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# Assessment of heavy metals and aflatoxin levels in export quality Indica rice cultivars with different milling fractions

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**The present study was designed to characterize the Pakistani rice varieties (Basmati and coarse), along with their milling fractions intended for export in Europe and other regions for heavy metals and aflatoxins content. The content of heavy metals, that is, arsenic, cadmium, lead and mercury varied significantly among the rice varieties. The highest content of cadmium (0.63 mg kg$^{-1}$) and lead (0.27 mg kg$^{-1}$) was found in the rice variety IRRI-6, whereas the highest mercury level was found in KS-282 (0.075 mg kg$^{-1}$). The content of lead in IRRI-B, IRRI-W and brown rice of Basmati 2000 was higher than that allowed by European Union (EU) legislation, whereas the contents in milling fractions SB-B, SB-W KS-B, KS-W and B2-B were within the EU prescribed limits. The highest arsenic content was found in the rice variety IRRI-6 while Basmati 2000 had the lowest contents. The content of aflatoxins, that is, B1, B2, G1 and G2 varied significantly among rice varieties and between rice milling fractions, but the total aflatoxin content in all varieties was within the prescribed limits.**

**Key words:** Export quality rice with milling fractions, heavy metals, aflatoxins, high power liquid chromatography.

## INTRODUCTION

Rice is the leading food grain crop which has been reported to be used a human food for the last almost 5000 years (International Rice Research Institute (IRRI), 1997). The major rice growing Asian, American and African regions are in more than a hundred countries. The major rice exporting countries are the Thailand, United States, Vietnam, Pakistan and India. Pakistan falls in the region, where 90% of the world's rice is produced as the second biggest cash crop Government of Pakistan (GOP, 2013). The quality characterization of rice in relation to consumers and export is also attributed to chemical and biochemical characteristics like heavy metal contents, aflatoxin level and microbiological parameters.

These quality parameters are big threat to rice exporting countries. The Codex Alimentarius commission (CAC) has set a specific codex standards for rice (CODEX STAN 198-1995) including the general quality factors (classification of rice types and qualities), specific quality factors (moisture content, filth and other matter) and contaminants (heavy metals and pesticide residues). The main components of the European Union (EU) food legislation for rice contaminants, that is, nitrates, aflatoxins, heavy metals like lead, cadmium, mercury and 3-monchloropropane 1,2 diol. The mycotoxin contamination, particularly aflatoxin, is commonly found

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**Table 1.** Sample codes of rice varieties with milling fractions.

| S/N | Sample code | Name of sample |
|---|---|---|
| 1 | IRRI-B | Brown rice of IRRI-6 variety |
| 2 | IRRI-W | White rice of IRRI-6 variety |
| 3 | KS-B | Brown rice of KS-282 variety |
| 4 | KS-W | White rice of KS-282 variety |
| 5 | B2-B | Brown rice of Basmati 2000 variety |
| 6 | B2-W | White rice of Basmati 2000 variety |
| 7 | SB-B | Brown rice of Super Basmati variety |
| 8 | SB-W | White rice of Super Basmati variety |

in locally produced agricultural crops. The aflatoxins are designated as B1, B2, $G_1$, and $G_2$. Aflatoxins are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (Mishra and Das, 2003). Aflatoxin $B_1$ is the most compelling carcinogen known compound (FAO/WHO, 2004). The aflatoxin in food has been recognized as a latent hazard in humans. This health hazard may be caused by direct contamination through contaminated grains or it's by products (Kotsonis et al., 2001). The numeral death cases have been associated by the consumption of aflatoxin infected food in humans (Moss, 1996). The intake of heavy metals in food makes up a considerable amount of contamination in humans. The ingestion of heavy metals in humans may be through food chain via directly or indirectly and to some extent it is also accumulated in the human body. Lead and cadmium are among the most abundant heavy metals and are particularly toxic. Exceeding level of these toxic metals may affect the health of individuals. Cadmium has shown adverse effects on kidney's role in human body and some studies have reported it as a carcinogenic element. There are various sources of contamination of these heavy metals in food grains especially the use of contaminated water in rice fields may enhance the level of cadmium contents in rice grains. A number of cases have been reported that if rice is major food of different countries than their cadmium intake might be due to the consumption of that infected rice. The 50% cadmium intake in Indonesia has been reported due to the consumption of contaminated rice and this amount is ranged from 40 to 60% in Japan (Suzuki, 1988).

