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Binding of Zearalenone, Aflatoxin B_1, and Ochratoxin A by Yeast-Based Products: A Method for Quantification of Adsorption Performance

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ABSTRACT

A methodology was developed to quantify the efficiency of yeast-based products for adsorption of three mycotoxins: zearalenone (ZEA), aflatoxin B_1 (AFB_1), and ochratoxin A (OTA). Eight products were tested (yeast cell wall or inactivated yeast). The described experimental protocol based on in vitro tests provided reliable isotherms for each mycotoxin. The most suitable models were the Hill model for ZEA, the Langmuir model for AFB_1, and the Freundlich model for OTA. From these models, original mathematical affinity criteria were defined to quantify the product adsorption performances for each mycotoxin. The best yeast product, a yeast cell wall from baker's yeast, can adsorb up to 68% of ZEA, 29% of AFB_1, and 62% of OTA, depending on the mycotoxin concentrations. The adsorption capacity largely depended both on yeast composition and mycotoxin, but no direct correlation between yeast composition and adsorption capacity was found, confirming that adsorption of mycotoxin on yeast-based products involves complex phenomena. The results of this study are useful for comparing the adsorption efficiency of various yeast products and understanding the mechanisms involved in adsorption.

Mycotoxins are secondary metabolites produced by several fungi, specifically those of the genera *Aspergillus, Penicillium,* and *Fusarium*. These toxins may be carcinogenic, mutagenic, teratogenic, estrogenic, neurotoxic, or immunotoxic for animals or humans and can be found in cereals, wine, spices, coffee, beer, and animal feeds. The most common mycotoxins found in animal feed and human food are aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids *(5)*. For livestock, the contamination of feed with mycotoxins impairs animal health, welfare, and productivity, causing economic losses *(16),* and is an indirect source of exposure for humans by the carryover of mycotoxins and their metabolites in animal tissues, milk, or eggs.

One of the most promising and economical strategies for reducing animal (and thus human) exposure to mycotoxins is the utilization of adsorbents in animal feed to reduce gastrointestinal mycotoxin absorption. This technique has been given considerable attention over the last two decades. Several chemical adsorbents such as activated charcoal and aluminosilicates (e.g., zeolites, hydrated sodium calcium aluminosilicate, clays) have been tested *(10)*. However, most of these inorganic adsorbents cannot adsorb a wide range of mycotoxins *(14)* and may have adverse nutritional effects. The large amounts of these chemicals that must be added to obtain a perceptible effect also may reduce the bioavailability of minerals or vitamins in the diets *(30)*. Some binders are not biodegradable and may accumulate in manure and then in fields were manure is spread for fertilizer. The risk of natural clays becoming contaminated with dioxins also must be considered *(14)*. Faced with these drawbacks associated with inorganic treatments, treatments with yeasts and yeast products recently have been proposed. In addition to their excellent nutritional value, yeasts and yeast cell walls are potential mycotoxin binders. The yeast cell walls, which harbor polysaccharides (mannans and glucans), proteins, and lipids, have many different and easily accessible adsorption centers, including different adsorption mechanisms such as hydrogen binding and ionic or hydrophobic interactions. Use of yeast cell walls instead of whole yeast cells could enhance the adsorption of mycotoxins *(14)*. Devegowda et al. *(6)* observed that glucomannans extracted from the external part of yeast cell walls were able to bind a large range of mycotoxins in vitro. In some animal feeding experiments with whole yeast cells and yeast cell walls or extracts, the addition of *Saccharomyces cerevisiae* to the

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TABLE 1. *Composition of the eight yeast-based products*

Product code	Origin	Dry matter (g/100 g)	Proteins (g/100 g)	Lipids (g/100 g)	Mannans (g/100 g)	Glucans (g/100 g)	Mannans/ glucans ratio
Y1	YCW[a] from brewer's yeast	94.8	31.0	3.3	15.4	20.6	0.75
Y2	YCW from brewer's yeast	96.8	30.9	4.19	13.9	42.7	0.33
Y3	Inactivated baker's yeast	95.7	58.5	6.39	8.7	13.1	0.66
Y4	YCW from baker's yeast	97.1	22.9	17.2	21.3	23.7	0.9
Y5	Inactivated baker's yeast	96.7	59.9	4.83	11.6	13.1	0.89
Y6	YCW from baker's yeast	95.5	22.3	15.5	25.5	27	0.94
Y7	YCW from brewer's yeast	90.6	22.4	7.67	7.5	29.8	0.25
Y8	Alcohol yeast	93.1	43.7	2.47	13.7	25.9	0.53

[a] YCW, yeast cell wall.

diet resulted in reduced mycotoxin toxicities, indicating the stability of the yeast-mycotoxin complex through the gastrointestinal track in broilers, pigs, or cows *(4, 19, 22, 25, 27)*. These organic binders seem to be effective against a larger range of mycotoxins than are affected by inorganic binders, which make yeasts more useful for treatments in the most frequent cases of feeds with multiple contaminants. Yeasts are biodegradable and do not accumulate in the environment after being excreted by animals *(11)*.

Toxin binding depends on strain and pretreatment *(24)*, but mechanisms of binding for different mycotoxins to cell walls and the identification of cell surface binding structures are still unknown. Thus, a reliable screening method is needed to evaluate the adsorption performance of yeast by-products for a wide range of mycotoxins. In some in vitro studies, the adsorption capacity of yeast products for several mycotoxins have been compared. Most of these studies involved a single test, i.e., determination of adsorption for only one mycotoxin concentration *(2, 8, 21, 23)*. However, single concentration studies do not allow a comparison of different in vitro experiments, and extrapolation for other concentrations is difficult. Isotherm adsorption studies are preferred because they give a more complete and reliable picture of adsorption *(7)*. A few other studies have established isotherm curves for yeast products, but no methodology has been provided to compare these isotherms for different yeast products *(20, 21)*. To our knowledge, only Yiannikouris et al. *(31)* have proposed a methodology for isotherm comparison for zearalenone based on the Hill model.

The goal of this study was to develop a reliable methodology to quantify and compare the binding performance of yeast products for three mycotoxins: zearalenone (ZEA), aflatoxin B_1 (AFB_1), and ochratoxin A (OTA). A standardized in vitro protocol of adsorption was proposed for each mycotoxin, isotherm curves were established and modeled for eight yeast-based products, and original mathematical criteria were proposed to classify the yeast product adsorption performance. The adsorption capacity of the eight yeast products was compared for each mycotoxin.

