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Distribution of Total Aflatoxins in Milled Fractions of Hulled Rice

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Two varieties of hulled rice artificially contaminated with aflatoxins at five different levels were processed by dehulling and polishing methods. Contamination levels ranged from 356 to 818 μg/kg and from 244 to 645 μg/kg in medium and long grain rice, respectively. After physical processing, four different milled fractions were obtained (hull, bran, polished broken grains, and polished whole kernels). The fractions were analyzed for total aflatoxins (B_1, B_2, G_1, and G_2) by enzyme-linked immunosorbent assay (ELISA). Aflatoxins were removed in fractions intended for human consumption (polished broken grains and polished whole kernels) at rates up to 97%. They were found throughout all fractions, but higher contamination levels were detected in hull and bran fractions than in unprocessed kernels and polished fractions. Regardless of the rice variety, the aflatoxin distribution pattern depended on the initial contamination level and type of milled fraction but not on the duration of polishing.

KEYWORDS: Aflatoxins, dehulling, rice varieties, whitening

INTRODUCTION

Aflatoxins are highly toxic, mutagenic, teratogenic, and carcinogenic fungal secondary metabolites produced by species of *Aspergillus*, of which *Aspergillus flavus* and *Aspergillus parasiticus* are by far the most important (*1*). They are of economic and health importance because of their ability to contaminate agricultural commodities worldwide, in particular cereals and oilseeds, resulting in contaminated human food and animal feeds (*4*−*6*). Consumption of food contaminated with aflatoxins has been shown to produce human hepatic and extrahepatic carcinogenesis (*2*). The International Agency for Research on Cancer (*3*) has classified naturally occurring mixtures of aflatoxins as carcinogenic to humans (group 1). In Malaysia, aflatoxins have been detected at levels up to 96 μg/kg and 436 μg/kg in rice and wheat samples, respectively (*7*). Lower levels of 2.7 and 8.7 μg/kg aflatoxins in polished and brown rice from Philippines, respectively, have been found by Sales and Yoshizawa (*8*). Regarding AFB_1 and AFG_1, levels up to 185 and 963 μg/kg, respectively, have been detected for parboiled rice samples in Sri Lanka (*9*). Rice is the most important food in the world. Around 500 million tons of paddy rice are produced, providing the main source of nutrition for three-quarters of the Earth's population. Rice grains are used not only as food ingredients but also as ingredients for making noodles, snacks, pasta, chips, breakfast cereals, and alcoholic beverages (*10*). The European Union has established maximum admissible levels of aflatoxins (B_1, B_2, G_1, and G_2) in cereals (4 μg/g) and in unprocessed products thereof intended for direct human consumption (4 μg/g) (*11*). Under certain environmental conditions, fungal growth and subsequent rice contamination is unavoidable. Thus different treatments, including chemical, biological, and physical strategies, are receiving a great deal of attention because of their potential to improve the quality of the contaminated grains (*12*, *13*). Among these procedures, milling has been found to reduce the mycotoxin levels present in cereals such as rice, barley, wheat, and maize (*14*−*17*). Milling is a crucial step in postproduction of rice. Its basic objective is to remove the hull and the bran layers with minimum breakage of the endosperm and to produce an edible, white rice kernel that is sufficiently milled and free of impurities for human consumption. Regardless of the method used for milling, the process follows the same path of hull removal and subsequent bran removal (*18*). The first step through the milling process is known as dehulling, which consists of a shearing action created by two rubber rollers that rotate in opposite directions, separating the hull from the rough rice. The resulting brown kernel containing the bran layer is thereafter processed following a whitening step (polishing), which is carried out by a friction whitener that consists of a ribbed steel cylinder that rotates inside a perforated steel plate cylinder. As the ribs force the grains against each other and against the steel, the bran layer is separated from the broken and white kernels (*19*). The aim of this study was to determine how total aflatoxins would be distributed among the milled fractions during the dehulling and whitening processes of two hulled rice varieties.

