

Parboiled rice: chemical composition and the occurrence of mycotoxins

Arroz parboilizado: composição química e ocorrência de micotoxinas

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Abstract

The objective of this work was to evaluate the incidence of aflatoxin B₁ (AFB₁), deoxynivalenol (DON), ochratoxin A (OTA), and zearalenone (ZEA) in parboiled rice with respect to its chemical composition. Eight lots from five different brands of parboiled rice were collected in four samplings, at different seasons, until the amount of 32 lots. It was observed that: DON was present in 22% of the samples (from 180 to 400 ppb); ZEA in 19% (from 317 to 396 ppb); OTA in 12.5% (from 13 and 26 ppb); and AFB₁ in 9% (from 11 to 74 ppb). The results of the chemical composition were not different from those previously mentioned in the literature concerning parboiled rice. The ash and phenol levels in the contaminated parboiled rice samples suggested that those compounds had a relation to the occurrence of OTA, DON and ZEA mycotoxins.

Keywords: *deoxynivalenol; ochratoxin; physical-chemical characterization; zearalenone.*

Resumo

O objetivo deste trabalho foi avaliar a incidência de aflatoxina B₁ (AFB₁), deoxinivalenol (DON), ocratoxina A (OTA) e zearalenona (ZEA) em arroz parboilizado, relacionando com a composição química. Foram coletados oito lotes de cinco marcas em quatro amostragens, realizadas em diferentes épocas, perfazendo um total de 32 lotes. Foi observado que DON estava presente em 22% das amostras (180-400 ppb), ZEA em 19% (317-396 ppb), OTA em 12,5% (13-26 ppb) e AFA B₁ em 9% (11-74 ppb). Os resultados da composição química não foram diferentes dos citados em literatura sobre arroz parboilizado. Porém, os teores de cinzas e fenóis, nas amostras contaminadas, sugerem que esses compostos estavam relacionados com a ocorrência das micotoxinas OTA, DON e ZEA.

Palavras-chave: *deoxinivalenol; ocratoxina A; caracterização físico-química; zearalenona.*

1 Introduction

Rice is a cereal consumed by great part of the human population throughout the world in many kinds of products, such as white rice, parboiled rice, meal rice and rice bran (FOOD..., 2009).

Toxigenic molds are the main risk among the contaminants of rice products, because they produce several mycotoxins that can cause problems in humans and animals that consume different fractions of rice grain (OSBORNE, 1982; UENO, 1986; PLACINTA; D'MELLO; MACDONALD, 1999; YOSHISAWA, 2001).

It has been mentioned that the occurrence of mycotoxins in cereal and other products is correlated to many environmental conditions in a complex way (OSBORNE, 1982; UENO, 1986; PLACINTA; D'MELLO; MACDONALD, 1999; HUSSEIN; BRASEL, 2001). The chemical composition of cereals is a factor that can influence the development of mold and/or toxin production. Besides, the industrial process can affect the level of several chemical components (EGGUN, 1979; AMATO; ELIAS, 2005).

The fungi *Aspergillus flavus* and *Aspergillus parasiticus* produce a group of mycotoxins called Aflotoxins, whose main toxic effect is carcinogenicity - being the liver the first target.

Ochratoxins are substances of the coumarin type, produced mainly by the species *Aspergillus ochraceus* and *Penicillium verrucosum* and others of the same genus - being the kidney the main target (UENO, 1986; HUSSEIN; BRASEL, 2001; MELLO; MACDONALD, 1997; YOSHISAWA, 2001; MURPHY et al., 2006; RICHARD, 2007).

Tricothecene is a class of mycotoxin whose toxic effect is caused mainly due to the epoxide ring found in the molecule. The principal toxic effects of this family of toxins affect the immunological and blood systems. Zearalenone is produced by *Fusarium graminearum* and it causes hyperestrogenism, miscarriage, stillbirth, false estrus, rectal and vaginal prolapse, infertility, effeminacy of males with the development of breasts and other changes in the reproduction system (SNYDER, 1986; UENO, 1986; PLACINTA; D'MELLO; MACDONALD, 1999; MELLO; MACDONALD, 1997; MURPHY et al., 2006; RICHARD, 2007).