MATERIALS AND METHODS

Adsorbents. The eight adsorbent materials tested in this study were yeasts or yeast cell walls (YCW) from yeast industries. Each product was labeled with a letter code (Y1 to Y8), and some of their characteristics are summarized in Table 1. Experiments were carried out with adsorbents at a level of 5 mg ml^{-1}.

Mycotoxin stock solutions. ZEA, AFB_1, and OTA were purchased from Sigma (St. Louis, MO). Stock solutions for each mycotoxin were prepared at 10 mg ml^{-1} separately in methanol. These stock solutions were diluted with methanol to the desired concentrations to allow the addition of 10 µl in 990 µl of citrate buffer. The citrate buffer was composed of 29.41 g of Tris-sodium 2-hydrate dissolved in 900 ml of ultrapure water, the pH was adjusted to 3 by adding citric acid, and then the volume was increased to 1 liter.

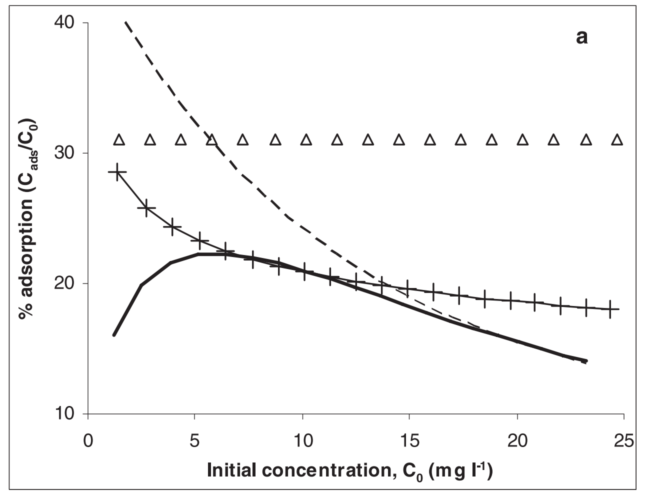
Adsorption experiments. Five milligrams of yeast products was added to 990 µl of citrate buffer (pH 3) in an 1.5-ml Eppendorf safe-lock tube. The suspension of adsorbents was shaken in a thermostatically controlled shaker (Ping-Pong 74582, Fisher Bioblock Scientific, Illkirch, France) at 37°C for 5 min at 175 rpm. Then 10 µl of mycotoxin solutions was added to obtain a range of final concentrations from 0.5 to 80 µg ml^{-1} for ZEA, from 0.005 to 10 µg ml^{-1} for AFB_1, and from 0.005 to 10 µg ml^{-1} for OTA. The final incubation volume was 1 ml. When no precision is given in the text, the mycotoxins were added separately for the adsorption tests. One experiment was carried out with the three mycotoxins together with initial concentrations of 20 µg ml^{-1} for ZEA, 0.5 µg ml^{-1} for AFB_1, and 0.5 µg ml^{-1} for OTA to study possible competition during adsorption. The suspensions were shaken in a thermostatically controlled shaker at 37°C for 15 min at 175 rpm and then centrifuged at 9,200 $\times$ g for 10 min at 37°C. The supernatants were collected and used for high-performance liquid chromatography (HPLC) separation.

For each experiment, a control treatment without adsorbent (blank control) was included. All experiments were performed in duplicate.

Chromatographic analysis. HPLC analytical methods were used to determine mycotoxin concentration. An HPLC system (ICS, Bruges, France) equipped with a 20-µl injector loop, a C_{18} spherisorb column (Prontosil 120-3-C18, 25 by 0.4 cm), and a fluorescence detector (Shimadzu RF-10AXK) was run in a temperature-controlled room (25°C).

For ZEA evaluation, the mobile phase was acetonitrile-water (70:30, vol/vol) at a flow rate of 0.5 ml min^{-1}. The spectrofluorimetric conditions for ZEA were 275 nm for excitation and 450 nm for emission *(15)*.

The mobile phase for separation of OTA was methanol–acetonitrile–0.005 M sodium acetate (0.68 g $liter^{-1}$ in water) (300:300:400, vol/vol/vol) at a flow rate of 0.5 ml min^{-1}. The spectrofluorimetric conditions for OTA were 330 nm for excitation and 465 nm for emission *(17)*.



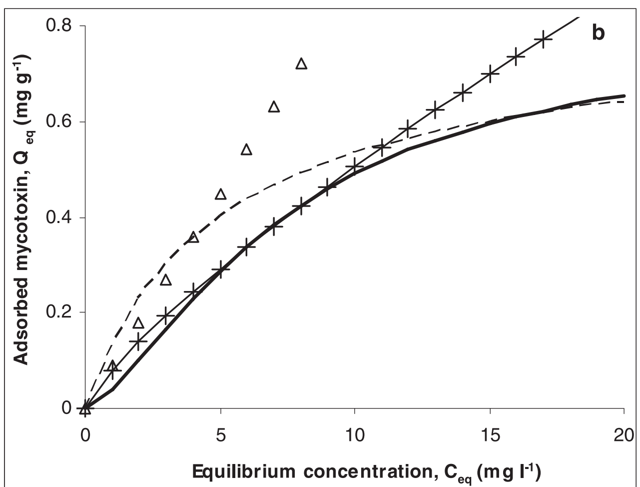


FIGURE 1. *Theorical percentage of adsorption (a) and isotherm shape (b) for four models: linear (△△△), Freundlich (+++), Langmuir (−−−), and Hill (—).*

AFB_1 was detected after derivatization in Kobra cells. The mobile phase was methanol-acetonitrile-water (200:200:600, vol/vol/vol) with 119 mg liter^{-1} potassium bromide and 350 μl liter^{-1} 4 M nitric acid at a flow rate of 0.5 ml min^{-1}. The spectrofluorimetric conditions for AFB_1 were 362 nm for excitation and 425 nm for emission *(18)*.

Adsorption calculation. The percentage of adsorbed mycotoxins was calculated from

$$\%\ \text{adsorption} = \frac{C_{\text{ads}}}{C_0} \times 100 = \frac{(C_0 - C_{\text{eq}})}{C_0} \times 100 \qquad (1)$$

where C_{ads} is the concentration of adsorbed mycotoxins (milligrams per liter), C_0 is the concentration of mycotoxins in the supernatant of the blank control (with no adsorbent) (milligrams per liter), and C_{eq} is the residual mycotoxin concentration in the solution at equilibrium (milligrams per liter).