MATERIALS AND METHODS

Rice Varieties. Paddy rice was supplied by Dacsa-Maicerías Españolas, S. A., Valencia, Spain. Two different varieties were used for the study: Bahia variety (medium-size kernels) harvested in Valencia, Spain, and Puntal variety (long-size kernels) harvested in Sevilla, Spain.

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Table 1. Initial Total Aflatoxin Concentrations for Two Hulled Rice Varieties Inoculated with *A. flavus*[a]

incubation period, days	Bahia variety,[b] μg/kg (mean (SD)	Puntal variety,[c] μg/kg (mean (SD)
7	355.9 ± 37.2 A, a	244.2 ± 90.7 A, a
9	784.3 ± 150.3 BC, a	323.5 ± 86.5 AB, b
11	818.5 ± 103.1 C, a	250.7 ± 102.4 A, b
13	767.2 ± 235.5 B, a	645.1 ± 167.5 C, a
15	585.3 ± 178.8 B, a	406.8 ± 112.4 B, a

[a] Means within each rice variety (A–C) or within each incubation period (a, b) with different letters are significantly different ($P < 0.05$), according to ANOVA and Duncan's test. [b] Medium-size kernels. [c] Long-size kernels.

Inoculation of Aflatoxigenic *A. flavus* in Hulled Rice and Moisture Content Adjustment. Previous unpublished data from experiments conducted in this laboratory showed that *A. flavus* (CECT 2687), provided by the Spanish Type Culture Collection (Colección Española de Cultivos Tipo, Valencia, Spain), in hulled rice at $0.90a_w$ and in an incubation period of less than 15 days at 30 °C produced amounts high enough so that aflatoxins could be subsequently detected in all fractions after processing, while fungal growth was not observable to the naked eye. According to a moisture curve previously obtained, $0.90a_w$ was observed to be equivalent to 18% moisture content, therefore a sufficient amount of distilled water was added to paddy rice in order to reach this moisture level.

Contamination was induced in both rice varieties by adding spore suspensions to autoclaved (121 °C, 15 min) 1-L flasks containing 900 g of hulled rice each. After an incubation period of 7 days at 25 °C of *A. flavus* cultures on malt extract agar (MEA), spores were suspended in sterile distilled water solutions containing 0.005% Tween 80. A Thoma chamber was used to determine the final spore concentration, which was in the range $(1-5) \times 10^6$ spores/mL. Aliquots (27 mL) of these spore suspensions were used to inoculate rewetted paddy rice flasks (900 g) that were then incubated at 30 °C and periodically hand-shaken. Inoculated flasks were removed at incubation periods of 7, 9, 11, 13, and 15 days so that different aflatoxin concentrations were obtained (**Table 1**).

Once flasks were removed from the incubator and before the dehulling and whitening processes, contaminated hulled rice at 18% moisture content was dried at 60 °C for 6 h, so that rice humidity was decreased to 13%, and then stored at 4 °C until processing.

Experimental Design. The study was conducted at Dacsa-Maicerías Españolas, S. A., Valencia, Spain. For each hulled rice variety, five different batches with different initial contents were obtained (**Table 1**). From each batch, four replicate 100-g samples were first dehulled with a model MC-120 laboratory mill (Nuova Emmebienne S. n. c., Mezzomerico, Italy), with brown rice and the hull fraction collected separately. Thereafter, brown rice replicates underwent the whitening process for a contact time of 50 s with a model Resatrice Nuova Universale laboratory whitener (Baragioli Mario & C., Vercelli, Italy). As a result of the whitening, the bran and germ were removed (named as bran fraction) from the grains and the remaining polished broken grains and polished whole kernels were finally separated on the basis of kernel size with a model 300 laboratory separator (Industrias Luis Peris S. A., Valencia, Spain). Therefore, four different fractions were obtained from each replicate. Another four replicates from each batch were processed as mentioned above but with a whitening duration of 60 s.

