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Evaluations of Different Sources of *Saccharomyces cerevisiae* to Binding Capacity of Aflatoxin B_1 Utilizing their Adsorption Isotherms

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Abstract

The aim of the present study was to determine the ability of four different sources of *Saccharomyces cerevisiae* (autolyzed yeast, brewer's yeast, cell wall yeast and inactivated yeast) to adsorb aflatoxin B_1 through adsorption isotherms. For this, a phosphate buffered saline solution containing 2 µg of AFB_1/ml was prepared and the yeast with a variant concentration of 0.05 g to 1.0 g was added and incubated at 10, 20 and 30 mins. After the incubation time the quantification was made by high performance liquid chromatography. It was verified through the isotherms that the absorption of aflatoxins increased as a result of yeast concentration and that the best time for action was 20 mins. Also, the application of the extended Langmuir model was more adequate for brewer's yeast and cell wall, and these were more effective in the adsorption. In contrast, the autolyzed and inactivated yeast presented an inverse behavior, reducing the percentage of adsorption by increasing the amount of free aflatoxin; being able to be recurrent of the reversibility of the reaction (30 min) and reduction in the adsorptive capacity (10 min); related to the state of the β-D-glucans present in the cell membrane and the occupation of the yeast binding sites; adapting better to the Freundlich model.

Keywords

Isotherm, *Saccharomyces cerevisiae*, Aflatoxin B_1, Decontamination

Abbreviations

AFB_1: Aflatoxin B_1; AFM_1: Aflatoxina M_1; IWPC: Cell Wall Yeast; RYLA: Autolyzed Yeast; RLC: Brewer's Yeast; SYL: Inactivated Yeast

Introduction

Mycotoxins are a result of secondary metabolism of molds, have high toxicity, low molecular weight and are thermally stable [1, 2]. Various genera of molds can produce mycotoxins in foods under favorable conditions. The factors that affect the development of mycotoxins are relative humidity (must be greater than 80%) and temperature (varies according to the fungal species) [3, 4]. The presence of mycotoxins in foods is a serious problem from both a public health concern and for industry and consumers because of the reduced quality of the food [5].

Aflatoxins are of great importance in feed and food (peanuts, beans, corn, rice, wheat, etc.). They are produced by different genera of fungi, *Penicillium*, *Fusarium* and especially *Aspergillus* (*flavus*, *parasiticus* and *nomius*) [6-9]. Currently, there are 18 similar compounds known as aflatoxin, however, aflatoxin B_1 (AFB_1), aflatoxin

B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) are the most relevant public health concerns [10].

Aflatoxin B_1 is the most important metabolite due to its higher toxicity and is considered to be the most potent hepatocarcinogen in mammals. This mycotoxin which has received increased attention because it has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen and is responsible for the formation of hepatocellular carcinoma in humans [11-13]. When ingested by animals, especially dairy cattle, aflatoxin B_1 is absorbed from the gastrointestinal tract and biotransformed by the hepatic system, into aflatoxin M_1 (AFM_1), a compound that can be excreted in milk and biological fluids of these animals [14-16].

Chronic exposure to aflatoxins can cause reduced productivity in animals and agricultural inputs, resulting in decreased quality of products which poses a great risk to consumers. Therefore, to reduce the exposure of consumers, methods of decontamination are needed, that can be chemical, physical or biological methods [17, 18].

The research on detoxification procedures for aflatoxins in contaminated food products have historically been conducted by using two main approaches, one aiming to modify the chemical structure of the toxin into less toxic compounds (decontamination), and another aiming to remove the unaltered aflatoxins from the product or reduce its bioavailability in the food or feed. In both cases, the procedures may be based on physical (for example inactivation of the aflatoxins by high temperatures and removal by extraction with solvents), chemical (for example degradation of aflatoxin molecules by means of acids, bases, aldehydes) and biological (involve the use of bacteria, yeast or respective enzymes) methods [19, 20].

Currently, biological methods are being widely studied as a means of controlling aflatoxins to protect the quality of food or feed because they are considered safer. This is because most inorganic adsorbents cannot adsorb a wide range of mycotoxins and may have adverse nutritional effects, need to be used in large quantities to obtain a noticeable effect and may also reduce the bioavailability of minerals or vitamins in diets. In addition, due to consumer resistance to physical and chemical treatments [17, 21-23].

