Journal Name	Manuscript No.	B	Dispatch: 5.12.12	Journal: JAM	CE: Priya Lekshmi S.G.
JAM 12082			Author Received:	No. of pages: 8	PE: Kaviarasi

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

The corn influence on the adsorption levels of aflatoxin B_1 and zearalenone by yeast cell wall

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Keywords
adsorption, aflatoxin B_1, corn, mathematic models, yeast cell wall, zearalenone.

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2012/1003: received 1 June 2012, revised 18 October 2012 and accepted 10 November 2012

doi:10.1111/jam.12082

Abstract

Aims: To evaluate the influence of the corn on the adsorption levels of aflatoxin B_1 (AFB_1) and zearalenone (ZEA) by yeast cell walls (YCWs), *in vitro*.
Methods and Results: Two commercial YCWs were studied. The YCWs contain different percentages of polysaccharides. YCW1 and 2 contain 5·9 and 21% of mannans and 17·4 and 23% of β-glucans, respectively. Each YCW was resuspended at pH 2 and pH 6 solutions. Corn was used to study the matrix influence. An aliquot of 500 μl YCW concentration was added to each microtube containing 500 μl of 0·1, 0·25, 0·5, 1, 2·5 and 5 μg ml^{-1} AFB_1 and 0·5, 5, 10, 20 and 50 μg ml^{-1} ZEA. Microtubes were introduced into a centrifuge with mechanical agitation at 37°C for 30 min and then centrifuged for 10 min at 14 000 rpm; the supernatants were quantified by high-pressure liquid chromatography. The amount of bound toxin was plotted as a function of the amount of added toxin according to mathematical expressions proposed by three theoretical models. Both YCWs were capable of adsorbing AFB_1 and ZEA in amounts from 0·061 to 0·40 and from 0·10 and 0·26 g g^{-1}, respectively. In the presence of the matrix, both adsorbents were not able to adsorb AFB_1. However, they could adsorb ZEA at levels from 0·03 to 0·23 g g^{-1}.
Conclusions: Both YCWs adsorbed ZEA in the presence of corn and also under simulated gastrointestinal pH conditions. These results suggest that the studied YCWs are potential candidates for ZEA adsorption.
Significance and Impact of the Study: Several *in vitro* assays have informed the ability of different substrates including yeast walls to adsorb AFB_1 and ZEA; none of them have evaluated their ability to adsorb AFB_1 and ZEA in the presence of the corn. The matrix as corn can influence the adsorption phenomena of these mycotoxins.

Introduction

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as grain contaminants. They can cause serious problems in livestock resulting in substantial economic losses (Huwig *et al.* 2001). Two of the most common mycotoxins found in animal feed, aflatoxin B_1 (AFB_1) and zearalenone (ZEA), cause food- and feed-borne intoxications called mycotoxicoses (CAST 2003). Aflatoxin B_1 is a carcinogenic metabolite produced primarily by *Aspergillus flavus* and *A. parasiticus* (IARC 2002). It is a potent liver toxin that can either be lethal when consumed at large doses or induce cancer after chronic exposure. (Khanafari *et al.* 2007). ZEA, produced by a number of *Fusarium* species mainly by *F. graminearum*, binds to oestrogen receptors producing functional and morphological alterations in reproductive systems. Among farm animals, pigs are most sensitive to ZEA

intake, being reported clinical signs such as ovarian atrophy, prolonged oestrus intervals, persistent corpora lutea, decreased fertility and stillbirth (Fink Gremmels and Malekinejad 2007).

Physical, chemical, physicochemical and biological approaches have been developed to reduce the impact of mycotoxins. One of the most efficient prevention strategies to prevent mycotoxicoses is the dietary supplementation with materials that reduce the toxin bioavailability in the digestive tract and, therefore, their adverse effects on animals. Basic ingredients and dietary supplements such as the yeast *Saccharomyces cerevisiae* may have functional properties in the diet and show satisfactory results when added to feedstuff either as active cells or as cell wall components (Shetty and Jespersen 2006). α-D-mannan and β-D-glucan are the two major polysaccharides present in *S. cerevisiae*. They constitute up to 90% of the cell wall dry weight and have remarkable properties to interact with the host immune system and constitute a good source of adsorbent (Shetty and Jespersen 2006; Kogan and Kocher 2007). Besides its excellent nutritional value, yeasts produce a high quantity of biomass that is used in a large variety of industrial processes. The ability of the *S. cerevisiae* cell wall to bind ZEA has been reported recently (Yianninkouris *et al.* 2003, 2004a; Yiannikouris *et al.* 2004b). Yiannikouris *et al.* (2004b) and Yianninkouris *et al.* (2003, 2004a) tested different mathematical models to describe the ability of *S. cerevisiae* cell wall to adsorb ZEA. However, there is little information on the influence of the corn on the adsorption levels of AFB_1 and ZEA by yeast cell walls (YCWs). The aim of the present study was to evaluate the influence of the corn on the adsorption levels of AFB_1 and ZEA by YCW.