The quality characterization of rice in relation to consumers' use and export requires the assessment of the levels of potential contaminants, such as heavy metals and aflatoxin (Santini and Ritieni, 2013). Failure to meet these quality parameters can be a big threat to rice exporting countries. Pakistan is the major rice exporting country. Basmati rice produced in Pakistan is exported to more than 80 countries mainly to Gulf and European Countries. The Pakistani Basmati rice varieties have special pleasant aroma, long slender grain and with soft texture on cooking. Thus, it is of great importance to analyze the Pakistani rice varieties for their quality parameters.

The objective of this study was to determine occurrence and level of aflatoxin and heavy metals in export quality Indica rice varieties for the better understanding of world rice exporters and consumers.

## MATERIALS AND METHODS

### Samples

Four Rice varieties (IRRI-6, KS-282, Basmati 2000 and Super Basmati) were procured from Rice Research Institute, Kala Shah Kaku, Pakistan. The paddy of each variety was dehulled and milled by passing through stake sheller. The McGill laboratory mill (Rapsco, Inc, Brookshire, TX) was used to obtain two fractions of each rice variety, that is, brown rice and white rice. A portion of rice fractions each of white and brown rice was milled by passing through cyclone mill (Udy Corp, Fort Collins, Co) to get rice flour for further studies. The code number was assigned to each sample as described in Table 1.

### Heavy metals analysis

The analyses of heavy metals were performed by following the method given in AOAC (2000). One gram sample of each fraction of rice flour was taken in a conical flask. The samples were fist digested with 10 ml $HNO_3$ at a temperature of 60 to 70°C for 20 min and then digested with 5 ml $HCLO_4$ at a temperature of 60 to 70°C for 20 min and subsequently raising the temperature to 195°C till the volume was near to dry or until a clear solution was obtained. For arsenic determination, the 5 ml of $H_2SO_4$ was also added. The digested samples were transferred to 100 ml volumetric flask and volume was made with distilled deionized water and then filtered and stored in air tight bottles for analysis of heavy metals with the help of atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus) at wavelengths: 228.8 nm for cadmium, 283.3 nm for lead, 254 nm for mercury and 193.7 nm for arsenic.

### Aflatoxin analysis

The level of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ in rice flour samples was estimated by running samples through HPLC according to the procedure described by Alberts et al. (2006) with some modifications.

### Sample extraction and clean-up

The extraction and clean-up of flour samples was carried out by following the procedure mentioned by Romar Labs Inc (2003) and described in Figure 1.

### HPLC conditions

The levels of aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ in standards and test rice flour samples were estimated by running the extracted samples through HPLC (Perkin Elmer Series 200) equipped with an auto sampler (Perkin Elmer Series 200) using a UV detector and standard chromatogram is presented in Figure 2. The

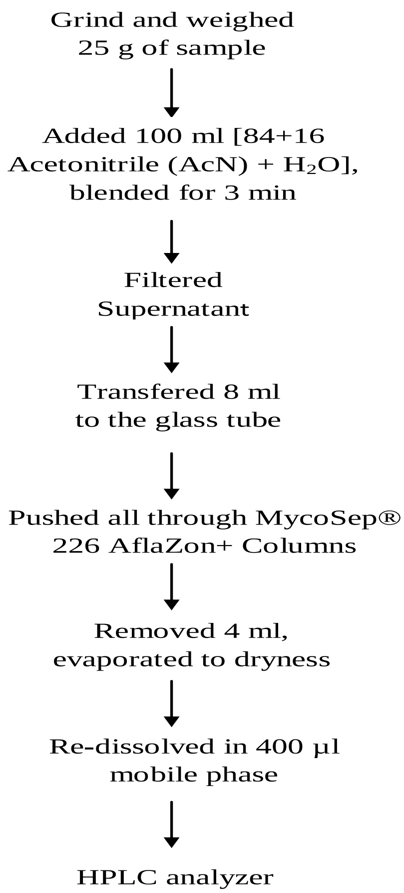


**Figure 1.** Sample extraction and clean-up for aflatoxin ($B_1$, $B_2$, $G_1$, $G_2$).

standard Mycotoxin mix 1(Aflatoxins) was obtained from Biopure Referenzsubstanzen GmbH (Technopark1, Tulln, Austria). A Phenomenex Luna C18 column (25 cm × 4.6 mm I.D.) was used to perform the HPLC analysis. A mobile phase of water/acetonitrile/methanol (57:17:26 v/v/v) was used for the separation of aflatoxins through the column at a flow rate of 0.5 ml/min and UV wavelength at 365 nm. The column temperature was maintained at a temperature of 30°C. The sample dilution was done 1.5 ml with water and injection volume was 100 µl.