As reported by Joannis-Cassan et al. [24], several studies have tested the adsorption capacity of yeast products for various mycotoxins. However, most of these studies involved a single test, that is, the determination of the adsorption in only one concentration. These single concentration studies do not allow comparison of different experiments *in vitro* and extrapolation to other concentrations is difficult.

According to the literature, the isotherms were used in the evaluation of a number of mycotoxin sequestering agents [25, 26]. This kind of study (isothermal adsorption) are preferred because they give a more complete and reliable image of adsorption [25].

In this context, the objective of this study was to evaluate the ability of different sources of *Saccharomyces cerevisiae* to adsorb aflatoxin, by analyzing their behavior at different incubation times, through their adsorption isotherms.

Materials and Methods

Sources of *Saccharomyces cerevisiae*

The four different sources of *Saccharomyces cerevisiae* used in the experiment were immuno wall (prebiotic additive and functional fiber consisting of MOS and ß-glucans), autolyzed yeast (soluble solids of fermentation - dry yeast together with the fermentative medium), inactivated yeast (inactivated yeast from the fermentation of sugarcane ethanol) and brewer's yeast (100% natural yeast by *S. cerevisiae*) were provided by ICC® (Brazil) to the Laboratory of Food Microbiology and Mycotoxicology, College of Animal Science and Food Engineering of University of Sao Paulo, Brazil.

The number of yeast cells in the products was determined by light microscopy using a modified Neubauer chamber. The products were weighed to reaching a cell concentration of 1.0×10^{10} cells mL^{-1}. All SC cells were heat-killed, being inactivated by autoclaving at 121 °C for 10 mins before the binding assays, to avoid any possible fermentation during the contact time.

Aflatoxin adsorption trials

AFB_1 was purchased from Sigma Aldrich Company (St. Louis) and dissolved in acetonitrile to get a stock solution containing 100 µg AFB_1/mL. The stock solution was used to prepare the working standard solution, containing 2 µg AFB_1/mL. The trials intended to evaluate the affinity of the different sources of yeast to adsorb aflatoxin were carried out using adsorption isotherms. Four isotherms were made to each type of yeast, aiming to evaluate the influence of the incubation time on the adsorption capacity.

The yeast products were prepared by weighing from 0.05 to 1.0 g (of each yeast) and 3 mL of working standard solution were added to them. Samples were subjected to three different incubation periods (10, 20, 30 mins), then placed on a shaker and incubated at 25 °C and then centrifuged at 4000 rpm for 20 mins. The supernatant was filtered through a PTFE filter (0.45 mm) and analyzed using high-performance liquid chromatography.

Aflatoxin quantification using HPLC

To evaluate the AFB_1 samples a Shimadzu® High Performance Liquid Chromatograph (HPLC) system (Tokyo, Japan) was used, consisting of an RF-10A XL fluorescence detector (Shimadzu®) equipped with a Synergy Fusion 4 µm C18 column 4.6 × 150 mm (Phenomenex®, Torrance, USA) and the SIL-10AF autosampler (Shimadzu®). A flow rate of 1 ml/min with a mobile phase containing water, acetonitrile and methanol (60: 20: 20) was used. Detection was done at an excitation wavelength of 360 nm and emission at 440 nm. The limit of detection (LOD) was calculated based on a signal/noise ratio of 3:1, its value being 0.5 µg/kg.

$$A = \{[B-C-D] / B\} * 100 \qquad \text{Eq.(1)}$$

With a being the percentage of AFB_1 adsorbed by the yeast products. B, concentration of the buffered standard of AFB_1. C, the concentration in the yeast + solution of AFB_1 and D concentration of interferences- negative control (buffer solution + yeast).

Statistical analysis

The results of the experiment were submitted to analysis of variance according to the procedures established in the *General Linear Model of SAS* [27] to verify statistically significant differences between the means of the studied variables.