The parboiling process has been used as a way to minimize grain loss during processing and avoid the excessive removal of compounds that are nutritiously important, resulting in a product with better conservation conditions. The increase of nutrients and functional compounds detected in parboiled rice

Recebido para publicação em 2/4/2009

Aceito para publicação em 31/10/2009 (004095)

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justifies the expansion of this product's market along the past years. In spite of these beneficial effects, some changes due to the processing are undesirable, such as the development of unpleasant taste, texture, and color in the opinion of traditional white rice consumers (HORIGANE et al., 2006; LAMBERTS et al., 2008). Another possibility to consider is the migration of contaminants during the rice grain soak (COELHO; BADIALE-FURLONG; ALMEIDA, 1999; BADIALE-FURLONG, 2005; DORS; PINTO; BADIALE-FURLONG, 2009).

The Mycotoxins Laboratory at "Fundação Universidade Federal do Rio Grande" has studied food products contaminated by mycotoxins. The results demonstrated that the parboiled rice and rice bran were the most frequently contaminated products (FURLONG et al., 1999; NUNES et al., 2003). Coelho, Badiale-Furlong and Almeida (1999) and Dors, Pinto and Badiale-Furlong (2009) and they also demonstrated that the parboiling process allowed mycotoxin migration from the outside to the inner layer of the rice grain.

In view of the described evidences, the objective of this work was to evaluate the incidence of aflatoxin B₁ (AFB B₁), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEA) in parboiled rice, with respect to its chemical composition, in products meant for human consumption.

2 Material and methods

2.1 Chemical and solvents

All reagents (analytical grade) were obtained from Merck. Standards aflatoxin B₁ (AFB B₁), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEA) were purchased from Sigma Chemical Company.

The stock solutions were prepared considering the AOAC (ASSOCIATION..., 2000) procedure and the concentration was measured through the spectrophotometric method described by Bennett and Shotwell (1990) in a Varian model Cary 100 spectrophotometer.

2.2 Samples

Four sampling operations were conducted during the year observing the seasonal variation of the available parboiled rice (February, May, August, and November). Five parboiled rice types and three parboiled whole-rice types were selected in each sample, which represents 70-80% of the brands of processed rice available in the Brazilian market.

Samples of 1 kg package of each type were collected at random, representing 1% of the amount available in the sales points. The samples were manually homogenized, reduced by quartering and milled in a paddle mill. The fraction with the 32 mesh (0.5 mm) was separated for analytical procedures. The types were codified as A, B, C, D, E, F, G and H, with the last three representing parboiled whole-rice.

2.3 Mycotoxins determination

The mycotoxins extraction was conducted through the Tanaka (2001) method, where 10 g of sample were weighed, stirred with 100 mL of acetonitril:water (3:1) solution in a vertical stirrer (1.8 g, 25 °C) for 30 minutes. The extracts were filtered and washed twice with 20 mL of hexane. Two grams of sodium chloride was added during the acetonitril:water phase, which was evaporated under reduced pressure at 60 °C. The residue was dissolved in 3 mL of methanol and 27 mL of chloroform in ultra-sound bath. After centrifugation, the supernatant (1700 g) was divided in three 10-mL portions, stored in an amber glass for trial, confirmation and quantification, respectively.

AFB B₁ and OTA mycotoxins were determined through the multi-method described by Soares and Rodriguez-Amaya (1989). The extracts were submitted to screening, confirmation by chemical derivation, and quantification by contrasting the spot fluorescence intensity with standards under UV light.

DON and ZEA mycotoxins were extracted according to Tanaka (2001), purified in a chromatographic column containing 3 g of silica:alumina:celite mixture at 7:5:3 proportion. The extract was eluted with 35 mL of chloroform:methanol solution (3:1) and evaporated (FURLONG; SOARES, 1995). The confirmation and quantification were carried out through gas chromatography, as described by Garda-Bufferon and Badiale-Furlong (2008). The performance of the methods was carried out according to Ribani et al. (2004); limit detection and recovery were evaluated at three levels for each mycotoxin (1.5; 3.0 and 5.0 limit detection).