The isotherm curves present the variation of Q_{eq} in relation to C_{eq}, where Q_{eq} is the quantity of adsorbed mycotoxin per gram of adsorbent (milligrams per gram), calculated as

$$Q_{\text{eq}} = \frac{(C_0 - C_{\text{eq}})}{m} \cdot V \qquad (2)$$

where V is the volume of the solution (liters) and m is the mass of the adsorbent (grams).

Isotherm curve fitting. Three equations were tested to fit the data obtained for isotherm curves: Freundlich, Langmuir, and Hill. The Freundlich model is based on the sorption onto a heterogeneous surface and described by

$$Q_{\text{eq}} = K_F \cdot C_{\text{eq}}^{1/n_F} \qquad (3)$$

where K_F is a constant related to the capacity of the adsorbent for the mycotoxin (milligrams$^{1-(1/n_F)}$·liter$^{1/n_F}$per gram) and n_F is a constant related to the affinity of the adsorbent.

The Langmuir model describes a monolayer sorption to a surface with a finite number of identical sites and is described by

$$Q_{\text{eq}} = \frac{Q_{\max} \cdot K_L \cdot \text{C}_{\text{eq}}}{1 + K_L \cdot C_{\text{eq}}} \qquad (4)$$

where K_L is the Langmuir adsorption constant related to affinity (liters per milligram) and $Q_{\max}$ is the capacity of the adsorbent to absorb the mycotoxin (milligrams per gram).

The Hill model is used to describe the binding of different species onto a heterogeneous substrate:

$$Q_{\text{eq}} = \frac{Q_{H\max} \cdot C_{\text{eq}}^{n_H}}{K_D + C_{\text{eq}}^{n_H}} \qquad (5)$$

where $Q_{H\max}$ is the maximal mycotoxin adsorption corresponding to the site saturation (milligrams per gram), K_D is the Hill constant (milligrams per liter), and n_H is the cooperativity coefficient of the binding interaction.

The mathematical shapes of these models (from equations 3 through 5) for $1/n_F < 1$, and $n_H > 1$, and the linear model are shown in Figure 1b. The shape of the adsorption percentage related to the initial concentration is presented in Figure 1a. Contrary to the linear model, these models allow description of a decrease in the adsorption percentage with an increase in the initial concentration of mycotoxin.

Two techniques could be used to determine the model parameters: linearization of the isotherm equation or nonlinear resolution by minimizing the sum of normalized errors *(20)*. In this study, a nonlinear method was used because linearization of nonlinear models could distort the fit, resulting in predicting errors. Nonlinear optimization provides a more complex yet mathematically rigorous method for determining isotherm parameter values *(26)*. The sum of the squares of errors (ERRQS) was minimized with a nonlinear method (generalized reduced gradient, GRG2) with Excel solver (Microsoft, Redmond, WA). The ERRQS was calculated as

$$\text{ERRSQ} = \sum_{i=1}^{p} (Q_{\text{eq,exp}} - Q_{\text{eq,calc}})_i^2 \qquad (6)$$

where $Q_{\text{eq,exp}}$ is the experimental value for Q_{eq}, $Q_{\text{eq,calc}}$ is the value of Q_{eq} calculated by the model, and p is the number of experimental values of Q_{eq} for the isotherm curves.

To compare the different models for a given mycotoxin, the total ERRQS for all the adsorbents was calculated as

$$\text{ERRSQ}_t = \sum_{i=1}^{n} (\text{ERRSQ})_i \qquad (7)$$

where n is the number of adsorbents (8) and ERRSQ_i is the ERRSQ for one absorbent for this mycotoxin.

Statistical methods. All experiments were carried out in duplicate, and the values are given as mean ± standard deviation.

The means were compared with an analysis of variance, with the significance level set at 5% ($P < 0.05$).

RESULTS AND DISCUSSION

Adsorption tests. The experimental conditions for reported in vitro experiments often are not sufficiently described to be reproduced or are so different (e.g., pH regulation or not, filtration versus centrifugation for adsorbent separation, and equilibrium time) that comparison of the results is difficult. No official method has been available to correctly define the adsorption of yeast products. Some guidelines have been published recently by the European Food Safety Authority *(7)*. The protocol described above in the ''Materials and Methods'' section was based on these recommendations, providing a standardized method for adsorption studies. The development of this protocol particularly focused on the following important points.

(i) The level of adsorbent (5 mg ml^{-1}) was sufficient to adsorb at least 20% of the mycotoxins.

(ii) The range of mycotoxin concentrations covered more than two or three orders of magnitude.

(iii) Six to eight points spread over the large range of concentrations were used for isothermal curves.

(iv) Centrifugation instead of filtration was used to separate adsorbent because this method provided better recovery of mycotoxin *(9)*.

(v) Experiments were carried out in centrifugation tubes to avoid loss during transfer.

(vi) The temperature was fixed at 37°C, including during centrifugation.

(vii) The pH was fixed with a buffer, and pH at equilibrium time was checked. A pH of 3 allowed sufficient adsorption of the three mycotoxins tested (data not shown).

(viii) The equilibrium time was previously determined by kinetic studies. The adsorption was very rapid, with no change after 15 min *(9)*.

(ix) The mycotoxin concentration was measured after centrifugation in both supernatant and solid phases after extraction to verify the mass balance. Because mass balances were satisfying at ±10%, only the concentration in supernatant was measured for adsorption calculation *(9)*.

(x) Preliminary tests were carried out to ensure that mycotoxins in buffer solutions were stable during adsorption tests (no degradation and no adsorption on tube walls).

The adsorption percentages obtained with this protocol in relation to the initial concentration of mycotoxin are presented in Table 2 for ZEA, AFB_1, and OTA. As expected, the adsorption percentage differed with the type and initial concentration of mycotoxin from 72% ± 3% for OTA with an initial concentration of 7.256 μg ml^{-1} to 2.5% ± 0.5% for AFB_1 with an initial concentration of 2.47 μg ml^{-1}. Regardless of the percent adsorption, standard deviations were quite low with only some exceptions, probably due to experimental errors. These standard deviations are the same order of magnitude as those previously reported under similar experimental conditions *(21)*.