Extraction and Determination of Aflatoxins. Samples of hulled rice and its byproducts (hull, bran, and both polished broken grains and polished whole kernels) were analyzed for aflatoxin content by Ridascreen aflatoxin total enzyme-linked immunosorbent assay (ELISA) (R-Biopharm, Darmstadt, Germany). Briefly, ground samples (2 g) were extracted with 10 mL of methanol/distilled water (70/30) for 10 min in a shaker. Extracts were filtered, diluted with dilution buffer contained in the kit, and assayed. The detection limit for the assay was 1.75 μg/kg. Cross-reactivity of the kit as described by the manufacturer is 100%, 200%, 15%, and 16% for aflatoxins B_1, B_2, G_1, and G_2, respectively. Mean recovery rate of aflatoxins was 85% with a coefficient of variation of 15%. In our laboratory, mean recovery rate of aflatoxin B_1-spiked rice samples (5, 50, and 500 ng g^{-1}) was found to be 85.1% with a coefficient of variation of 12.4%.

Note: Decontamination of the glassware and aflatoxin solutions is best carried out with a sodium hypochlorite (bleach) solution [10% (v/v)] overnight (adjust solution with HCl to pH 7).

Statistical Analysis. Analysis of variance (ANOVA) was performed with the SAS program (Statistical Analysis System) version 8.2 (SAS Institute Inc., Cary, NC) to evaluate the effect of initial aflatoxin concentration, type of fraction, and whitening contact time on the final aflatoxin content for each studied rice variety ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Contamination of Rice Samples. From **Table 1** it is evident that the longest inoculation time did not lead to the highest aflatoxin content in hulled rice; the highest aflatoxin levels were observed after 11 and 13 days of inoculation for the medium- and long-size kernels, respectively. In both varieties, the lowest aflatoxin contamination for the time frame of the experiment was observed after 7 days of incubation.

Even though the flasks containing rice of both varieties were inoculated with the same spore inoculum, initial aflatoxin concentrations were found to be statistically higher in medium-size kernels than in long kernels when rice was incubated for 9 and 11 days. By contrast, initial concentrations reached after 7, 13, and 15 days of incubation were not significantly different between the two varieties.

Removal of Aflatoxin Initial Content during Dehulling and Whitening. The effects of dehulling and whitening on the aflatoxin levels of contaminated rice are shown in **Figure 1**. These procedures effected an average removal of 96% and 97% of the initial aflatoxins present in polished broken grains and polished whole kernels processed from medium-size kernels, respectively (**Figure 1A**). Similar percentages were observed in fractions from long-size kernels (**Figure 1B**); 92% and 97% of the initial aflatoxins was removed in polished broken grains and polished whole kernels, respectively. Similar removal rates were also observed for dehulling of aflatoxin-contaminated maize; an average removal of 92% of initial contamination was found (*17*). The polishing of barley brought reductions in nivalenol, deoxynivalenol, and zearalenone contents up to 94%, 100%, and 100%, respectively (*20*), while zearalenone was highly removed in flour fraction upon processing of wheat (*21*). Lower decontamination rates were found for fumonisins, which were removed by 57−65% after dehulling of maize (*22*) and for deoxynivalenol in barley and wheat, which was eliminated by ca. 40% and 52%, respectively (*23*).

Apart from its main goal of removing the outer portions of the grains, rice milling incidentally also reduced initial contamination by more than 90%; therefore, dehulling and whitening processes could be considered good processes to improve the safety of the final fractions.

Distribution of Aflatoxins in Milled Fractions. Aflatoxins were distributed into milled fractions depending on the initial contamination level and type of milled fraction. Statistical analysis showed that those factors, as well as their interaction, had a significant effect on aflatoxin distribution ($P < 0.05$), while whitening contact time did not have a significant influence on the fate of aflatoxins, which means that they were concentrated in each fraction regardless of the duration of polishing of brown kernels. However, the narrow time period set up leading to a slight 10-s difference could not have been enough to find significant differences between samples processed for 50 and 60 s. Contrary conclusions were reached in previous studies, where it was demonstrated that mycotoxin levels in

