Comparing Efficiency of Rice Washing and Soaking Processes in Reducing the Amount of Aflatoxin B1

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ABSTRACT

Contamination of some agricultural products by mycotoxins has exposed the human and animal health to serious concerns. Aflatoxin B1 is the most toxic type of aflatoxins, which is of the highest amount in different foods, including rice, among various types of aflatoxins. The present study was aimed to evaluate the amount of this toxicant in the consuming rice in Yazd city and investigate the effect of rice washing and soaking process on reduction of the mean concentration of aflatoxin B1.

The present study was a descriptive-analytical one, in which 36 rice samples (18 domestic types and 18 foreign types of rice) were randomly selected from the rice distribution centers in Yazd city. The Standard No.6872 of the Institute of Standards & Industrial Research of Iran (ISIRI) was used for measuring the amount of aflatoxin B and G toxicants using HPLC (high pressure or performance liquid chromatography) method and purifying by immune affinity column. Analysis of the collected data was performed using SPSS-22 statistical software as well as the statistical methods of one-way ANOVA and Tukey HSD.

The mean concentration of aflatoxin B1 in domestic and imported rice was measured equal to 1.461 and 0.508ng/g, respectively. The contamination rate in the imported rice was lower than that in the domestic one, while there was no significant relationship between them ($p \geq 0.166$); furthermore, the effect of soaking process on reduction of the toxicant amount in one and ten hours of soaking was statistically insignificant. There was no significant relationship between the effect of a single time of washing and reduction of the amount of toxicant; however, in case of three and five times of washing, the amount of toxicant exhibited a reduction of 68.2% and 98.7%, respectively, which was statistically significant ($p<0.05$).

Results of the present study indicated that the rice washing process can significantly reduce the amount of aflatoxin B1, whereas the rice soaking process has no significant effect on reduction of the amount of toxicant.

Key words: Aflatoxin, Contamination, HPLC, Rice, Soaking, Washing

INTRODUCTION

Despite the increasing growth of population in developing countries and the increasing need for healthy foods, many type of the available foods, such as cereals, beans, spices, and fruits, are contaminated by harmful substances like mycotoxins. Besides contaminating agricultural products and animal foods, mycotoxins are associated with numerous economic disadvantages and losses [1-5]. These toxicants are indeed products with low molecular weight, which has one or more heterocyclic rings in their structure and is known as the secondary metabolites of mold [6,7]. One of the most harmful types of these toxicants is the aflatoxins, which are produced by *Aspergillus Flavus* and *Aspergillus parasiticus* molds [6-9]. Acute complications of Aflatoxin consumption as well as the chronic complications emerge in the form of carcinogenic, mutagenic, and teratogenic complications [2, 10]. Aflatoxin B1 is one of the most powerful hepato carcinogenic factors, which prevent activity of the DNA-polymerase and RNA-polymerase enzymes through binding to DNA and cause reduced protein synthesis [8, 9, 11]. The IARC (International Agency for Research on Cancer) has categorized aflatoxin B1

in the first group of the human mutagens [12]. The CODEX Committee has determined the maximum concentration of aflatoxin and aflatoxin B1 in rice equal to 4ng/g and 2ng/g, respectively [13-15]. Moreover, ISIRI (Institute of Standards and Industrial Research of Iran) has determined the minimum levels of 5 and 30ng/g for Aflatoxin B1 and Aflatoxin in rice, respectively [4, 14, 15].

One of the best methods for reducing the aflatoxin level in food and, consequently, the aforementioned complications is to prevent mold growth in agricultural products. Since rice is one of the most widely used food products, particularly in Asian countries, numerous studies have been conducted in this regard [8, 16-18]. A study conducted on the consumed rice in five Vietnamese states showed that 51% of the samples contained aflatoxin AFB1, and also the toxicant level in rainy seasons was higher than that in the dry ones [19]. Furthermore, another study conducted in India on 1200 various types of rice indicated contamination of these products by aflatoxin [20, 21].

In a study on 110 types of rice and corn in China, the mean concentration of aflatoxin in corn, rice, and whole grain brown rice was measured as 0.99, 3.87, and 0.88ng/g, respectively [1]. Another study reported the toxicant contamination rate of rice in China and India equal to 0.92% and 67.8%, respectively [4]. In another study conducted in Austria, aflatoxin was observed in 24 samples out of the total 81 samples, and also the toxicant concentration in 3 samples was higher than the European standard level [3]. Studies have shown that aflatoxin contamination rate in imported and domestic rice types was 0.011-30 and 0.8-6.3ng/g, respectively [15].

Results of Najafian's study indicated that aflatoxin level in the imported rice was higher than the domestic rice [22]. Therefore, the present research was aimed to determine the amount of aflatoxin B1 in the consuming rice in Yazd city as well as the effect of washing and soaking processes on reduction of this toxicant.