For ZEA experiments, a decrease in the adsorption percentage was noted with the increasing initial concentration, indicating that sorption was not linear (see Fig. 1a). This finding also was reported by Yiannikouris et al. *(31)* at 37°C for initial ZEA concentrations of 1 to 20 μg ml^{-1}. This nonlinearity of the adsorption makes the comparison of the adsorption percentages among studies difficult. To our knowledge, only Sabater-Vilar et al. *(21)* have undertaken a study with conditions similar to ours. These authors reported adsorption percentages ranging from 3% for purified mannoproteins from yeast to 71% for YCW and up to 88% for purified β-glucans from yeast under conditions of pH 2.5, initial ZEA concentration of 1 μg ml^{-1}, and adsorbent concentration of 5 mg ml^{-1}. For similar initial concentrations, we found an adsorption percentage of 22% ± 9% to 62% ± 1%.

For AFB_1, adsorbance was globally inferior to that of ZEA; adsorption ranged from 2.5% ± 0.5% to 49.3% ± 0.5% depending on the AFB_1 concentration and the yeast product. A decrease in adsorption when the initial concentration increased also was observed for higher adsorption percentages, as was previously reported by Shetty et al. *(23)*. These authors studied the sorption of AFB_1 by 18 strains of *Saccharomyces* at pH 6, 25°C, and 30 mg ml^{-1} adsorbent. For an initial AFB_1 concentration of 5 μg ml^{-1}, Shetty et al. reported that 15 of the 18 strains adsorbed less than 40% of the initial AFB_1. For the strain with the best adsorption capacity, experiments were carried out by incubating with AFB_1 at 1 to 20 μg ml^{-1}. The strain bound 69.1% of the added toxin with 1 μg ml^{-1} AFB_1, 41% with 5 μg ml^{-1} AFB_1, and 34% with 20 μg ml^{-1} AFB_1. In 2010, Gallo and Masoero *(8)* reported adsorption percentages ranging from 32 to 54% for an initial AFB_1 concentration of 0.82 μg ml^{-1}.

For OTA, results indicated better adsorption than that for AFB_1, ranging from 14% ± 10% to 72% ± 3%. In addition, variation in the adsorption percentage with the initial concentration of OTA was observed. These results were similar to those of Ringot et al. *(20)*, who reported the sorption of this toxin by three YCW derivatives at 25°C and 50 mg ml^{-1} adsorbent (pH was not specified). The authors also found variation in adsorption with initial concentrations ranging from 0.5 to 10 μg ml^{-1}, but the adsorption percentages were not specified.

These results indicate that the sorption capacity of yeast products depends greatly on the initial concentration of the mycotoxin. Therefore, a comparison from single tests, frequently used in previous studies by assuming the linear sorption of the mycotoxin, is not adequate. When the isotherms are not linear, the comparison of adsorption capacity of yeast products could lead to opposite conclusions, depending on the initial mycotoxin concentration tested. We compared the adsorption of AFB_1 and OTA by Y1. For an initial concentration of about 0.05 μg ml^{-1} (0.058 μg ml^{-1} AFB_1 and 0.054 μg ml^{-1} OTA), adsorption was significantly higher for AFB_1 than OTA ($P = 0.0002$), whereas for an initial concentration of about 2.3 μg ml^{-1} (2.47 μg ml^{-1} AFB_1 and 2.23 μg ml^{-1} OTA), no significant difference in adsorption percentage was observed ($P =$

TABLE 2. *Percentage of three mycotoxins adsorbed onto eight yeast-based products*[a]

Zearalenone

	Initial concn ($\mu g\ ml^{-1}$):							
	0.59	1.24	5.82	23.15	48.22	56.51	64.77	72.9
Y1	32 ± 1	22 ± 9	30 ± 9	32 ± 3	51 ± 32	16 ± 14	24 ± 3	30 ± 3
Y2	47 ± 0.9	30 ± 3	30 ± 6	35 ± 2	30 ± 5	22 ± 1	17 ± 23	28 ± 12
Y3	65 ± 5	53 ± 2	56 ± 3	49 ± 4	44 ± 4	*28 ± 4*	*17 ± 1*	*38 ± 20*
Y4	68 ± 3	62 ± 1	62 ± 1	66 ± 1	53 ± 0.4	39 ± 4	27 ± 11	30.5 ± 0.1
Y5	62 ± 3	54 ± 1	47 ± 2	57 ± 0.2	46 ± 3	26.6 ± 6	*10.9 ± 0.5*	*16 ± 0*
Y6	60 ± 0.7	54 ± 2	55 ± 2	60 ± 1	46 ± 4	30 ± 5	*26 ± 4*	*20 ± 10*

	Initial concn ($\mu g\ ml^{-1}$):					
	0.41	0.966	4.979	27.74	45.3	64.9
Y7	23 ± 9	44 ± 4	41 ± 6	30 ± 1	28 ± 12	23 ± 7
Y8	31 ± 2	44 ± 8	49 ± 2	34 ± 9	32 ± 6	28 ± 14

Aflatoxin B_1

	Initial concn ($\mu g\ ml^{-1}$):						
	0.0058	0.0091	0.043	0.5	0.97	2.47	6.35
Y1	49.3 ± 0.5	41 ± 7	43 ± 3	40 ± 1	29 ± 0.3	20.4 ± 3	7.6 ± 0.9
Y2	10 ± 7	14 ± 6	19 ± 5	17 ± 1	29 ± 0.3	20 ± 3	7.6 ± 0.5
Y3	23 ± 16	24 ± 4	19 ± 5	17 ± 1	20.6 ± 0.8	13.6 ± 0.6	10 ± 4
Y4	15 ± 7	21 ± 2	18.1 ± 0.1	25 ± 0.1	13 ± 4	*4 ± 2*	29 ± 2
Y5	23 ± 4	26.5 ± 0.5	40 ± 2	44 ± 1	30 ± 11	17 ± 2	19 ± 3
Y6	7 ± 2	17 ± 2	14 ± 8	17 ± 8	13 ± 1	2.5 ± 0.5	3 ± 4

	Initial concn ($\mu g\ ml^{-1}$):						
	0.009	0.013	0.056	0.821	1.1337	1.170	4.571
Y7	31.2 ± 0.8	25 ± 3	25 ± 6	35 ± 1	23 ± 5	24.5 ± 0.5	25 ± 0.7
Y8	44 ± 2	33 ± 6	34 ± 1	28.7 ± 15	27 ± 5	29 ± 5	30 ± 1

