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Advances in Food Processing and Technology

Prietto L, et al. Adv Food Process Technol 1: 123.

Research Article

DOI: 10.29011/2639-3387.1000123

Domestic Hydrothermal Treatment of Rice and the Residual Aflatoxins Levels

Luciana Prietto, Verônica Simões de Borba, Kelly Cristina Massarolo, Eliana Badiale-Furlong*

Laboratório de Micotoxinas e Ciência de Alimentos, Escola de Química e Alimentos, Universidade Federal do Rio Grande - FURG, Avenida Itália km 8, Campus Carreiros, 96203-900, Rio Grande, Rio Grande do Sul, Brazil

***Corresponding author:** Eliana Badiale-Furlong, Eliana Badiale-Furlong, Laboratório de Micotoxinas e Ciência de Alimentos, Escola de Química e Alimentos, Universidade Federal do Rio Grande - FURG, Avenida Itália km 8, Campus Carreiros, CEP: 96203-900, Rio Grande, Rio Grande do Sul, Brazil

Citation: Prietto L, Simões de Borba V, Massarolo KC, Badiale-Furlong E (2019) Domestic Hydrothermal Treatment of Rice and the Residual Aflatoxins Levels. Adv Food Process Technol 1: 123. DOI: 10.29011/2639-3387.1000123

Received Date: 02 November, 2019; **Accepted Date:** 25 November, 2019; **Published Date:** 29 November, 2019

Abstract

This study evaluated the effect of three thermal sources for preparing rice (cooking) on the levels of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2), with the intention to evaluate if the domestic preparing food could contribute to mitigate aflatoxin exposure. White polished rice samples contaminated with aflatoxins (AFs) at three different levels (4, 5 and 6 µg kg^{-1}) were submitted to hydrothermal treatment in microwave, electric plate and autoclave. The residual AFs were determined by previously validated method. Microwave heating considerably reduced each aflatoxin by 80%, electric plate treatment reduced around 34% for AFB_1, 13% for AFB_2, 57% for AFG_1 and 29% for AFG_2, while autoclaving reduced 29% of AFG_1; 3% AFG_2; AFB_1 and AFB_2 were not affected by this treatment. Therefore, preparing food by microwave heating may be promising to mitigate AFs exposure.

Keywords: Cooking; Home preparation; Mycotoxin; Oriza sativa

Introduction

Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are responsible for a set of diseases, including gastrointestinal manifestations that may progress to liver cancer, according to the conditions and acute or chronic exposure levels [1]. Aflatoxins are produced by some toxigenic Aspergillus species on grains, such as peanut, corn, wheat and rice, under ideal moisture and temperature conditions [2-4]. Aflatoxin contamination has been reported even in commercially available grain-based products and many times at levels not acceptable by health regulation organization [2,4-7].Several practices such as using resistant varieties, crop protection, and careful harvesting, storage, and processing have been adopted with the hope of intending to prevent contamination by fungal and aflatoxins [8].The gap is that aflatoxins are stable compounds, heat resistant and soluble in organic solvents, thus making difficult their mitigation by processing or by hydrothermal treatment for foods preparing [1,6]. Another aspect is that the exposition to level lower than the recommended may promote chronic exposure, especially if this occurs in grains frequently consumed, as rice is around the world [9].

The information about aflatoxin contamination is mainly related to crude grains or their products. Therefore, it is important to emphasize the concern about daily food consumption in Brazil and in many Western countries, such as cooked rice, whose eating habits can favor chronic exposure [5,10]. Grain processing conditions do not always provide fully effective decontamination, heat treatment used in domestic or industrial preparation, from defined conditions, may be an alternative to mitigate aflatoxins exposure. Some authors have found that heat treatments promote aflatoxin degradation in peanut, corn and rice, once the contamination levels were more than 40% lower in peanut and corn subjected to conventional roasting [11] and 61.6- 89.7% of aflatoxins reduction in peanuts when roasted at 160-200 ˚C [12]. The cooked rice contaminated with AFB_1 resulted in a 34% loss of toxin [13] and even greater reductions (78-88%) when the grain was cooked under pressure [14]. The hydrothermal treatment reduced to about 80% the detected level of fumonisin B_1 in rice [10] and 84.5% and 87.2% aflatoxins (B_1, B_2, G_1 e G_2) reduction when the rice was submitted of the microwave oven and electric plate treatments [5].

