



Migration of mycotoxins into rice starchy endosperm during the parboiling process

Giniani Carla Dors*, Luiz Antônio de Almeida Pinto, Eliana Badiale-Furlong

Laboratory of Food Biochemistry and Mycotoxins, Department of Chemistry, Federal University Foundation of Rio Grande (FURG), P. O. Box 474, CEP 96201-900, Rio Grande, RS, Brazil

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ABSTRACT

Considering the occurrence of rice contamination by mycotoxins and the increase in rice consumption, the present work had the objective of assessing the migration of mycotoxins into the starchy endosperm during the parboiling process, as to propose conditions that provide lower contamination levels. The newly harvested rice grain sample was examined for the natural occurrence of mycotoxins (aflatoxin B₁, aflatoxin B₂, deoxynivalenol, ochratoxin A, and zearalenone); only the presence of aflatoxin B₁ was found (17 ng/g). The samples were then artificially contaminated with deoxynivalenol and zearalenone, and the parboiling process was conducted according to a 2³ factorial planning with central point, having as variables the contamination level deoxynivalenol 720, 1440, and 2160 ng/g, and zearalenone 476, 952, and 1428 ng/g the soaking time (4, 5, and 6 h) and autoclave time (15, 22.5, and 30 min). Mycotoxins aflatoxin B₁ (AFA B₁), deoxynivalenol (DON), and zearalenone (ZEA) were confirmed and determined through gas chromatography. Findings showed a lower migration trend for AFA B₁ under 6 h of soaking and 30 min of autoclaving, for DON under 6 h of soaking regardless of the autoclaving time, and for ZEA under 4 h of soaking and 15 min of autoclaving. This information can contribute to the choice of process parameters that limit the migration of these mycotoxins if they happen in the raw material.

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1. Introduction

According to data from FAO (2006), about 15–16% of the rice harvest is lost every year, mainly during critical post-harvest operations. Inappropriate grain storage and processing may provide favorable conditions to contamination by deleterious agents, such as fungi, which besides causing nutritional and sensorial changes in the product can produce mycotoxins (Patkar, 1994). Such toxins are associated with the occurrence of pathological effects in both animals and humans alike (Mello & Macdonald, 1997; Sweeney & Dobson, 1998).

In the case of irrigated rice, studies involving newly harvested grains or it's products available in shops and the occurrence of mycotoxins are still scarce, but those already concluded showed the mycotoxins like AFA B₁ (Coelho, Badiale-Furlong, & Almeida, 1999; Furlong, Soares, Vieira, & Dadalt, 1999) DON and ZEA (Badiale-Furlong, 2005; Nunes, Magagnin, Bertolin, & Badiale-Furlong, 2003), beside toxigenic fungal species (Bianchini, 2003; Nunes et al., 2003).

The parboiling is defined as a “hydrothermal process in which the unpeeled rice is submerged in drinking water at a temperature

above 58 °C, followed by a partial or full gelatinization of the starch and later drying”. Parboiled rice is then the “product whose grains upon processing show a yellow, steady color, due to the parboiling process used to elevate the vitamin and mineral salt content” (Amato & Elias, 2005).

During the soaking of unpeeled rice, the water migrates to the inner portion of the grain, carrying water-soluble compounds, also providing an appropriate environment for starch gelatinization, which should take place during cooking. The gelatinization “welds” any fissures present and fixates the water-soluble compounds in the grain's inner portion, notably reducing the loss of such nutrients. With the later drying, the rice grain becomes more resistant to physical effects during processing, thus increasing the efficiency in terms of unbroken grains (Bello, Tolaba, & Suarez, 2007; Bhattacharya & Ali, 1985; Luz & Treptow, 1994; Martinez, 1984; Singaravadivel & Anthoni Raj, 1984). However, beside the nutrients the contaminants may migrate too.

Coelho et al. (1999) studied the migration of mycotoxins (aflatoxins B₁, B₂, G₁, and G₂ and ochratoxin A) during the parboiling process of contaminated rice cropped in Rio Grande do Sul state, Brazil. It was demonstrated that 32% (4.7 ppm) for aflatoxin B₁, 44% (4.8 ppm) for aflatoxin B₂, 36% (3.6 ppm) for aflatoxin G₁, and 22% (0.6 ppm) for aflatoxin G₂ migrated from the outer to the inner layer of the rice grain. Such results suggest the need of studying the

* Corresponding author. Tel.: +55 53 3233 8663.