Ochratoxin A

	Initial concn ($\mu g\ ml^{-1}$):								
	0.0054	0.0107	0.0415	0.643	0.969	2.234	3.482	7.256	10.05
Y1	19 ± 0.4	14 ± 10	19 ± 9	25 ± 2	16 ± 0.7	18 ± 5	28 ± 8	47 ± 6	42 ± 6
Y2	27 ± 2	19.5 ± 1	22.4 ± 5	26 ± 5	22 ± 0.5	23 ± 2	31 ± 8	32 ± 16	45 ± 5
Y3	37 ± 2	54 ± 2	56 ± 3	59 ± 0.5	56 ± 1	33 ± 4	38 ± 20	40 ± 2	65 ± 6
Y4	46 ± 4	48 ± 2	48 ± 2	52 ± 1	47 ± 1	48 ± 2	50 ± 1	50 ± 17	62 ± 1
Y5	58.5 ± 0.5	59 ± 0.4	62 ± 2	61 ± 1	56 ± 2	61 ± 3	58 ± 3	72 ± 3	64 ± 3
Y6	44 ± 1	45.7 ± 0.2	42.9 ± 1	49.4 ± 1	34 ± 2	48 ± 1	52 ± 1	62.2 ± 5	55 ± 13
Y7	39 ± 2	44 ± 0.5	48 ± 1	44 ± 1	35 ± 1	25 ± 1	40 ± 3	54 ± 12	38 ± 17

	Initial concn ($\mu g\ ml^{-1}$):							
	0.006	0.015	0.045	0.859	1.315	2.025	4.542	6.31
Y8	57.9 ± 4	73 ± 1	69 ± 1	66 ± 1	63 ± 3	63 ± 2	62 ± 4	52 ± 22

[a] Results are mean ± standard deviation adsorption percentages for each of the eight products (Y1 through Y8). The values in italics were not taken into account for isotherm model fitting.

0.65). We also compared the adsorption of ZEA by two adsorbents, Y1 and Y6. For 0.59 $\mu g\ ml^{-1}$ ZEA, the adsorption capacity of Y6 was significantly higher than that of Y1 ($P = 0.0004$), whereas at 72.9 $\mu g\ ml^{-1}$ no significant difference was observed between the two products ($P = 0.49$).

Thus, adsorption of mycotoxin by yeast products was not a linear phenomenon, which means that isotherm studies

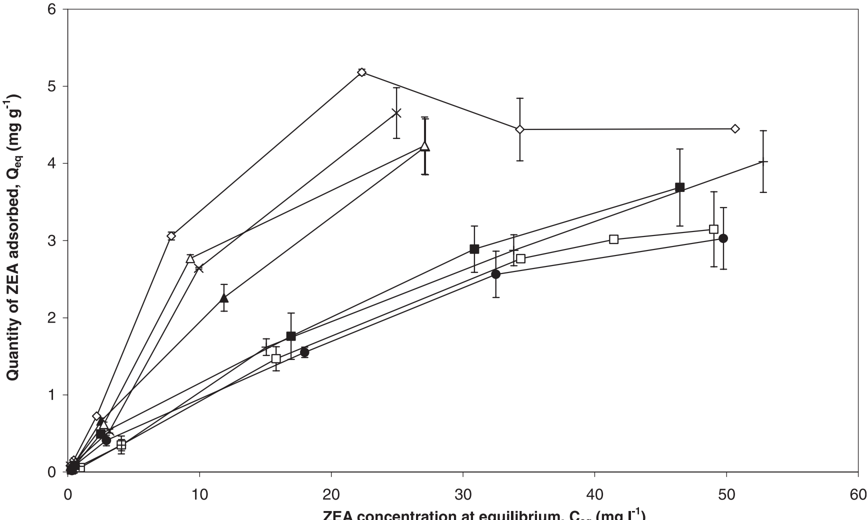


FIGURE 2. *Isotherm curves from experimental data for ZEA adsorption for Y1 (□), Y2 (+), Y3 (▲), Y4 (◇), Y5 (×), Y6 (△), Y7 (●), and Y8 (■). The bars are standard deviations.*

are necessary to compare the sorption capacity of yeast products.

Isotherm curves. Isotherm curves were plotted from each mycotoxin (Figs. 2 through 4). For ZEA (Fig. 2), some experimental data (in italics in Table 2) for Y3, Y5, and Y6 were removed from the graph because decreasing isotherms were observed for the higher equilibrium concentration that could not be explained. At low concentration, adsorption is independent of the initial concentration and could be considered linear, but up to some value (depending on the adsorbent), adsorption isotherms seem to indicate that the binding of ZEA is a process reaching saturation.

For AFB_1, this phenomenon was also observable for Y1, Y2, Y3, and Y6 but not for other adsorbents (Fig. 3). For OTA (Fig. 4), the process reaches saturation for most of the adsorbents. The isothermal curves do not have the same shape, suggesting that different mechanisms are involved in adsorption for the three mycotoxins. Therefore, different isothermal models must be used to fit these curves. In other studies, the most frequently used isotherm models for inorganic adsorbents are the Freundlich and Langmuir models *(7)*. For organic products, isothermal studies are very scarce, and the models used to describe sorption experimental isotherms of mycotoxins on yeast products are summarized in Table 3. The three models tested in the present study, Freundlich, Langmuir, and Hill, are the most frequently used in previous studies.

Tables 4, 5, and 6 present the values of the calculated parameters for the three models with the ERRSQ values for each adsorbent for ZEA, AFB_1 and OTA, respectively. For AFB_1 (Table 5) and OTA (Table 6), some parameter values given for the Hill model are outliers for Y5 and Y7 for AFB_1 and for Y8 for OTA. This finding corresponds to no apparent saturable isotherms on Figures 3 and 4, so the convergence of the model was difficult. The Hill model does not seem to be suitable for mycotoxins AFB_1 and OTA.

To compare the three models (except Hill for AFB_1 and OTA), the best model was determined in relation to each mycotoxin rather than to each adsorbent. Although the adsorbents have different compositions (Table 1), they are all yeast products and a single model for a given mycotoxin is needed to compare the adsorption capacity of several adsorbents.