Materials and methods

Yeast cell walls, suspension corn and reagents

Two commercial YCWs were studied for toxins' binding ability: YCW1 and YCW2. Their compositions are described in Table 1. Each YCW was resuspended in buffer solution at pH 2 (50 ml of potassium chloride 0·2 mol l^{-1} and 13 ml of hydrochloric acid 0·2 mol l^{-1}) and pH 6 (100 ml of potassium phosphate bi acid 0·1 mol l^{-1} and 11·2 ml of sodium hydroxide 0·1 mol l^{-1}) for the subsequent uses. The pH was adjusted by adding hydrochloric acid of 0·2 mol l^{-1} or sodium hydroxide of 0·1 mol l^{-1} using a pH meter (model 250A; Orion Research Inc., Boston, MA, USA). The pH was confirmed using a pH meter (model 250A; Orion Research Inc.) and it was adjusted using hydrochloric acid 0·2 mol l^{-1} or sodium hydroxide 0·1 mol l^{-1} solutions as appropriate.

Table 1 Composition (%) of the main carbohydrates in yeast cells dry mass

	Composition (%)	
Ingredients	YWC1*	YWC2*
β-glucans	17·4	23
Mannans	5·9	21
Total of polysaccharides	23·3	44

*Commercial yeast cell walls.

Corn, ground and sieved, was utilized to study the matrix influence on toxin–adsorbent interactions. The extraction and detection of AFB_1 and ZEA corn samples in this test to discard the presence of the same in this substrate were performed. The AFB_1 and ZEA levels were below the detection limit. The methodology for the detection and quantification of these mycotoxins was performed according to the proposed Trucksess *et al.* (1994) and Cerveró *et al.* (2007). The detection limits of the used method were 0·4 and 3 ng g^{-1} for AFB1 and ZEA, respectively.

Adsorption test

Different concentrations of each adsorbent were tested to obtain an adequate relation between adsorbent and toxin. Seven (7) suspensions of each YCW (2, 5, 10, 25, 50, 100 and 500 μg ml^{-1}) were tested against AFB_1 (2 μg ml^{-1}), and five (5) suspensions of each YCW (10, 50, 75, 100 and 200 μg ml^{-1}) were used for ZEA (1 μg ml^{-1}) adsorption.

An aliquot of 500 μl of the corresponding toxin (2 μg ml^{-1} AFB_1 or 1 μg ml^{-1} ZEA) was added to each microtube containing 500 μl of each YCW suspension. The microtubes were introduced into a shaker Labor 2K15 centrifuge (Sigma) at 37°C with mechanical agitation for 30 min. Microtubes were then centrifuged for 10 min at 14 000 rpm, and the supernatant was taken and evaporated to dryness under gentle stream of nitrogen gas. Each adsorption test was performed in duplicate, and control tests were performed. The extracts were quantified by high-pressure liquid chromatography (HPLC).

Calibration curves were plotted in a range from 0·125 to 2 μg ml^{-1} of each toxin (AFB_1 and ZEA). Data were plotted for choosing the appropriate adsorbent mass. This methodology allowed obtaining greater reproducibility in the results.

Suspension corn–adsorbent preparation

Considering that, in general, the adsorbent is commercially used at 2% weight of finished feed and that preliminary test suspensions were prepared from

50 μg ml^{-1} for each YCW, and 1 mg of YCW and 50 mg of ground corn were dissolved in 20 ml of the buffer solution at pH 2 or pH 6.

