Detection and quantification of heavy metals and toxins in rice bran related products

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Abstract

This study was aimed to investigate heavy metals and toxins effect on rice bran related industrial products such as rice bran (RB), rice bran oil (RBO) and deoiled rice bran (DORB). Heavy metals such as As, Pb, Cd, Cr, Ni, bacteriological assay such as bacterial load (cfu/g), fungal assay such as yeast and molds load (cfu/g) and fungal toxins specially aflatoxins (B1, B2, Gl and G2) were investigated for RB, RBO and DORB samples. RB and DORB samples were found positive for bacteria, yeast and mold population, and aflatoxin B1 for fresh (0), 7, 14 and 21 days after storage at ambient temperature. RB and DORB samples for 28 days after storage found lower load in bacteria, yeast and mold but completely negative for aflatoxin B1. We have examined all the above mentioned quality parameters for RBO and found negative for bacteria, yeast and mold load and even aflatoxin B1.

Key words: Heavy metals, toxins, rice bran (RB), rice bran oil (RBO) and deoiled rice bran (DORB)

Introduction

Referring to our previous study on post-harvest loss minimization of rice bran for quality bran oil (Haque and Shozib, 2018), we concluded that heat treatment at 130-135°C for two hours, found suitable for stabilizing rice bran from increasing free fatty acid (FFA%) and lowering oil% for at least 28 days and it is expected that lipase activity might possibly inhibited or at least slow down their activity by heat treatment. We recommended adopting heat treatment as physical treatment, soon after harvesting bran from brown rice in this regards. This particular physical treatment should be applied in auto rice mill promises soon after harvesting fresh bran. RBO industries are facing challenges with lack of fresh bran, high FFA% containing bran with lower percentage of oil content, artificial crisis of bran even at harvesting season. So we assumed that our findings could assist these RBO industries to stabilize rice bran with attainable quality up to 28 days after harvesting bran at parboiled milling condition. Considering the above mentioned research findings we wanted to examine quality parameters of rice bran (RB), rice bran oil (RBO) and deoiled rice bran (DORB) in terms of heavy metal and toxins.

RBO has an increasing trend of consumption in local market along with RB and DORB, which has a huge market in cattle, fish and poultry related feed industries. In addition, SBE has a little exposure in insulation brick industries in our country. Value chains have developed in association with the growth of the formulated feed industry and now widely connect feed suppliers with farmers, though more remote farmers still lack access to formulated feed. Bangladesh produces 50-55% of fish feed ingredients, with the remainder imported. These products flow from producers or importers via feed processors to reach farmers through various channels. Raw materials such as rice bran require processing in mills before incorporation into formulated aquaculture feeds. The main ingredients used for fish feed production are rice bran (20-50% inclusion), maize (5-20% inclusion), soybean meal (10-30% inclusion), mustard oil cake (10-25% inclusion), fish meal (5-15% inclusion) and meat and bone meal (10-20% inclusion). Rice bran is mainly produced locally and is derived from rice milling. It accounts for 8-10% of rough rice grain by weight. Three types of rice bran are used: deoiled rice bran (DORB), grade A rice bran (comprising of 85-90% bran and 15-10% husk) and grade B rice bran (comprising of 50-60% bran and 40-50% husk). The protein content of DORB and grade A rice bran is approximately 12-17% and 10-13% respectively. Only 30% of total rice bran production in Bangladesh is used for commercial animal feed products. Three main factories produce approximately 0.13 million tons of DORB per year during production of rice bran oil (Mamun-Ur-Rashid et al., 2013).

Rice bran (RB) is a byproduct of rice milling industry which converts into DORB when its oil content is extracted in oil industry. Its composition varies according to the type of milling but it contains 15-25% oil, 11-17% protein, 6-14% fibre, 10-15% moisture and 8-17% ash (Sharif et al., 2014; Haque and Shozib, 2018). It is an abundant source of antioxidant compounds such as tocopherols, γ-oryzanol and other phenolics (Aguilar-Garcia et al., 2007), which helps in health benefits including lowering blood cholesterol, decreasing platelet aggregation and anti-inflammation (Lai et al., 2009). It is a good source of lysine and methionine (Dale, 1997). As such RB is considered a suitable feed ingredient for livestock and fish. During oil extraction chemical and heat treatments applied to RB. It seems reasonable to conclude that, those treatments may change the quality of the nutrients presence in the bran that need further investigations. On the other hand, some nutrient profile would proportionately be higher in the DORB than RB due to oil extraction from RB (Houston, 1972; Warren and Farrell, 1990). Other benefits include less susceptibility of DORB to the rancidity. Some researchers found that the nutritional value of DORB is equal to the value of RB in broiler diets when diets are adjusted for metabolizable energy by adding oil (Farrell, 1994). Islam et al., (2018) conducted a study on the performance of broiler feed on iso-caloric and iso-nitrogenous diets containing either RB or DORB and assessed the economics of broiler diet.

