Zearalenone Removal by Using Banana Peel as an Adsorbent

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Received 27 January 2020, Revised 04 November 2020, Accepted 05 November 2020

Abstract
Zearalenone (ZEA) is the most occurring Fusarium toxin in animal feed causing reproductive disorders and results in severe economical losses. A renewable bio adsorbent sourced from banana peel was tested for in vitro removal of ZEA from liquid mediums at different pH values. Efficacy of banana peel to sequester ZEA was evaluated by varying its pH, adsorbent dosage, equilibration time and quantified by using UHPLC-MS/MS. Adsorption was found to be efficient and completed in fifteen minutes with highest adsorption at alkaline pH (9). The adsorption and desorption studies have demonstrated the adsorption was strong enough to sustain the pH changes (3-9). Fourier transforms infrared spectroscopy (FTIR) and scanning electron microscope (SEM) was used to characterize the surface of bio sorbent to explain the mechanism of adsorption. Langmuir and Freundlich isotherm was found to be best fitted model with maximum monolayer capacity (Q0) 8 ng/mg. The results of this study suggested that dried banana peel may be an effective low cost decontamination method to be incorporated in animal feed.

Keywords: Zearalenone, Bio adsorbent, Banana peel, Animal feed

Introduction

Mycotoxins are toxic secondary metabolites produced by filamentous fungi. These fungi are ubiquitous in nature and can grow on various cereal products and may cause economic losses at all levels of food and feed production [1]. Nearly twenty five percent of the agricultural products are contaminated with mycotoxins and declared unfit for human consumption. Due to strict regulations for human food, these contaminated cereal products find its way to livestock industry and posses heavy economical losses and exposed humans to toxic effects through contaminated animal products [2].

Zearalenone (ZEA) is a 6-(10-hydroxy-6-oxo 6 \textit{trans}-1- undecenyl)-b-resorcylic acid lactone, it is a non steroid estrogenic compound produced by various Fusarium species and its structure is shown in Fig. 1. Due to polarity of ZEA, this toxin is easily absorbable and available in blood of animal within 30 min of its ingestion [3]. Recently, ZEA has gained much attention due to its transference to humans through the animal products like meat, eggs and milk [4]. It produces various estrogenic effects in humans including decrease fertility, reduced litter size, changes normal function of different glands and results in increased level of progesterone and estradiol in humans [5].
Different strategies have been tested to minimize the effect of ZEA in animal feed. Use of selective adsorbent for the purpose to absorb the toxin without affecting the other nutrients in GI tract of animal is most studied approach to mitigate the toxic effects of ZEA. Unlike to other mycotoxins, ZEA has polar nature and no any adsorbent found to be effective for its complete removal in the gut of animal without affecting the health of animal. Few mineral adsorbents and specialized polymers have been reported for the sequestration of ZEA which include, activated charcoal, aluminosilicate and other specific polymers [6-11]. Utilization of mineral adsorbents is limited due to either high cost or non-specificity toward the ZEA and may absorb some essential micro and macro nutrients beside the ZEA and have negative impact over the animal health. Furthermore its use in larger amount is restricted due to their environmental disposal problem when excreted in large amounts as manure [12].

Alternative to mineral adsorbents, a cheap bio waste as a bio adsorbent gaining much importance for being used as a feed additive to sequester ZEA in the gut of the animal [13-19]. Use of dietary fibres to counteract toxic effects of mycotoxins in animals have been reported [20]. Undegradable fibre has proved its efficacy in overcome the toxic effects of ZEA in rats and swine [21-23]. Alfalfa has decreased the toxicity of T2 toxin in rats [24] and micronized wheat fibres has shown its efficacy to decrease the ochratoxin A toxicity [25, 26]. Use of bio adsorbent are beneficial in many ways, firstly it make the utilization of available bio-waste which become otherwise discarded and secondly these are rich with minerals and provide dietary fibre to the animal [27, 28]. This study was designed to assess the efficacy of banana peel for in vitro sequestration of ZEA. This study results may provide basis for further research on the use of fibrous bio sorbents as an alternative to mineral adsorbent for the removal of mycotoxins.

Materials and Methods

Chemicals and Reagents

ZEA standard was purchased from Biopure (Tulln, Austria) and its stock solution (1000 µg/L) was prepared by dissolving it in acetonitrile. Other chemicals ammonium formate was obtained from Sigma Aldrich, formic acid was purchased from Fluka, phosphate buffer saline (PBS) was purchased from Sigma Aldrich (St. Louis, MO, USA). Buffer solutions were prepared in the range of pH 3-9 by using citric acid and disodium orthophosphate. pH measurement was done with pH meter (Thermo orion model 420). Ultra-pure RO water (Milli Q) were utilised for all analysis.