In vitro adsorption capacity

An aliquot of 500 μl of YCW concentration was added to each microtube containing 500 μl of 0·1, 0·25, 0·5, 1, 2·5 and 5 μg mL^{-1} of AFB_1 and 0·5, 5, 10, 20 and 50 μg ml^{-1} of ZEA. Microtubes were introduced into a centrifuge (Labor 2K15 centrifuge, Sigma) at 37°C with mechanical agitation for 30 min. Microtubes were then centrifuged for 10 min at 14 000 rpm, and the supernatant was taken and evaporated to dryness under gentle stream of nitrogen gas. Each adsorption test was performed in duplicate, and control tests were performed. The extracts were quantified by HPLC.

The study of the *in vitro* corn influence on toxin–adsorbent interaction was developed in the same way as previously described. The concentration of toxins was the same. The suspension corn–adsorbent used was performed as previously described.

Detection and quantification of aflatoxin B_1

Aflatoxin B_1 detection and quantification from each sample were performed by HPLC according to the methodology proposed by Trucksess *et al.* (1994). An aliquot (200 μl) was derivatized with 700 μl trifluoroacetic acid/acetic acid/water (20 : 10 : 70 v v^{-1}). Chromatographic separations were performed on a reverse-phase column (Silica Gel, 150 × 4·6 mm id., 5-μ particle size; VARIAN, Inc., Palo Alto, CA, USA). Acetonitrile/methanol/water (1 : 1 : 4 v v^{-1}) was used as mobile phase at a flow rate of 1·5 ml min^{-1}. Fluorescence of AF derivatives was recorded at excitation and emission wavelengths of λ 360 and λ 460 nm, respectively. Quantification of AFB_1 was performed by measuring the area and its extrapolation to a calibration curve obtained using solutions of AFB_1 standards. The detection limit of the used method was 0·4 ng g^{-1}.

Detection and quantification of zearalenone

The used methodology was described by Cerveró *et al.* (2007). Extracts were resuspended in mobile phase methanol/water (70 : 30 v v^{-1}) and injected into the HPLC. Detection and quantification of ZEA were performed using a fluorescence detection system on Hewlett Packard 1100 Series. The chromatographic separations were carried out on a C18 reverse-phase column (150 × 4·6 mm, 5-μm particle size; Phenomenex, Luna), connected to a Supelguard LC-ABZ column (20 × 4·6 mm particle size; Supelco). The flow of mobile phase was 1 ml min^{-1}. The wavelengths of excitation and emission used were 280 and 460 nm, respectively. Quantification of ZEA was performed by measuring the area and its extrapolation to a calibration curve obtained using solutions of ZEA standards. The limit of detection of the technique used was 3 ng g^{-1}.

Curve fitting and data processing

The amount of bound toxin (AFB_1 and ZEA) was plotted as a function of the amount of added toxin according to the mathematical expressions proposed by three theoretical models (Langmuir, Frumkin-Fowler-Guggenheim and Hill) and selected according to the form of isotherms. Mathematical equations and parameters of Langmuir, FFG and Hill models are shown in Table 2. The adjustment was made with the program Origin® version 6·1 software. The errors that affected the estimated parameters were calculated by propagation of errors in the corresponding adjustment parameters fitted by the method of adjustment. The quantity of toxin adsorbed was determined by the following equation:

$$\mathrm{Toxin}_{ads} = [(\mathrm{Toxin}_0 - \mathrm{Toxin}_{eq}) \times V]/m$$

Toxin_{ads} = quantity of toxin adsorbed per gram of adsorbent (μg μg^{-1}).

Toxin_0 = initial concentration of toxin in solution (μg mL^{-1}).

Toxin_{eq} = residual toxin concentration at equilibrium (μg ml^{-1}).

V = volume of solution (ml).

m = mass adsorbent (μg).

Results

Yeast cell wall concentrations

The concentration 50 μg ml^{-1} of each YCW was used to determine the adsorption capacity (data not shown).

Table 2 Mathematical equations and parameters of Langmuir, FFG and Hill models

Models	Mathematical expression	Parameters
Langmuir	$\beta = \frac{\Gamma}{(\Gamma max - \Gamma)[Tox]}$	Γ_{max}, β
FFG	$\beta = \frac{\Gamma}{(\Gamma_{max} - \Gamma)[Tox]} \exp[-2a\Gamma/\Gamma_{max}]$	Γ_{max}, β, a
Hill	$\frac{1}{KD} = \frac{\Gamma[Tox]^n}{(\Gamma max - \Gamma) + [Tox]^n}$	Γ_{max}, KD, n

Γ: amount of adsorbed toxin per unit weight of biomass, [Toxin]: residual toxin concentration in solution at equilibrium, Γ_{max}: maximum amount of adsorbed toxin per unit weight of biomass, β: adsorption constant, a: parameter measuring the interaction between both adsorbed and in solution toxins, KD: Hill constant, n: Hill cooperativity coefficient of the binding interaction.