### Statistical analysis

The analysis of variance was used to analyze the data (two factor factorial) according to the method described by Steel et al. (1997). Completely randomized design (CRD) was applied on the data to assess the significance level and differences among means were compared with Duncan's multiple range test (DMRt).

## RESULTS AND DISCUSSION

The flour samples of different rice varieties were analyzed for different parameters to assess their quality characteristics. The data obtained for different parameters were subjected to statistical analyses and the results are interpreted and discussed following.

### Heavy metals content

The ingestion of heavy metals in humans may be through food chain via directly or indirectly and to some extent it is also accumulated in the human body. Some countries have set standards for permissible limits of heavy metals in rice. In case of rice it is very important that rice must possess heavy metals within the prescribed permissible limit. Cadmium, lead, mercury and arsenic level were estimated in different brown and white rice sample. The mean squares for cadmium, lead, mercury and arsenic concentration showed significant variation among milling fractions and rice cultivars.

The results pertaining to the cadmium content of different rice varieties and milling fractions have been presented in Table 2. The contents of cadmium ranged from 0.08 to 0.63 mg/kg among different rice varieties (when milling fractions were pooled). The cadmium content was found significantly higher in brown rice as compared to white rice. The highest cadmium level was found in rice variety IRRI-6 followed by KS-282, Basmati 2000 (B2) and Super Basmati (SB), in an ascending order. The cadmium level was found to be higher in brown rice of IRRI-6 (0.71 mg/kg) followed by white rice of IRRI-6 (0.55 mg/kg), KS-B (0.47 mg/kg), KS-W (0.41 mg/kg), B2-B (0.15 mg/kg), B2-W (0.12 mg/kg), SB-B (0.09 mg/kg). The lowest cadmium content was recorded in white rice of Super Basmati (0.07 mg/kg). The results showed that cadmium content was found significantly higher in coarse varieties as compared to Basmati varieties. In the present study, the cadmium content was found higher in brown rice samples and lower in white rice milling fractions. The higher level of cadmium in brown rice fractions may be present in the outer parts of the grain which is removed in the milling process. The interactive effect of brown rice of IRRI-6 and KS-282 varieties may be due to the uptake efficiency of element from soil to the grain which may vary depending on the type of soil. The findings of the present study are in the line with Meharg et al. (2013) who found the rice samples each of milled and brown rice had higher cadmium content of 2.2 and 3.1 mg/kg respectively. The findings of the present study were in concordance with the results observed by Kikuchi et al. (2002) and Frazzoli et al. (2007) who found the range of cadmium concentrations in different rice samples from 0.004 to 0.38 and 0.005 to 0.49 mg/kg, respectively. The cadmium concentration of different rice flour samples is much lower than the study of Chen et al. (1994) who reported that the mean level of cadmium in different samples of brown rice was (2.5 mg/kg). The variation in cadmium content may be ascribed to genetic as well as non genetic factors in different studies which have great impact on cadmium of a rice variety. Cadmium is the most abundant heavy metal and particularly toxic. The exceeding level of

