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Occurrence of aflatoxins in cashew nuts produced in northeastern brazil

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ABSTRACT

Samples of cashew nuts produced in northeastern Brazil were evaluated using monitoring system by Enzyme-linked Immunosorbent Assay – ELISA for detection and quantification of the level of total aflatoxins (B_1, B_2, G_1 and G_2). The data show that a total of 70 samples analyzed, only two (2.8%) showed contamination to levels above EU MRL (4 $\mu g\,kg^{-1}$). Only one sample analyzed showed level of total aflatoxins above the MRL (20 $\mu g\,kg^{-1}$) established by Brazilian Regulations. According to the results the incidence of aflatoxin contamination in Brazilian cashew nuts has decreased during the period 2010–2012. Programs for monitoring the level of mycotoxins in foods should be implemented continuously to ensure food security to the population.

1. Introduction

Mycotoxins are secondary metabolites produced by certain filamentous fungi (Luttfullah & Hussain, 2011; Ritter, Hoeltz, & Noll, 2011). Aflatoxins are considered one of the main types of mycotoxins produced by fungi of the genus *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus* species. Are known, currently 20 compounds designated by the term aflatoxin, however, the main types of sanitary interest are identified as B_1, B_2, G_1 and G_2 (EMAN, 2013; Inan, Pala, & Doymaz, 2007). These compounds are characterized by high toxicity that they present. Aflatoxin B_1 (AFB_1) is considered the most toxic, followed by G_1, B_2 and G_2 (Bennett & Klich, 2003; Oliveira & Germano, 1997).

Aflatoxins are genotoxic and carcinogenic compounds, and already in 1998 the European Union (EU) introduced maximum levels for certain food commodities, based on the principle as low as reasonably achievable (ALARA) (EFSA, 2013). In Brazil, the incidence and frequency of nut contamination by aflatoxin had been monitored by the *Ministério da Agricultura, Pecuária e Abastecimento – MAPA* (Freitas-Silva & Venâncio, 2011).

Brazil almonds and nuts are very susceptible to attack by fungi, due to inadequate production, transport and storage, favoring the production of mycotoxins. Some cultures also become contaminated in the field before harvest, while the other become contaminated after harvest when stored under conditions of high humidity and temperature. In the case of almonds cashew, for example, one of the most common routes of infection in Brazil is confirmed by the invasion of flowers (Costa, Freire, Vieira, Andrade, & Mendes, 2009; EMBRAPA, 2007).

The nuts are considered the major export products in Brazil. The Brazilian Northeast occupies a prominent position in the production of cashew nuts: the states of Ceará, Rio Grande do Norte and Piaui recorded 99.9% of total sales of cashew nuts to the exterior, in the year 2012 (IPECE, 2013).

However, the product has suffered restrictions in these markets due to aflatoxins contamination above the permitted level. This framework brings a great concern with respect to food safety and consumer health in relation to contamination of the products consumed in Brazil. They may constitute a major risk to the health of the population that consumes the nut and its products.

In Brazil the occurrence of aflatoxins has been observed frequently, and at high levels, especially in the state of São Paulo, in food used for human and animal consumption, as corn, peanuts and derivatives. The contamination control of nuts and derivatives takes public health relevance (Arrus, Blank, Clear, Holley, &

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Abramson, 2005). Research and monitoring programs have been developed in several countries in order to investigate the levels of aflatoxins in foods for human consumption (Abdulkadar, Al-Ali, & Al-Jedah, 2000; Caldas, Silva, & Oliveira, 2002; Georgiadou, Dimou, & Yanniotis, 2012; Leong, Ismail, Latif, & Ahmad, 2010; Prado et al., 2000; Prandini et al., 2009).

Aflatoxins have been investigated by different physical chemical methods such as thin layer chromatography-TLC, High Performance Liquid Chromatography-HPLC and LC-Mass spectrometry (Bankole, Adeadenus, Lawal, & Adesanya, 2010; Dini et al., 2013; Ezekiel et al., 2013; Pacheco, Lucas, Parente, & Pacheco, 2010; Set & Erkmen, 2010). However, the need for rapid decisions use has led to the use of screening methods such as immunoassays. *Enzyme-linked Immunosorbent Assay* – ELISA have been widely used in research aflatoxins, due to their sensitivity, specificity, quickness, simplicity and low cost, using antibodies specific for detecting mycotoxins in foods (Aydin, Erkan, Baokaya, & Ciftcioglu, 2007; Liu, Hsu, Lu, & Yu, 2013).

Oliveira et al. (2010) evaluated the contamination of total aflatoxins and zearalenone in maize varieties in the state of Parana – Brazil. The method used for quantification of aflatoxins was enzyme immunoassay ELISA. Two hundred and twenty-three samples of dairy products marketed in Ankara, Turkey were monitored using ELISA, during 2002–2003, by Aycicek, Aksoy, and Saygi (2005). The techniques of enzyme immunoassay (ELISA) and thin layer chromatography (TLC) was used by Amaral, Nascimento, Sekiyama, Janeiro, and Machinski (2006), in determining total aflatoxin in food products from corn and Marialva marketed in Maringa, Parana, Brazil. Leong et al. (2010), analyzed products marketed them Penang, Malaysia through enzyme immunoassay (ELISA) for rapid detection and reverse phase chromatography of High Performance Liquid (HPLC) for the quantification and confirmation.