E-mail addresses: dorsgi@yahoo.com.br, dqmebf@furg.br (G.C. Dors).

parboiling conditions and their effects on the distribution of other mycotoxins in rice products that are common in the human diet. Dors (2006) evaluated the occurrence of DON and ZEA in 32 samples of parboiled rice found 45% contaminated with DON (180–400 ppb) and 47% with ZEA (317–396 ppb). As it was demonstrated by the authors, the chronic contamination risk exists and the knowledge about the behavior of the mycotoxins during the parboiling process is the best form of preventing the damages for the human health. The knowledge about mycotoxins behavior during a process is the best way to prevent the damage to human health.

The present work had the objective of assessing the migration of deoxynivalenol and zearalenone into the starchy endosperm during the parboiling process, in order to propose process conditions to control the contamination in the inner grain to allow levels that guarantee the food safety.

2. Material and methods

2.1. Mycotoxin standard

The standards for aflatoxin B₁ (AFA B₁), aflatoxin B₂ (AFA B₂), deoxynivalenol (DON), ochratoxin A (OTA), and zearalenone (ZEA) were acquired from Sigma Chemical Company. The stock solutions were prepared and quantified through the procedure described by Bennett and Shotwell (1990) by using the standard molar absorptivity of the mycotoxins under UV light.

2.2. Sample

The rice sample used was a newly harvested, unpeeled sample from the 2004/2005 harvest, cultivated in Taim – RS and belongs to a specie recommended to cultivate in Brazil. It was dried until 13% humidity (dry basis), through a parallel air flow drier at 60 °C for 3 h. Later on, it was subjected to the parboiling process and milled in a Susuki laboratorial mini-mill, removing the husk. Every portion was ground and was sifted in a Tecnal knife-mill (model TE-631) and the portion passing through the 32 mesh (0.5 mm) was collected.

2.3. Mycotoxin determination

The mycotoxins AFA B₁, AFA B₂, AFA G₁, AFA G₂, and OTA were determined before the parboiling study, employing the multi-method described by Soares and Rodriguez-Amaya (1989). The AFA B₁ was determined after parboiling process too. DON and ZEA were determined before and after parboiling process as procedure adapted from Tanaka (2001), 10 g of sample rice was weighed, then stirred with 100 mL of an acetonitril:water (3:1) solution in a vertical stirrer (200 rpm = 1.8 g, 25 °C) for 30 min. The extract was filtered and washed twice with 20 mL of hexane. Then, 2 g of sodium chloride was added to the acetonitril:water phase, which was evaporated under reduced pressure at 60 °C. The residue was dissolved in 3 mL of methanol with the help of ultra-sound bath and 27 mL of chloroform. The residue was dissolved in 3 mL of methanol with the help of ultra-sound bath and 27 mL of chloroform, after it was centrifuged at 3000 rpm (1610 g) per 10 min. The supernatant was divided in three 10-mL portions, then stored in an amber glass for trial, confirmation, and quantification, respectively.

The extracts were purified through a silica:alumina:celite (7:5:3) mini-column, amounting to a total of 3 g of stationary phase. The extracts were eluted with 35 mL of a chloroform:methanol (3:1) solution and evaporated until drying under N₂. The derivation of the dry residue was carried out using 1 mL of 1.5 mg/mL pyridine in toluene:acetonitrile (95:5) and 300 µL of trifluoroacetic anhydride (TFAA), treated at 60 °C for 60 min. Seven hundred microlitres of toluene and 2 mL of 0.1 M pH 7 phosphate buffer, shaken in and

frozen. Four hundred microlitres were removed from the organic stage and dried in an N₂ current, 1 µg of araquidic acid was then added as an internal standard dissolved with 200 µL of n-hexane and DON and ZEA were determined by gas chromatography (Garda et al., 2005). Confirmation was conducted through chemical derivation specific for each mycotoxin in according to Furlong and Soares (1995).