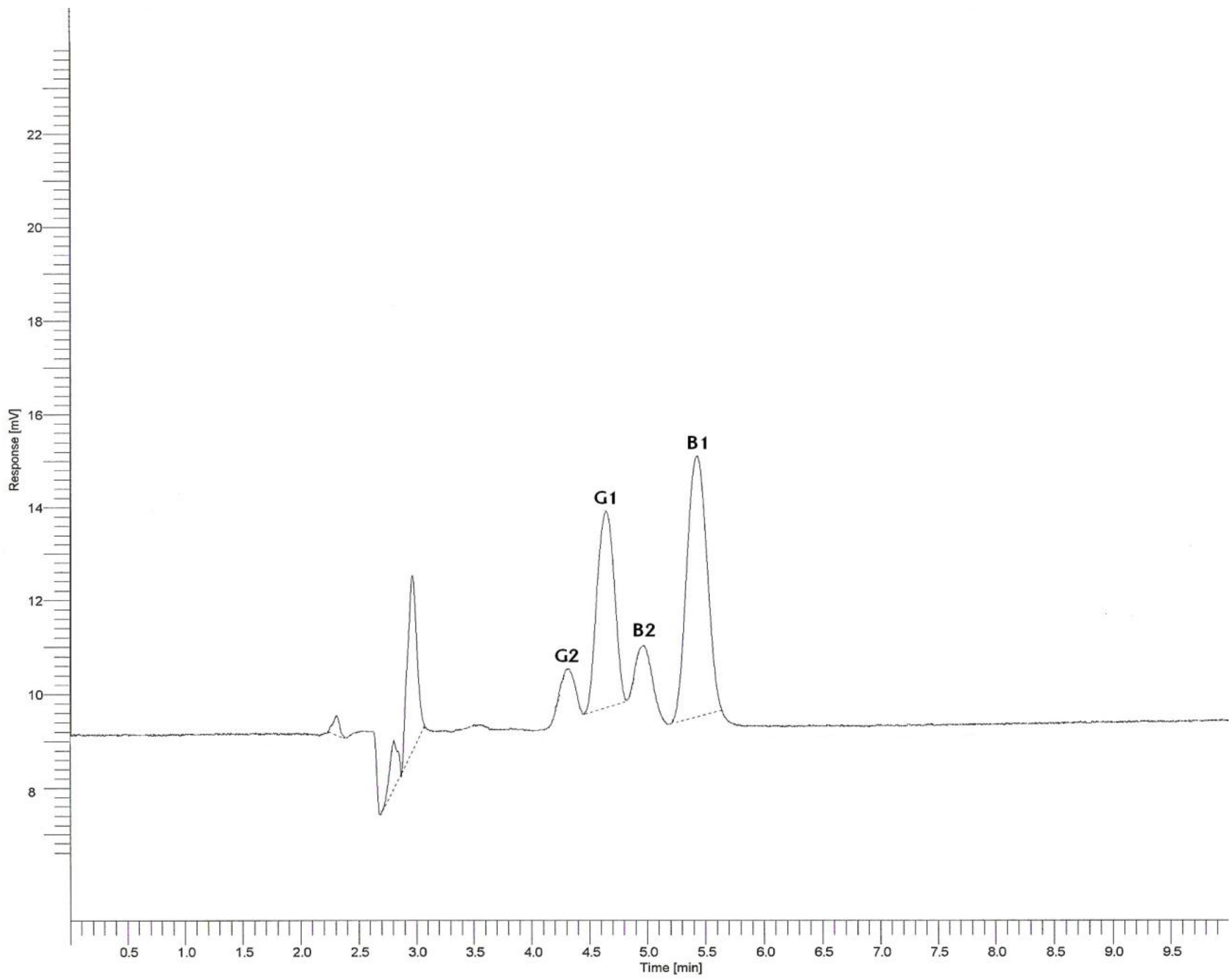


**Figure 2.** Representative standard chromatogram of aflatoxin ($B_1$, $B_2$, $G_1$ and $G_2$).

**Table 2.** Cadmium content (mg/kg) in different rice samples.

| Varieties/ fractions | Irri-6 | KS-282 | Basmati-2000 | Super Basmati | Mean |
|---|---|---|---|---|---|
| Brown | $0.71^{a}$ | $0.47^{c}$ | $0.15^{e}$ | $0.09^{fg}$ | 0.35 |
| White | $0.55^{b}$ | $0.41^{d}$ | $0.12^{ef}$ | $0.07^{g}$ | 0.29 |
| Mean | 0.63 | 0.44 | 0.14 | 0.08 | |

*Means carrying same letter in a row or column are not significantly differed ($P \geq 0.05$).

cadmium can affect the health. Cadmium can disturb kidney functions, and some studies indicate a cancerous effect. Research has proved that, those countries whose main food is rice, the consumption of this rice causes an intake of cadmium. 50% of cadmium intake comes from the rice consumption (Machiwa, 2010) and in Japan this amount was 40 to 60%. The Asian Governments have established critical maximum levels of heavy metals in rice to protect the health of their citizens. In Japan the maximum level of cadmium in unpolished rice is 1.0 mg/kg while in China the maximum permitted level is 0.4 mg/kg of polished rice (Chen, 2000). According to DOH/ROC (1988) the maximum permissible cadmium concentration in rice is only 0.5 mg/kg. European Union (EU) food legislation provides an overview of maximum permissible level of cadmium concentration in rice is 0.2 mg/kg. The standards for the cadmium level of different brown rice have been found within the safe limits prescribed by Japanese Standards. However, according to EU standards the cadmium content found in coarse varieties (KS-282 and IRRI-6) exceeds the prescribed limits. However, rice varieties Basmati 2000 and Super Basmati contained cadmium contents within the permissible limits prescribed by European Union