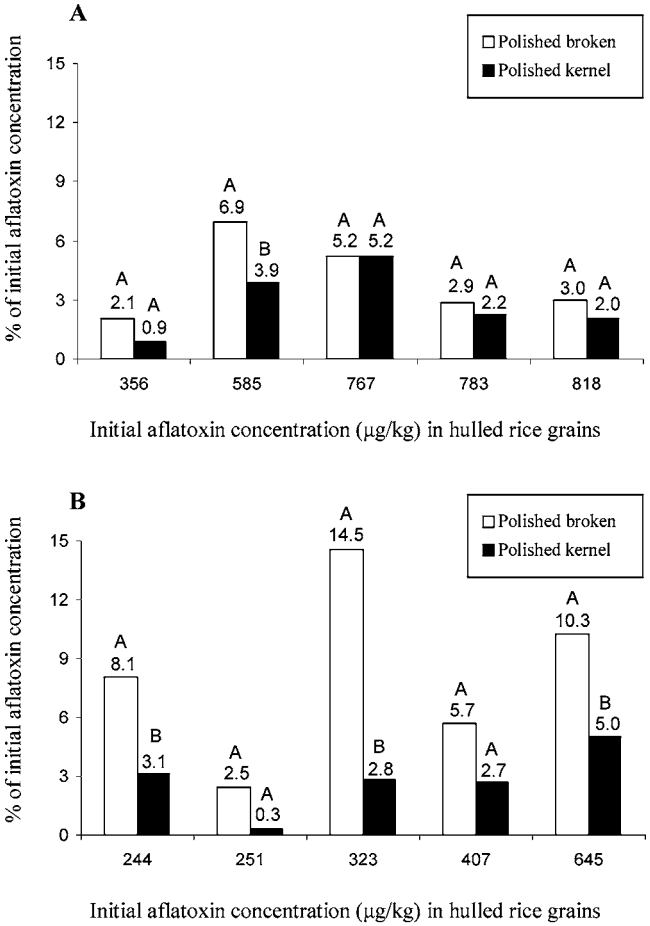

Figure 1. Remaining total aflatoxins (percent) after dehulling and whitening in final fractions processed from (**A**) medium- (Bahia variety) and (**B**) long- (Puntal variety) size rice kernels. Numbers above bars indicate the percent of initial aflatoxins in polished broken grains and polished whole kernels. Bars with different letters (within each initial concentration level) are significantly different ($P < 0.05$).

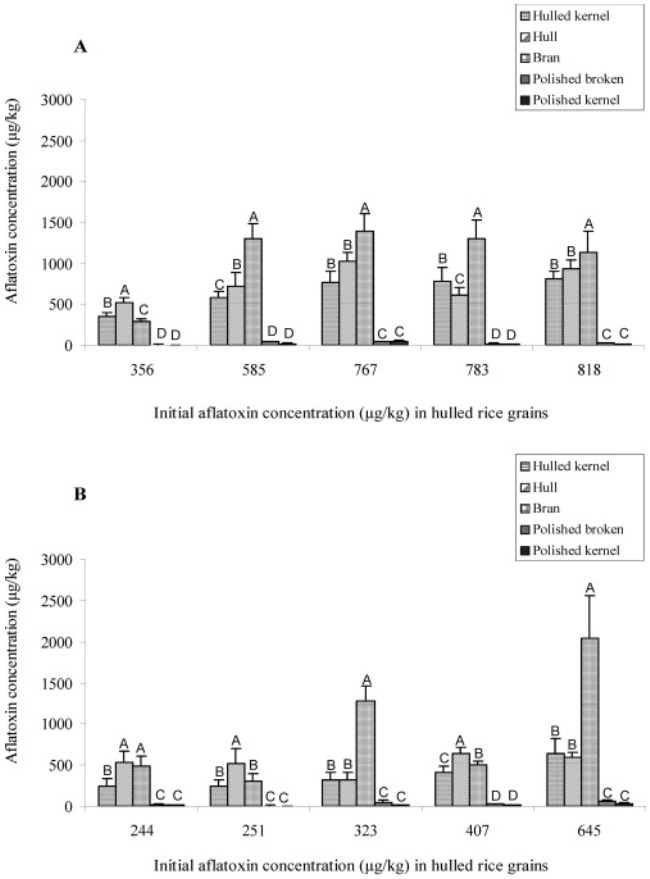

Figure 2. Levels of aflatoxins (micrograms per kilogram, mean ± standard deviation) in hulled unprocessed kernels and milled fractions processed from (**A**) medium- (Bahia variety) and (**B**) long- (Puntal variety) size rice kernels. Bars with different letters (within each initial concentration level) are significantly different ($P < 0.05$).

