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Fate of Aflatoxin B_1 during the Cooking of Korean Polished Rice

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ABSTRACT

The fate of aflatoxin B_1 (AFB_1), a mycotoxin known to occur in polished rice, during rice cooking was evaluated to determine reduction in AFB_1 residues and mutagenic potentials. The amounts of AFB_1 in three lots of naturally contaminated polished rice from Korea were analyzed by high-performance liquid chromatography after washing and after steaming. An in vitro mutagenicity assay with *Salmonella* Typhimurium TA100 was used to confirm the results of the chemical analyses. Cooking significantly reduced AFB_1 (mean reduction, 34%) in naturally contaminated polished rice and reduced mutagenicity by ca. 27%. Processing factors (reflecting the removal of AFB_1 residues during processing) for cooked rice were estimated at 0.66 to 0.73, as determined chemically and toxicologically, respectively. The revised Korean provisional daily intake of AFB_1 from consumption of rice as a dietary staple (0.58 to 3.94 ng/kg of body weight per day) is still higher than that reported for foods in general in the United States (0.26 ng/kg of body weight per day). Thus, Koreans probably consume higher amounts of foodborne AFB_1 than do Americans and thus are at higher risk for AFB_1-induced health consequences.

Aflatoxins are mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus (17).* Among these toxins, aflatoxin B_1 (AFB_1) is usually found in the greatest concentration in food, is known to be the most toxic, and is a group 1B human carcinogen based on evidence of carcinogenicity in humans *(3).* The primary agricultural commodities associated with aflatoxin contamination are peanuts, corn, and cereals; rice grains are occasionally contaminated *(1, 12, 14).* These foods could act as dietary sources of aflatoxin, e.g., from consumption of processed foods produced from naturally contaminated commodities.

Rice has been the most important crop in the Korean food supply for thousands of years. The daily intake of rice by the average Korean in 1998 was estimated as 246.1 g, which accounts for about 60% of the total caloric intake *(7).* Polished rice is sequentially subjected to rinsing with water and steaming prior to being consumed as cooked rice, the staple food in Korea. In a previous survey of aflatoxins in polished rice consumed by Koreans, aflatoxins were found at concentrations below the residue limit (10 ng/g) established in Korea *(13).* Because of the large amount of rice consumed by Koreans, even low concentrations of AFB_1 in rice could pose a potential health risk *(13).* However, there has been no consideration of the effect of cooking on AFB_1 in rice. More researchers have assumed that there would be no loss of AFB_1 during cooking because aflatoxins are relatively heat stable once formed. Some reports have revealed the effect of cooking, baking, and frying on aflatoxin residues. Stoloff and Trucksess *(16)* found that thermal processes, i.e., frying or baking for making pan-fried grits or corn muffins, reduced concentrations of aflatoxins by 53 and 13%, respectively. The loss of AFB_1 during rice cooking was reported as being between 6 and 88%, depending on the rice-to-water ratios used and whether the rice was cooked under pressure *(15).* Elias-Orozco et al. *(2)* found that losses of aflatoxins exceeding 46% occurred during extrusion, and substantial losses of aflatoxins also occurred after the nixtamalization process, which involves cooking and steeping naturally contaminated corn in lime water. The concentration of AFB_1 in cooked rice that reaches the consumer may be considerably lower than that in raw polished rice. Thus, the estimated probable daily intake of AFB_1, based on the concentration of AFB_1 in raw material such as polished rice, may be overestimated. It would be appropriate to adjust the estimates of AFB_1 intake to reflect the effect of processing in commodities that are always processed before consumption. Especially, this processing factor, which reflects the removal of aflatoxin residues during the processing of raw commodities, could be used to formulate legal tolerances and to estimate accurate AFB_1 intakes from processed food, which are required for the assessment of dietary exposure to aflatoxin.