Rice, rice polish and deoiled rice bran along with maize, starch, wheat, wheat bran, wheat grain (milled to flour), molasses, cassava meal etc. are used as energy source of fish feed in Bangladesh (Table 1).

Table 1 Identification of feed ingredient (Rice related only source BFRI online report)

Identification of feed Ingredient		**Nutrient content (%)**			**Gross Energy, GE* kJ.g^{-1}**
Name of Ingredients (Plant sources)	Physical properties	Crude Protein (%)	Crude Fat (%)	Carbohydrate NFE (%)	
Rice bran (Traditional milling)	Powder	7-10	10-12	56	15-16
Rice bran (Auto, boiled)	Powder	10-12	10-15	45	13-16
Rice bran (Auto, *atob*)	Powder	10-14	10-18	45	13-17

Around 100 commercial feed mills are currently in operation in Bangladesh. About 600 of small-scale noncommercial and on-farm feed manufactures produced feed for their own consumption. About 6-8 large feed mills account for 60-70% of market share. In case of fish feed, most of the mills produce pangas, koi, shing, magur and tilapia feeds. Only few feed mills produce feed for shrimp and carps. There are several types of fish feeds such as nursery (Mesh/Fine Crumbles), Starter-1 (Crumbles), Starter-2 (Crumbles), Starter-3 (1.8-2.5 mm diameter), Grower (2.0-4.0 mm), Finisher (2.0-4.0 mm). Distributions of feed production are as nursery 5%, starter 25% and grower 70%. Species wise feed productions are pangas 60%, tilapia 28%, koi and shing 8%, carp 2% and shrimp 2% (Zulfikar, BFRI). In feed mills especially fish feed mills a wide range of 5-30% RB and DORB are being used as active plant feed ingredient. DORB is used as high protein source (>18%) and RB is used for high fat source (>20%).

The lipid fraction of rice bran contains mainly oleic, linoleic and palmetic acids which are excellent nutrient source. Rice bran oil contains the lowest hypho cholestrolaemic activity (Rukmini, 1985). Deoiled bran contains less fat and high protein that is used in the cattle. Almost all commercial feed mills also produce poultry feeds. In 2009, Jayaraman and Kalyanasundaram reported the toxigenic fungi presence in the rice bran oil and deoiled bran samples which were classified as *Aspergillus glaucus*, *A. flavus*, *A. niger*, *A. nidulans*, *A. candidus*, *A. fumigatus*, *Penicillium* spp. and *Gliocladium viride*. Since rice bran has now achieved considerable importance as a source of oil, which besides being edible, also has several industrial applications. Besides, rice bran has always been an inexpensive and easily available source of nutritious cattle feed for the farmer. A preliminary survey on some samples of unparboiled rice bran and parboiled rice bran in 1990, Jayaraman and Kalyanasundaram reported that 35% of the samples contained aflatoxin B1 (AFB). When oil was extracted from contaminated bran, a considerable amount of the toxin came out in the crude oil but the refined oil was free from toxins. The residual bran after oil extraction which generally goes into processed cattle feed however, still contained a considerable amount of aflatoxins (Jayaraman and Kalyanasundaram, 1994). Aflatoxin B1 is one of the most hepatocarcinogenic naturally occurring compounds which is produced by toxic species of fungi in different types of food including rice. The contamination of food with this toxin could lead to a series of health hazards and huge economic losses.

Rice is the second largest quantity staple food and internationally traded cereal. Aflatoxin is produced in areas where climatic conditions are favorable to fungal growth. The production of aflatoxin affects plant growth and rice yield.

Elangovan and Kalyanasundaram (1999) surveyed on the prevalence of aflatoxin B1 (AFB) in rice bran and collected 142 samples from 55 rice mills in coastal and interior districts of Tamil Nadu, Andhra Pradesh and Karnataka. It was reported that 62% of the samples contained AFB and the levels far exceeded the permissible limit of 50 ppb. Among them 60% of the positive samples having 50-500 ppb and 30% up to 2000 ppb. The mycoflora of bran included over 20 osmophilic species, of which *Aspergillus flavus* link showed the highest frequency (75%) as well as abundance (37.6%). The high frequency and high levels of AFB showed no correlation with moisture content of the samples, climatic variations, or with the unparboiled or parboiled status of the rough rice but with the maintenance of sanitation in the rice mills, which were, therefore, suspected to be a major source of contamination of bran with aflatoxigenic fungi.