Results and Discussion

In order to obtain the isotherms, it was necessary to calculate the amount of aflatoxin adsorbed by the percentage resulting from the calculation with the areas; using Equation 2. By means of the study of the adsorption isotherms of the different sources of yeasts, some analyzes were done in relation to the binding capacity of AFB_1 with *Saccharomyces cerevisiae*, being observed a variation in the behavior according to the time of contact (10, 20 and 30 mins) and with the yeast concentration used (0.05 to 1.0 g).

$$AFB_1^{absorbed}\left(\frac{mg}{g}\right) = 2\left(\frac{ug}{mL}\right) AFB_1 * \frac{3(ml)}{[yeast](mg)} * \% \quad \text{Eq.(2)}$$

In the isotherms, the adsorption model was applied, which presented the best coefficient of determination for the adjustment of non-linear models (X^2), so that the models used were extended Langmuir and Freundlich classic and extended models. The Langmuir model (equation 3 and 4) considers homogeneous surface and the formation of only one layer, occurring then an absorption in only a finite number of sites. In the derivation of this model, homogeneous adsorption occurs in which each molecule has an activation and enthalpy energy [28]. Thus, the adsorption isotherms were obtained according to the equations that govern the model, being observed below:

$$Cads = \frac{\alpha\beta Ceq}{(1 + \alpha \text{Ceq})} \quad \text{Eq. (3)}$$

$$Cads = \frac{\alpha\beta Ceq^{1-c}}{1 + \alpha Ceq^{1-c}} \quad \text{Eq.(4)}$$

In which Cads represents the concentration of mycotoxin adsorbed in equilibrium, Ceq is the concentration of mycotoxin in the solution, α is the adsorption constant K_L (L/mg) and β is the maximum amount retained on the surface Qmax (mg/g). Therefore, by applying the model to the isotherms, one can obtain the parameters K_L and Qmax, and thus compare the behavior of yeasts and their absorptive potential [28].

However, the Freundlich model (equations 5 and 6) considers the formation of several layers and the logarithmic decrease of adsorption energy according to this increase [29]. The equations describing the classical and extended model are shown below, respectively; where corresponds to the Freundlich exponent and K_F is constant (mg/g). Both models cited for single-component $1/\eta_F$ systems only, are the most used since multicomponent mixtures can be complex to characterize the adsorption equilibrium [30].

$$\frac{1}{\eta_F} * Q_{eq} = K_F * C_{eq} \quad \text{Eq.(5)}$$

$$\left\{\left(\frac{1}{\eta_F}\right) * C_{eq}\right\}^{-c} * Q_{eq} = K_F * C_{eq} \quad \text{Eq.(6)}$$

Thus, it is possible to subdivide the isotherms according to the behavior of the different *Saccharomyces cerevisiae* sources (adsorption rate, how much aflatoxin was adsorbed and the amount of aflatoxin in solution) during the incubation time. For the time of 10 mins, brewer's yeast (RLC) and autolyzed yeast (RYLA) can be grouped because they have a lower spectrum (0.0012 - 0.0024 mg/L) relative to cell wall yeast (IWPC) and inactivated yeast (SYL), which presented a higher amount of AFB_1 in solution (0.005 - 0.025 mg/L), as can be seen in figure 1.