At this concentration, the YCW maintains the equilibrium conditions where coexist free and occupied sites on the adsorbent and the toxin in the supernatant.

Aflatoxin B_1 adsorption

Figure 1 shows AFB_1 adsorption isotherms on YCW1 and YCW2 at both tested pH value. Isotherms, slightly sigmoid, were adjusted by both the Hill and FFG model, revealing a cooperative adsorption. The setting parameters for both models are shown in Tables 3 and 4. Both YCWs adsorbed similar amounts of AFB_1 at pH 2 and pH 6. Values of the adsorption constant were similar for both YCWs. Furthermore, the cooperativity index (n) obtained for both adsorbents was higher at pH 2.

Zearalenone adsorption

Figure 2 shows ZEA adsorption isotherms for YCW1 and YCW2 at both tested pH value. The curves correspond to the setting according to the Hill model. The setting parameters by the Hill and FFG models are shown in Tables 3 and 4. The settings were comparable (R^2), although the Hill model appears to represent better the adsorption process.

At pH 2, the YCW2 had a cooperativity index (n) of approximately 1, and in these conditions, there was no cooperativity. This fact agrees with a reasonably good fit using the Langmuir model. In relation to the effect of pH on YCW1, the results showed that at pH 2 there was a much more pronounced cooperative effect than that at pH 6, where the cooperativity index (n) tended to be 1. It is the opposite for the YCW2. Considering the influence of pH on the adsorption capacity (Γ_{max}) for each YCW, it was observed to be doubled at pH 6 compared with that observed at pH 2. At pH 2, the adsorption capacity for YCW1 was 0·10 (g g^{-1}) and the YCW2 was 0·14 (g g^{-1}), while at pH 6, it was 0·25 and 0·26 (g g^{-1}), respectively. There were no appreciable differences in the adsorption capacity for both adsorbents. The affinity of ZEA measured by the association constant (β) was greater for YCW 2 (3·12 μmol l^{-1}) at pH 2.

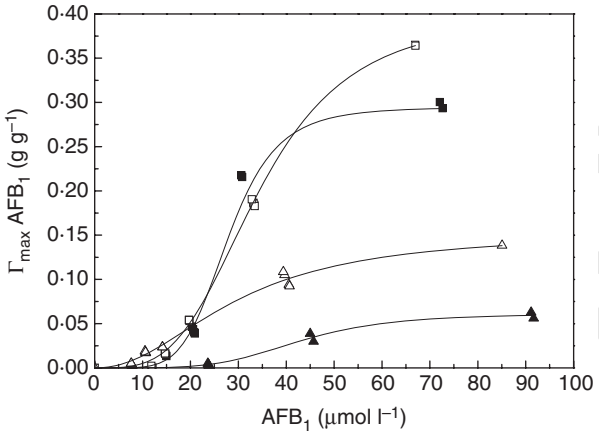

Figure 1 Aflatoxin B_1 adsorption (Γmax) isotherm by commercial yeasts cells walls (YCWs) using the theoretical mathematic model of Hill. YCW1 – pH 2 (■), YCW2 – pH 2 (□), YCW 1 – pH 6 (▲), YCW 2 – pH 6 (Δ).

Adsorption of aflatoxin B_1 in the presence of the corn

None of the tested adsorbents were able to adsorb AFB_1 at appreciable amounts in the presence of corn.

Adsorption of zearalenone in the presence of the corn

Figure 3 shows ZEA adsorption isotherms of YCW1 and YCW2 at both tested pH value in the presence of corn. The graph shows that the isotherms were sigmoid, more pronounced at pH 6 and were adjusted with the Hill model, revealing a cooperative adsorption. Parameters settings are shown in Table 5. The settings to the Hill model were comparable (R^2). With respect to the pH on both YCWs, result shows that at pH 6, there was a cooperative effect much more pronounced than that at pH 2,

Table 3 Set-up parameters obtained from AFB_1 and zearalenone (ZEA) adsorption isotherms by yeast cell wall (YCW) using the Hill model