The chromatography used was carried out in Varian (model 3400) chromatograph, equipped with flame ionization detector and split/splitless injector. A 30-m × 0.248-mm I.D. fused silica column was used, with 0.25-µm DB-17 (50% Phenyl – Methylpolysiloxane) film. Hydrogen was used as carrier gas. Chromatographic conditions used were injector temperature 250 °C, valve opening at 0.75 min, injector cleaning flow of 75 mL/min, detector temperature 300 °C, and attenuation 8 × 10¹². Programming for the chromatographic column was 50 °C/min up to 200 °C, keeping for 2 min, then increasing 4 °C/min up to 250 °C, keeping for 14 min, amounting to a total of 32.5 min (Nunes et al., 2003).

The performance of the methods for determining all the mycotoxins was carried out in according to Ribani, Botolli, Collins, Jardim, and Melo (2004).

2.4. Experimental parboiling

Experiments were conducted according to three factors (X₁, X₂, X₃) and two-levels (–1, +1) with central composite in order to study the migration of DON and ZEA into the starchy endosperm during the parboiling process. The studied factors of variation were contamination level, time soaking and autoclaving time. The levels of DON and ZEA in the starchy endosperm were the answer. From the combination of treatment conditions defined in the planning, 11 experiments result were performed in a random order in which 8 factorial points and 3 center points (8 + 3 = 11). The factorial planning was repeated two times and Table 1 shows the independent variables (X_i) with their levels and the actual experiments that were carried out for developing the model. The unpeeled rice samples were artificially contaminated at the levels established in the planning, 24 h before the parboiling process.

The unpeeled rice samples were soaked to 1:2 (rice:water) proportion, remaining in a 60 °C bath for 4, 5, and 6 h. The soaked unpeeled rice underwent autoclaving at manometric pressure of 1 × 10⁵ Pa (121 °C) during times of 15, 22.5, and 30 min. Each combined condition was conducted in a specific recipient. The samples of each experiment were dried in aluminum trays, with a parallel air flow drier at 60 °C during 3 h until 13% humidity (dry basis). From the combination of treatment conditions defined in the planning, 11 experiments resulted, as described in Table 1.

Table 1

Matrix of 2³ factorial planning with central point in codified and noncodified forms to assess the migration of deoxynivalenol (DON) and zearalenone (ZEA) to the starchy endosperm during the parboiling process

Experiments	Soaking time		Autoclaving time		Contamination levels		
	X _S	(h)	X _A	(min)	X _C	DON (ng/g)	ZEA (ng/g)
1	–1	4	–1	15	–1	720	476
2	+1	6	–1	15	–1	720	476
3	–1	4	+1	30	–1	720	476
4	+1	6	+1	30	–1	720	476
5	–1	4	–1	15	+1	2160	1428
6	+1	6	–1	15	+1	2160	1428
7	–1	4	+1	30	+1	2160	1428
8	+1	6	+1	30	+1	2160	1428
9	0	5	0	22.5	0	1440	952
10	0	5	0	22.5	0	1440	952
11	0	5	0	22.5	0	1440	952

X_S = codified soaking time; X_A = codified autoclaving time; X_C = codified contamination levels.

2.5. Statistical analysis of results

The software *Statistica* version 6.0 for Windows in its “experimental design” mode was used to analyze the influence of variables soaking time, autoclaving time, and contamination levels upon the migration of the mycotoxins studied, based on variance analysis (ANOVA/MANOVA) at a 95% significance level.

3. Results and discussion

3.1. Mycotoxins found in newly harvested rice

The performance of the methods employed to mycotoxin determination is described in Table 2, and the results showed that it was possible to employ it to evaluate the effect of planning experiments in the mycotoxins levels.

3.2. Mycotoxin behavior during the parboiling process

3.2.1. Aflatoxin B₁ (AFA B₁)

The rice sample collected was naturally contaminated with 17 ng/g of AFA B₁. After the process it was verified that AFA B₁ migrated in every condition studied being about 100%, when the sample was not contaminated with DON or ZEA artificially. However, an 82% reduction could be seen when the soaking time was increased from 4 to 6 h, at both autoclaving conditions. Coelho et al. (1999) concluded, when studying AFA B₁, AFA B₂, AFA G₁, AFA G₂, and OTA, that the shorter soaking time (4 h) provided a significant increase in the rate of AFA B₁ in the starchy endosperm, when compared to the longer soaking time (6 h) in a similar way that it was observed in this work.