milled fractions depended on processing time. When hulled barley was processed by an abrasive type of milling, it was found that the longer the pearling time, the greater the reduction in deoxynivalenol content (*24*). The wider range of contact time (15−120 s), the presence of the hull, and the different type of grain could be the reasons why processing time was significant in earlier experiments but not in ours. In another study, the decrease of deoxynivalenol, nivalenol, and zearalenone levels increased with the increasing degree of polishing when hulled and dehulled barley samples underwent the polishing process (*20*).

Initial concentration had a significant effect on the distribution of aflatoxins into the different milled fractions in both rice varieties. However, no general trend was found to explain how aflatoxins present in processed fractions varied depending on the initial concentrations for both varieties (**Figure 2**). The effect of initial mycotoxin concentration on its distribution among the processed fractions is still unclear; while some authors observed that the distribution pattern depended on the initial concentration (*25*, *26*), others stated that the distribution was not affected by the mycotoxin content of the whole kernels (*27*).

The results also revealed a significant effect of type of fraction on the distribution of aflatoxins in the grain mass, which was observed not to be uniform. Even though aflatoxins were found throughout all the milled fractions in both rice varieties, in all cases, hull and bran fractions were found to be more heavily contaminated with aflatoxins than were polished broken grain and polished whole kernel fractions. Young et al. (*25*) reported on the correlation between ergosterol and deoxynivalenol levels and concluded that mycotoxin was produced at the site of fungal growth rather than transported from the kernel surface to the interior. On the basis of this theory, aflatoxins would not be transported to the interior of the kernels, thus the low levels found in the polished fractions would be the result of the penetration of few fungal mycelia into the endosperm, as reported in previous studies (*17*, *28*). Furthermore, the low penetration of fungal mycelia leading to higher aflatoxin levels in ca. 30% of total grain mass (hull and bran), could be explained by the fact that *A. flavus* may not had enough time to reach the endosperm. This distribution pattern could be also attributed to the fact that hull, as the outermost layer, may not act as a strong barrier to the growth of *A. flavus* while the bran layer may behave as a barrier, preventing the mycelia from penetrating further in the kernel structure. Moreover, the high content in fats of outer layers may be favorable to the attack of molds (*29*); thus growth of *A. flavus* occurred mostly in surface layers.

In the case of medium-size kernels, bran fraction was found to have the highest aflatoxin content, except in the case of bran fractions processed from the whole kernels with the lowest initial contamination, where bran was less contaminated than the hull and the whole unprocessed kernel (**Figure 2**). Generally, both bran and hull fractions were more contaminated than the whole

unprocessed kernels, as concluded by Trigo-Stockli et al. (*30*). This is presumably because of a concentration phenomenon that occurred when most of the aflatoxins were concentrated in ca. 30% of the total grain mass. Similarly, earlier studies concluded that nivalenol, deoxynivalenol, and zearalenone in the bran fractions increased severalfold over the original barley and wheat, respectively (*20*, *21*).

The same distribution pattern was observed for the long-size kernels since bran and hull fractions showed higher aflatoxin levels than whole unprocessed kernels (**Figure 2**). Hull was found to be more contaminated than bran when fractions were processed from whole kernels with low and medium initial aflatoxin concentrations, while bran was significantly more contaminated than hull when unprocessed whole kernels showed medium and high initial aflatoxin levels. As observed for the medium-size kernels, generally fractions coming from the outer layers of the grains had higher aflatoxin content than the unprocessed kernels, presumably because most of the aflatoxins were concentrated in 30% of the total grain mass.