However, few studies cite the incidence of aflatoxin contamination in cashew nuts produced in Brazil. Furthermore, due to continental dimensions of the country, there is the possibility of different levels of mycotoxins in foods produced is detected in several regions. The main objective of this study was to investigate the occurrence and distribution of aflatoxin contamination in cashew nuts produced in the state of Ceara, northeastern Brazil to assess whether levels of these aflatoxins are affecting the health of the population.

2. Materials and methods

2.1. Chemicals and reagents

All reagents and solvents used in the analyses were of analytical grade (Vetec, Brazil). Ultrapure water (Millipore, Brazil) was used in the preparation of solutions and samples. Kits for analyzes of aflatoxins B_1, B_2, G_1 and G_2 by immunoassay, were purchased from Beacon Analytical Systems Inc.

2.2. Sampling

Samples of cashew nuts produced in the state of Ceara, Brazil were collected during 2010–2012. Different brands of roasted and salted cashew nuts sold in the region were analyzed for determination of the total concentration of aflatoxins (B_1, B_2, G_1 and G_2). Packaging of 500 g were obtained from different lots and were stored at room temperature until the time of analysis.

2.3. Aflatoxin determination by immunoassay

Total Aflatoxin analysis was performed using a commercial competitive *Enzyme-linked Immunosorbent Assay* – ELISA kit

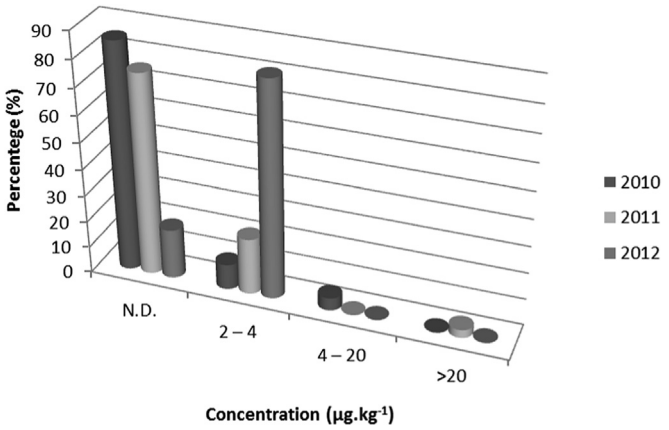

Fig. 1. Incidence of total aflatoxins (%) in samples of Brazilian cashews nuts monitored during 2010–2012.

(Beacon Analytical Systems Inc.) that is specific for the quantification of aflatoxin in nuts, grain and grain products. The kit has been certified as a Performance Tested Method by the AOAC (License 081003). The samples were milled and homogenized in a knife mill (Tecnal-TE 631) and passed on 10 mesh sieves. The extraction was performed using 50 g of the ground sample and adding methanol: water (80:20 v/v) and 5 g NaCl. The extract was then filtered and the extract tested in the immunoassay.

Aliquots of 100 μL of the sample extracts or the standard (0, 2, 5, 20 50 $\mu g\,L^{-1}$) were added to 200 μL of aflatoxin–HPR enzyme conjugate in the mixing plate wells. After careful mixing, 100 μL of the contents of each well were transferred to the test wells coated with aflatoxin antibodies. After 10 min incubation at room temperature, the wells were washed (four times) with deionized water to remove any unbound toxin or enzyme-labeled toxin. After this, 100 μL of substrate were added to each well and any bound enzyme–toxin conjugate caused the conversion to a blue color. After 5 min incubation at room temperature (±28 °C) , was added 100 μL of stop solution (1 $mol\,L^{-1}$ HCl) in each well. Quantitative measurements were made based on a standard curve constructed by plotting the absorbance (*X* axis) versus the log of concentration (*Y* axis) of the standard. The measurements were done using a plate reader Stat Fax 303 Plus (Awareness technology, Inc. – USA) and a 450 nm filter. The color of unknown samples was compared to the color of the standards and the total aflatoxin concentration of the samples was determined. All analysis were done in duplicate.

3. Results and discussion

Immunological methods have been widely used for the determination of aflatoxins, due to their sensitivity, specificity, quickness, simplicity and low cost (Aydin et al., 2007; Liu et al., 2013). The ELISA test detects and amplifies the antigen–antibody reaction by covalent bonds between the enzyme–antibody or enzyme–analyte whose presence is subsequently determined by adding the enzyme substrate. The levels of total aflatoxins ($\mu g\,kg^{-1}$) in samples of cashew nuts produced in Ceará, Brazil, were determined by ELISA using a commercial and certified kit.

The imunoassays (ELISA) were done using a commercial kit specific for the quantification of aflatoxin in nuts, grain and grain products. The kit has been certified as a Performance Tested Method by the AOAC (License 081003) presenting good recovery, a limit of detection (LOD) of 0.6 $\mu g\,L^{-1}$ and a limit of quantification (LOQ) of 1.8 0.6 $\mu g\,L^{-1}$. The quantification was done based on a calibration curve obtained with known concentrations of a standard aflatoxin