Heavy metal pollution of soil affects the nature of the environment leading to serious consequences. Heavy metals group includes Ag, Ba, Cd, Co, Cr, Mn, Hg, Mo, Ni, Pb, Cu, Sn and Zn and some metalloids such as As, Sb, Bi and Se. Arsenic, for example, is often considered as a heavy metal due to the similarity of its chemical properties and behavior with the other heavy metals. Heavy metals accumulation in soil and in the environment in general, may be related to the phenomenon of bioaccumulation ability of living organisms that is heavy metal may be increased in human organism due to industrial activities and the food chain. The main sources of heavy metal pollution in soil are irrigation, especially with sewage; solid-waste disposal, for example, sludge and compost refuse; the use of pesticides and fertilizers; and atmospheric deposition (Fabjola et al., 2015).

Plants acquire the necessary nutrients, such as N, P and K from the soil. However, they may also accumulate unnecessary and toxic metals, such as Pb and Cd. Several plants have the ability to accumulate high metal concentrations (Watanabe, 1997). Many studies have reported data for the transfer of heavy metals from soil to plants and vegetables through roots and shoot (Uchida et al., 2009). Therefore, toxic metals such as As, Cd and Pb can be taken up from cereal crops and transferred to their grains (Hu et al., 2014). Toxic metals may be classified according to their capability of being transferred from soil to plants in mobile metals, such as Cd and slow moving metals, such as Pb. This property may affect their bioaccumulation in plants (Sekara et al., 2005). So finally, in this study, we focused our research activities on the detection of heavy metals and toxins specially aflatoxins.

Materials and methods

Heavy metal detection: After drying to a constant weight, RB and DORB samples were grounded into powder. Approximately 0.25 g of samples (RB and DORB) weighed and added into the polytetrafluoroethylene digestion vessel with seven mL of concentrated HNO: and one mL of hydrogen peroxide (H_2O_2). Subsequently, the samples were digested using two-step temperature program. During the first step, the temperature was linearly increased to 190°C over 10 minutes; the maximum power of the rotating magnetron was 1000W. During the second step, the temperature was maintained at 190°C for 30 minutes. After digestion and cooling, each solution was evaporated to ~2 mL and diluted with deionized water and filtered through Whatman No. 1. The filtrate was used for the determination of Pb, Cd, Cr and Ni by Atomic

absorption spectroscopy with the graphite furnace for the GFAAS analysis (AA6800). The results were reported as the average of three repeated measurement and all digestions were conducted in triplicate. All the solutions were made from analytical reagent grade chemicals and distilled and millipore filtered water with resistance of 18.2 MQcm.

A total of five mL of the reducing agent, 3% (NaBHs) solution (with 1% m/v NaOH), were added to one g aliquot sample in a conical flask and left for about 10 s. The arsine vapor was generated then directed to the heated quartz T-tube cell connected to the AAS. Calibration was carried out using aqueous standard solutions of As (III) in the concentration range of 1.0×10^{-4} - 4.0 μg L^{-1}. All measurements were carried out using an Analyst 100 atomic absorption spectrophotometer (Perkin Elmer) equipped with a deuterium for background correction with an arsenic cathode lamp at the wavelength of 193.5 nm. Quartz T-tube cell with a path length of 166 mm and diameter of 12 mm was heated to about 900°C in an air-acetylene flame with gas flow rates of 6.0 L min^{-1} (air) and 1.8 L min^{-1} (acetylene). The reaction conditions were optimized using three of the certified reference result.

Isolation of microbes from RB and DORB: Two types of samples such as rice bran (RB) and deoiled rice bran (DORB) of BRRI dhan28 were used to isolate microbes. Both types of bran were stored for 28 days at normal condition. Every seven days of intervals (1^{st} sample was used at same days of oil extraction before storage) one g of each sample was added to nine mL of sterilized water in 150 mL Erlenmeyer flask and was shaken for two minutes. After settled down five folds (10^{-1} to 10^{-5}) serial dilutions of the supernatant were made in test tubes. Small aliquots (50 μL) from the 10^{-1} and 10^{-5} dilutions were pipetted and lawned onto nutrient agar (NA, Oxoid) medium in petri dish (9 cm) with the help of angled glass rods. The plates were incubated in growth chamber at 28±2°C for 24hrs for colony formation. Colonies of bacteria formed on the plates were enumerated and expressed as cfu/g. Single bacterial colonies were selected based on morphologically different from each other. Selected bacteria were sub-cultured onto NA medium. The plates were incubated for three days. The pure culture was maintained in NA slants in a refrigerator at 4°C for further use.