Polished fractions are the result of the whitening procedure, which consists of removing the bran layer from the brown kernels. The fact that the levels of aflatoxins in those fractions were statistically lower than the concentrations found in the rest of the fractions provides strong evidence that whitening, as a physical process used to convert the grain in an edible food intended for human consumption, was effective in avoiding the transference of high aflatoxin quantities to those final fractions. This is in agreement with the results obtained by Clear et al. (*15*), who observed that polishing of hulless barley to 60% of the original weight of kernels significantly reduced deoxynivalenol initial concentration. Moreover, scouring of wheat, which consists of the removal of outer layers of the kernel, leaving the entire endosperm for milling, resulted in a substantial reduction in ochratoxin A content (*16*).

The fact that aflatoxins were heterogeneously accumulated in the grains of both varieties is in agreement with many studies where it was demonstrated that higher mycotoxin levels were found in outer layers of kernels such as bran, hull, and shorts, whereas lower levels were in inner layers such as flours and grits. One of the earliest studies about the effect of milling on aflatoxin contamination was performed by Schroeder et al. (*14*), who observed that the bran and polished fractions contained aflatoxins in a concentration more than 10 times that of the milled rice kernels. Dehulling of maize also showed that bran fraction contained the highest aflatoxin levels, whereas grits contained lower levels (*17*). Similar distribution pattern of aflatoxins was reported by Njapau et al. (*31*), who demonstrated that the content was significantly higher in bran than in endosperm.

Comparable results were observed for different mycotoxins that were also found to accumulate in fractions coming from the outer layers of kernels rather than in fractions coming from the internal parts. Bran and short milled fractions processed from wheat (*21*, *32*) and from dehulled barley (*20*) had higher deoxynivalenol, nivalenol, and zearalenone contents than the fractions containing the inner parts of the kernels such as flours. Several studies reported on the fate of deoxynivalenol present in hulled barley (*24*), wheat (*25*, *27*, *30*, *33*, *37*), and barley (*15*). Processing made deoxynivalenol accumulate in the outer layers of kernels. The same effect of processing was observed for zearalenone present in wheat since it was also more concentrated in the bran and short fractions than in the inner portions (*30*).

Similarly, most of the fumonisin B_1 contained in maize was found to concentrate in the outer fractions (*29*). Fumonisins B_1 and B_2 were also more concentrated in the germ, bran, animal flour, and fine than in the inner portions such as grits and flours (*34*). Results obtained by Scudamore et al. (*35*) showed that ochratoxin A was concentrated in the surface layers.

Even though the majority of studies revealed higher concentrations of different mycotoxins in portions processed from outer layers of kernels, research conducted by Chelkowski et al. (*36*) demonstrated that surface portions were not the most contaminated fractions. It was demonstrated that levels of ochratoxin A in wheat and barley were not significantly affected by milling so that ochratoxin A levels in flour and bran were not statistically different. Bandara et al. (*9*) concluded that aflatoxin B_1 was not most concentrated in the bran and dehulling was found to reduce the initial content only by 5%.

It must be taken into account that rice was artificially contaminated through inoculation leading to high initial aflatoxin concentrations in rough rice; different patterns of decontamination could have been observed for naturally contaminated rice. Even more, despite the high sensitivity and specificity of the ELISA method, it is known that compounds with similar chemical composition can also interact with antibodies, resulting in an overestimation or underestimation of mycotoxin concentrations (*38*), thus results of this study should not be taken as absolute values of concentration but as a mean of comparison among the aflatoxin contents in the different rice fractions.

In conclusion, it can be stated that a combination of dehulling and polishing of two different hulled rice varieties is an attractive method of decontamination of rice-based foods intended for human consumption. However, since bran and hull fractions have been found to be highly contaminated with aflatoxins, special attention should be given to animal feeds because of the possible residue of aflatoxins in edible tissues of farm animals that may cause a secondary health hazard in humans.

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