MATERIALS AND METHODS

Sample provision

The present study was a descriptive-analytical one, which was conducted on 36 types of rice (18 domestic and 18 imported types) consumed in Yazd in 2014. The sample size was determined based on the similar studies as well as the research limitations. In the present study, samples were selected randomly from all the rice distribution centers in Yazd; then, in order to perform the experiments, the samples were transferred to the food control laboratory of Shahid Sadoughi University of Medical Sciences in Yazd.

Sample preparation: In the washing process, 50g of contaminated rice was poured in a beaker containing 200 ml of water, and then was stirred for one, three, and five times by a stirrer with speed of 50 rps. At the end of each time of washing, the water was discharged. On the other hand, in the process of soaking, 50g of the sample was placed in a beaker containing water for one and ten hours.

Aflatoxin extraction

For this purpose, the Standard No.6872 of ISIRI was used in order to measure the aflatoxins of groups B and G through HPLC method and purification by immunoaffinity column. The samples were ground by a dry grinder, so that the particle size of <2mm was obtained in order for the particles to pass a mesh with a pore diameter of 2mm. Then, they were mixed with 5g of salt and then poured in a mixer, followed by adding 200 ml of methanol-80% solvent in water; next, the mixture was mixed well for 3min. The resulted extract was passed through a grade-1 Whatman filter paper. The filtered samples were used in order to measure aflatoxin level [23].

Aflatoxin measurement: The filtered extract was stirred well with distilled water in a capped glass container in order to obtain a diluted extract. The diluted extract was passed through filter paper (microfiber); next, the extract was passed through the immunoaffinity column, which had antibodies of groups B and G, at speed of one drop per second. By passing the diluted extract through the column, the antigen in the extract was bound to the column antibody. By passing methanol through the column, the resulted compound was washed, collected in a vial, and then diluted with water. Injection, isolation, recognition, and amount determination by reverse-phase method of HPLC was calculated in ng/g using reverse-phase column, derivative tool and fluorescence detector, comparison of sub-surface of the standard curve with unknown sample, and dilution coefficient of contamination rate, respectively. In order to provide the standard calibration curves, prior to injecting the sample, primarily certain concentrations of the measured standard aflatoxin solutions were injected into the HPLC device [23].

Statistical analysis: Statistical analysis of data was performed using SPSS-22 software. The one-way ANOVA was used to compare the samples in terms of aflatoxin level; subsequently, Tukey HSD was used for a multiple comparison between various groups. The values with $p<0.05$ were considered statistically significant.

RESULTS

Results of investigating 36 types of domestic and imported rice in the present study showed that the aflatoxin level was above the standard level (5ng/g) only in one sample; besides, in 5 samples, this level was above the European standard level (2ng/g). The mean level of aflatoxin B1 in the domestic and imported rice was estimated 1.461 and 0.508ng/g, respectively. Furthermore, the contamination level in the imported rice was lower than that in the domestic one, which indicated no statistically significant relationship ($p \geq 0.166$).

The following table represents the mean concentration of residual aflatoxin in the tested samples in one 1 and 10 hours of soaking in water. Comparing the mean concentrations in 1 and 10 hours of soaking indicated no significant reduction in the aflatoxin level in both methods, which was not statistically significant.

Table1: Comparing reduction rate and SD of aflatoxin B1 in rice in soaking and washing processes

Process	Duration time	Basic density (ppb)	Final density (ppb)	Reduction amount (ppb)	p-value
Soaking	One hour Ten hours	2.04±0.132 2.04±0.132	1.72±0.057 1.73±0.063	0.32 0.31	0.269 0.291
Washing	One time Three times Five times	23.32±1.31 23.32±1.31 23.32±1.31	15.25±2.12 7.4±0.71 0.3±0.02	8.07 15.92 23.02	0.150 0.018 0.005

Based on the data in the above table, the toxicant level was reduced by 34.6% in a single time of washing, which was not statistically significant; besides, in 3 and 5 times of washing, this level was reduced by 68.2% ($p<0.005$) and 98.7% ($p<0.018$), respectively, which was statistically significant. Fig.1 and Fig. 2 represent the percentage of aflatoxin reduction by washing and soaking processes.