About the hydrothermal treatment parameters to prepare rice around the world there is little information, however it may be a good strategy to decrease the risk of chronic damage caused by eating habits, and this may be by recommendation about preparing the product for consumption. However, it would be necessary to evaluate if the water added to cook or the matrix where the mycotoxin is found determine the effect of the heat treatment. The present study evaluated the stability of aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 in isolated form and in white polished rice employing different hydrothermal treatments (Electric hot-plate, pressure and microwave) commonly used in domestic preparation in order to find parameters that could be adopted to mitigate exposure to these fungal contaminants.

Materials and Methods

Material

White polished rice (type 1) was purchased in a local market in the city of Rio Grande-RS, Brazil. Acetonitrile and methanol (Backer, >99.99% purity) previously filtered through Millipore filter (Supelco, 58067) with pores of 0.45 µm, degassed an ultrasonic bath and used as mobile phase. The ultrapure water was obtained by Direct-Q UV3® system (Millipore, Bedford, USA) with resistivity of 18.2 MΩ cm^{-1}. Glacial acetic acid (>99% purity) was purchased from Merck (Darmstadt, Germany). Celite (Nuclear), ammonium sulfate and chloroform (Synth) and potassium chloride (Vetec) all with 99% purity were used. High performance liquid chromatography system (Shimadzu, Kyoto, Japan) coupled to a fluorescence detector (HPLC-FL) was used for AFs identification and quantification. A C18 Nucleosil column (10 x 4.6 mm, 3 µm, Bellefonte, PA, USA) was used. The equipment control and data processing were performed through LC Solution software. The aflatoxins standards solutions were purchased from Sigma Aldrich with purity > 99% and prepared according the AOAC Official Method [15].

Thermal Stability of Aflatoxin Standards

The effects of heat treatment in autoclave (0.5 and 1.1 kgfcm^{-2} for 10 and 30 min), microwave (320 and 800 W for 1 and 5 min), and by heating electric plate (50 and 150 ˚C for 10 and 30 min) on the degradation of AFB_1, AFB_2, AFG_1 and AFG_2 were investigated. AFs standards were placed in amber vials (12 ng ml^{-1} AFB_1 and AFG_1, and 5 ng ml^{-1} $AFB_2$and AFG_2) and dried under N_2, prior to thermal treatment. The dry residue resulting from each treatment was re-dissolved in 1 ml mobile phase and injected into the HPLC-FL. Reduction percentage were calculated by the ratio between the initial aflatoxin mass in the flasks and the remaining after heat treatments. The same experiment was conducted in water presence and for this the vials containing the standards were dried and after water was added (5 ml).

Hydrothermal Treatment (Cooking) By Different Heat Sources

White polished rice samples without natural contamination were spiked with 4, 5, and 6 µg kg^{-1} of each aflatoxin (AFB_1, AFB_2, AFG_1, and AFG_2) and allowed to stand for 24 h, these levels were the concentration found in rice samples, that were evaluated in change to a routine analysis carried out in the laboratory of Mycotoxin and Food Science, FURG (data not show) from 2007 to 2014. The spiked samples were subjected to cooking by microwave, autoclave and heating electric plates according to the following conditions: 20 g sample; 0.2 g NaCl; 0.4 g commercial soybean oil and 50 ml water, with the exception of the microwave treatment, in which 180 ml water were used [10].