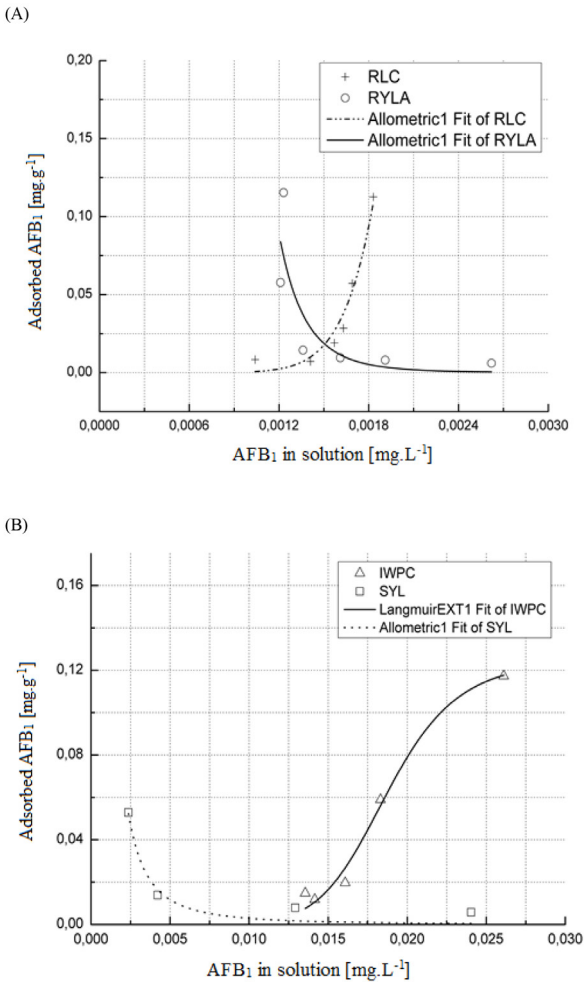

Figure 1: AFB_1 adsorption isotherm applying the classic Freundlich model **(A)** and the extended Langmuir and Freundlich classic **(B)** in time of 10 minutes.

It can be observed that lower values of aflatoxin in solution indicate faster adsorption, thus occurring in beer yeast and autolyzed. However, IWPC and SYL showed an unusual behavior, with the reduction of adsorption according to the increase of aflatoxin in solution, which may be the result of the reversion of the yeast binding with aflatoxin. The RLC and IWPC showed the increase of the adsorption according to the increase of aflatoxin in solution.

For the time of 20 mins, the yeasts SYL, RLC and RYLA showed the expected behavior, that is, increased adsorption of aflatoxins indicating the application of the Langmuir model as the most appropriate for the time. By the isotherms, it is observed that the application of cell wall yeast was more effective, since it indicated larger amounts of aflatoxins adsorbed in lower concentrations. For the IWPC, a separate isotherm was applied, since it presented different behavior from the others, resulting in lower values of free AFB_1 (0.0003 - 0.0012 mg/L), as can be seen in figure 2.

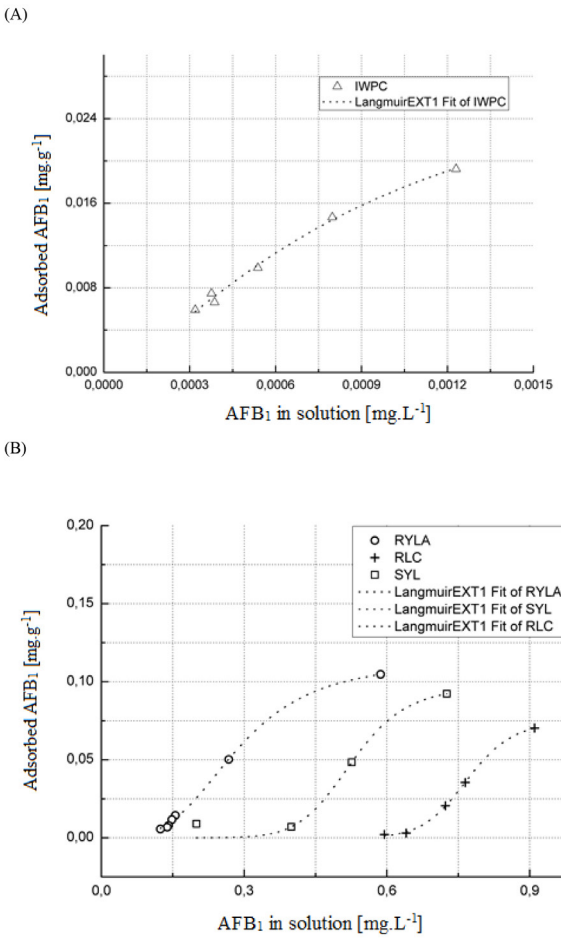

Figure 2: AFB1 adsorption isotherm applying the extended Langmuir model **(A)** and the extended Langmuir model **(B)** in the time of 20 minutes.

Then, for the longer incubation time, all yeasts showed a concentration of aflatoxin in a similar solution (0.2-0.8 mg/L). However, two isotherms were constructed to facilitate visualization of yeast behavior (Figure 3). It was observed that the autolyzed and inactivated yeast presented a behavior similar to the autolyzed yeast in the time of 10 mins, that is, decrease of the adsorption of 0.45 mg/L of free AFB_1 in the medium, being able to indicate that this yeast is not ideal for application in aflatoxin adsorption.

