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In-house method validation, estimating measurement uncertainty and the occurrence of fumonisin B_1 in samples of Brazilian commercial rice

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ABSTRACT

Fumonisin B_1 was investigated in samples of rice intended for human consumption, including polished parboiled rice, whole grain rice and whole grain parboiled rice. Until the present, no studies on the occurrence of fumonisin B_1 have been performed on these types of rice that are commercially available in the south-eastern region of Brazil. A careful intralaboratory validation was carried out to demonstrate the fitness-of-purpose of the applied method for determining fumonisin B_1 in the three studied rice types. The performance criteria – selectivity, reliable limits of detection (50 μg kg^{-1}) and quantification (100 μg kg^{-1}), linearity (range 100–2500 μg kg^{-1}), precision (RSD values ≤ 17.0%) and recovery (71.7–112.0 %) were evaluated, and the expanded measurement uncertainty was estimated by using the data obtained from precision and recovery experiments. Matrix-matched calibration standards were employed to quantify the mycotoxin levels in the rice samples, in which the residual normality, homoscedasticity and independence were confirmed. In addition, the measurement uncertainty values are consistent with the maximum acceptable uncertainty established by European Union regulation for analytical methods for controlling mycotoxins in foodstuffs. Among the thirty-one commercial samples of rice analysed in the present study, five samples presented detectable levels of the mycotoxin, and these levels ranged from 64.8 to 163.0 μg kg^{-1}.

1. Introduction

Fumonisin B_1, a secondary metabolite of the *Fusarium* and *Alternaria* fungal genera, has been associated with the incidence of human oesophageal cancer in areas of the Transkei region of South Africa (Shephard et al., 2007) and with neural tube defects along the Texas–Mexico border (Missmer et al., 2006). This mycotoxin is classified under group 2B as a possible human carcinogen (IARC, 2011). In animals, fumonisin B_1 has been shown to cause porcine pulmonary oedema and equine leukoencephalomalacia, as well as species-specific targeted tissue damage, such as hepatotoxicity in rodents and nephrotoxicity in rabbits and sheep (Smith, 2007).

The highest levels and incidence of fumonisin B_1 have been reported in corn and corn-based foods (Soriano & Dragacci, 2004); however, this mycotoxin has also been associated with other cereals, such as rice (Tanaka, Sago, Zheng, Nakagawa, & Kushiro, 2007). Because of the toxicity of this mycotoxin and its processing stability (Bullerman & Bianchini, 2007), the occurrence of fumonisin B_1 in rice implies a potential risk to populations in regions of the world in which rice is a dietary staple. Specifically, in Brazil, the average daily consumption of rice is approximately 160 g per person (IBGE, 2011), and rice production was estimated to be 12,151.5 thousand tonnes in 2015 (CONAB, 2015).

Recently, we detected fumonisin B_1 in one sample of rice that was purchased at a local retail store in the south-eastern region of Brazil; however, that study was restricted to polished rice samples (Petrarca, Rodrigues, Rossi, & Sylos, 2014). Until the present, no studies on the occurrence of fumonisin B_1 have been performed on other types of rice intended for human consumption such as the polished parboiled rice, whole grain rice and whole grain parboiled rice that are commercially available in the south-eastern region of Brazil. Parboiled rice is the product that is obtained from the parboiling process, in which unpeeled rice is submerged in drinking water at a temperature above 58 °C, followed by partial or full gelatinization of its starch and then drying. Whole grain rice is the product that results when only the husk of the grain has been removed, and it can also be subjected to a parboiling process. The maximum moisture content permitted in these products is 14% (MAPA, 2009).

Several analytical methods have been explored to investigate fumonisin B_1 levels in rice and its products (Khayoon et al., 2010;

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Kim, Scott, Lau, & Lewis, 2002; Lombaert et al., 2003; Park, Choi, Hwang, & Kim, 2005; Scott, Lawrence, & Lombaert, 1999; Seo et al., 2009). Many of these methods include laborious sample preparation steps as well as considerable amounts of sample and extraction solvent, and these methods require solid phase extraction (SPE) cartridges or immunoaffinity columns, which makes them costly. However, simple and low residue generation methods, which are based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure, have been successfully applied to determine fumonisin B_1 in barley, corn, oats and wheat (Vaclavik, Zachariasova, Hrbek, & Hajslova, 2010; Yang & Wu, 2012; Zachariasova et al., 2010), rice (Becker-Algeri, Heidtmann-Bemvenuti, Hackbart, & Badiale-Furlong, 2013; Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2014; Petrarca et al., 2014), cereal flours (Desmarchelier et al., 2010), and other food matrices and feed (Mol et al., 2008; Trebstein, Lauber, & Humpf, 2009). High performance liquid chromatography (HPLC), with fluorescence detection or coupled to mass spectrometry (MS), have been extensively employed for identifying and quantifying the mycotoxin in food matrices (Arranz, Baeyens, Van der Weken, De Saeger, & Van Peteghem, 2004; Köppen et al., 2010; Maragos & Busman, 2010).