In the presence of DON and ZEA the same migration reduction trend was found with the increase in soaking time. Table 3 shows the percentage results of AFA B₁ migration into the rice starchy endosperm during the parboiling process, obtained with the experiments according to a 2³ factorial planning with central point. Such results were subjected to variance analysis, concluding in the case of AFA B₁ that there was a significant difference in the migration percentage, at the 95% level ($p < 0.05$), for the main effects of factors soaking time (X_S), autoclaving time (X_A), and contamination level (X_C); the interaction between soaking time and autoclaving time ($X_S X_A$); the interaction between soaking time and contamination level ($X_S X_C$); and the interaction between soaking time, autoclaving time, and contamination level ($X_S X_A X_C$), as shown in Table 4.

By considering only the significant effects in Table 4 in the statistical analysis, the determination coefficient for the mathematical theoretical model was obtained ($R^2 = 0.689$) along with its respective equation that cannot predict the percentage of AFA B₁ migration into the starchy endosperm (Eq. (1)).

Table 2

Performance of the method employed to mycotoxin determination in starchy endosperm

Mycotoxin (method)	Detection limit (ng/g)	Recovery (%) ^a	Multimethod linearity (ng/g)
AFA B ₁ (TLC)	2.6	86	2.6–52
AFA B ₂ (TLC)	4.0	86	4.0–78
OTA (TLC)	6.5	86	6.5–65
DON (TLC)	180	82	180–2700
ZEA (TLC)	40	61	40–1170
DON (GC)	59	90	150–1800
ZEA (GC)	195	75	150–1800

^a Three-level mean: AFA B₁ = (32, 64 and 96 ng/g); OTA = (39, 78 and 117 ng/g); DON = (480, 960 and 1440 ng/g); ZEA = (616, 1232–1848 ng/g).

Table 3

Migration percentage for AFA B₁, DON, and ZEA into the starchy endosperm resulting from the 2³ factorial planning with central point

Experiments	Migration into starchy endosperm (%) ^a		
	AFA B ₁	DON	ZEA
1	38 ± 1.0	94 ± 2.0	46 ± 1.5
2	11 ± 1.0	8 ± 1.0	65 ± 1.0
3	35 ± 1.0	100 ± 2.5	96 ± 2.0
4	8 ± 0.5	3 ± 1.0	82 ± 2.5
5	7 ± 0.5	39 ± 1.0	64 ± 1.0
6	15 ± 1.5	41 ± 1.5	58 ± 1.0
7	11 ± 1.0	36 ± 1.5	61 ± 1.5
8	8 ± 0.5	39 ± 1.0	58 ± 1.0
9	32 ± 0.5	47 ± 0.5	64 ± 2.0
10	32 ± 0.5	48 ± 1.0	61 ± 1.5
11	31 ± 1.0	45 ± 1.5	62 ± 1.0

^a Mean of two repetitions.

$$\begin{aligned} (\%) \text{AFA B}_1 \text{ migration} = & 20.73 - 6.12X_S - 1.12X_A - 6.37X_C \\ & - 1.38X_S X_A + 7.38X_S X_C - 1.38X_S X_A X_C \end{aligned} \quad (1)$$

Observing the migration percentage of AFA B₁ into the starchy endosperm as a function of soaking time and autoclaving time it is possible to see that the lowest migration takes place in the longest times, 6 h and 30 min, respectively. It may be seen that in the highest contamination levels, both of DON and ZEA (1440 and 952 ng/g, respectively), for the naturally AFA B₁-contaminated samples, the lowest migration also takes place at the longest soaking time (6 h).

3.2.2. Deoxynivalenol (DON)

The percentage results of DON migration into rice starchy endosperm during the parboiling process, obtained through the experiments according to a 2³ factorial planning with central point and found in Table 3, were subjected to variance analysis. In the case of DON, it was concluded that there was a significant difference in the migration percentage at the 95% level ($p < 0.05$), for the main effects of factors soaking time (X_S) and contamination level (X_C) and the interaction between both ($X_S X_C$), as shown in Table 5.