2.4 Physical-chemical characterization

Moisture, ashes and protein (conversion factor 5.7) were determined by AOAC procedures (ASSOCIATION..., 2000). Fiber determination was conducted through a chemical method based on the determination of the insoluble organic residue, after acid and alkaline digestion, discounting ash scores (BRASIL, 1991).

Amylose was determined through the procedure described by Sowbhagya and Bhattacharya (1979), reaction of amylose with iodine through the spectrophotometric method.

Phenol compounds were cold-extracted with methyl alcohol purified and quantified by spectrophotometry using Folin-Ciocalteu reagent (BADIALE-FURLONG et al., 2003).

2.5 Statistical analysis

The occurrence of mycotoxins was evaluated by the frequency of occurrence in each sampling. The assessment of physical-chemical characteristics was examined with Statistica 6.0 Software through variance analysis (ANOVA) and Tukey's test, comparing the sampling periods and the types of parboiled rice.

3 Results and discussion

The performance of the multi-method for mycotoxins determination is showed at Table 1. The confirmed samples were

quantified comparing the fluorescence with standard solutions of AFB₁, OTA and ZEA, considering that the performance indicators were similar to those mentioned in the literature and the ones recommend by the European Community's rules (FURLONG; SOARES, 1995; SOARES; RODRIGUEZ-AMAYA, 1989). The limits of detection are enough in view of the Brazilian legislation to mycotoxins. DON quantification was carried out through CG using a standard curve, where the peak areas of DON were relative to the internal standard arachidonic acid methodology, according to Garda-Buffon and Badiale-Furlong (2008). The recovery of zearalenone was lower using the TLC method than using the GC method. The observed behavior of zearalenone during the GC method was expected, because this compound may be affected by the derivation reaction. The extract of suspected samples were concentrated and derived before the GC determination in order to confirm the occurrence of zearalenone.

Table 2 presents the results found in the research of parboiled rice samples meant for human consumption. Figure 1 shows the contamination frequency of each mycotoxin during of the sampling intervals.

The results of the 32 samples analyzed are as follows: 22% were contaminated above the limit of detection with DON; 19% with ZEA; 12.5% with OTA and 9% with AFB₁. *Fusarium* toxins were found contaminating 41% of the samples, being ZEA the most frequent in samples of parboiled whole-rice (42%). These results suggest that the handling of the rice crops could be revised, because *Fusarium* genus is frequently associated with field contamination. These results were in agreement with other researches performed in the region, considering white rice, whole rice and rice bran. The parboiling process could be associated to the frequency of contamination found in this work and others, where the *Fusarium* toxins were the most frequent ones (COELHO; BADIALE-FURLONG; ALMEIDA, 1999; FURLONG et al.,

1999; OLIVEIRA et al., 2004; BADIALE-FURLONG, 2005; DORS; PINTO; BADIALE-FURLONG, 2009).

AFB₁ occurrence was observed in the first sampling operation (February) and OTA in the second. This is in accordance with the behavior of the species of molds, which are the producers of AFB₁ and OTA that contaminated the grain during the harvesting (RICHARD, 2007; TURNER; SUBRAHMANYAM; PILETSKY, 2009). Bandara, Vithanege and Bean (1991) studied the occurrence of AFB toxins in parboiled and white rice and also found the parboiled rice more contaminated than the white rice.

Coelho, Badiale-Furlong and Almeida (1999) demonstrated that the parboiling process allowed the migration of mycotoxins from the outside to the inner layer of rice grain. The authors observed that OTA migration was determined by the soaking time and temperature and that the AFB₁ migration was influenced by autoclave temperature as well.

In this work, the chemical composition of the parboiled rice samples was evaluated with the intention to relate it to the mycotoxin contamination. The results of the chemical composition during the sampling operation are presented in Table 3, including the phenolic compounds and amylose levels.