The rice was cooked by heating in electric plates for 10 min at a temperature of approximately 100 ˚C until water was either absorbed by the grain change to or evaporated. Rice was cooked by autoclave at 1.1 kgfcm^{-2} for 2.5 min and the cooking by microwave the conditions were 800 W power output for 10 min. All heat treatments were compared with a non-spiked sample (control). AFs reduction percentage were estimated by the difference between initial levels and the remaining ones after hydrothermal heat treatments [10].

AFs Determination in Rice

The sample (10 g) were weighed and mixed with 60 ml methanol; 4% potassium chloride solution at a ratio of 9:1 and stirred in a blender for 2 min and filtered. After, 30 ml filtrate was transferred to an Erlenmeyer flask, and 30 ml of 30% ammonium sulfate solution, 1 cm^3 celite were added to the mixture followed by gentle stirring and standing for 5 min. After filtration, extract (30 ml) was transferred to a separator funnel, and mixed with distilled water (30 ml). The extract was partitioned in 10 ml aliquots of chloroform by 3 times. Fractions of 10 ml chloroform were placed in amber vials with a screw cap and dried in a water bath at 80 ˚C for 25 min [16].

The residual aflatoxins in the extracts were determined by HPLC-FL using as eluent ultrapure water acidified with 1% glacial acetic acid (v v^{-1}), acetonitrile, and methanol in the ratio 60:8:32 (v/v/v). The chromatographic conditions were: mobile phase flow rate 0.4 $mlmin^{-1}$; column temperature 45 ˚C resulting in retention time of 12.5; 15.0; 18.5; and 22.4 min for AFG_2, AFG_1, AFB_2, and AFB_1, respectively, and total run time 25 min. The dried residue was re-dissolved in 1 ml mobile phase and injected into the equipment. AFs concentrations were estimated on dry basis.

Statistical Analysis

Statistical analysis was performed using Statistica 7.0 and ANOVA to compare the level of each AF (B_1, B_2, G_1, and G_2) under treatment the microwave, autoclave, and heating electric plate treatments. Tukey's test was used with 95% confidence to evaluate significant differences.

Results and Discussion

According to the indicative of merit recommended by Na-

tional Health Surveillance Agency, National Institute of Metrology, Quality and Technology and Codex Alimentarius, the method used for AFs quantification was reliable. The correlation coefficients of the analytical curves were greater than 0.99, the limits of quantification were 0.3 μg kg^{-1} for AFB_1 and AFG_1, and 0.07 μg kg^{-1} for AFB_2 and AFG_2. The AFs extraction from the hydrothermally treated standard and nature rice was performed according to Prietto, et al. [4] and the method presented recoveries and repeatability acceptable (97% for AFB_1, 99% for AFB_2, 83% for AFG_1 and 100% for AFG_2). In order to verify the effects of water and temperature on aflatoxin degradation, as well as to promote a better evaluation of the effect of matrix (rice) on the levels of these mycotoxins, the temperature, time and pressure conditions adopted were the most drastic in three evaluated hydrothermal treatments (electric hot-plate, autoclaving and microwave). The percentage of degradation of the aflatoxin's standards levels were estimated by the difference between the initial and residual concentration (Table 1).

