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Post-harvest operations and aflatoxin levels in rice (*Oryza sativa*)

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ABSTRACT

This study evaluated the effect of post-harvest operations on the occurrence of aflatoxins in rice (*Oryza sativa*) samples. Newly harvested rice was subjected to stationary, intermittent, or combined drying, and stored in concrete storehouses during 10 months. The aflatoxins were extracted with methanol: potassium chloride and quantified by HPLC-FL. After ten months of storage, the highest aflatoxin levels were found in rice bran, of which the values were above 25 μg/kg for the sum of the aflatoxins ($B_1 + B_2 + G_1 + G_2$). The rice fractions of the endosperm and husk from the intermittent drying showed the lowest aflatoxin levels during the storage.

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1. Introduction

Rice (*Oryza sativa*) production in Brazil is concentrated in the Rio Grande do Sul state, where cultivation is carried out under an irrigated form, which may favor fungal growth and mycotoxin contamination during cultivation or storage (EMBRAPA, 2010). Aflatoxins (AFLAs) are produced by fungi of the genus *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*, which preferentially develop during grain storage. Drying and storage conditions can play an important role in the growth of these fungi in storage (Pereira et al., 2002). Aflatoxins comprise a set of coumarin-based compounds, known as $AFLAB_1$, $AFLAB_2$, $AFLAG_1$ and $AFLAG_2$ and other derivates (Rocha et al., 2014), the first being classified as the most toxic and carcinogenic mycotoxin to humans by the International Agency for Research on Cancer (IARC, 1993).

Many countries have established maximum limits for aflatoxins in food and feed; thus the contamination should be evaluated in all productive chains (Liu et al., 2006). This fact demands technological alternatives during production, handling, storage, processing, and packaging aimed to prevent commercialization and consumption of contaminated food, or at least, to ensure that aflatoxin levels remain below safe limits (ANVISA, 2011; Hoeltz et al., 2009).

Although mycotoxins in rice are less common and their levels are lower when compared to other cereals such as wheat and maize, there occurrence would be a serious problem considering the daily intake in some regions, where the consumption is at chronic levels, resulting in a long-term health risks, being very difficult to associate with a specific cause (Reiter et al., 2010). During the rice post-harvest operations, the combination of several factors such as humidity, temperature, storage time, physical conditions of the grain, O_2 and CO_2 levels, inoculum level, prevalence of toxigenic strains, and presence of impurities may favor fungal contamination, mainly through the genus *Aspergillus* (Tanaka et al., 2007). The drying and storage may contribute to aflatoxins contamination in rice grain and their products. Thus, the effect of three drying techniques commonly employed in the food industry on aflatoxin contamination has been studied (Bullerman and Bianchini, 2007; Hoeltz et al., 2009).

In this study, the effect of stationary, intermittent, and combined drying operations on the occurrence of aflatoxins in rice and its derivatives, during storage in concrete storehouses, was investigated.

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2. Material and methods

2.1. Materials

Acetonitrile and methanol (Baker) with a purity degree more than 99.9% were used as the mobile phase in the chromatographic step. Both reagents were filtered through a Millipore membrane filter (Supelco, 58067) with pores of 0.45 μm. All components of the mobile phase were previously degassed using an ultrasonic bath 1600 W (Ultrasonic Cleaner Unique, Indaiatuba, São Paulo). Ultrapure water was obtained by a Direct-Q System UV3® resistivity of 18.2 MΩ cm^{-1}, (Millipore, Bedford, USA). Glacial acetic acid (>99% purity) was purchased from Merck (Darmstadt, Germany). Celite (Nuclear), ammonium sulfate, chloroform (Synth), and potassium chloride (Vetec) were used in the experiments, all with a 99% purity grade.

A high performance liquid chromatography coupled with a fluorescence detector (HPLC-FL) (Shimadzu, Kyoto, Japan) was used for determination of the mycotoxins. It had a system of pumps (model LC-AT), an oven, a mobile phase degassing instrument (model DGU), a controller (CBM-20A), a manual injector with 20 μL sample loop (7725i), and a fluorescence detector (model FL – 10AXL). A Nucleosil C18 column (10 cm × 4.6 mm and 3 μm particle size) was used for the stationary phase (Bellefonte, PA, USA). The equipment control and data processing were performed using LC Solution software.

2.2. Sampling

Long grain rice produced in an irrigated cropping system and harvested with a moisture content around 24% was used in the study. After harvesting (harvest 2012–2013), the grains were taken to the pilot plant at the experimental station of the IRGA (Riograndense Rice Institute), located in Cachoeirinha-RS, Brazil. The rice grains were discharged in a hopper, passed through the cleaning step, and subjected to stationary, intermittent, or combined drying. After reaching 12.5% moisture, the rice grains were stored for 10 months.