Table 3 presents the mean results of proximal composition determination for the 32 lots collected. The variance analysis showed that there was a significant difference between the samples. Through Tukey's test, it was possible to find that: the moisture rate means differed significantly, mainly in the 3rd and 4th samplings (from 10.7 to 12.8%); the ash rates in samples A, D, G, H and I differed significantly, with values ranging from 0.5 to 3.1% between parboiled rice and parboiled whole-rice; parboiled whole-rice also differed significantly in protein level (from 7.3 to 12.8%) when the sampling period was observed; and fiber rates did not present significant differences during distinct samplings.

The rice moisture of the last sampling was significantly different from the others - being higher in the last two samplings. These differences occurred in August and November, period in which the relative humidity is higher in the region (~80%). Results also suggest that the quality of packaging used by the manufacturers should be assessed concerning its ability to avoid changes in the moisture of the products.

The parboiling process promotes an increase in ash, compared to the white rice (of 30%) (DORS; HEIDTMANN PINTO; BADIALE-FURLONG, 2009), due to the migration of mineral salts during the soaking and boiling steps. The parboiled whole-rice presented higher levels of ashes and proteins when compared to the parboiled rice. This may be explained by the fact that the parboiled whole-rice is not submitted to milling,

Table 1. Performance of thin-layer chromatography multimethod for mycotoxins.

Mycotoxin (method)	Detection limit (ng.g ⁻¹)	Recovery (%)*	Multi-method linearity (ng.g ⁻¹)
AFA B ₁ (TLC)	2.6	86	2.6 to 52
OTA (TLC)	6.5	86	6.5 to 65
DON (TLC)	180	82	180 to 2700
ZEA (TLC)	40	61	40 to 1170
DON (GC)	59	90	150 to 1800
ZEA (GC)	195	75	150 to 1800

*three-level mean: AFA B₁ = (32, 64 and 96 ng.g⁻¹); OTA = (39, 78 and 117 ng.g⁻¹); DON = (480, 960 and 1440 ng.g⁻¹); ZEA = (616, 1232 to 1848 ng.g⁻¹); TLC = thin layer chromatography; GC = gas chromatography.

Table 2. Incidence of AFA B₁, DON, OTA and ZEA in parboiled rice and parboiled whole rice commercialized in south brazilian region.

Kind of rice	Number brands (sampling)	AFA B ₁ (ng.g ⁻¹)	DON (ng.g ⁻¹)	OTA (ng.g ⁻¹)	ZEA (ng.g ⁻¹)
Parboiled rice	5 (4)	11, 53 and 74	200, 320 and 400	26	396
Parboiled whole rice	3 (4)	ND*	180, 180 and 400	13, 26 and 26	317, 317, 396, 396 and 396

*ND - below detection limit.

which removes the surface layers of the grain, particularly germ and bran, where there is a higher concentration of ashes and proteins (DAMIR, 1985; SILVEIRA FILHO, 1986).

The Amylose and phenols rates were significantly different in the four samplings, including in samplings of the same type of parboiled rice. According to Salunkhe, Chavan and Kadam (1999), the amylose content in rice varies between 12.0 and 35.0%, and waxy varieties contain lower amylose rates. In the present research, the mean value was 10%. The results

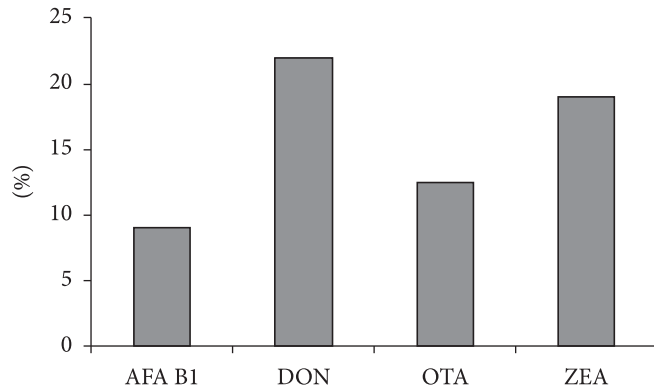


Figure 1. Frequency distribution of each mycotoxin during the sampling operation.

of the effect of the process on the total phenol levels are shown in Table 3. Comparing to the level in white rice founded by Oliveira and Badiale-Furlong (2008), of 40.0 $\mu\text{g}\cdot\text{g}^{-1}$, the average has increased tree times for parboiled rice.