	Heat treatments		Degradation of aflatoxin (%)			
			AFB_1	AFB_2	AFG_1	AFG_2
Microwave	1' 320 W*	Dry	0^{Ec}	19.0^{CDEb}	24.1^{Ea}	0^{Ec}
		Aqueous	2.0^{10c}	20.0^{6b}	25.0^{11a}	2.0^{8c}
	5' 320 W	Dry	0^{Eb}	17.0^{DEa}	20.2^{Ea}	0^{Eb}
		Aqueous	2.0^{10c}	18.0^{7a}	16.0^{12b}	2.0^{8c}
	1' 800 W	Dry	14.2^{Eb}	$15.1D^{Eb}$	$34.8D^{Ea}$	0^{Ec}
		Aqueous	16.5^{7b}	15.0^{8c}	32.3^{9a}	2.0^{8d}
	5' 800 W	Dry	3.1^{Ec}	12.0^{EFb}	25.4^{Ea}	0^{Ec}
		Aqueous	4.3^{9c}	3.6^{9b}	27.5^{10a}	3.0^{7c}
Autoclave	10' 0.5kgf cm^{-2}	Dry	27.0^{EDab}	18.4^{CDEb}	35.6^{EDa}	23.8^{Db}
		Aqueous	25.6^{6b}	19.8^{6d}	34.9^{7a}	22.2^{6c}
	30' 0.5kgf cm^{-2}	Dry	47.4^{BCb}	43.0^{Ab}	80.4^{Aa}	70.4^{Aa}
		Aqueous	43.7^{4c}	43.2^{1c}	76.2^{3a}	68.5^{2b}
	10' 1.1kgf cm^{-2}	Dry	15.0^{EFb}	2.5^{Ec}	31.2^{Ea}	0^{Ec}
		Aqueous	15.0^{8b}	3.2^{10c}	33.4^{8a}	3.0^{7c}
	30' 1.1kgf cm^{-2}	Dry	23.4^{EDc}	16.4^{DEc}	77.9^{ABa}	52.5^{Bb}
		Aqueous	25.0^{6c}	18.6^{7d}	78.8^{2a}	50.3^{2b}
Hot plate	10' 50 ˚C	Dry	39.1^{DCb}	24.8^{BCDc}	55.8^{BCDa}	25.8^{CDbc}
		Aqueous	40.0^{5b}	23.6^{5c}	50.2^{6a}	22.6^{6d}
	30' 50 ˚C	Dry	56.6^{Bab}	43.9^{Ab}	68.5^{ABCa}	46.5^{Bb}
		Aqueous	54.0^{2b}	40.2^{2c}	65.6^{4a}	40.5^{3c}
	10' 150 ˚C	Dry	52.4^{BCa}	28.7^{BCb}	52.9^{DCa}	26.3^{CDb}
		Aqueous	51.6^{3a}	28.0^{4b}	54.3^{5a}	24.7^{5b}
	30' 150 ˚C	Dry	88.2^{Aa}	34.0^{ABb}	87.7^{Aa}	32.3^{Cb}
		Aqueous	86.0^{1a}	37.8^{3b}	86.6^{1a}	34.0^{4c}

Table 1: Degradation of aflatoxin standards under different heat treatments.

Results expressed as mean.': Minutes; *W: power informed by the manufacturer. Different superscript capital letters and number in the same column indicate significant differences ($p<0.05$). Different superscript lower case in the same line indicate significant differences ($p<0.05$). Results expressed as mean. ': Minutes; *W: power informed by the manufacturer. Different superscript capital letters and number in the same column indicate significant differences ($p<0.05$). Different superscript lower case in the same line indicate significant differences ($p<0.05$).

The treatment under microwave of the standard dry and in aqueous solutions show the best reducing effect. The AFB_1 was the mycotoxin in most susceptible to treatment when the standard dry was treated. The treatment of the standard under autoclave was less efficient, since it would be AFG_1 and AFG_2 remained stable. As shown in (Figure 1), lowest level of the contaminants after

treatment was observed when the rice was cooked in a microwave with an average of 80% reduction in the level of each of aflatoxins, considering the three levels of contamination studied. The aflatoxin reduction average value was adopted because the sample initial contamination levels were around that found in our laboratory during control quality of this crop asked of by regional industries (data not shown). Treatment in the hot-plate reduced 34% of $AFLAB_1$ detected, 13% to $AFLAB_2$, 57% to $AFLAG_1$ and 29% for $AFLAG_2$, and in the autoclave reduced 29% of $AFLAG_1$, 3% to $AFLAG_2$, while AFLAS B_1 and B_2 were not affected.