The purpose of this study was to evaluate typical processes used in Korea for making cooked rice for their effects on naturally occurring aflatoxin residues. Four lots of Korean polished rice *(13)* were examined using high-performance liquid chromatography with fluorescence detection (HPLC-FD) and HPLC coupled with mass spectrometry (HPLC-MS). The effects of processing were also biologically evaluated using the *Salmonella* mutagenicity assay *(11)* to determine the extent to which rinsing or cooking of aflatoxin-contaminated rice reduced in vitro toxic potential. The estimated exposure of Koreans to AFB_1 through rice consumption was reformulated by providing adequate

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processing factors calculated for the processing of polished rice to cooked rice.

MATERIALS AND METHODS

AFB_1 standard was purchased (Sigma, St. Louis, Mo.), and a stock solution was prepared in acetonitrile-methanol (1:1, vol/vol) and stored in an amber vial in a freezer (ca. −18°C). HPLC grade solvents and analytical grade reagents (or better) were used for all purposes.

Polished rice. Four lots (each 1 kg) of polished rice from our previous study *(13)* were used in this experiment. All were harvested in 2002 in Korea and were obtained from several grain wholesale markets in Seoul. They were of acceptable visual quality and intended for human consumption. Three lots (1, 2, and 3) consisted of rice naturally contaminated with AFB_1 at 1.8, 4.5, and 7.3 ng/g, respectively. An aflatoxin-free rice lot (<0.8 ng/g) served as a control for recovery tests. Lots were stored in sealed plastic bags under refrigeration (4°C).

Preparation of cooked rice. The cooking procedure was performed by a modification of the method of Yeom et al. *(18).* One hundred grams of polished rice from each lot was rinsed three times with 200 ml of distilled water to eliminate some portions of pericarp and then drained on a screen. Rice grains remaining were combined and kept overnight at −70°C (rinsed rice). The rinsed rice was again mixed with 200 ml of distilled water and then cooked at 160°C for 20 min in a commercial electronic cooker (SJ-F104, Samsung Electronics, Seoul, Korea). After cooling, the cooked rice was frozen overnight at −70°C (cooked rice). All the samples were freeze dried with a laboratory lyophilizer (Millrock Technology Inc., Kingston, N.Y.) and ground with a mortar and pestle to pass at least a no. 20 sieve. The powdered samples were weighed and then analyzed for AFB_1 residues. The following experiments were done in two series of three replicates.

Aflatoxin analysis. All samples were extracted and cleaned as previously described *(13).* Five grams of the sample was extracted with 25 ml of methanol-water (8:2, vol/vol) by shaking for 30 min in an orbital shaker and then filtered (no. 4 filter, Whatman, Clifton, N.J.). The remaining solid was extracted again and filtered again. The first and second extracts were combined and partitioned with dichloromethane followed by a cleanup stage using Sep-Pak silica SPE tubes (Waters, Milford, Mass.). These purified extracts were analyzed by HPLC at a flow rate of 1 ml/min. Precolumn derivatization was performed with trifluoroacetic acid using FD with excitation set at 360 nm and emission set at 440 nm (fluorescence detector Model 474, Waters). The analytical column was a Nova-Pak C18 column (3.9 by 150 mm; Waters), and the mobile phase was methanol-acetonitrile-water (17:17:70, vol/vol/vol). Recoveries of AFB_1 from the spiked aflatoxin-free rice lot were 89 ± 4, 79 ± 9, and 82 ± 7% for samples A, B, and C, respectively, based on triplicate analyses at a spiking concentration of 10 ng/g.

To confirm the AFB_1 residues in some samples, HPLC-MS analysis was performed according to the procedure described recently *(9),* with a VG Biotech platform (VG Biotech, Cheshire, UK) mass spectrometer with electrospray ionization in positive ion mode; the flow rate of electrospray ionization nebulizing gas (N_2) was 2.5 liters/min. Each cleaned-up extract that contained AFB_1, as determined by LC, was reevaporated and then dissolved in an appropriate mobile phase of acetonitrile, methanol, and 10 mM ammonium acetate (2:6:15, vol/vol/vol); the flow rate was 0.2 ml/min. The MS platform interfaced with an HP 1100 LC system (Agilent Technologies, Palo Alto, Calif.), which was equipped with an UltraCarb ODS 30 column (5 μ, 2 by 150 mm; Phenomenex, Torrance, Calif.). The detection limit for AFB_1 by HPLC-FD was 0.80 ng/g and that with HPLC-MS was 0.10 ng/g, at a signal-to-noise ratio of 5:1.