Toxin	Adsorbent	pH	K_D (μmol l^{-1})	β (μmol l^{-1})	$\Gamma_{máx}$ (g g^{-1})	n	N	R^2
AFB_1	YWC 1	2	27·9 ± 0·9	0·035 ± 0·001	0·29 ± 0·01	5·5 ± 0·7	15	0·981
		6	43·1 ± 1·8	0·023 ± 0·001	0·061 ± 0·003	4·5 ± 08	13	0·975
	YWC 2	2	34·3 ± 0·7	0·029 ± 0·001	0·40 ± 0·1	3·5 ± 0·1	18	0·998
		6	29·7 ± 2·7	0·034 ± 0·003	0·15 ± 0·01	2·1 ± 0·2	14	0·989
ZEA	YWC 1	2	0·66 ± 0·09	1·52 ± 0·21	0·10 ± 0·01	2·5 ± 0·8	12	0·972
		6	1·25 ± 0·18	0·80 ± 0·12	0·25 ± 0·02	1·96 ± 0·31	12	0·980
	YWC 2	2	0·32 ± 0·15	3·12 ± 1·46	0·14 ± 0·03	1·30 ± 0·58	13	0·898
		6	2·72 ± 1·16	0·37 ± 0·15	0·26 ± 0·11	2·81 ± 1·84	12	0·954

K_D, dissociation constant; β, association constant; Γ_{max}, maximum amount of bound toxin; n, cooperativity coefficient; N, number of points.

Table 4 Set-up parameters obtained from AFB_1 and zearalenone (ZEA) adsorption isotherms by yeast cell wall (YCW) using the Langmuir and FFG models

Toxin	Adsorbent	pH	β (μmol l)$^{-1}$	Γ_{max} (g g^{-1})	a	*N*	R^2
AFB_1	YWC 1	2	0·014 ± 0·001	0·33 ± 0·01	1·13 ± 0·02	6	0·999
		6	0·004 ± 0·001	0·07 ± 0·001	1·71 ± 0·13	6	0·998
	YWC 2	2	0·005 ± 0·0001	0·41 ± 0·001	1·83 ± 0·002	6	0·999
		6	0·008 ± 0·001	0·15 ± 0·001	1·40 ± 0·01	7	0·999
ZEA	YWC 1	2	0·77 ± 0·14	0·103 ± 0·012	0·51 ± 0·34	7	0·993
		6	0·22 ± 0·03	0·26 ± 0·01	1·31 ± 0·16	9	0·998
	YWC 2	2	4·34 ± 1·34	0·14 ± 0·01	0	8	0·898
		6	0·12 ± 0·03	0·26 ± 0·18	1·1 ± 0·9	6	0·982

B, adsorption constant; $\Gamma_{máx}$, maximum amount of adsorbed (g toxin per g adsorbent); 'a', is the FFG parameter measuring the interaction between adsorbed AFB_1 and ZEA molecules with the ones in solution; *N*, number of points. Each point is the average of two replicates.

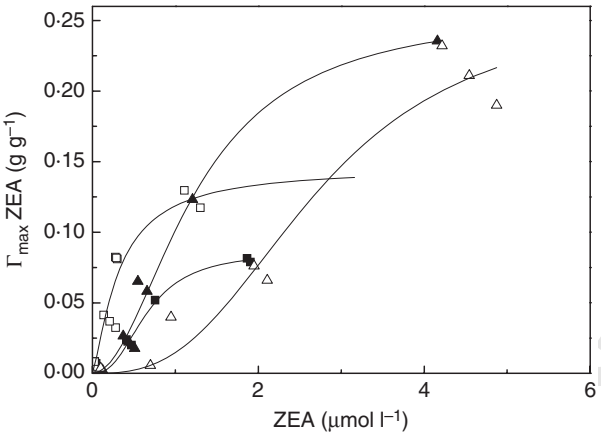

Figure 2 Zearalenone adsorption (Γmax) isotherm by commercial yeasts cells walls (YCWs) using the theoretical mathematic model of Hill. YCW1 – pH 2 (■), YCW2 – pH 2 (□), YCW 1 – pH 6 (▲), YCW 2 – pH 6 (Δ).