Quantification of ZEA

UPLC-ESI-MS/MS (Waters, Milford, MA, USA) was used for the quantification of ZEA. Separation was achieved on Acquity H-Class HSS T3 column (1.8μm 100nm x 2.1 mm,) with gradient mixture of mobile phase A (H2O/ HCOOH, 99/1 v/v, and 10 mM ammonium formate and mobile phase B (CH3OH/H2O/HCOOH, 97/2/1v/v/v, and 10 mM ammonium formate) at a flow rate of 0.5 mL/min as described [29].

Preparation of Adsorbent

Full ripe banana peel was separated from the banana fruit sourced from the local market. Peel was washed thoroughly in order
to remove the dirt and oven dried for 48 h. Oven dried (OD) peel were cut into small pieces and ground by using grinder, sieved by using 500 μm mesh screen.

**Characterization of Adsorbent**

Surface morphology and functional groups available on the surface of any material are driving factors for the adsorption. In order to understand the mechanism of the adsorption of ZEA on the surface of the banana peel FTIR and SEM spectra were collected. IR Spectra were recorded by using FT-IR (Thermo Nicolet) equipped with a ZnSe crystal controlled by OMNIC software. Surface structure and morphology were determined by using Scanning electron microscope (Zeiss, JEOL-JSM-35CF, UK), operating at invariable pressure (VP) mode at 0.3 tor and 20 kV accelerating voltage. SEM spectra were recorded at 5 kV.

**Adsorption Process**

OD banana peel was weighed and placed in micro tubes already containing half millilitre of ZEA (1 µg/mL) standard in different buffers. The suspension was mixed and shaked for thirty min on reciprocating pump at the speed of 100 rpm (at room temperature). The suspension was allowed to settle and centrifuges for two min at 18000 g. Supernatant was filtered through 0.2 μm GHP syringe filter and subjected UPLC-MS/MS. A control was prepared by using ZEA standard in buffered solution without banana powder. Percent adsorption calculated by subtracting residual ZEA from control samples. All analyses were carried out in triplicate.

**Optimization of Parameters**

**Adsorbent Dosage**

In order to select proper dosage of sorbent, different dosages of adsorbent tested for the removal of ZEA. Five different dosages 3, 5, 15, 30 and 60 mg/mL were selected while keeping temperature at 295 K, time 30 min and initial concentration of ZEA 1 µg/mL. All analyses were done in triplicate.

**Time Study**

Time required for equilibration was determined allowing shaking for 5, 15, 30 and 60 min by selecting optimized adsorbent dosage (30 mg). All other parameters were kept constant sorbent dosage 30 mg, temperature 295 K and pH 7. Analysis was carried in triplicate.

**Effect of pH**

To check the effect of the pH over the removal of ZEA; different buffers including citrate buffered pH (3-6) and phosphate buffered (7-9) were tested in batch adsorption experiments by keeping other parameters constant (temperature 295K, contact time 15 min and adsorbent dosage 30 mg and ZEA concentration was 1 µg/mL). Analyses were carried in triplicate.

**Desorption Study**

To check weather sorption was strong enough to sustain the pH changes, adsorption and desorption were carried out at different pHs. Optimized adsorbent dosage (30 mg) were weighed into 2 mL screw-cap test tubes and mixed with 1 mL of ZEA working solution (pH 3), the suspension was mixed and shaked for 30 min on reciprocating pump at the speed of 100 rpm. The suspension was allowed to settle and centrifuges for 2 min at 18000 g. Supernatant was filtered thru 0.2 μm GHP syringe filter and subjected UHPLC-MS/MS for residual ZEA. Adsorbent pellets were washed with 1 mL of buffer at pH 8 (30 min shaking time in all cases). Suspensions in buffer were centrifuged and the supernatants were analysed to assess ZEA desorption. Values for ZEA adsorption (pH 3) and desorption (pH 8) were calculated for ZEA and
expressed as a percentage. Similarly adsorption carried at pH 8 desorbed at pH 3. Adsorption and desorption experiments carried out in triplicate.

