Talanta 152 (2016) 423–431

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rice husk as an adsorbent: A new analytical approach to determine aflatoxins in milk

Priscila Tessmer Scaglioni *, Eliana Badiale-Furlong

Post Graduate Program in Engineering and Science of Food, School of Chemistry and Food, Laboratory of Food Science and Mycotoxins, Federal University of Rio Grande (FURG), Itália Avenue, km 8, Bairro Carreiros, Rio Grande CEP 96203-900, RS, Brazil

ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form
16 February 2016
Accepted 18 February 2016
Available online 20 February 2016

Keywords:
Adsorbent
Aflatoxin B_1
Aflatoxin M_1
Rice husk

ABSTRACT

Aflatoxins determinations are usually expensive and employ environmentally unfriendly procedures, thus, the search for new materials and technologies, that are both ecologically safe, inexpensive and able to fulfill its role with little pre-processing is growing. One interesting approach is employing by-products as adsorbents during the extraction step of aflatoxins especially in products such as milk and dairy that are so important in basic dietary. Thus, a method to use rice husk, an agroindustry residue that is a promising material to adsorb aflatoxins to enable further analysis steps, is proposed by applying a Plackett–Burman design followed by 2^2 central composite rotational design. Rice husks were prepared by washing the husk with a solvents sequence. The washed particles were analysed by scanning electron microscopy, characterized by an elemental analyser and analysed for the presence of pesticides and mycotoxins. The rice husks contained 41% carbon, 4.3% hydrogen and 0.2% nitrogen, without mycotoxins and pesticides. The adsorptions were conducted using 0.5 g of rice husk, with 42 mesh, and 10 mL of milk contaminated with several know levels of aflatoxins M_1 and B_1. The solution was filtrated trough the adsorbent layer using a pressure of 10 in. Hg. The adsorbed mycotoxins were removed with 6 mL of methanol:chloroform (80:20). This condition achieved recovery of around 100% for both mycotoxins, with the average quantity of mycotoxin adsorbed equal 0.0150 $\mu g\ g^{-1}$ of afla B_1 and 0.0174 $\mu g\ g^{-1}$ of afla M_1.

1. Introduction

The most frequent mycotoxins that contaminates foods and feeds are aflatoxins B_1 (afla B_1), B_2 (afla B_2), G_1 (afla G_1) and G_2 (afla G_2) [1]. These are toxic compounds produced by the fungi *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius.* They can cause severe issues and need to be eliminated or diminished in foods and feeds to protect the consumer's health.

Aflatoxins M_1 (afla M_1) and M_2 (afla M_2) are hydroxylated metabolites of afla B_1 and afla B_2, respectively, that can be found in dairy products produced from milk of animals fed with contaminated feed [2]. These compounds are resistant to high temperatures, some of them decompose only above 300 °C [3,4]. Milk processing techniques do not involve drastic enough conditions for aflatoxins to degrate. Thus, efficient detection methods to ensure safe levels of them in milk and its derivatives are an important step towards food safety, beside process conditions that reduce the availability of these mycotoxins.

It is important to mention that the methods for the aflatoxins determination involves high solvents volumes to guarantee extraction, many steps are necessary to eliminate interfering, preconcentration of the analyte to reach detectable levels, among other sample preparation steps. Through these stages a significant amount of toxic wastes is produce. This increases the technique overall price, both due to the analytical compound starting price but also due to necessary subsequent waste treatment. One promising procedure to diminish these problems is applying sorbents to retain the contaminant to further elution, for example by the method of matrix solid phase dispersion (MSPD) followed by chromatographic techniques to determinate the contaminant. However this procedure presents some limitations like sorbent adsorption and desorption ability, and it's high cost.

Procedures to adsorb contaminants to reduce their level in foods or as analytical tools must be versatile and accessible. This is especially true in the analytical determination of trace compounds where interfering compound concentrations must be decreased [5]. However, when applied in industrial operations, the adsorbent materials can hold contaminants and also nutrients. Generating an

* Corresponding author.
E-mail addresses: priscilascaglioni@gmail.com (P.T. Scaglioni), dqmebf@furg.br (E. Badiale-Furlong).

http://dx.doi.org/10.1016/j.talanta.2016.02.042
0039-9140/

increase in operational cost and an inferior final product [6]. Nevertheless, in analytical determinations, these characteristics can be dealt as an advantageous to the technique.

Sorbents from plant origin are mainly constituted of macromolecules such as humic substances, lignin, cellulose, hemicellulose, and proteins. These structures usually possess chemical groups, such as carbonyl, carboxyl, amine, and hydroxyl groups, possessing adsorptive properties. They are capable of adsorbing contaminants by ion exchange or other interactions processes [7].

The sorbent choice is an important step to consider within the analytical procedure. A high selectivity, holding capacity, and stability in several use cycles are the most important characteristics. In order to diminish the analysis overall cost, the sorbent should also be available in large quantities at low cost and be environmentally friendly [8,9].

Rice husks, a residue of the rice milling process, accounts for approximately 23% of the total weight of rice, and has low nutritional value and high ash content, where silica is the principal component. This waste, very abundant in grain-producing regions [5], may be applied in the adsorption of different compounds, such as heavy metals in wastewater (Ni^{2+}, Cu^{2+} and Zn^{2+} ions) [7] and Remazol 5R red textile dye [5]. However, there is currently no information in the literature about aflatoxins adsorption by rice husks, neither for analytical determination nor to reduce the presence of this mycotoxin in industrial products.