In stationary drying, a reinforced concrete silo-dryer with a capacity up to 4.4 tons was used, in which the psychrometric air conditions were modified with the burning of liquefied petroleum gas (LPG). This followed the principle of equilibrium moisture content for rice grains, set at 12.5% moisture content. The intermittent drying was performed in a pilot dryer (Ferrabil SA1000-LAB, Frederico Westphalen, Brasil) with a capacity up to 1 ton, using an air drying temperature of 45 °C until the grains reached a moisture content between 12 and 13%. A combination of intermittent and stationary drying was carried out, using intermittent drying to reduce the initial moisture from 24 to 16%. After that, the grains were kept under stationary drying to reduce up to 12.5% moisture.

The samples were collected from the upper, middle, and lower layers of each storehouse, in May, August, and November 2013, and February 2014; and these were subsequently processed by the traditional milling process of polishing white rice (Zaccaria, Limeira, Brasil). The endosperm, bran and husk fractions were separated, stored in polyethylene bags, and sent to the Laboratory of Mycotoxins at FURG. The fraction samples were homogenized and ground in a knife mill to less than a 32 mesh particle size.

2.3. Chromatographic conditions

For a determination of aflatoxins, an HPLC-FL chromatograph was used with ultrapure water acidified with 1% glacial acetic acid, acetonitrile, and methanol in the ratio 60:8:32 (v/v/v) as the mobile phase. The chromatographic parameters were a flow rate for the mobile phase 0.4 mL/min, and a column temperature of 45° C, resulting in a retention time of 12.5; 15.0; 18.5; and 22.4 min for $AFLAG_2$, $AFLAG_1$, $AFLAB_2$ and $AFLAB_1$, respectively, at 25 min as a total running time. For the quantitation of the aflatoxins, the dry residue resulting from the extraction was dissolved with 0.2 mL of the mobile phase and manually injected on the HPLC-FL (Hackbart et al., 2014).

2.4. Extraction of aflatoxins (B_1, B_2, G_1 and G_2)

For the extraction procedure, a 10 g sample was homogenized in a blender with 60 mL of a mixture of methanol: 4% potassium chloride (9:1 v/v) and filtered. After filtration, 30 mL of the filtrate were collected, to which 30 mL of 30% ammonium sulfate and 1 cm^3 of diatomaceous earth were added. The mixture was stirred manually and allowed to stand for 5 min, and then filtered again. In sequence, 30 mL of filtrate were collected into a dropping funnel with 30 mL of distilled water. Partition was performed three times with 10 mL of chloroform and then homogenized. Each 10 mL of the chloroformic extract fraction was placed in amber vials and dried in a water bath at 80 °C for 25 min under nitrogen (Soares and Rodriguez-Amaya, 1989; modified) for a subsequent resuspension in the mobile phase for quantitation by HPLC-FL.

2.5. Method validation

The method was validated in according to the National Health Surveillance Agency (ANVISA, 2003), the National Institute of Metrology, and the Standardization and Industrial Quality (INMETRO, 2003).

To construct the curves, standard solutions were prepared in triplicate at concentrations of 0.3; 2.5; 5.0; 8.0; 10.5 ng/mL for $AFLAB_2$ and $AFLAG_2$, and 1.0; 6.0; 12.0; 16.0; and 20.0 ng/mL for $AFLAB_1$ and $AFLAG_1$. Linear regression equations for the quantification of the aflatoxins were obtained with the aid of LC Solution software.

The limits of detection (LOD) and limits of quantification (LOQ) were determined by injecting standard solutions of each aflatoxin in descending order of concentration. The LOD was considered as the concentration corresponding to the peak that represented 3 times the signal-to-noise ratio of the base line of the equipment. The LOQ was estimated from the concentration that would generate the peak compared with 10 times the signal/noise.

Accuracy was determined by the percent recovery of the spiked analytes. The endosperm, bran, and husk samples were fortified with three levels of aflatoxins (2.5; 5.0; and 10.0 ng/g) AFLAs (B_1, B_2, G_1 and G_2), and then left to stand for 24 h for complete evaporation of the solvent prior to the extraction.

2.6. Sample characterization

The moisture content of the samples was determined for each grain fraction by the gravimetric method according to AOAC (2000). The temperature of the stored grain mass was monitored by five monthly measurements on the upper, middle, and lower layers of the storehouse. The temperature values of the grain mass were recorded and estimated as monthly averages.

2.7. Statistical analysis

The software Statistica 7.0 and OriginPro 8 were used for data analyses. The differences between the means were verified by ANOVA, and the Tukey's test was used as a post hoc test. The relationship between the contamination by aflatoxins and the

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