Mutagenicity assay. The Ames test was performed with *Salmonella* Typhimurium TA100 *(11).* The effects of rinsing with water and cooking on the mutagenicity of AFB_1 residues in polished rice were determined using a preincubation procedure in the presence of a rat liver S9 mix as an external enzymatic metabolizing system. The methanol-water extracts (equivalent to 5 g of dry weight) obtained from powdered samples in each lot were evaporated to dryness under nitrogen gas, and the residues were taken up in 1 ml of dimethylsulfoxide (DMSO). An extract from the aflatoxin-free rice lot served as a solvent control and was evaporated and reconstituted in DMSO. These extracts were preincubated at 37°C for 30 min with the bacteria (about 10^8 CFU/ml) and S9 mix. After the preincubation period, the mixtures were diluted with soft agar and subsequently plated onto minimal glucose agar (Difco, Becton Dickinson, Sparks, Md.) plates. The number of histidine-positive revertants was counted after 2 days of incubation at 37°C. A doubling of the number of spontaneous revertants was considered a positive mutagenic response using this bioassay.

Statistical analyses. Statistical analyses were performed with Sigma Stat (version 3.0, Jandel Scientific, San Rafael, Calif.). The AFB_1 concentrations and the number of revertants were evaluated using a one-way analysis of variance followed by Duncan's multiple-range test. Differences among sample groups were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Pronounced induction of histidine-positive revertants was obtained with AFB_1 (Fig. 1). The presence of AFB_1 significantly increased the number of revertants as compared with the solvent control. AFB_1 had a mutagenic effect on *Salmonella* Typhimurium TA100 when mixed with the S9 enzymes. There was a significant dose-response relationship ($R^2 = 0.99$) between AFB_1 concentrations of 15 to 60 ng per plate and the mutagenic potential. This finding confirms that of a study reported by Koch et al. *(6).* The relevance of the dose response was discussed previously in the report of a study *(5)* in which the mutagenic response of *Salmonella* Typhimurium TA100 to AFB_1 had a positive mutagenic tendency with metabolic activation.

The reduction in AFB_1 after washing with water ranged from 20 to 24%, with an average of 22% (Table 1). When rinsed rice samples were brought to a temperature at 160°C, i.e., during the steaming step, additional losses of AFB_1 were significant, with a mean loss of 34% AFB_1 (31 to 38%) compared with the amount present in the starting raw polished rice samples. AFB_1 concentration in the raw rice appeared to have no discriminating effects on the results when the three lots were compared. Water washing and steaming at 160°C had effects on the AFB_1 recovered from the cooked rice, a finding that is in agreement with the work of L'vova et al. *(10),* who reported that AFB_1 concentrations in cooked rice were considerably lower than those in raw samples. They found that 37% losses of AFB_1 occurred during cooking at a rice-to-water ratio of 1:8, and pressure cooking of rice resulted in further aflatoxin losses.