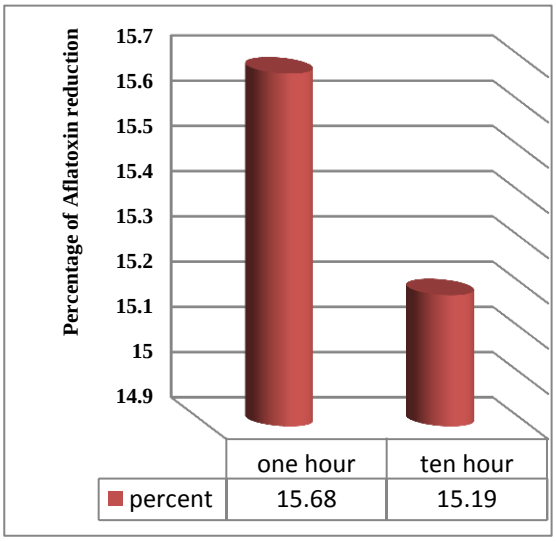

Fig.1: Reduction percent of Aflatoxin by means of washing process

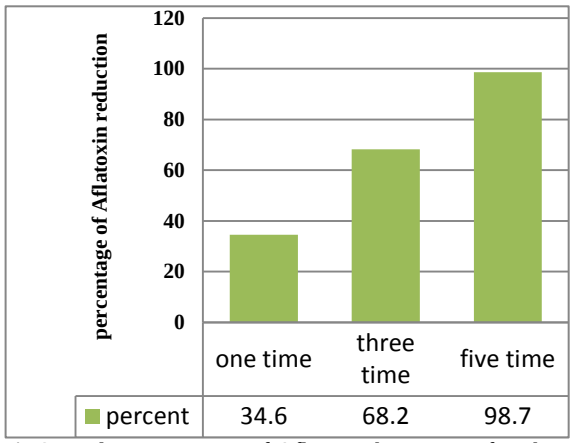

Fig.2: Reduction percent of Aflatoxin by means of soaking process

DISCUSSION

Results of the present study on the effect of rice washing and soaking on reduction of aflatoxin in rice indicated that soaking the rice has no effect on reducing the level of aflatoxin in rice; furthermore, in this study, no considerable difference was observed between the amounts of toxicant in the two types of rice. Although 1 and 10 hours of soaking resulted in no significant reduction in the level of aflatoxin in rice, the residual toxicant level was very low in the case of 3 and 5 times of washing. The obtained results on the amount of toxicant in the imported rice were consistent with those of Mazaheri *et al.* In their study, the mean aflatoxin level in all the imported samples was calculated 2.09ng/g, which was below the Iranian standard level (5g/g) similar to the present

study [1]. Mohammadi Sani *et al.* conducted a study to measure the aflatoxin level in the imported and domestic types of rice, in which the mean aflatoxin level in the imported and domestic rice was 1.89±0.87 and 1.08±0.02, respectively. On this basis, in none of the evaluated samples, the aflatoxin level was above the Iranian standard level, which is consistent with results of the present study. Furthermore, in this study, no significant difference was observed between the imported and domestic rice in terms toxicant level, which is consistent with the present study [24]. In another study conducted by Najafian, the aflatoxin level of the imported rice was higher than the domestic rice by 31.7%, which indicated a significant difference between the two types of rice. Results of this study are not consistent with those of the present study [22]. In these studies, the rice was of various types; thus, regarding the fact that these types of rice were imported from different countries, they had relative contamination origins and had not identical transportation conditions. Therefore, it was expectable to have different rates of contamination in the imported rice.

All the previous studies around the world were focused on the effects of the method of cooking rice on reduction of the toxicant. Fasiha *et al.* studied three different methods of rice cooking, including normal cooking, cooking with additional water, and under-pressure cooking, indicating that the aflatoxin B1 reduction rate (82%) in cooking with additional water was higher than other methods, while this value in the normal cooking method (49%) was lower than other method [25]. In several studies, effect of washing process on reduction of the toxicant in corn and wheat was investigated. Fandohan *et al.* investigated the effects of different processes on aflatoxin level in corn and observed that washing for 15 min could reduce the aflatoxin level in corn up to 91%; accordingly, they concluded that washing process would reduce the aflatoxin level significantly [26]. Hwang *et al.* conducted a study, entitled reducing aflatoxin B1 in wheat using various methods, in South Korea in which the wheat washing process in wheat was investigated. Results of this study indicated that washing the wheat could reduce the aflatoxin level B1 by 46-58% [27]. In the present study, the process of rice washing was studied for the first time, the results of which showed that washing the rice could reduce the aflatoxin B1 level significantly. Regarding mold activity on the seed's surface and production of aflatoxin at this surface, the washing process can be effective in reducing the toxicant level; thus, duration of the washing process and the number of washing times would be effective as well. There is no other study on the effect of seed soaking on reducing the toxicant so far; however, based on the results obtained from soaking the rice for 1 and 10 h, it can be inferred that rice soaking with different duration can reduce the aflatoxin contamination by 15%, which can be attributed to the short-run washing of rice and isolation of aflatoxin by water, because mere soaking cannot be much effective in reducing the aflatoxin level in rice.

CONCLUSION

Based on the reports and results of the present study, it can be concluded that different processes, including washing and soaking, affect reduction of toxicant in rice. With regard to the high consumption rate of rice and its derivatives in the society's food ration, the present study was conducted to recognize the aflatoxin B1 contamination of the rice consumed in Yazd in order to determine the contamination rate and, if not in the permitted range, provide it to the relevant authorities and offices for controlling and preventing the contamination. In the present study, due to the shortage of financial resources and high cost of performing the experiments by the HPLC method, it was impossible to measure a higher number of samples.

ETHICAL ISSUES

Ethical issues such as plagiarism have been observed by the authors.

CONFLICT OF INTEREST

There was no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally.

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