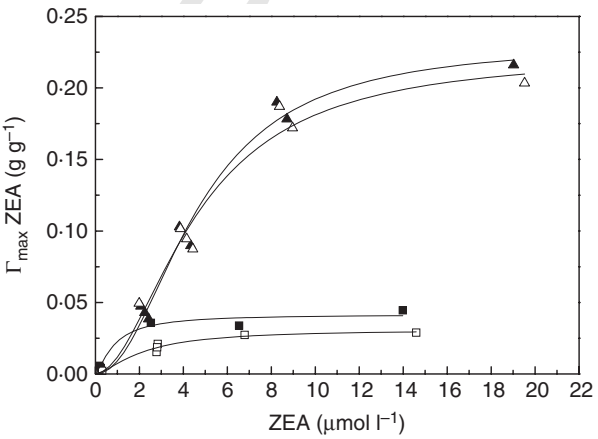

Figure 3 Zearalenone adsorption (Γmax) isotherm by commercial yeasts cells walls (YCWs) with matrix influence using the theoretical mathematic model of Hill. YCW1 – pH 2 (■), YCW2 – pH 2 (□), YCW 1 – pH 6 (▲), YCW 2 – pH 6 (Δ).

where the cooperativity index (*n*) tended to be 1. The effect of pH on the association constants was similar for both YCWs. None of the significant differences were observed for both adsorbents in its adsorption capacity. ZEA affinity of the surface of the adsorbents was greatest for the YCW 1 at pH 2 with a value of 1·12 ± 0·52 μmol l^{-1}.

In the presence of the corn, the maximum adsorption capacity (Γ_{max}) for each YCW was similar at both pH values. The presence of the corn decreases the adsorption capacity of ZEA at pH 2 compared with pH 6.

Discussion

The present study evaluated the influence of the corn on the adsorption levels of AFB_1 and ZEA by YCWs, and the results were interpreted by three different mathematical models.

Langmuir is the simplest theory for the adsorption and assumes both the equivalence of a finite number of sites on the adsorbent surface and the lack of interactions between adsorbed molecules. The FFG theory assumes adsorption on a heterogeneous surface and is applied to either sigmoid, Langmuir or high-affinity isotherms. The adsorption model proposed by FFG assumes cooperative, repulsive or not interacting adsorption mechanisms depending on the sign of the adjusting parameter 'a'. It must be considered that β values obtained by FFG model represent the adsorption constant extrapolated to coating zero. In such, all sites are equivalent and no cooperativity is observed in the system. The adsorption constant β differs from the true thermodynamic constant at the adsorption equilibrium, which includes the concentration of displaced solvent from sites at the adsorbent surface (β = Kas $[H_2O]^{-1}$). The Hill mathematical expression includes the dissociation constant (K_D), the maximum

Table 5 Set-up parameters obtained from zearalenone (ZEA) adsorption isotherms by yeast cell wall (YCW) with corn influence using the Hill model

Adsorbent	pH	K_D (μM)	β (μmol l)$^{-1}$	$\Gamma_{máx}$ (g g^{-1})	*n*	*N*	R^2
YWC 1	2	0·89 ± 0·42	1·12 ± 0·52	0·04 ± 0·01	1·37 ± 0·43	7	0·969
	6	4·66 ± 0·46	0·21 ± 0·02	0·23 ± 0·02	2·08 ± 0·28	12	0·986
YWC 2	2	2·18 ± 0·38	0·46 ± 0·08	0·03 ± 0·01	1·5 ± 0·6	7	0·964
	6	4·63 ± 0·81	0·22 ± 0·04	0·22 ± 0·03	1·88 ± 0·54	8	0·951

K_D, dissociation constant; β, adsorption constant; $\Gamma_{máx}$, maximum amount of adsorbed ZEA (g toxin per g adsorbent); *n*, Hill cooperativity coefficient of the binding interaction; *N*, number of points. Each point is the average of two replicates.

adsorption (Γ_{max}) and the minimum number (n) of binding sites required for cooperative adsorption. The inverse of K_D is precisely the adsorption constant that is called β. Both theoretical models can therefore explain S-type isotherms through cooperate adsorption; however, curve fitting will provide either $n > 1$ (Hill equation) or 'a' >0 (FFG equation).