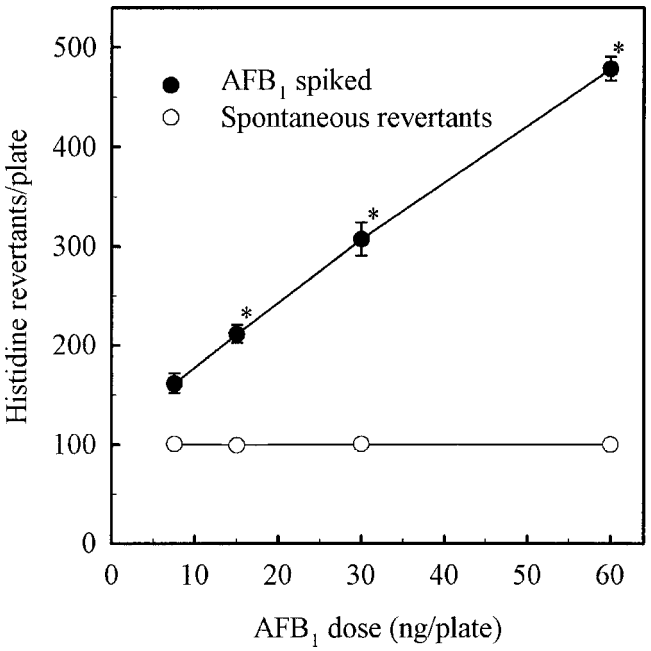

FIGURE 1. *Effect of different amounts of AFB_1 added to the extract from aflatoxin-free rice on the induction of histidine-positive revertants in* Salmonella *Typhimurium TA100 with metabolic activation. The spontaneous reversion rate was 102 ± 8 CFU per plate. Each of the points and bars represent the mean and standard deviation of two series of three replicate assays.* * $P < 0.01$, *value is significantly different from the corresponding value for the solvent control.*

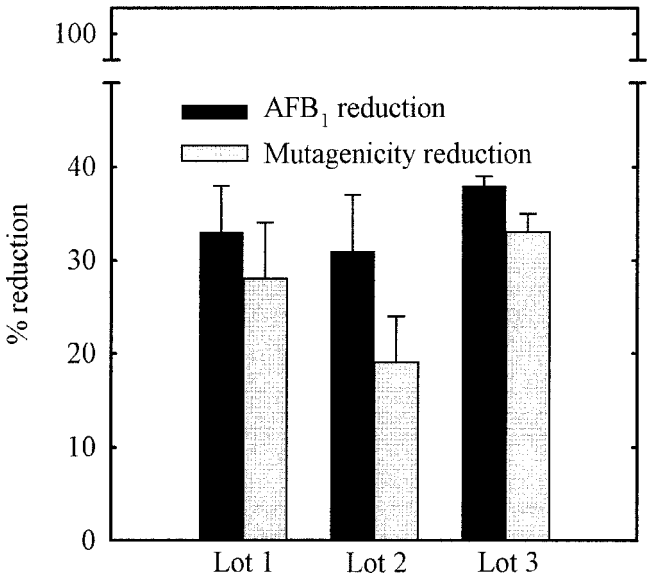

FIGURE 2. *Comparison between reduction in AFB_1 concentration and mutagenic potential for AFB_1-contaminated rice that was washed with water and steamed. Results are presented as mean and standard deviation for two series of three replicates.*

However, residual AFB_1 was still present in the final food products. Conditions typically used for cooking rice appear not to be stringent enough to completely decompose or remove naturally incurred AFB_1 in rice. These steps also could modify the toxin chemically so that it cannot be detected by fluorescence detection. However, reduction in fluorescence does not always mean reduced toxicity. The bioassay with *Salmonella* Typhimurium TA100 was used to determine whether the reduction of AFB_1 concentration in cooked rice also decreased the mutagenic potential of AFB_1-contaminated rice samples and to estimate possible interactions in the biological system, although there were no decomposition products found in the mass range of the HPLC-MS analysis. The measurement of the number of revertants has the advantage of reflecting biologically active aflatoxin, such as analogues or decomposition products, in cooked foods.

When comparing reduction of AFB_1 concentrations based on both HPLC-FD and HPLC-MS analyses and reduction of mutagenic potential for the naturally contaminated rice samples during cooking, the greater the percent loss of AFB_1, the lower the *Salmonella* mutagenicity (Fig. 2). In the three lots of cooked rice, all had 31 to 38% reductions in AFB_1 concentrations as determined by both HPLC analyses. There also was a marked reduction (19 to 33%) in mutagenic potential. These findings indicate that detoxification of AFB_1-contaminated rice was partially achieved during the cooking procedure evaluated in this study. Thus, the analytical results were consistent with the in vitro bioassay findings. However, about two thirds of the AFB_1 toxicity remained in the cooked rice; more than 60% of AFB_1 survived after production of typical Korean steamed rice, whatever measurement was used. These results prompted us to reconsider the Korean probable daily intake of AFB_1 estimated in our previous report *(9)*. Because of the heat stability of AFB_1, we overlooked the fact that rice grain is consistently processed before reaching the consumer. Thus, a processing factor that can indicate how concentrations of AFB_1 in raw commodities change throughout processing is needed.