By considering only the significant effects in Table 5 in the statistical analysis, the determination coefficient for the mathematical theoretical model was obtained ($R^2 = 0.998$) along with its respective equation to predict the percentage of DON migration into the starchy endosperm (Eq. (2)).

$$(\%) \text{DON migration} = 45.45 - 22.25X_S - 6.25X_C + 23.50X_S X_C \quad (2)$$

Fig. 1 represents the response surface of the percentage of DON migration into the starchy endosperm as a function of soaking time and contamination level. It is possible to see that in the lowest

Table 4

Analysis of variance of AFA B₁ migration into starchy endosperm

Factors	QS	DF	QM	F	p
(X_S) Soaking time	300.12	1	300.12	900.38	0.0011
(X_A) Autoclaving time	10.12	1	10.12	30.38	0.0314
(X_C) Contamination level	325.12	1	325.12	975.38	0.0010
$X_S X_A$	15.12	1	15.12	45.38	0.0213
$X_S X_C$	435.12	1	435.12	1305.38	0.0008
$X_A X_C$	1.12	1	1.12	3.38	0.2076
$X_S X_A X_C$	15.12	1	15.12	45.38	0.0213
Lack of fit	493.64	1	493.64	1480.92	0.0007
Pure error	0.67	2	0.33		
Total QS	1596.18	10			

QS = quadratic sum; DF = degrees of freedom; QM = quadratic mean; F = Fischer test; p = confidence level.

Table 5
Analysis of variance of DON migration into the starchy endosperm

Factors	QS	DF	QM	F	p
(X_S) Soaking time	3960.50	1	3960.50	1697.36	0.0006
(X_A) Autoclaving time	2.00	1	2.00	0.86	0.4523
(X_C) Contamination level	312.50	1	312.50	133.93	0.0074
$X_S X_A$	12.50	1	12.50	5.36	0.1467
$X_S X_C$	4418.00	1	4418.00	1893.43	0.0005
$X_A X_C$	4.50	1	4.50	1.93	0.2994
$X_S X_A X_C$	18.00	1	18.00	7.71	0.1089
Lack of fit	6.06	1	6.06	2.59	0.2484
Pure error	4.67	2	2.33		
Total QS	8738.73	10			

QS = quadratic sum; DF = degrees of freedom; QM = quadratic mean; F = Fischer test; p = confidence level.

contamination level (720 ng/g) with the shortest soaking time (4 h), the highest DON migration takes place to the starchy endosperm. However, variables soaking time and autoclaving time are more important in terms of industrial application. The results from the experimental planning showed that it is possible to identify the best condition so that there is the lowest migration of DON into the starchy endosperm, by applying the longest soaking time (6 h).

3.2.3. Zearalenone (ZEA)

Table 3 also shows the percentages of ZEA migration into the starchy endosperm. The analysis of variance performed showed that there was a significant difference in the migration percentage at the 95% level ($p < 0.05$) for the main effects of factors autoclaving time (X_A) and contamination level (X_C), the interaction between soaking time and autoclaving time ($X_S X_A$), the interaction between autoclaving time and contamination level ($X_A X_C$), and the interaction among all three factors ($X_S X_A X_C$), as shown in Table 6.