Determination of aflatoxins (B1, B2, G1 and G2) in RB, RBO and DORB: Agilent 1260 Infinity system, consisting of solvent rack, quaternary pump (with built-in degasser), standard autosampler, column compartment and fluorescence detector was used for separation and quantification. R-Biopharm electrochemical derivatization kit, including KOBRA® cell, variable control current source, 0.5 mm i.d. peek tubing (at least 34 cm long) and so on, was employed for derivatization. HPLC conditions are Column: Zorbax Eclipse Plus C18, (4.6 x 150 mm x 5 um, Column Temp.: 40°C Mobile Phase A: 1L water containing 238 mg KBr and 700 μL 4M HNO_3, Mobile phase B: MeOH, Isocratic: A : B= 50:50, 12 min., Flow rate: 1.0 mL/min, Detection: Ex: 362 nm, Em: 455 nm. Weigh 25g (mL) of sample and 2 g of sodium chloride into high speed blender jar. Add 125 mL of HPLC grade methanol:distilled water (60:40, v:v) into the jar, cover and blend for one minute at high speed. Dilute the extract with 125 mL of distilled water. Mix well by swirling followed by filtering approximately 40-50 mL of sample extract through Whatman No. 4 filter paper immediately. Transfer 10 mL of the filter (equivalent to 1 g of sample) into the glass syring barrel for passage through the prepared immune affinity column at a flow rate of 2-3 mL/min. Then add 10 mL of distilled water to the glass syring for washing the column. Expel the residual water from the column and transfer accurate 1 mL of HPLC grade methanol to elute aflatoxins from the column. It could be back-flushed with the methanol solution for completely release of aflatoxins from the monoclonal antibody if necessary. Collect

all of the methanol elution and dilute with 1 mL of distilled water before injection into HPLC system.

Toxicity analysis: Experimental rat model was used for toxicity analysis of RB, RBO and DORB samples. Increment of body weight, mortality, activity and diarrhea occurrence were measured for toxicity analysis.

Results and discussion

Study on heavy metals and toxins in rice bran related industrial products. A total of 33 commercial mills including nine auto rice mills, 12 RBO and 12 feed industries were visited to get rice bran related information from industries. In this regard, RBO mills including KBC Agro Products Private Limited (Savar, Dhaka), Ali Natural Oil Mills and Agro Industries Limited (Sonotia Bazar, Jamalpur), AM Bran Oil Company Ltd (Ghatail, Tangail), Rashid Oil Mills Limited (Ishwardi, Pabna), Majumder groups of industries (Chanka, Sherpur, Bogura), Jamuna Agro products Ltd (Godagari, Rajshahi) and Tamim Agro Industries Ltd (Bogura) etc; feed mills including Krishibid Feed Limited (Bhaluka, Mymensigh), Saudi Bangla Fish Feed Ltd (Bhaluka, Mymensigh), Provita Feed Ltd (Bhaluka, Mymensigh), Nourish Poultry and Hatchery Ltd. (Bhaluka, Mymensigh), Chhuya Agro Products Ltd. (Kapasia, Gazipur) and Aristocrat Agro Ltd (Bogura) etc as well as and auto rice mills including Haji Auto Rice Mill (Shombugong, Mymensingh), Yamin Auto Rice Mill (Shombugong, Mymensingh), Ma Auto Rice Mill (Kashigong, Mymensingh), Akashi Auto Rice Mill (Shombugong, Mymensingh), Krishani Auto Rice Mill (Dhamrai, Dhaka) and Mordern Auto Rice Mill (Sherpur) etc were visited. We have analyzed RB, RBO and DORB samples for heavy metal detection such as As, Cd, Pb, Cr and Ni and found negative results for these repeated samples (three replications). The detection limit of As, Cd, Pb, Cr and Ni are 5.0 ppb, 0.05 ppm, 0.5 ppm, 0.2 ppm and 0.5 ppm respectively. In both crude and refined RBO samples were free from bacterial and fungal growth specially aflatoxin B1 (Table 2). We also analyzed RB and DORB samples from freshly harvested (0) to 28 days of storage samples at ambient temperature with a period of seven days interval. We took only freshly extracted and purified samples regarding crude and refined RBO samples. The detection limit of heavy metals such as As, Cd, Pb, Cr and Ni in crude and refined RBO was similar with RB and DORB (Table 2). Irrespective of storage duration bacterial colonies were found ranging from $1x10^4$ to $2.75x10^4$ cfu/g and $1.16x10^4$ to $5.9x10^4$ cfu/g in RB and DORB respectively. In case of RB, the highest number of colony ($2.75x10^4$ cfu/g) was found at 7 days of storage followed by 14 days ($1.85x10^4$ cfu/g) and 21 days ($1.68x10^4$ cfu/g). The lowest number of colony ($1x10^4$ cfu/g) was recorded at 0 day of storage. In case of DORB, the highest number of colony ($5.9x10^4$ cfu/g) was recorded at 14 days of storage which was followed by 21 days ($1.72x10^4$ cfu/g), 0 day ($1.16x10^4$ cfu/g) and 28 days ($0.93x10^4$ cfu/g) (Table 3). The lowest number was at 28 days ($0.93x10^4$ cfu/g). In both types of rice bran like RB and DORB, yeast and molds (cfu/g) were positive for all samples and those ranges from 50 to 12500 cfu/g and 36 to 14700 cfu/g for RB and DORB respectively (Table 3). These RB and DORB samples were also tested for toxicity test on experimental rat model and found negative in toxicity but toxins such as aflatoxin B1 was found positive for all RB and DORB samples ranges from 9.79 to 40.21 ppb and 9.43 to 57.74 ppb respectively except 28 days of storage samples (Below detectable limit). RB samples of 14 and 21 days of storage had showed higher load of aflatoxin B1 of 40.21 and 33.24 ppb respectively, which are over MRL (maximum residual limit of >20.0 ppb). On the other hand, DORB samples of 7, 14 and 21 days of storage had showed higher load of aflatoxin B1 of 40.98,

