quality of rice samples was the presence of aflatoxin AFB_1

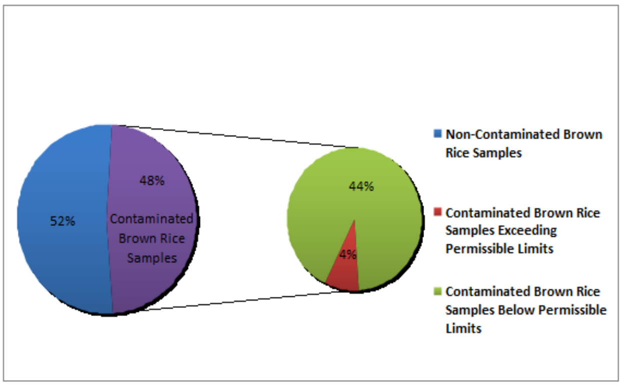

Figure 2. Percentage analysis of imported brown rice

and the complete absence of AFG_2 despite the fact that the concentration of aflatoxins B_1 in the local quality brown rice samples was higher than those in the import quality brown rice samples. Through careful analysis of the Import quality brown rice samples had about 48% contamination of which 44% were non-contaminated and the remaining 4% were contaminated. 52% of the rice samples had no aflatoxin contamination in them (Figure 2). Of many commodities like brown rice samples which have been tested to detect aflatoxins in them, AFB_1 aflatoxins are most abundant and very carcinogenic in foodstuffs (Nisa *et al.*, 2012; Iqbal *et al.*, 2014).

Through these experimental observations the results depict that since different developed countries have put restrictions on the supply of aflatoxin contaminated food import beyond the permissible limits, so the suppliers tend to take the preventive measures to improve the import quality of the brown rice samples. However, local quality needs the same consideration as that of the import quality of brown rice samples.

Conclusion

From Investigational analysis it can be inferred that aflatoxins are frequently found in local quality brown rice samples than those of import quality brown rice samples. The local quality brown rice samples consumed by the local people in Pakistan contained large quantity of aflatoxins; whereas, the import quality is safe. For the import quality of rice, the permissible limits provided by the European Commission are usually followed but there is no proper safety regulation authority in Pakistan regarding locally available rice. There should be precautionary and preventive measures against aflatoxins to save people's life by assuring healthy and safe food.

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