The values of $ERRSQ_t$ for each mycotoxin are presented in Tables 4 through 6. The most suitable model

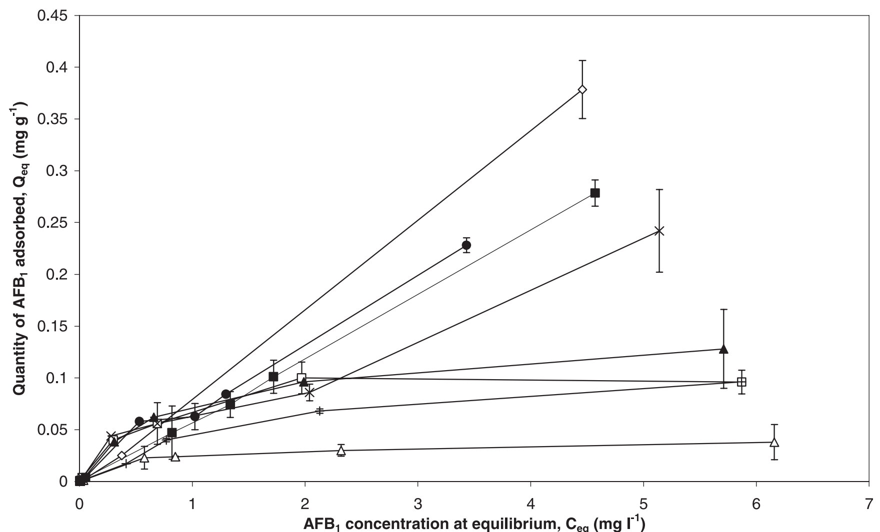


FIGURE 3. *Isotherm curves from experimental data for AFB_1 adsorption for Y1 (□), Y2 (+), Y3 (▲), Y4 (◇), Y5 (×), Y6 (△), Y7 (●), and Y8 (■). The bars are standard deviations.*

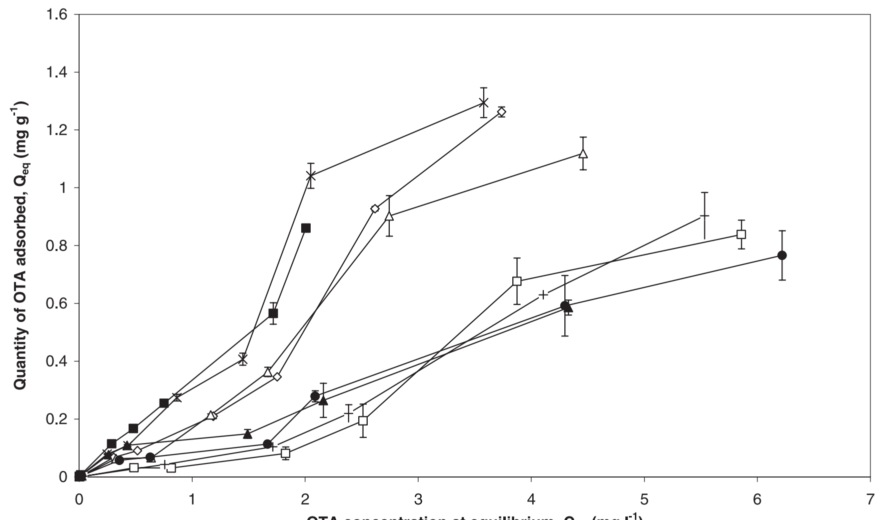


FIGURE 4. *Isotherm curves from experimental data for OTA adsorption for Y1 (□), Y2 (+), Y3 (▲), Y4 (◇), Y5 (×), Y6 (△), Y7 (●), and Y8 (■). The bars are standard deviations.*

was the Hill model for ZEA (with $n_H > 1$), the Langmuir model for AFB_1, and the Freundlich model for OTA ($n_F < 1$).

Comparison with previously published studies is difficult because of the different experimental conditions (pH, concentrations, and temperature) used by other authors (Table 3). However, our results are similar to those of Yiannikouris et al. *(31)* for ZEA and Ringot et al. *(20)* for OTA, even though those authors presented a model for each yeast product tested.

Different models were deemed suitable for each of the three mycotoxins because adsorption depends largely on properties of both the mycotoxin and the yeast product. Yeast cells have three main constituents that are involved in adsorption: 30 to 60% polysaccharides (β-glucans and mannans), 10 to 15% proteins, most of them linked to mannans and so-called mannoproteins, and 5 to 20% lipids. The physical properties of mycotoxins, such as polarity, solubility, size, specific shape, and for ionized compounds charge distribution and dissociation constants, play a significant role in the adsorption processes. The complex mechanism of mycotoxin adsorption on YCW has not been well studied and still is not fully understood. Yiannikouris et al. *(29, 31)* and Jouany et al. *(12)* reported that for ZEA, β-D-glucans of YCW are the main organic components involved in adsorption. These authors found that hydroxyl, ketone, and lactone groups of the mycotoxin are involved in both hydrogen bonds and van der Waals interactions with the hydroxyl groups and rings in glucans, respectively. These authors also obtained similar results with AFB_1 *(28)*. However, the amount of β-D-glucans did not seem to be the only explanation, because adsorption is also favored by a

TABLE 3. *Isotherm models used in other studies for the three mycotoxins*

Mycotoxin	Adsorbent	Experimental conditions	Model		Reference
			Choosen by the authors	Tested by the authors	
Zearalenone	Yeast cell walls 100 µg ml^{-1}	2–20 µg ml^{-1} pH not specified 27°C	Hill	Langmuir Hill	*31*
	Yeast cell walls 5 mg ml^{-1}	1–80 µg ml^{-1} pH 3 37°C	Hill	Langmuir Freundlich Hill	This work
Aflatoxin B_1	Yeast cell walls 5 mg ml^{-1}	0.05–10 µg ml^{-1} pH 3 37°C	Langmuir	Langmuir Freundlich Hill	This work
Ochratoxin A	Yeast by-product EX16 50 mg ml^{-1}	0.5–10 µg µl^{-1} pH not specified	Hill	Langmuir Freundlich	*20*
	Yeast by-product BETA 50 mg ml^{-1}	25°C	Freundlich	Hill Brunauer-Emmet-Teller	
	Yeast by-product LEC 50 mg ml^{-1}		Brunauer-Emmet-Teller	Redlich-Peterson Radke-Prausnitz Toth	
	Yeast cell walls 5 mg ml^{-1}	0.05–10 µg ml^{-1} pH 3 37°C	Freundlich	Langmuir Freundlich Hill	This work

TABLE 4. *Parameter values for isotherm models and mathematical affinity criteria* (A_H, A_L, A_F) *for ZEA adsorption by eight yeast-based products*