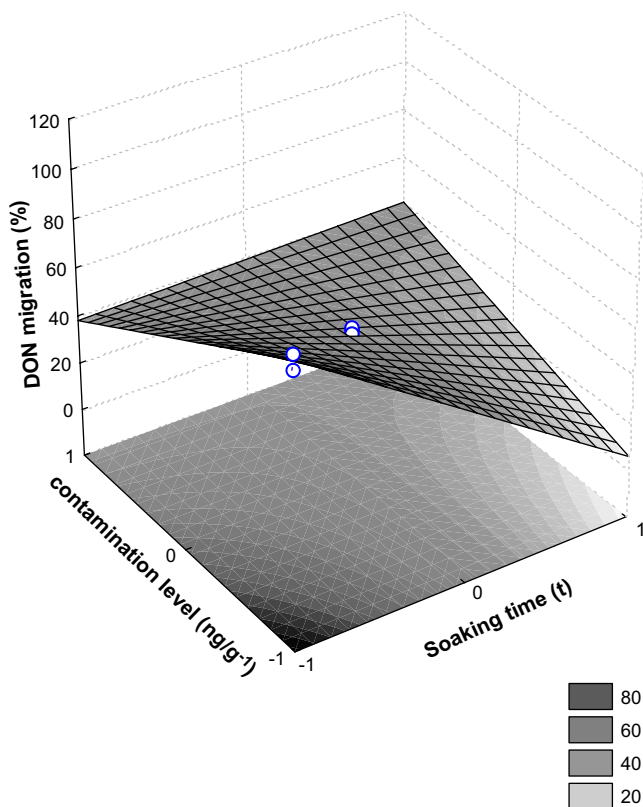


Fig. 1. Response surface representing the percentage of DON migration as a function of soaking time and contamination level after the parboiling process.

Table 6
Analysis of variance of ZEA migration into the starchy endosperm

Factors	QS	DF	QM	F	p
(X_S) Soaking time	2.00	1	2.00	0.85	0.4523
(X_A) Autoclaving time	512.00	1	512.00	219.43	0.0045
(X_C) Contamination level	288.00	1	288.00	123.43	0.0080
$X_S X_A$	112.50	1	112.50	48.21	0.0201
$X_S X_C$	24.50	1	24.50	10.50	0.0835
$X_A X_C$	612.50	1	612.50	262.50	0.0038
$X_S X_A X_C$	162.00	1	162.00	69.43	0.0141
Lack of fit	33.47	1	33.47	14.34	0.0632
Pure error	4.67	2	2.33		
Total QS	1751.64	10			

QS = quadratic sum; DF = degrees of freedom; QM = quadratic mean; F = Fischer test; p = confidence level.

By considering only the significant effects in Table 6 in the statistical analysis, the determination coefficient for the mathematical theoretical model was obtained ($R^2 = 0.978$) along with its respective equation to predict the percentage of ZEA migration into the starchy endosperm (Eq. (3)).

$$(\%)ZEA \text{ migration} = 65.18 + 8.00X_A - 6.00X_C - 3.75X_S X_A - 8.75X_A X_C + 4.50X_S X_A X_C \quad (3)$$

Fig. 2 represents the response surface of ZEA migration into the starchy endosperm as a function of soaking and autoclaving times. It is possible to see that the lowest migration takes place in the lowest times, 4 h and 15 min, respectively. In Fig. 3, the surface response which is a function of autoclaving time and contamination level, it is seen that the least migration also takes place in the lowest contamination levels (476 ng/g) with the shortest autoclaving time (15 min).

Therefore, the best condition to be employed in the parboiling process so that there is less migration, at a contamination level near 476 ng/g, includes the shortest soaking time (4 h) and autoclaving time (15 min).

It is important to highlight that the samples were simultaneously contaminated with DON and ZEA in the presence of AFA B₁.

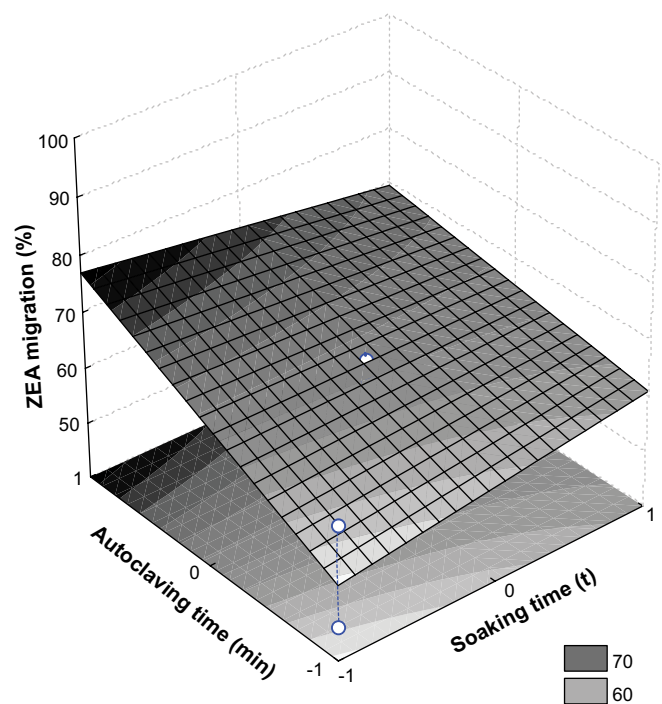


Fig. 2. Response surface representing the percentage of ZEA migration as a function of soaking time and autoclaving time after the parboiling process.