**Data Evaluation**

ZEA adsorbed on powder calculated by taking the difference between the blank tubes with no bio sorbent and supernatants with bio sorbent. Toxin adsorbed on sorbent calculated by taking the difference between the blank tubes with no bio sorbent and supernatants with bio sorbent. Toxin adsorbed on sorbent calculated by Eq. (1).

\[
q_e = \frac{(C_o - C_e)V}{m}
\]  

\(q_e\) is the ZEA adsorbed per unit mg (μg/mg); \(C_o\) is the initial concentration of toxins (μg/mL); \(C_e\) concentration of toxins available in tubes after adsorption (μg/mL); \(V\) is volume and \(m\) is mass of adsorbent (μg). Nature of adsorption were explained by plotting the Langmuir and Freundlich curve with linear fitting, Eq. (2) and (3), [30, 31].

\[
q_e = \frac{Q_0bC_e}{1 + bC_o}
\]  

\[
q_e = K_Fc_e^{1/n}
\]

\(Q_0\) (μg/mg), \(b\) (mL/mg), \(K_F\) and \(n\) are Langmuir and Freundlich isotherm parameters respectively. Separation factor which explains the favourability of adsorption were calculated by using equation Eq.(4)

\[
R_L = \frac{1}{1 + bC_o}
\]

Where \(b\) is Langmuir isotherm constant

**Statistical Analysis**

Simple statistics was used to check the variability between the replicates and precision was found as standard deviation of replicates. Single way ANOVA was used to check the significance of treatments as described in previous study [13].

**Results and Discussion**

**Characterization of Banana Peel**

Sorption of any molecule on the surface can be explained on the basis of various electrostatic interactions. In order to understand which factors played major role in the removal of the ZEA can be attributed to the nature of the functional groups available for the adsorption. IR spectrum of peel has shown a broad peak in range of 3200-3500 cm\(^{-1}\) which could be attributed to hydroxyl groups present in cellulose, pectin, hemicelluloses, lignin and adsorbed water (Fig. 2a). Peaks observed at 1734 and 1600 cm\(^{-1}\) were due to carbonyl (C=O) and carboxylate groups (COOR), respectively. Peaks at 1380-1300 cm\(^{-1}\) could be attributed to aliphatic and aromatic(C-H) groups of methyl and methylene [32].

SEM spectra has revealed the porous and rough structure with large edges which may help for adsorption of ZEA (Fig. 2b)
Effect of Amount of Adsorbent

As the amount of dosage increases; the adsorption increase linearly due to introduction of more binding sties for the adsorption (Fig. 3 a). ZEA adsorption increase linearly upto 30 mg/mL, further addition of sorbent did not contribute toward the adsorption due to aggregate formation with reduced the area at higher sorbent densities [33]. By taking into consideration it is deemed that it is not useful to increase bio sorbent amount beyond 30 mg. Therefore, for all subsequent experiments, OD peel dosage was fixed at 30 mg/mL.

Effect of Contact Time

Increasing the time to allow the ZEA for adsorption increased the total amount of adsorption. The effect of time for adsorption of ZEA over the surface was studied for a period of one hour (Fig. 3b). The Fig. 3b shows that adsorption at the initial stages is higher and decrease as the time increases due to coverage of all available sites. The maximum adsorption took place within the time period of 10-20 min and no further changes in the adsorption were noted beyond the 30 min. Previous studies of mycotoxins adsorption on bio adsorbents have reported 15-30 min required for maximum adsorption [11, 13, 34]. Smaller time for the adsorption is beneficial from point of view that it can sequester all of the toxin in GI tract before it enter into the blood of the animal and could be useful for vivo studies.

Effect of pH

Adsorption of any molecule on the surface is generally a complex phenomenon and is combination of non-electrostatic and electrostatic interactions, pH generally influenced both types of phenomenon and has ionizing effects on surface functional groups as well as on the ZEA. The ZEA adsorption increase with the increase of pH and highest adsorption was obtained at pH 8 and little effect has been observed beyond pH 8 (Table 1).

Table 1. Effect of pH on ZEA adsorption by OD banana peel.

| pH | Percent Adsorption by Banana Peel, (RSD) |
|----|------------------------------------------|
|    | ZEA                                      |
| 3  | 21±1.8                                   |
| 4  | 34±1.5                                   |
| 5  | 42±0.9                                   |
| 6  | 57±1.5                                   |
| 7  | 85±1.0                                   |
| 8  | 90±1.7                                   |
| 9  | 91±1.0                                   |

ZEA is a diphenolic compound with an estimated pKa of 7.6, at alkaline pH ZEA is present in phenolate ion. Ionization depends...
upon the pKa value; if pH<pK_a, anlayte will be in protonated form and if pH >pK_a it will be in deprotonated form. Adsorption at higher pH may be indicative of higher tendency of the phenolate ion toward the adsorbents. Similar results obtained in recent studies conducted on sugar beet pulp biomass in which higher adsorption was obtained at higher pH [34]. However in another study conducted on grapes pomace, author did not find any effect of pH change over the adsorption of ZEA [13]. This may be fact due to the different functional groups present on the grape pomace which contribute toward the adsorption.