Model	Variable	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8
Hill	$Q_{H\max}$	4.402	6.814	3.145	4.477	5.116	4.149	5.200	6.610
	n_H	1.432	1.188	1.983	2.234	1.842	1.941	1.080	1.105
	K_D	98.917	84.229	27.899	37.652	63.837	37.835	47.965	57.355
	ERRSQ	0.0123	2.2003	2.4323	0.8455	0.0160	0.3044	0.0335	0.0557
	ERRSQ_t				5.900				
	A_H	0.445	0.408	1.467	2.206	1.340	1.596	0.361	0.424
	Ranking[a]	3	3	2	1	2	1, 2	3	3
Freundlich	K_F	0.175	0.183	0.783	0.966	0.403	0.751	0.223	0.234
	n_F	1.319	1.285	2.724	2.212	1.347	2.192	1.476	1.385
	ERRSQ	0.130	2.272	4.649	3.298	0.373	2.249	0.070	0.025
	ERRSQ_t				13.070				
	A_F	0.175	0.189	0.296	0.521	0.397	0.411	0.201	0.224
	Ranking[a]	4	4	2, 3	1	2	2, 3	4	4
Langmuir	$Q_{\max}$	9.688	9.983	3.581	5.891	9.427	4.879	5.991	8.807
	K_L	0.0107	0.0182	0.1592	0.1245	0.0346	0.1111	0.0212	0.0155
	ERRSQ	0.0715	2.2504	2.9338	1.3073	0.1945	0.8830	0.0309	0.0335
	ERRSQ_t				7.704				
	A_L	1.002	1.814	2.041	4.323	3.079	2.646	0.759	1.205
	Ranking[a]	4	3	2, 3	1	1, 2	2	4	4
Experimental ranking[a]		3	3	2	1	1, 2	2	3	3

[a] Same ranking number means no significant difference ($P < 0.05$).

more flexible cell wall conformation, which differs by yeast strain. For AFB_1, the shape of the adsorbent is an important parameter because this mycotoxin is an aromatic planar molecule that exhibits very high affinity to planar adsorbents *(7)*. Other authors suggested that mannan components of the YCW play a major role in aflatoxin binding by *S. cerevisiae (6)*. Both glucans and mannans should be involved in AFB_1 adsorption; several authors have reported the high efficiency of a commercial glucomannan-containing yeast product for adsorption of AFB_1 *(6, 13)*.

In contrast to the situation for AFB_1 and ZEA, mannoproteins were reported to be responsible for OTA removal *(1–3)*. This finding was confirmed by Yiannikouris et al. *(28)*, who reported that adsorption of OTA on β-D-glucans was very low compared with adsorption of AFB_1

TABLE 5. *Parameter values for isotherm models and mathematical affinity criteria* (A_H, A_L, A_F) *for* AFB_1 *adsorption by eight yeast-based products*

Model	Variables	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8
Hill	$Q_{H\max}$	0.105	0.113	0.154	2.368	3,530.62	0.138	417.68	2.495
	n_H	1.302	1.217	0.921	1.164	0.986	1.895	0.956	1.136
	K_D	0.397	1.540	1.053	29.925	74,511.2	0.401	6,008.25	29.649
	ERRSQ	0.00020	0.00003	0.00002	2.10^{-8}	0.00158	0.00002	0.00050	0.00003
	ERRSQ_t				0.00236				
	A_H	0.53	0.20	0.36	0.32	0.10	0.56	0.12	0.32
Freundlich	K_F	0.060	0.040	0.065	0.073	0.061	0.022	0.071	0.081
	n_F	2.920	1.933	2.371	0.911	1.213	2.859	1.061	0.933
	ERRSQ	0.00111	0.00023	0.00046	7.10^{-8}	0.00125	0.00010	0.00050	0.00002
	ERRSQ_t				0.00366				
	A_F	0.020	0.026	0.031	0.097	0.066	0.007	0.085	0.106
	Ranking[a]	4	4, 5	4	1	3	5	2	1
Langmuir	$Q_{\max}$	0.112	0.129	0.147	3.594	1.794	0.040	1.927	4.452
	K_L	1.824	0.513	1.073	0.026	0.030	1.943	0.038	0.020
	ERRSQ	0.00024	0.00004	0.00003	0.00011	0.00154	0.00001	0.00055	0.00027
	ERRSQ_t				0.00279				
	A_L	0.023	0.009	0.023	0.339	0.096	0.003	0.142	0.402
	Ranking[a]	3	3	3	1	2	4	1, 2	1
Experimental ranking[a]		4	4, 5	4	1	3	5	2	1

[a] Same ranking number means no significant difference ($P < 0.05$).

TABLE 6. *Parameter values for isotherm models and mathematical affinity criteria (A_H, A_L, A_F) for OTA adsorption by eight yeast-based products*

Model	Variable	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8
Hill	$Q_{H\max}$	0.870	1.477	2.369	1.686	1.518	2.627	1.127	7,563.00
	n_H	5.362	2.574	1.368	2.954	2.712	3.072	1.875	1.226
	K_D	434.96	51.818	22.905	15.749	4.604	20.016	14.293	22,498.2
	ERRSQ	0.0034	0.0151	0.0099	0.0204	0.0587	0.0074	0.0083	0.0155
	$ERRSQ_t$				0.1387				
	A_H	0.701	0.797	0.600	1.659	2.163	2.477	0.683	5.333
Freundlich	K_F	0.0723	0.0593	0.1249	0.1978	0.3760	0.2365	0.1137	0.3510
	n_F	0.7022	0.6222	0.9620	0.6978	0.9871	0.9236	0.9404	0.8587
	ERRSQ	0.0495	0.0196	0.0074	0.0346	0.0999	0.0574	0.0120	0.0151
	$ERRSQ_t$				0.2955				
	A_F	0.111	0.096	0.160	0.305	0.474	0.312	0.148	0.484
	Ranking[a]	3	3	3	2	1	2	3	1
Langmuir	$Q_{\max}$	80.771	80.427	80.470	64.554	15.309	20.539	10.315	30.817
	K_L	0.0017	0.0018	0.0016	0.0049	0.0267	0.0133	0.0129	0.0127
	ERRSQ	0.0822	0.0662	0.0076	0.09687	0.0992	0.0620	0.0137	0.0192
	$ERRSQ_t$				0.4469				
	A_L	11.151	11.898	10.613	20.426	6.263	5.597	1.372	12.050
	Ranking[a]	2	2	2	1	3	3	4	2
Experimental ranking[a]		3	3	3	2	1	2	3	1

[a] Same ranking number means no significant difference ($P < 0.05$).

and ZEA. These authors preferred the exponential model for OTA rather than the Hill model.