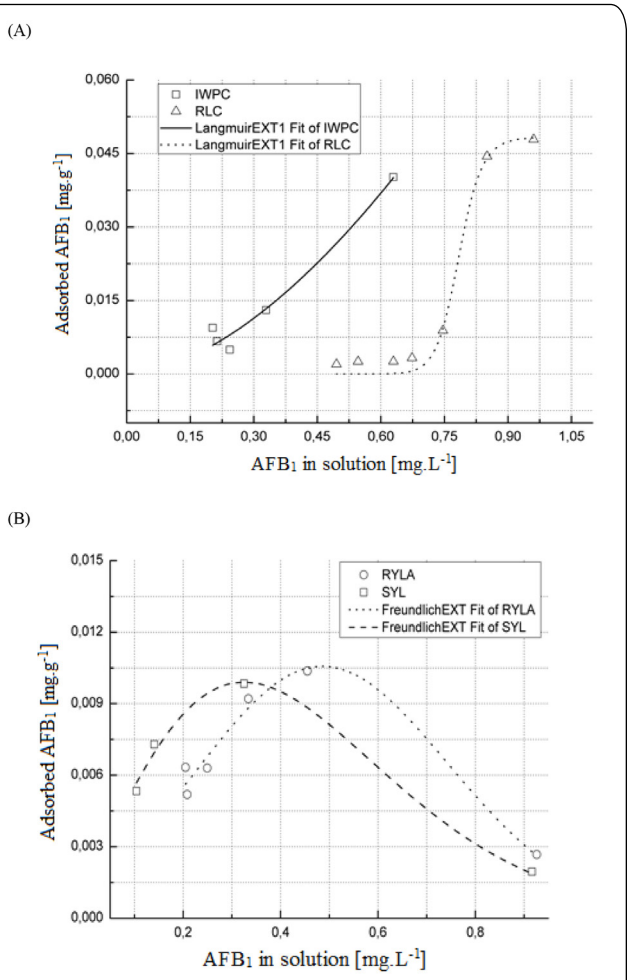

Figure 3: AFB_1 adsorption isotherm applying the extended Langmuir model **(A)** and Freundlich extended model **(B)** in the time of 30 minutes.

In addition, it is observed that although the IWPC and RLC present the expected increase in adsorption, the construction of the adsorptive curve was different; and therefore it will be necessary to evaluate the significance behind the behavior of the curves to understand which yeast obtained the greatest efficiency at this time. Also, it is noted that brewer's yeast required high concentrations of aflatoxin in the medium to start the adsorption process, however it presented the highest amount of adsorption among all yeasts at this time.

It is observed that each yeast tested showed a behavior at each incubation time. Thus, the best incubation time, at which time the yeast adsorbed the highest amount of aflatoxin at lower concentrations, differed among the yeasts. For cell wall yeast (IWPC), the best time was 10 min because, although it began to adsorb more rapidly in the time of 20 min, the adsorbed amount was lower. For inactivated (SYL), the best time was 20 mins, as well as for the autolyzed yeast (RYLA), since, during the time of 10 and 30 min, presented unusual

behavior. And for brewer's yeast (RLC), the best time was the lowest, 10 mins.

The Giles classification can be used to evaluate the isotherms and curves behavior, as it was observed that the yeasts presented different behaviors. In this, the isotherms are classified into four classes: "spherical", "langmuir", "high affinity" and "constant partition". Each class of these is divided into subgroups according to the shape of the curve, totaling twelve types of classification [31]. These can be seen in figure 4.

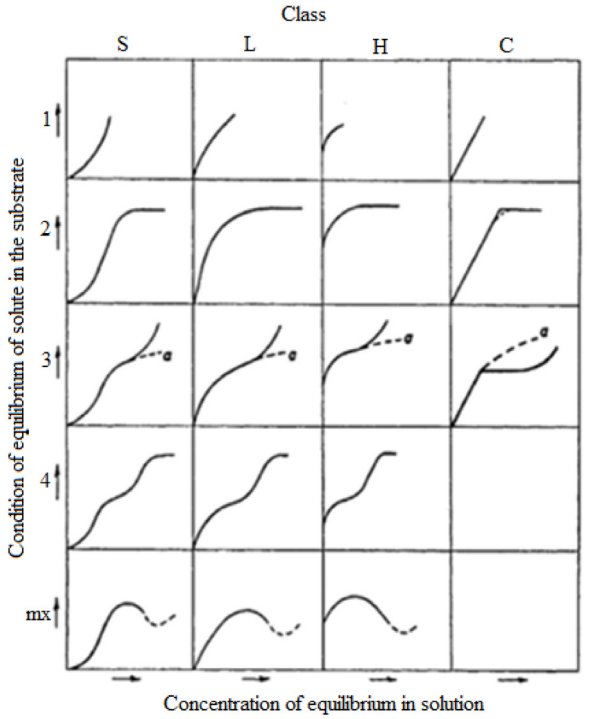

Figure 4: Adapted from Gyles classification for adsorption isotherms.