**Table 3.** Lead content (mg/kg) in different rice samples.

| Varieties/ fractions | Irri-6 | KS-282 | Basmati-2000 | Super Basmati | Mean |
|---|---|---|---|---|---|
| Brown | 0.31 | 0.16 | 0.25 | 0.20 | $0.23^{a}$ |
| White | 0.23 | 0.13 | 0.19 | 0.14 | $0.17^{b}$ |
| Mean | $0.27^{a}$ | $0.15^{d}$ | $0.22^{b}$ | $0.17^{c}$ | |

*Means carrying same letter in a row or column are not significantly differed (P ≥ 0.05).

**Table 4.** Mercury content (mg/kg) in different rice samples.

| Varieties/ fractions | Irri-6 | KS-282 | Basmati-2000 | Super Basmati | Mean |
|---|---|---|---|---|---|
| Brown | $0.01^{d}$ | $0.09^{a}$ | $0.03^{c}$ | $0.00^{e}$ | $0.03^{a}$ |
| White | $0.00^{e}$ | $0.06^{b}$ | $0.01^{d}$ | $0.00^{e}$ | $0.02^{b}$ |
| Mean | $0.005^{c}$ | $0.075^{a}$ | $0.020^{b}$ | $0.000^{c}$ | |

*Means carrying same letter in a row or column are not significantly differed (P ≥ 0.05).

standards.

The lead content ranged from 0.15 to 0.27 mg/kg among different rice varieties (Table 3). The brown rice yielded lead content 0.23 mg/100 g which reduced significantly to 0.17 mg/100 g in white rice milling fraction. The highest content of lead was found in rice variety IRRI-6 followed by Basmati 2000 (B2), Super Basmati (SB) and KS-282. The lead content was found significantly higher in brown rice than white rice. The lead content ranged from 0.13 to 0.31 mg/kg and found significantly higher in brown rice of IRRI-6 followed by B2-B, IRRI-W, SB-B, B2-W, KS-B and SB-W while the lowest lead concentration was found in white rice milling fraction KS-282. The results of the present study are much lower than the results of Lin (1991) and Bakhtiarian et al. (2001) who found higher lead concentrations (0.43 and 0.74 mg/kg, respectively) in brown rice of different rice varieties. These researchers indicated that many environmental factors and variable may have an effect on the absorption and storage of lead in the grains of rice. This may be the reason for variation in the results obtained in the present study. EU has set standards for maximum permissible level of lead concentration in rice and other cereals as 0.2 mg/kg. The lead concentration in rice variety IRRI-6 was 0.27 mg/kg and Basmati 2000 (0.22 mg/kg) which is higher than the prescribed limits set by EU standards. Rice variety Super Basmati (SB) and KS-282 was found within permissible limits set by EU. The lead concentration of IRRI-B, B2-B and IRRI-W was higher than EU standards and the lead contents of SB-B, SB-W, B2-W, KS-B and KS-W fall with in the limits set by EU standards. Higher concentration of lead may cause brain complications; coma and death may occur if not treated instantly (Environmental Protection Egency (EPA), 1986).

The mercury content of different rice varieties given in Table 4 indicated that the maximum level of mercury (0.09 mg/kg) was found in brown rice of KS-282 variety. The concentration of mercury ranged from 0.02 to 0.03 mg/kg between white and brown rice milling fractions, respectively. The highest mercury content was found in rice variety KS-282 followed by Basmati 2000 (0.020 mg/kg) and IRRI-6 (0.005 mg/kg) (when the fractions were combined). The highest mercury content (0.09 mg/kg) was found in brown rice of KS-282 followed by brown rice of Basmati 2000 (0.03 mg/kg) and brown rice of IRRI-6 (0.01 mg/kg). The mercury content could not be detected in white rice of IRRI-6 and white and brown rice of Super Basmati. Similarly in white rice fractions, the highest mercury content was found in white rice of KS-282 (0.06 mg/kg) followed by white rice of Basmati 2000 (0.01mg/kg) while mercury content could not be detected (ND) in white rice of IRRI-6 and Super Basmati. The content of mercury found in some rice varieties in the present study is lower than level of 0.71 µg/kg proposed by WHOM. Taiwan has also reported maximum permissible level of mercury in harvested rice to be 0.05 mg/kg In the present study the mercury content with exception of brown and white rice of KS-282 was found lower than those reported for Taiwan Standards as reported by Chen (2000).