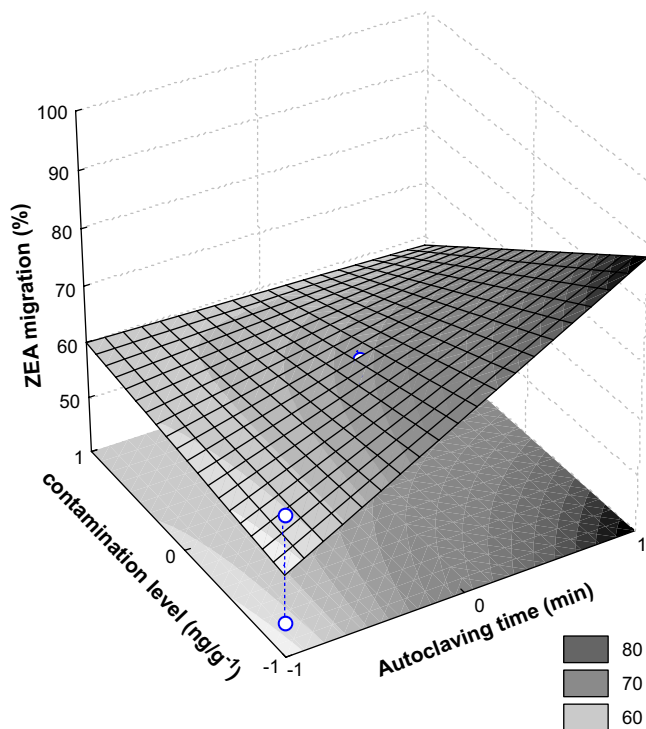


Fig. 3. Response surface representing the percentage of ZEA migration as a function of autoclaving time and contamination level after the parboiling process.

Besides the interference of one mycotoxin upon each other, there was interference of other compounds from the matrix. It is very important to evaluate the mycotoxins in the unpeeled grain before to realize the parboiling process. Therefore, the data obtained emphasize the importance for these studies to be continued, as there is a possibility of establishing conditions with lower risk of contamination of the inner grain during parboiling rice.

4. Conclusions

It was concluded that the processing conditions that promoted the lowest AFA B₁ migration into the starchy endosperm were the longer soaking time (6 h) and autoclaving time (30 min); for DON the longer soaking time (6 h) regardless of the autoclaving time; and for ZEA the shorter soaking time (4 h) and autoclaving time (15 min). Such finding occurred at the lowest contamination levels, which are closer to what is naturally found in surveys realized for such kind of product. It was possible to predict the percentage of DON and ZEA migration to the starch endosperm employing the equation obtained in the planning experiments.