Thus, yeasts or YCW should more efficient than purified extract for simultaneous adsorption of several mycotoxins.

Comparison of product adsorption capacities. Depending on these models, we put forward mathematical affinity criteria to quantify the adsorption capacity of the yeast products tested.

For the Hill model, the mathematical affinity criterion A_H was inspired by the work of Yiannikouris et al. *(31)*:

$$A_H = \frac{Q_{\max}}{2 \cdot \sqrt[n_H]{K_D}} \cdot \frac{m}{V} \quad (8)$$

This criterion is the ratio between concentrations (milligrams per liter) of adsorbed and free mycotoxin for the half-saturation. It takes into account the increase of adsorption with the increase of $Q_{\max}$ and the decrease of $\sqrt[n_H]{K_D}$.

For the Langmuir model, we proposed the mathematical affinity criterion A_L calculated as

$$A_L = {Q_{\max}}^2 \cdot K_L \quad (9)$$

This criterion takes into account the increase of adsorption with the increase of $Q_{\max}$ and K_L.

For the Freundlich model, the proposed mathematical affinity criterion A_F was calculated as

$$A_F = \frac{K_F}{2 \cdot (0.5)^{n_F}} \cdot \frac{m}{V} \quad (10)$$

This criterion is the ratio between concentrations (milligrams per liter) of adsorbed and free mycotoxin for the half-saturation. This criterion takes into account the increase of adsorption with the increase of K_F and n_F.

Values of A_H, A_L, and A_F are presented in Tables 4 through 6 for each mycotoxin. From these results, a classification of the performance of the adsorbent was established for each model (except for the Hill model for AFB_1 and OTA). In these tables, the ranking of each adsorbent is given from the best (1) to the worst (8). Products with different ranking numbers were considered significantly different in their adsorption ($P < 0.05$).

These rankings for individual models can be compared with the experimental ranking, which was based directly on the experimental data (Figs. 2 through 4). Similar values for experimental ranking and individual model ranking were obtained for the Hill model for ZEA, the Langmuir model for AFB_1, and the Freundlich model for OTA. These findings support the choice of these models to describe the adsorption of the three mycotoxins by yeast products.

The comparison of the adsorption performance of these yeast products revealed that for ZEA, Y4 was the most efficient followed by Y5, Y6, and Y3, even though these last three products are very different (Table 1) in terms of glucan, mannan, and protein composition. For AFB_1, Y4 and Y8 were the most efficient followed by Y7. For OTA, Y5 and Y8 were the most efficient followed by Y4 and Y6. For the three mycotoxins, there was no evidence that whole yeast products (Y3, Y5, and Y8) were more or less efficient than YCW products. No direct correlation was found between the adsorption capacity and the main characteristics of the yeast products as shown in Table 1 (data not shown). Yeast products (Y3, Y5, and Y8) and YCW were investigated both separately and combined. For yeast products, adsorption mechanisms are complex, probably because more than one site is available for mycotoxin and configuration and shape of the sorption site is important.

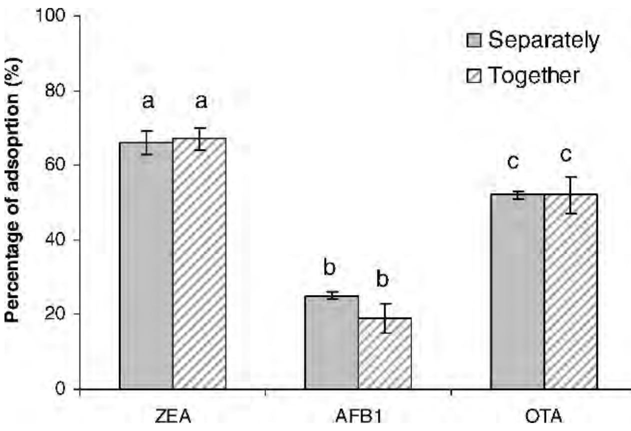


FIGURE 5. *Comparison of Y4 product adsorption percentages for the three mycotoxins separately or together. Initial concentrations were 20 μg ml^{-1} ZEA, 0.5 μg ml^{-1} AFB$_1$, and 0.5 μg ml^{-1} OTA. Same letter indicates no significant difference (*$P < 0.05$*).*

More investigations coupled with multiple regressions are needed to understand these mechanisms.

These results also indicate that the differences in product properties make it difficult to find a single yeast product that can efficiently adsorb all three mycotoxins *(14)*. Because of the low mycotoxin concentrations, competition was not expected. The possibility of a competition effect was tested in the laboratory by comparing the adsorption percentage of the three mycotoxins separately or together for an initial concentration of 20 μg ml^{-1} for ZEA, 0.5 μg ml^{-1} for AFB$_1$, and 0.5 μg ml^{-1} for OTA. Results for Y4 are presented in Figure 5. The adsorption of each mycotoxin was not significantly influenced by the presence of the others at the concentrations tested. We determined which yeast product was the best compromise for the simultaneous adsorption of ZEA, AFB$_1$, and OTA by multiplying the mathematical affinity criteria proposed here for each mycotoxin separately. Thus, Y4 was considered the best compromise for the simultaneous adsorption of the three mycotoxins followed by Y8 and Y5. Two of these three products are yeast cells (Y8 and Y5). Depending on the mycotoxin concentrations, Y4 could adsorb up to 68% $\pm$ 3% of the ZEA, 29% $\pm$ 2% of the AFB$_1$ and 62% $\pm$ 1% of the OTA (Table 2).

The nonlinear shape of the isotherm curves presented here for ZEA, AFB$_1$, and OTA indicates a more complex behavior than previously described for these mycotoxins. Our results suggest multisite adsorption by yeasts, with several compounds involved in the binding. Therefore, single tests must be used with caution when comparing the adsorption capacity of various products. The proposed methodology based on isotherm curves allows a reliable comparison of the adsorption capacity of yeast-based products. A better characterization of the yeast products will be necessary to identify the cell surface binding structures involved in adsorption of mycotoxins. The results of this study should make it possible to select the best performing yeast product or mixture of yeast products for adsorption of mycotoxins in food and feed.

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