57.74 and 38.11 ppb respectively (Table 3). Gourama and Bullerman (1995) verified a positive correlation between fungal growth and aflatoxin Bl production in rice stored under controlled conditions. Our data revealed similar result.

Table 2 Detection of heavy metals and toxins in RB, DORB and RBO samples

Parameters	As (ppb)	Cd (ppm)	Pb (ppm)	Cr (ppm)	Ni (ppm)	TVC (cfu/g)	Yeasts & Molds (cfu/g)	Microbiological effect	Toxicity	Aflatoxins (ppb)
RB-Fresh	<5.0	<0.05	<0.5	0.03	<0.5	10000	50	AQ	No	9.79
DORB-Fresh	<5.0	0.071	<0.5	<0.2	<0.5	11600	300	NAQ	No	9.43
RBO crude	<5.0	<0.05	<0.5	0.03	<0.5	Nil	Nil	AQ	No	Nil
RBO refined	<5.0	<0.05	<0.5	<0.2	<0.5	Nil	Nil	AQ	No	Nil

Note: RB= Rice bran, DORB= Deoiled rice bran, cfu = colony forming unit

Table 3 Detection of microbes and toxins in RB and DORB samples at different duration (0-28 days)

Parameters	TVC (cfu/g)	Yeasts & Molds (cfu/g)	Microbiological effect	Toxicity	Aflatoxins MRL 20 ppb
RB-Fresh	10000	50	AQ	No	9.79
RB-7 days	27500	12500	NAQ	No	12.15
RB-14 days	18500	1600	AQ	No	40.21
RB-21days	16800	923	AQ	No	33.24
RB-28 days	920	85	AQ	No	ND
DORB-Fresh	11600	300	NAQ	No	9.43
DORB-7 days	59000	14700	NAQ	No	40.98
DORB-14 days	17200	2000	AQ	No	57.74
DORB-21 days	9350	892	AQ	No	38.11
DORB-28 days	11.40	36	AQ	No	ND

Note: MRL: Maximum Residual Limit, AQ: Accepted Quality, NAQ: Not Accepted Quality

Note: ppm= Parts per million, ppb= Parts per billion, BDL= below detectable limit

Conclusion

Our data reveal that RB and DORB have immense potential use in RBO and feed mills in Bangladesh. Since long time storage of RB products produce toxins, so feed industries should be more attentive in dealing with RB and DORB as plant energy source. Since both crude and refined RBO do not possess toxins and heavy metals, so these products are safer for Bangladeshi population.

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