In any situation, it is critical to determine the optimal operation variables for adsorbing the target compound. This is particularly important when the target is to optimize analytical procedures. In this sort of activity, the analyte must be quantitatively removed from the adsorbent before the determination of its concentration. Considering this, it was studied, for the first time, the optimal operational conditions, in an apparatus Manifold (Phenomenex), to apply the rice husk as an adsorbent for afla B_1 and afla M_1 determination.

2. Materials and methods

2.1. Material

Afla B_1 and afla M_1 standards were purchased from Sigma Chemical Company. Working solutions (1 $\mu g\,mL^{-1}$) of both aflatoxins were prepared by diluting them in benzene:acetonitrile (98:2).

Raw milk samples (with mean composition 16.2% ($\pm$0.07) total solids; 5.5% ($\pm$0.02) lactose; 5.4% ($\pm$0.02) protein; 5.4% ($\pm$0.02) fat; and 10.8% ($\pm$0.05) non-fat dry extract) were purchased from local producers in the city of Rio Grande-RS, Brazil and were stored under refrigeration until analysis, which was performed within 24 h after their collection. The samples were contaminated with afla M_1 on average 0.84 $\mu g\,L^{-1}$ ($\pm$0.08), and no natural contamination with afla B_1 was observed.

The milk samples used for the adsorption assay were artificially contaminated with both studied aflatoxins, resulting in a total of 0.1 $\mu g\,mL^{-1}$ of each mycotoxin. Milk samples without natural contamination were used, in order to understand the adsorption ability of the rice rusk.

The rice husks (with mean composition: 8.1% ($\pm$0.1) moisture; 15.3% ($\pm$0.1) ash; 3.0% ($\pm$0.2) protein; and 0.9% ($\pm$0.2) fat) used in the present study were obtained from a rice milling industry located in Rio Grande do Sul state, Brazil.

2.2. Methods

2.2.1. Preparation and characterization of rice husks

The rice husks were treated before use as an adsorbent. The husks were washed three times with hexane at a ratio of 1:4 husk: solvent with stirring at 1600 rpm for 30 min. The resulting residue underwent the same treatment procedure using methanol and was dried in an oven at 90 °C [10].

Subsequently, the presence of the most common contaminants present in external fraction of grains was evaluated by a procedure adapted from the literature: the aflatoxins were extracted with methanol and 4% potassium chloride. The extract fraction was then clarified with 30% ammonium sulfate and celite, followed by the addition of water. The mixture was submitted to three partition with chloroform which was evaporated until dry under a nitrogen stream [11]. The mycotoxin concentrations were determined by high performance liquid chromatography with fluorescence detection (HPLC-FL).

The pesticides clomazone, epoxyconazol, pirimiphos-methyl and tebuconazole were extracted by QuEChERS method [12,13] and identified and quantified by liquid chromatography with electrospray source ionization and tandem mass spectrometry (LC-ESI-MS/MS). The LC column was a Kinitex C18 (50 × 3 mm, 2.6 μm). The gradient elution mode was chosen, since this facilitates the separation of compounds by varying the strength of the mobile phase, by varying the proportion of 20% methanol and 80% of water acidified with 0.1% acetic acid to reach 90% of methanol and mobile phase flow rate ranging between 0.2 and 0.4 mL min^{-1}. The determinations were performed in positive and negative mode simultaneously. The capillary voltage was 3.5 kV, the source block and desolvation temperatures were 120 °C and 450 °C, respectively. The desolvation and cone gas flows were set at 550 and 50 L h^{-1}, respectively. Nitrogen was used as nebulizing, desolvation and cone gas whereas argon was used as collision gas. For each pesticide, two transitions were optimized in the MRM mode. The transition that presented the most intense signal was chosen for quantification while the other was used for confirmation.

Scanning electron microscopy (SEM) (model JEOL JSM-6610LV) was used to observe the surface morphology of the rice husks. A dried rice husk was placed on an aluminium stub and coated with carbon using a vacuum sputter coater (model Denton Vacuum DESK V). The accelerating voltage was 15 kV.

The elemental composition of the rice husks was evaluated with a CHNS/O elemental analyzer (Model 2400 Series II Perkin Elmer). The equipment calibration was performed using a Certified Reference Material (acetanilide).

The rice husk ash were characterized according to their solubility in water, alkalinity and solubility in hydrochloric acid [14].

2.2.2. Establishment of adsorption and desorption parameters

Initially, the milk samples were heated to 37 °C for 15 min and centrifuged at 2.576 *g* for 15 min, separating the fat excess. Rice husks were packed into columns inserted in the vacuum manifold (Phenomenex, SPE12 – Position Vacuum Manifold Set) immediately below syringes containing the milk samples so that elution occurred through the adsorbent (Fig. 1a). Next, the columns were washed with a methanol:chloroform mixture to clean up the adsorbent and remove the adsorbed mycotoxins for further quantification (Fig. 1b).

To verify whether the aflatoxins remained adsorbed on the rice husks after washing with solvents, the mycotoxins were extracted conform the previous method [11].

A Plackett–Burman design was used to evaluate the main factors that affected the adsorption of afla B_1 and afla M_1 in milk by the rice husks. Sixteen runs were performed to evaluate the six input variables studied at two levels with three repetitions. The independent variables were defined as −1, 0 and +1 for the amount of rice husks (0.5; 1 and 1.5 g), husk diameter (24; 32 and 42 mesh), vacuum (5; 10 and 15 in. Hg), solvent mixture (60:40;