References

- Amato, G. W., & Elias, M. C. (2005). *Parboilização do arroz* (p. 160). Porto Alegre, Brasil: Editora Ricardo Lenz Ziede.
- Badiale-Furlong, E. (2005). E. Manejo operacional para micotoxinas em arroz. In M. C. Elias, & I. Lorini (Eds.), *Qualidade de arroz na pós-colheita. Anais do II Simpósio Sul – Brasileiro de qualidade de arroz* (pp. 660). Pelotas, Brasil: Abrapós/UFPel.
- Bello, M. O., Tolaba, M. P., & Suarez, C. (2007). Water absorption and starch gelatinization in whole rice grain during soaking. *LWT – Food Science and Technology*, 40, 313–318.
- Bennett, G. A., & Shotwell, O. L. (1990). Criteria for determining purity of *Fusarium* mycotoxins. *Journal Association of Official Analytical Chemists*, 73(2), 270–275.
- Bhattacharya, K. R., & Ali, S. Z. (1985). Changes in rice during parboiling, and properties of parboiled rice. *Advances in Cereal Science Technology*, VIII, 105–107.
- Bianchini, A. (2003). Estudo comparativo entre manejos de secagem e armazenamento de arroz na incidência de fungos toxigênicos e micotoxinas. Dissertação de mestrado apresentada ao Programa de Pós-graduação em Microbiologia Agrícola e do Ambiente, p. 103.
- Coelho, C. S., Badiale-Furlong, E., & Almeida, T. L. (1999). Migração de micotoxinas durante o processo de parboilização do arroz. *Brazilian Journal Food Technology*, 2(2), 43–50, Campinas – SP.
- Dors, G. C. (2006). Arroz parboilizado: situação micotoxicológica e suas relações com as condições de parboilização. Masters Dissertation presented to Mestrado em Engenharia e Ciência de Alimentos with FURG, Rio Grande, RS, Brazil.
- FAO. (2006). Produção mundial de grãos e arroz. <http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/j3877e/j3877e05.html>. Accessed 17.01.06.
- Furlong, E. B., Soares, L. A. S., Vieira, A. P., & Dadalt, G. (1999). Aflatoxinas, ocratoxina A e zearalenona em alimentos da região sul do Rio Grande do Sul. *Revista Instituto Adolfo Lutz*, 58(2), 105–111.
- Furlong, E. B., & Soares, L. M. V. (1995). Tricothecenes in wheat: a gas chromatographic method for quantitation and confirmation in wheat of deoxynivalenol, nivalenol, diacetoxyscirpenol, T₂ e HT₂ toxins, T₂ triol and T₂ tetraol. *Journal Association Official Analytical Chemistry, Estados Unidos*, 78(2), 386–396.
- Garda, J., Martins, R. M., Farias, R., Bernd, L., Dors, G., & Furlong, E. B. (2005). Alcoholic fermentation effects on malt spiked with tricothecenes. *Food Control*, 16, 423–428.
- Luz, M. L. G. S., & Treptow, R. O. (1994). Caracterização sensorial de arroz parboilizado submetido a diversas condições de secagem complementar. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, 28(1), 56–63.
- Martinez, E. S. M. (1984). Efeito de algumas variáveis operacionais nas características de qualidade do arroz parboilizado convencionalmente e por microondas. Campinas – SP, 1984. Dissertação de Mestrado, Faculdade de Engenharia de Alimentos – Universidade Federal de Campinas.
- Mello, J. P. F., & Macdonald, A. M. C. (1997). Mycotoxins. *Animal Feed Science Technology*, 69, 155–166.
- Nunes, I. L., Magagnin, G., Bertolin, T. E., & Badiale-Furlong, E. (2003). Arroz comercializado na Região Sul do Brasil: Aspectos Micotoxicológicos e Microscópicos. *Ciência e Tecnologia de Alimentos*, 23(2), 105–305, Campinas – SP.
- Patkar, K. L. (1994). Effects of spice oil treatment of rice on moulding and mycotoxin contamination. *Crop Protection*, 13(7), 519–524.
- Ribani, M., Botolli, C. B. G., Collins, C. H., Jardim, I. C. S. F., & Melo, L. F. C. (2004). Validação em métodos cromatográficos e eletroforéticos. *Química Nova*, 27(5), 771–780.
- Singaravadi, K., & Anthoni Raj, S. (1984). Minimizing losses in rice parboiling. *Indian Journal of Agricultural Science*, 54(3), 228–230.
- Soares, L. M. V., & Rodriguez-Amaya, D. B. (1989). Survey of Aflatoxins, Ochratoxin A, Zearalenone, and Sterigmatocystin in some Brazilian foods by using multi-toxin-layer chromatographic method. *Journal Association of Official Analytical Chemists*, 72(1), 22–26.
- Sweeney, M. J., & Dobson, A. D. W. (1998). Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *International Journal of food Microbiology*, 43, 141–158.
- Tanaka, T. (2001). *Analysis of mycotoxins. Textbook for country focused training course: Mycotoxins analysis for federative republic of Brazil* (p. 40). Hyogo: International Centre Japan International Cooperation Agency.