The arsenic concentration given in Table 5 indicated that it ranged from 0.09 to 1.00 mg/kg among different rice varieties and 0.39 to 0.78 mg/kg between milling fractions, that is, white and brown rice, respectively. The arsenic content was found to be the highest in rice variety IRRI-6 followed by KS-282 (0.65 mg/kg) and Super Basmati (0.60 mg/kg). The rice variety Basmati 2000 (B2) yielded the lowest content (0.09 mg/kg) of arsenic. The arsenic level was found higher in brown rice fraction of IRRI-6 (1.30 mg/kg) followed by SB-B (0.90 mg/kg), KS-B (0.80 mg/kg) and B2-B rice (0.10 mg/kg). Higher arsenic content was found in white rice of IRRI-6 (0.70mg/kg) followed by KS-W (0.50 mg/kg), SB-W (0.30 mg/kg) and it was the lowest in rice variety Basmati 2000 (0.07 mg/kg). The results in the present study are consistent

**Table 5.** Arsenic content (mg/kg) in different rice samples.

| Varieties/ fractions | Irri-6 | KS-282 | Basmati-2000 | Super Basmati | Mean |
|---|---|---|---|---|---|
| Brown | 1.30[a] | 0.80[c] | 0.10[g] | 0.90[b] | 0.78[a] |
| White | 0.70[d] | 0.50[e] | 0.07[g] | 0.30[f] | 0.39[b] |
| Mean | 1.00[a] | 0.65[b] | 0.09[c] | 0.60[b] | |

*Means carrying same letter in a row or column are not significantly differed (P ≥ 0.05).

with the previous findings of Das et al. (2004) and Bordajandi et al. (2004) who found variation in arsenic content from 0.04 to 0.27 mg/kg with mean value of 0.17 mg/kg in brown rice samples. With respect to rice milling fractions, the results are supported by the findings of Kokot and Phuong (1999) who found that the arsenic concentration in rice samples of each white and brown rice were 0.22 and 0.29 mg/kg, respectively. The arsenic content in the samples analyzed in this study did not exceed the permissible limit set by Food hygiene concentration limit of 1.0 mg/kg as reported by Das et al. (2004). The poisoning due to arsenic can cause serious health effects including cancers, restrictive lung disease, gangrene, diabetes mellitus and hypertension (Rahman, 2002). Generally, the crops do not accrue the adequate arsenic which may cause toxicity to man. However the arsenic contaminated soil and water may raise the level of arsenic in plant tissues of edible crops (Larsen et al., 1992). The findings of these researchers pointed out that the presence of arsenic contents in rice flour sample may be due to the contaminated soil and water. However, the present study suggested the level of arsenic content in different rice varieties are within safe limits.

## Aflatoxins content

Mycotoxin contamination, particularly aflatoxin contamination, is commonly found in agricultural crops such as corn, peanuts, wheat and rice. A number of mycotoxins have been investigated and their effects on human and livestock health problems are known (Hanak et al., 2002; Katiyar et al., 2000).

The aflatoxins, that is, $B_1$, $B_2$, $G_1$ and $G_2$ and totalaflatoxins detected in rice samples are given in Table 6. The samples of different rice varieties IRRI-6, KS-282 and Super Basmati (SB) were found to contain the level of aflatoxin $B_1$ ranging from 0.60 to 1.46 µg/kg. $AFB_1$ could not be detected (ND) in rice variety Basmati 2000. The highest $AFB_1$ content was found in rice variety IRRI-6 followed by KS-282 and Super Basmati (SB). The $AFB_1$ concentration ranged from 0.70 to 1.01 µg/kg between white and brown rice milling fractions, respectively (when varieties were pooled). The concentration of aflatoxin $B_1$ was found significantly higher in brown rice milling fraction of IRRI-6 (1.67 µg/kg) followed by KS-282 (1.63 µg/kg), Super Basmati (0.77 µg/kg) and Basmati 2000 (ND). The highest $AFB_1$ was found in white rice of IRRI-6 (1.24 µg/kg) followed by KS-282 (1.16 µg/kg), Super Basmati (0.43 µg/kg) and Basmati 2000 (ND).