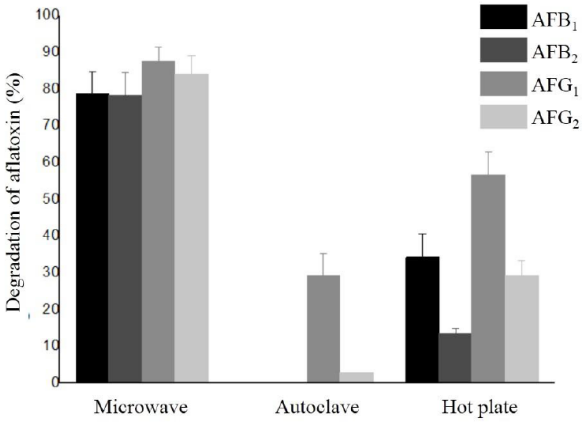

Figure 1: Hydrothermal treatment of rice and percentage reduction of AFs. Results expressed as mean for the three levels of rice sample spiked. Different letters indicate significant differences ($p<0.05$).

The parameters used for cooking rice were stablished to allowing that it presents appropriate texture for domestic consumption habits, evaluated as described by Silva, et al. [5]. Therefore, different times and water volumes were used for each treatment, with 10 min for microwave cooking, and 2.5 min for both heating electric plates and autoclave. Furthermore, the water volume used for microwave cooking was 3.6 times greater than the volume of the other treatments. This is probably due to the higher water evaporation during cooking, since heating is more homogeneous when compared to heating electric plate where the high temperature is produced only under recipient bottom. A sutured steam environment is formed in autoclave, creating homogeneous hot moisture medium, but this was not efficient to degrade the mycotoxins.

The use of microwave led to further aflatoxins degradation possibly due to the molecular friction generated by the dipole rotation of polar molecules in presence of modified electric field [17]. Furthermore, the ionic conduction can be another mechanism which has contributed to this response, since positive and negative ions of dissolved salts such as NaCl also permit electric charges to move from one region to another, breaking hydrogen bonds that form with water molecules to generate additional heat [5,18]. Silva, et al. [5], evaluated different hydrothermal treatment to cooking rice, including microwave oven, they explained that there were formed cross linking among the aflatoxins and macromolecules whose consequences were the reduction of the determined level.

Park, et al. [13] reported an average toxin degradation of 34% in rice spiked with AFB_1 and cooked by the conventional method (hot plate), which was also observed in this study. Becker-Algeri, et al. [10] adopting the same conditions evaluated the effect of heat treatment for rice spiked with fumonisin B_1 and also found no significant reduction when rice was subjected to hydrothermal treatment by autoclave. These findings are similar to that observed in our study, where the lowest degradation was observed for rice cooked in autoclave, and only AFG_1 was degraded (Figure 1). However, opposite results were obtained by Park and Kim [14], who found degradation between 78% and 88% for AFB_1 in rice cooked at 160 ˚C for 20 min under pressure fixed at 15 lb in^{-2} (0.10 MPa), in our study the cooking time under pressure was lower (2.5 min), therefore the time may be an important condition to promote the AF reduction.

Siminionato and De Sylos [19] evaluated the effect of cooking process on the AFB_1 and Ochratoxin A in white polished rice artificially contaminated, and subjected to conventional cooking, cooking in excess water, and microwave cooking. A cooking time of 9 min was performed in all treatments. The highest degradation was found in rice cooked in excess water (89.1%), followed by conventional cooking (82.3%), and microwave (72.5%). These results were similar to those found in this study for the microwave treatment, with opposite results observed in the conventional treatment, which showed degradation between 13% and 57%. It is important observe that in the treatment with microwave the water amount necessary was higher than the others and the early results suggested that it is important for decontamination.