The frequency of mycotoxin contamination did not allow the determination of any significant correlation to chemical composition. However, examining the physical-chemical characteristics of the contaminated samples, some interesting observations can be made. In 90.0% of the samples that presented ash rate above 0.7%, it was possible to confirm that mycotoxins remained in the grain. Therefore, rice polishing is a way to reduce contamination risks, as suggested by Osborne (1982).

Phenol rates in OTA contaminated samples were between 59.0 and 90.0 $\mu\text{g}\cdot\text{g}^{-1}$, the lowest contaminant levels detected in this research. This fact may be explained by the competitive migration, from the outer to the inner layer, between OTA and the phenolic structures, determined by their similar polarity (COELHO; BADIALE-FURLONG; ALMEIDA, 1999; DORS; PINTO; BADIALE-FURLONG, 2009).

Oliveira and Badiale-Furlong (2008) demonstrated that the phenolic extracts from rice were able to inhibit the production of AFB₁ by *Aspergillus flavus* and decrease the development of *Aspergillus oryzae* and *Rhizopus* sp. In relation to this research, another explanation is possible: the rice with the lowest level of

Table 3. Proximal composition for parboiled rice and parboiled whole rice.

Samplings	Physical-chemical characteristics	Samples							
		Parboiled rice				Parboiled whole rice			
		A	B	C	D	E	G	H	I
1 st	Humidity (%)	12.7 ^a	11.9 ^a	12.0 ^a	12.1 ^a	11.7 ^a	11.7 ^a	11.2 ^a	12.3 ^a
	Ash* (%)	1.0 ^a	0.6 ^a	0.6 ^a	0.5 ^a	0.7 ^a	0.9 ^a	0.7 ^a	0.5 ^a
	Protein* (%)	7.8 ^a	7.7 ^a	7.7 ^a	7.7 ^a	7.3 ^a	8.1 ^a	8.3 ^a	8.1 ^a
	Fibers* (%)	0.4 ^a	0.3 ^a	0.3 ^a	0.2 ^a	0.4 ^a	0.6 ^a	0.4 ^a	0.4 ^a
	Amylose (%)	10.6 ^a	12.9 ^a	13.7 ^a	16.7 ^a	11.3 ^a	12.0 ^a	9.1 ^a	13.4 ^a
	Phenol ($\mu\text{g}\cdot\text{g}^{-1}$)	108.1 ^a	136.0 ^a	114.8 ^a	137.9 ^a	102.6 ^a	120.1 ^a	146.2 ^a	126.4 ^a
2 nd	Humidity (%)	12.6 ^a	12.0 ^a	11.7 ^a	12.4 ^a	12.7 ^b	11.7 ^a	11.3 ^a	11.6 ^b
	Ash* (%)	0.5 ^b	0.8 ^a	0.7 ^a	0.7 ^a	0.9 ^a	3.1 ^b	2.0 ^b	1.5 ^b
	Protein* (%)	7.3 ^a	7.7 ^a	7.6 ^a	7.4 ^a	8.3 ^a	10.0 ^a	10.1 ^a	9.6 ^a
	Fibers* (%)	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.4 ^a	0.5 ^a	0.4 ^a
	Amylose (%)	10.9 ^a	8.7 ^b	9.0 ^b	10.3 ^b	10.3 ^a	11.1 ^a	9.9 ^a	7.4 ^b
	Phenol ($\mu\text{g}\cdot\text{g}^{-1}$)	71.4 ^b	63.6 ^b	52.0 ^b	175.5 ^b	84.8 ^b	156.0 ^b	144.0 ^a	88.5 ^b
3 rd	Humidity (%)	12.8 ^a	12.2 ^a	10.9 ^b	12.6 ^b	12.1 ^a	12.4 ^b	12.0 ^b	11.5 ^c
	Ash* (%)	0.6 ^c	0.6 ^a	0.6 ^a	0.6 ^a	0.7 ^a	1.0 ^a	1.0 ^a	1.0 ^a
	Protein* (%)	8.9 ^a	9.0 ^a	8.5 ^a	8.6 ^a	8.8 ^a	11.8 ^b	11.4 ^a	11.7 ^b
	Fibers* (%)	0.3 ^a	0.2 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.5 ^a	0.4 ^a	0.5 ^a
	Amylose (%)	7.4 ^a	7.8 ^c	7.4 ^c	7.7 ^c	6.4 ^b	5.5 ^b	6.1 ^a	6.9 ^c
	Phenol ($\mu\text{g}\cdot\text{g}^{-1}$)	39.1 ^c	46.1 ^c	41.8 ^c	36.0 ^c	40.8 ^c	70.3 ^c	89.8 ^b	79.1 ^c
4 th	Humidity (%)	11.5 ^b	10.7 ^b	11.6 ^a	10.7 ^c	11.0 ^c	11.4 ^a	10.9 ^a	11.2 ^d
	Ash* (%)	0.9 ^a	0.8 ^a	0.8 ^a	0.8 ^b	0.8 ^a	1.5 ^c	1.3 ^c	1.5 ^c
	Protein* (%)	8.4 ^a	8.5 ^a	8.4 ^a	8.6 ^a	8.8 ^a	12.0 ^c	12.1 ^b	12.8 ^c
	Fibers* (%)	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.5 ^a	0.4 ^a	0.4 ^a
	Amylose (%)	6.4 ^b	6.8 ^d	6.1 ^d	6.3 ^d	5.4 ^c	4.1 ^c	4.1 ^b	3.7 ^d
	Phenol ($\mu\text{g}\cdot\text{g}^{-1}$)	59.9 ^d	53.3 ^d	64.3 ^d	139.5 ^a	157.3 ^d	182.8 ^d	194.1 ^c	131.4 ^a