TABLE 1. *Aflatoxin B_1 in raw polished rice, rinsed rice, and cooked rice from three lots of naturally contaminated polished rice grains, as determined by HPLC-FD*[a]

	Lot 1		Lot 2		Lot 3	
Sample	AFB_1 (ng)	% of residue	AFB_1 (ng)	% of residue	AFB_1 (ng)	% of residue
Polished rice	176 ± 15	100	439 ± 46	100	714 ± 51	100
Rinsed rice	133 ± 20	76	350 ± 25	80	546 ± 33	76
Cooked rice	118 ± 20[b]	67	303 ± 58[b]	69	445 ± 38[b]	62

[a] All the values are corrected for recovery. Amount of AFB, was determined by multiplying AFB_1 concentration (ng/g) by total dry weight (g) of each sample from each lot ($n = 6$).

[b] Significantly different from the amount present in the other rice lots, $P < 0.05$.

TABLE 2. *Revision of the estimated daily exposure of Koreans to AFB_1 through consumption of cooked rice, based on the processing factors for polished rice*

Commodity	Mean AFB_1 (ng/g)	Mean AFB_1 intake (ng/kg of body weight/day)
Polished rice[a]	0.20–1.20	0.89–5.37
Cooked rice[b]		
HPLC-FD	0.13–0.79	0.58–3.53
Ames test	0.15–0.88	0.67–3.94

[a] Values on our previous report *(13)*.

[b] Processing factors for AFB_1 during cooking of polished rice were determined by HPLC-FD (66%) and by the Ames test (73%).

Table 2 illustrates that Korean daily intake of AFB_1 diminished when the processing factors for AFB_1 during cooking of rice were applied to the AFB_1 concentrations in polished rice based on survey data; 0.66 and 0.73 are the values determined chemically and toxicologically, respectively. According to these new calculations, the Korean probable daily intake of AFB_1 is lower than the prior estimate. However, the intake figures are still higher than that reported for Americans (0.26 ng/kg of body weight per day) *(4)*, indicating that Koreans probably consume higher amounts of AFB_1 than do Americans. These amounts may be as high as the provisional maximum tolerable daily intake of AFB_1 of 0.40 ng/kg of body weight per day for adults with hepatitis B, as reported by Kuiper-Goodman *(8)*. Throughout the world, especially in developed countries, there has been increasing consumer pressure for high-quality food with the lowest possible level of mycotoxins, and statutory limits for mycotoxins have been set. Further legislation is needed to cover a wide range of mycotoxins in more food commodities. Therefore, we must determine how mycotoxins survive processing so that this information can be taken into account when setting consumption guidelines. The present study gives us considerable information on the fate of AFB_1 during the cooking of rice.

Aqueous processes such as water washing and steaming caused loss of AFB_1 (27 to 34%), probably by extraction into the drained water or by decomposition of the toxin in the raw commodities. The processing factors for AFB_1 during rice cooking were proposed to be 0.66 and 0.73 as determined chemically and toxicologically, respectively. However, results provided no evidence for the complete removal of AFB_1 by cooking, suggesting that the decrease in AFB_1 concentrations achieved by cooking of such food products is insufficient to reduce the AFB_1-related risk to Korean consumers that depend on rice as a dietary staple. The best protection would be to prevent AFB_1 formation by careful handling of the foodstuff. More efforts are needed to further reduce AFB_1 residues in cooked rice without the loss of flavor and nutrients, e.g., leaching of AFB_1 into drained water by changing the ratio of rice to water or by increasing the number of washes to more than three times versus decomposition of AFB_1 by steaming under pressure. Revisions of typical cooking processes may decrease the risk to Koreans of health consequences associated with AFB_1 in cooked rice.

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