The European Communities (EC) has set the limits of 2 µg/kg for aflatoxin $B_1$, 4 µg/kg for total aflatoxins in cereals, including rice and cereal products intended for direct human consumption or use as an ingredient in foodstuffs.

The amount of aflatoxin $B_1$ found in the present study for all the rice flour samples was within the permissible limits of 2 µg/kg for $AFB_1$ prescribed by the EC and Ministry of Agriculture in Turkey (Official Journal of Turkish Republic, 2002).

The $AFB_2$ was detected only in rice variety Super Basmati. The content of $AFB_2$ could not be detected in all other rice varieties IRRI-6, KS-282 and Basmati 2000 and in their different milling fractions. The highest concentration of $AFB_2$ was found in brown rice fraction of Super Basmati (0.95 µg/kg) and its lowest content was found in the same variety but in white milling fraction (0.66 µg/kg).

The results for the contents of $AFG_1$ of different rice varieties revealed that the concentration of $AFG_1$ ranged from ND to 2.16 and 1.21 to 1.54 µg/kg among the rice varieties and between the milling fractions of white and brown rice, respectively. The content of $AFG_1$ was found to be significantly the highest in Basmati 2000 (B2) followed by IRRI-6, KS-282 while it was not detected in Super Basmati (SB). When the milling fractions were compared, the $AFG_1$ concentration was found higher in brown rice milling fraction of Basmati 2000 (2.43 µg/kg) followed by coarse varieties IRRI-6 (1.99 µg/kg), KS-282 (1.72 µg/kg) while in white rice fractions the highest $AFG_1$ content was found in white rice of Basmati 2000 (1.88 µg/kg) followed by IRRI-6 (1.64 µg/kg) and KS-282 (1.33 µg/kg).

The results of the $AFG_2$ concentrations indicated the range from ND to 0.52 µg/kg and 0.28 to 0.44 µg/kg among the rice varieties and between milling fractions of white and brown rice, respectively. The highest $AFG_2$ content was found in rice variety IRRI-6 followed by Basmati 2000 (B2), KS-282 while it could not be detected in Super Basmati (SB). Among milling fractions the $AFG_2$ content was found higher in brown rice than white rice milling fraction. The highest content of $AFG_2$ (0.64 µg/kg) was found in brown rice fractions of Basmati 2000 followed by IRRI-6 and KS-282.

The brown and white rice milling fraction of Super Basmati did not contain detectable amount of aflatoxin

**Table 6.** Aflatoxin level (µg/kg) of different rice cultivars with their milling fractions.

| Sample | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ | Total AFs |
|---|---|---|---|---|---|
| IRRI-B | 1.67 ± 0.05 | ND | 1.99 ± 0.08 | 0.61 ± 0.01 | 4.27 ± 0.07 |
| IRRI-W | 1.24 ± 0.08 | ND | 1.64 ± 0.02 | 0.42 ± 0.02 | 3.30 ± 0.09 |
| KS-B | 1.63 ± 0.05 | ND | 1.72 ± 0.02 | 0.49 ± 0.02 | 3.84 ± 0.06 |
| KS-W | 1.16 ± 0.04 | ND | 1.33 ± 0.06 | 0.37 ± 0.01 | 2.86 ± 0.11 |
| B2-B | ND | ND | 2.43 ± 0.03 | 0.64 ± 0.03 | 3.07 ± 0.03 |
| B2-W | ND | ND | 1.88 ± 0.05 | 0.33 ± 0.005 | 2.21 ± 0.02 |
| SB-B | 0.77±0.02 | 0.95±0.04 | ND | ND | 1.72±0.01 |
| SB-W | 0.43±0.01 | 0.66±0.006 | ND | ND | 1.09±0.02 |
| **Pooled mean fractions** | | | | | |
| Brown | $1.01^a$ | $0.24^a$ | $1.54^a$ | $0.44^a$ | $3.23^a$ |
| White | $0.70^b$ | $0.17^b$ | $1.21^b$ | $0.28^b$ | $2.37^b$ |
| **Pooled mean varieties** | | | | | |
| IRRI-6 | $1.46^a$ | $0.00^b$ | $1.82^b$ | $0.52^a$ | $3.79^a$ |
| KS-282 | $1.39^a$ | $0.00^b$ | $1.53^c$ | $0.43^b$ | $3.35^b$ |
| Basmati 2000 | $0.00^c$ | $0.00^b$ | $2.16^a$ | $0.49^a$ | $2.64^c$ |
| SuperBasmati | $0.60^b$ | $0.81^a$ | $0.00^d$ | $0.00^c$ | $1.41^d$ |

Means carrying same letter in a column are not significantly differed at $P \geq 0.05$; $AFB_1$ = Aflatoxin $B_1$, $AFB_2$ = Aflatoxin B2, $AFG_1$ = Aflatoxin $G_1$, $AFG_2$ = Aflatoxin $G_2$; Total AFs = Total aflatoxin, ND = Not Detected.