Considering that the same sample preparation method is employed to investigate fumonisin B_1 in the rice samples that were selected for the present study, the co-extractives and their amounts may vary between the analysed rice types, and consequently may affect the method performance characteristics and interfere with the generation of quantitatively accurate results. Thus, estimating the measurement uncertainty for each matrix studied is important to ensure the quality of the analytical results and to demonstrate the suitability of the analytical method (Boleda, Galceran, & Ventura, 2013). Different procedures have been applied to calculate the measurement uncertainty associated with analyses of antibiotics (Borecka et al., 2013), chlorides and fatty acids (Quintela, Báguena, Gotor, Blanco, & Broto, 2012), ochratoxin A (Fernandes, Barros, & Câmara, 2013), pharmaceuticals (Boleda et al., 2013), pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Aslan-Sungur, Gaga, & Yenisoy-Karakas, 2014; Kmellár et al., 2008; Planas, Puig, Rivera, & Caixach, 2006) in environmental and food matrices. However, some of these models require certified reference material, which is not always available. Therefore, the procedure proposed by Boleda et al. (2013) based on single-laboratory validation was selected to calculate the expanded measurement uncertainty in this study. Thus, the uncertainty was estimated by using the data obtained from precision and recovery experiments, i.e., within-laboratory repeatability and reproducibility standard deviations.

In the present study, fumonisin B_1 was analysed in samples of commercial rice that were available in the south-eastern region of Brazil; these types of rice have not been studied until the present, and they include polished parboiled rice, whole grain rice and whole grain parboiled rice. We evaluated the performance criteria, namely, the selectivity, limits of detection and quantification, linearity, matrix effects, extraction efficiency and precision of a simple and cost-effective method of sample preparation based on the QuEChERS procedure for each matrix. In-house validation data were used to estimate the expanded measurement uncertainty for the mass fraction of the mycotoxin detected in the rice samples.

2. Material and methods

2.1. Standard and chemicals

A fumonisin B_1 standard (98% purity) was obtained commercially from Sigma–Aldrich, Inc. (St. Louis, MO, USA). A fumonisin B_1 stock solution was made in acetonitrile: water (1:1, v/v) at 1000 μg ml^{-1}, and standard working solutions were prepared at 20 μg ml^{-1}. All solutions were kept in amber flasks at −18 °C.

Ortho-phthaldialdehyde (OPA) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA), sodium tetraborate was purchased from LabSynth (Diadema, SP, Brazil) and 2-mercaptoethanol was purchased from Vetec (Rio de Janeiro, RJ, Brazil). To prepare the derivatisation reagent, OPA (40 mg) was dissolved in 1 ml of methanol, and then 5 ml of 0.1 M sodium tetraborate solution (pH 9.0 ± 0.1) and 50 μl of 2-mercaptoethanol were added (Trucksess, 2005). This solution was prepared weekly and stored at room temperature in an amber flask.

HPLC-grade acetonitrile, glacial acetic acid and methanol were obtained from J.T. Baker, Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). The water used in the chromatographic analyses was prepared by using a Milli-Q system (Millipore, Milford, MA, USA). Anhydrous sodium sulphate was purchased from Qhemis (Indaiatuba, SP, Brazil), diatomaceous earth (Celite) was purchased from Almeria S.A. (Guadalajara, Mexico), phosphoric acid was purchased from LabSynth (Diadema, SP, Brazil), and monosodium phosphate 1-hydrate and sodium chloride were bought from Merck S.A. (Rio de Janeiro, RJ, Brazil).

2.2. Sampling

Samples of rice intended for human consumption were purchased in the commercially available size of 1 kg from 5 supermarkets, 1 grain store and 1 natural foods store in the city of Araraquara, SP, in the south-eastern region of Brazil, between September and October of 2011. A total of 31 different brands of three rice types were randomly collected, including 10 brands of polished parboiled rice, 6 brands of whole grain rice and 15 brands of whole grain parboiled rice. These cereals were ground in a food processor (Arno, SP, Brazil) to obtain homogenous samples, sieved through a 0.84 mm mesh and stored in polypropylene flasks until the time of analysis. The analyses were performed on the same day the cereal was ground.

2.3. Determination of fumonisin B_1

The sample preparation method applied in this study was optimized to determine fumonisin B_1 in polished rice, and it is described elsewhere (Petrarca et al., 2014). In this method, 10 g of ground sample, 20 mL of 50% acetonitrile aqueous solution and 0.2 mL of glacial acetic acid were added to a 50 mL polypropylene centrifuge tube (Nalgene, Thermo Scientific, Rochester, NY, USA) and vortexed for 1 min. Then, 2.5 g of anhydrous sodium sulphate and 0.5 g of sodium chloride were added to mixture and vortexed again for 1 min and centrifuged at 7500 rpm for 2 min. After the centrifugation step, 5 mL of supernatant, 0.3 g of anhydrous sodium sulphate and 0.1 g of diatomaceous earth were added to a 50 mL polypropylene centrifuge tube and this mixture was vortexed for 30 s, and then centrifuged at 7500 rpm for 2 min.

The final extract was filtered through a 0.22 μm syringe filter, and then the precolumn derivatisation reaction was performed. A 225 μl aliquot of the derivatisation reagent was mixed with 25 μl of filtered extract for 30 s at room temperature and protected from light, and then 10 μl of this mixture was injected into the HPLC system within 2 min of adding the derivatisation reagent to the extract (Trucksess, 2005).

2.4. Chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC-10AT VP quaternary pump, a SIL-10A automatic injector and an RF-10A XL fluorescence detector (FLD) that was set at excitation