The dependence of adsorption on pH may be helpful from the point view that monogastric animals such as pigs, pH is not constant but it changes along the GI tract of the animal. It is low in stomach (1.5-4.5) and increasing through the tract and reaches upto 7.5 in small intestine. An efficient adsorbent must absorb ZEA in all pHs and retain it throughout the GI tract as the bolus pass through the compartment. Adsorption of ZEA at alkaline pH may be beneficial from the point of view that in animal gut absorption takes place at alkaline pH and adsorbent which absorb ZEA in this region has advantage to be employed as a feed additive.

**Desorption Studies**

Adsorption and desorption were carried at two different pH in order to check the effect of pH changes over the sorbent capacity (Table 2). Adsorption takes place at pH 3 was strong enough that it remains on surface when adsorbate was washed with pH 8. While adsorption takes place at pH 8 were strong enough to be on surface when washed with pH 3. In both cases less than five percent was removed by changing the pH. This showed that mostly ZEA and adsorbent interaction were of non electrostatic and microporous and rough structure of the adsorbent was responsible for its capture of toxins and it was strong enough to sustain the changes in pH.

| Toxin name | Adsorption (%) pH3 | Adsorption (%) pH8 | Desorption (%) pH3 | Desorption (%) pH8 |
|------------|-------------------|-------------------|-------------------|-------------------|
| ZEA        | 21±1.8            | 90±4.7            | 1±0.3             | 2±0.8             |

These results are advantageous from point of view of being used as feed additive. The pH of bolus is not constant and depends upon the GI compartment. In poultry pH ranges from 2.5-3.5 in stomach and 5.5 to 7.5 in intestinal lumen where absorption of nutrients takes place. Banana peel works best at alkaline and it will decrease the bio availability of this toxin for animal absorption.

**Adsorption Isotherm**

Langmuir and Freundlich isotherm were tested by linear fit model and adsorption parameters determined and presented in Table 3.

| Toxin | Langmuir parameters | Freundlich parameters |
|-------|---------------------|----------------------|
|       | Monolayer capacity Q_0 (ng/mg) | Constant b (mL/mg) | Separation factor K_L | R^2 | Constant n | 1/n | Constant K_F (ng/mg) | R^2 |
| ZEA   | 8                   | 1.21                | 0.45                | 0.96 | 0.29       | 3.38 | 0.08                 | 0.91 |
Langmuir plot based upon an assumption of monolayer formation to compare and contrast adsorbent capacity of any material (Fig. 4). Maximum amount of toxins to be captured on the surface maximum monolayer capacity \( (Q_0) \) was found to be 8 ng/mg which is much higher than previously reported bio adsorbents for ZEA. Separation factor provide the information about favourability of the adsorption and found greater than 0 but less than 1 which showed adsorption is favourable [35].

![Langmuir plot](image)

Information about the heterogeneity of surface and based upon the assumption of multilayer formation was obtained from Freundlich isotherm. slope value \((1/n)\) provide basic information about surface heterogeneity, \(1/n\) calculated for ZEA adsorption was greater than 1 which proved that cooperative adsorption has been takes place. This supports our FTIR, and SEM studies which suggest that the functional groups at the surface of the banana peel and heterogeneous micro structures of absorbent surface may contribute toward the adsorption of the toxins.

**Conclusion**

In vitro studies have shown that dried banana peel is an effective bio-sorbent in the removal of ZEA from solution. Time study has demonstrated that banana peel can sequester the ZEA in just 15 min. Furthermore, the interaction of aflatoxins with the banana peel was strong enough to sustain changes in pH. This study suggests that bio-sorbent (banana peel) has potential as a cheap bio-sorbent for removal of mycotoxins and may have an application as a feed additive.

**Conflict of Interest**

No potential conflict of interest was reported by the authors.

**Funding**

The authors would like to acknowledge with great thanks the Higher Education Commission (HEC), Pakistan, for providing the financial support to carry out this research work under project no. 6713/Sindh/NRPU/R&D/HEC/2015.

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