*dry basis. Obs: Different letters for same sample indicate significant differences in distinct samplings and same letters indicate similarity for each parameter assessed.

phenolic compounds may be more susceptible to toxigenic mold species in the field and during the harvesting. If the molds find conditions that allow the production of mycotoxins, they may migrate from the outer to the inner layer of the rice during the parboiling process. The parboiling process allows the migration of the OTA to the amilaceous endosperm, as demonstrated by Coelho, Badiale-Furlong and Almeida (1999), as well as DON, as demonstrated by Dors, Pinto and Badiale-Furlong (2009). There is little information about the effect of phenol compounds level and the development of *Fusarium* genera and the results of this research; no correlation was determined.

The Brazilian legislation is not specific to rice products and only AFB₁, B₂, G₁ and G₂ are considered to other kind of food products (RDC protocol # 274, ANVISA, October 15, 2002). The research showed that the risk of chronic contamination might be considered in parboiled rice consumption. This is especially important if we note that the level of AFB₁ found was higher than the recommended limit to other products (20 ppb) that are less frequent in the dietary routine. The levels of DON and ZEA in cereals, recommended by the European Community Legislation, are of 1,250 and 100 ppb, respectively (FONSECA, 2009).

4 Conclusions

The occurrence of mycotoxins was observed in 22.0% of the samples with deoxynivalenol (from 180 to 400 ppb), 19.0% with zearalenone (from 317 to 396 ppb), 12.5% with ochratoxin A (from 13 and 26 ppb), and 9.0% with aflatoxin B₁ (from 11 to 74 ppb). The average results of the chemical composition were not different from those previously mentioned in the literature concerning parboiled rice. The ash and phenol levels in the contaminated samples suggest that these compounds had a relation to the occurrence of OTA, DON and ZEA mycotoxins in parboiled rice samples. The ash presented the highest value, while the phenolic compounds showed an inverse relation in the contaminated samples.

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