In this work, nevertheless, comparable adjustments were obtained with different models for comparison purposes. Hill's model was chosen for further discussions. This model has been previously proposed to explain the shape of adsorption isotherms on YCW and extracts derived from them (Yianninkouris *et al.* 2003, 2004a; Yiannikouris *et al.* 2004b). In one of the scarce studies on the adsorption of AFB_1 using YCW, Yiannikouris *et al.* (2006) found that 6177 μg ml^{-1} by AFB_1 was adsorbed for YWC. Regarding the maximum coating (Γ_{max}) obtained by the two YCW on AFB_1, the values were similar to those obtained by Galvano *et al.* (1997) with activated carbon (0·12 g g^{-1}) and Dakovíc *et al.* (2008) with copper modified montmorillonite (0·066 g g^{-1}) as adsorbents. 14

Devegowda and Castaldo (2000) explained that the interaction of AFB_1 with glucomannan of YCW was presumably through hydrogen bonds. On the other hand, the adsorption capacity in this study was found over than those observed by Decker and Corby (1980), Phillips *et al.* (1990), Natour and Yousef (1998), Schall *et al.* (2000), Howes and Newman (2000), Desheng *et al.* (2005), Dakovíc *et al.* (2008), who worked with different adsorbents such as activated carbon, aluminosilicates, diatomaceous earth, bentonite, modified extracts of yeast wall, montmorillonites modified and montmorillonites, respectively. The adsorption of the toxin was mainly cooperative and pH independent. 15 16

In the present work, both YCWs adsorbed ZEA at the studied pH conditions. These results agree with those reported by Yianninkouris *et al.* (2004a), who attributed ZEA adsorption in the presence of β-glucans in the walls. Values of the association constants in our assay were high, indicating the high affinity of these adsorbents for ZEA. The effect of pH on the adsorption constant was systematic, and a similar effect was observed with both YCWs. 17

Yiannikouris *et al.* (2004b) studied the influence of pH on the complex β-glucans–ZEA and found that under acidic and neutral conditions, there was an affinity percentage higher than that found under alkaline conditions, which could make the active participation of the conformation of the β-glucans difficult. It is known that the three-dimensional structure of the polysaccharides that constitute the YCW allows the adsorption of mycotoxins or its metabolic derivatives. The tested YCWs differ in their chemical compositions. YCW2 contains a higher percentage of mannans (21%) and β-glucans (23%), while the YCW1 contains 5·9 and 17·4% of mannans and β-glucans, respectively. Yeasts cell wall was composed mainly by polysaccharides, proteins and lipids that offer numerous functional groups for the interaction, such as carboxyl, hydroxyl, phosphate and amine groups, as well as hydrophobic adsorption sites, such as aliphatic chains and aromatic carbon rings (Jouany *et al.* 2005; Ringot *et al.* 2005). For these reasons, the efficiency to adsorb mycotoxins is a complex function of the following three factors: chemical structure of the toxin, the adsorbent composition and the pH of the medium. The spectroscopic studies of surface techniques on the complex toxin-YCW or toxin-derivatives wall could contribute to the explication of the molecular level interaction. 18

The influence of a solid matrix such as ground corn on AFB_1 and ZEA adsorption on the surface of the YCW was studied. In this study, the presence of the corn impedes the adsorption of AFB_1; however, in its absence, it adsorbed large amounts of this toxin. These results agree with many studies reported by other authors such as Karaman *et al.* (2005), Raju and Devegowda (2000), Aravind *et al.* (2003), Diaz *et al.* (2005) and Dvorska *et al.* (2003) who used dry yeast from beer fermentation and found that *in vitro* studies without the presence of some type of matrix were capable of adsorbing AFB_1. In contrast, Jansen van Rensburg *et al.* (2006) used the same adsorbent on *in vivo* studies with broiler chickens and 19

did not observe any decrease in the toxic effects produced by the toxin. Baptista *et al.* (2004) studied the ability of mannooligosaccharides, thermolysed yeast and active yeast to reduce the toxic effects in rats fed diets contaminates with AFB_1. These authors reported that mannooligosaccharides and thermolysed yeast did not suppress the effect of AFB_1, while active yeast reduced the aflatoxin symptoms.

In relation to ZEA, both YCWs adsorbed ZEA at simulated gastrointestinal pH conditions and under the presence of ground corn. These results suggest that both YCWs are future candidates for ZEA adsorption. Several *in vitro* assays have informed the ability to adsorb ZEA by different substrates including YCW; however, none of them was evaluated for the ability to adsorb ZEA in the presence of corn.

In vitro evaluations of the corn influence could be useful as a screening method. They provide an idea of the affinity for the toxins in a relatively short time and with a very small cost. Future studies should be conducted *in vivo* to determine the YCWs detoxification on animal production.

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