Rehana, et al. [20] also studied the effect of conventional cooking, cooking in excess water, and cooking under pressure on the AFB_1 levels in artificially and naturally contaminated rice. Cooking under pressure resulted in 70.7% and 70.5% degradation for artificially and naturally contaminated samples, respectively. Conventional cooking for 30 min resulted in an average degradation of 47.5% for naturally contaminated samples, and 49% for spiked samples. Cooking in excess water resulted in higher toxin losses, with reductions of 75.7% and 74.5% for naturally and artificially contaminated rice, respectively. Rehana, et al. [20] also found the presence of AFB_1 in wastewater for this type of treatment. Again, the results are different from those obtained by cooking under pressure, as in the present study the lowest AFs degradation was observed for cooking in autoclave, while those authors found degradations close to 70%, probably because the molecules compaction has also affected the kinetics response.

The effect of different heat treatments on mycotoxin level had been reported in many studies, with values ranging from 0 to

100%, and often presenting conflicting results, once many factors may affect aflatoxins degradation or instead of affect their recovery from the matrix, as it was also demonstrated in this study [14,19-21]. Our results suggest that the treatment time is another important parameter for aflatoxins degradation. According to Buser and Abbas [21], the free water content is a key factor for AFs deterioration, since the free water is required to hydrolyze the lactone ring during thermal treatment. A higher degradation was observed for AFG_1, regardless of treatment, indicating its lower thermal stability when subjected to any heat source (Table 1). Elisaldes-González, et al. [22] studied the influence of temperature on aflatoxins degradation in which the effect of the thermal treatment was evaluated by immersing aflatoxin solution in a bath at 100 ˚C for different time intervals. The results showed that 20% degradation was reached after 3 min for both fractions AFG_1 and AFB_2. The authors observed higher degradation (50%) in the treatment at 100 ˚C for 40 min, which is similar to the results found in this study, because aflatoxins degradation was higher when a great time interval was used for different treatments.

Hwang and Lee [3] evaluated the influence of water content in the aflatoxin's degradation in Sujebi (soup with wheat flakes) and bread baked with steam, two traditional products in Korean cuisine. The degradation was around 43% in bread, while no aflatoxins were detected in Sujenbi after heating. The authors have reported that this difference was related primarily to the free water during heat treatment, which may explain the results in this study, since the water volume used for cooking rice in a microwave was 3.6 times higher than the other treatments. Samarajeewa, et al. [23] reported that moisture is required to hydrolyze the lactone ring of the AFs with heating at home cooking temperature (85-95 ˚C). So far, for detoxifying AFs it is the most important step to open the lactone ring of the AFs [21]. The greater degradation of AFG_1 in most treatments is due to the greater susceptibility of the heterocyclic ring to oxidation by the action of the temperature in aqueous medium.

As it was expected that the aflatoxins submitted to heat treated in dry or in aqueous solution in an isolated manner shown different residual levels behavior when compared to a matrix containing different AFs. When rice matrix is submitted to heat treatment for cooking, the starch granules absorb water and swell in size during heating, becoming completely gelatinized, because the hydrogen bonds between starch micelles are converted into water molecules, changing the heating transfer in the matrix inner [24].

The matrix containing aflatoxins, besides water content, is another important variable, once degradation in naturally contaminated food can be more complex and time consuming when compared to artificially contaminated food. These mycotoxins are attached differently in food macromolecules, and thus more protected against degradation [24]. Although the heat treatment decreased the initial AFs levels, it cannot prevent exposure in case of large food consumption, because gelatinized starch can retain contaminants after heating, releasing them during the digestive process. Therefore, experiments involving variables that may affect these compounds interactions are decisive for reducing the risk of chronic exposure of the population to dietary xenobiotics.

Conclusion

The heat exposure time is decisive for the reduction of AFs levels in their pure form. The hydrothermal treatment using microwave as a heat source was more effective to reduce the residual level (around 80%) in white rice, although the other heat treatments of this study have also reduced the AFs levels with minor efficiency (0- 57%). The information about treatments that could be applied in domestic environment is not frequent therefore this study may be important to deep the knowledge about cooking strategy to mitigate aflatoxins exposure.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES, Brazil, Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS).

Declaration of Interests

The authors declare no conflict of interest.

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