$G_2$. Total concentration of aflatoxin of different rice varieties and milling fractions (white and brown) have been presented in Table 6 which ranged from 1.41 to 3.79 and 2.37 to 3.23 µg/kg, respectively. The total aflatoxin content was found higher in rice variety IRRI-6followed by KS-282, Basmati 2000 (B2) and its lowest content was found in Super Basmati (SB). Among milling fractions the total aflatoxins content was found significantly higher in brown rice than white rice and total aflatoxin content ranged from 1.09 to 4.27 µg/kg in different milling fractions of rice varieties. The highest content of total aflatoxin was found in IRRI-B followed by KS-B, IRRI-W, B2-B, KS-W, B2-W, SB-B and the lowest aflatoxin content was observed in white milling fraction SB-W.

European Union (EU) food legislation in regulation (EC) No. 466/2001 and in the subsequent amendments the maximum levels for certain contaminants in food stuffs are set in order to protect public health. EU food legislation provides an overview of maximum permissible level of $AFB_1$ concentration and total aflatoxins in cereals including rice is 2 and 4 µg/kg, respectively.

The aflatoxin contents of all the rice varieties along with their milling fractions have been found lower than those previously described by Saleemullah et al. (2006) who estimated the aflatoxin content (mean value 17.7 µg/kg) of different rice flour samples. All the rice samples of brown and white rice did not exceed the maximum limits permitted by EU standards except the brown rice milling fraction of IRRI-6 which exceeded to these limit. The individual aflatoxin and total aflatoxins content in the rice samples analyzed in this study have been also found under the permissible limits recommended by the United States of Food and Drug Administration (USFDA) and FAO which adopted the maximum allowed levels of 10 and 50 µg/kg for all cereals, respectively (Saleemullah et al., 2006).

The findings of the present study are in close agreement to the findings found earlier by Noorlidah et al. (1998) who showed the positive report of aflatoxin $G_1$ and $G_2$ concentration (mean, 3.69 µg/kg) in different rice samples and such results are also consistent with previous findings of Food Standard Agency (2002) who reported the aflatoxin contents range from 0.2 to 1.8 µg/kg in Basmati rice, long grain rice, brown and white rice and also observed that the highest level of aflatoxin was found in brown rice which support to the present findings.

Toteja et al. (2006) observed the presence of aflatoxin $B_1$ at levels of 5 µg/kg in 38.5% of total number of rice samples and 17% of the total samples presence of aflatoxin $B_1$ above the Indian regulatory limit of 30 µg/kg.

Yoshihashi et al. (2004) observed that the incidence percent of positive samples of aflatoxin in polished and brown rice were 94 and 100%, respectively and the aflatoxin levels decreased as the rice progressed through various milling stages. The results of the present study regarding aflatoxin content in rice flour samples are also well in agreement with the findings of Toteja et al. (2006) and Yoshihashi et al. (2004). The variation between milling fractions of different rice varieties may be due to the presence of bran portion in brown rice which may increase the level of aflatoxin as reported by Castells et al. (2007) who found aflatoxin contents in all rice milling fractions but higher contamination levels was detected in bran portion as compared to white rice. The results of this study demonstrated that the concentrations of aflatoxins in all the tested rice samples are almost under the permissible levels. However, the mycotoxin contamination should be monitored routinely for food safety.

## Conclusions

Though the presence of aflatoxin and heavy metals content were observed but mostly these contents were under the safe limits. It is also important to establish the permanent controlling and monitoring program from the production until consumption of cereals in order to minimize the contamination problem of AFs. On the other hand, the training programs on this problem should be developed especially for farmers and agriculturists. Using the optimum techniques for harvesting, handling and storage and selection of proper time for harvesting reduce or eliminate this problem for foods and prevent the threat to human health and the risk of great economic loss. Government must fix a legislative limit for major threats like aflatoxin, heavy metals in various foods and their products. Further work should be carried on aflatoxin and heavy metals in various foods/feeds to check health problems for humans and animals.

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