When analyzing the obtained isotherms, it can be observed that the cell wall and the brewer's yeast resemble the "spherical" class, in the time of 10 mins and 20 mins for the RLC and 30 mins for the IWPC. This type of curve indicates that the adsorption increases according to the number of molecules adsorbed, according to [24, 32], a cooperative interaction between the adsorbed molecules may have occurred, resulting in a low affinity between yeast and aflatoxin. However, the autolyzed, inactivated and cell wall ferment for the time of 20 mins and the brewer for 10 and 30 mins can be classified as Langmuir, since a behavior close to a concavity can be observed. The isotherm for inactivated and autolyzed yeasts at maximal tested time cannot be analyzed because their behavior does not fit those described by [31].

These differences may have occurred because the comparison of single tests, frequently used in previous studies, assuming linear mycotoxin adsorption, is inadequate. When isotherms are not linear, comparing the adsorption capacity of yeast products can lead to opposite conclusions, depending on the initial concentration of mycotoxins tested [24].

From the application of the Langmuir model, the following data were obtained from the parameters calculated with the aid of the program Origin, found in table 1 below.

Table 1: Application parameters of the extended Langmuir model.

Yeast	IWPC			RYLA	RLC		SYL
Time	10 min	20 min	30 min	20 min	20 min	30 min	20 min
K_L	3E + 14	2E + 04	1E - 02	76	65	1061	366
Q_{max}	0.124	0.031	7.288	0.113	0.075	0.048	0.097
-c	7.444	0.449	0.704	2.461	14.938	27.727	8.200
x^2	0.972	0.991	0.947	0.998	0.998	0.986	0.951

Therefore, it can be verified that, although the previous analysis indicates that brewer's yeast was closer to the other classification, the application of the Langmuir model generated a high coefficient of determination, with 20 mins the time that generated the largest X^2. The maximum amount adsorbed was very high for the cell wall yeast in the 30 mins, with 7.288 (mg/g), which may be due to the high contact time and the high value of the Langmuir coefficient, since smaller K_L values indicate that the absorbance has higher affinity with the absorbent [33].

In the isotherms in which the Freundlich model was applied, it was observed that the value of the determination coefficient for autolyzed yeast in 10 mins was lower than those observed in this experiment, justified by its adsorption reduction behavior that does not apply to the adsorptive models. Furthermore, the negative values in the Freundlich exponent were also the result of the non-adsorbing behavior of the yeasts autolyzed and inactivated at most times. The parameter c, used in the extended model, was only necessary for these to yeast in 30 mins, since at this time both aflatoxin uptake and expulsion can be observed (Table 2).

Table 2: Application parameters of the classic and extended Freundlich model.

Yeast	RYLA		RLC	SYL	
Time	10 min	30 min	10 min	10 min	30 min
1/η	-6.98	-7.07	9.16	-2.00	-5.00
K_F	3.49E - 22	0.00161	1.35E + 24	2.78E - 07	0.00125
x^2	0.622	0.949	0.962	0.927	0.977
-c	-	1.385	-	-	0.889

In general, it is noticed that the time of 20 mins presented the best development of the absorptive curve and the best adaptation to the Langmuir model; indicating therefore that this is the best time of application. Also, it was necessary to apply the extended Langmuir model to most of the yeasts, since the great dispersion of the values indicated the requirement of a more complex model, for a multicomponent system.

Regarding yeast binding capacity, [34] observed that the adsorption by yeast application showed a higher efficiency when smaller concentrations of mycotoxin were used. Also, *in vitro* studies indicated that the adsorption is concentration-dependent and reversible, which may justify the behavior

of the autolyzed and inactivated yeast in 30 mins, in which absorption decreased [35].

This behavior was observed by the study of [36], in which a proportional inverse relationship between the toxin concentration and the adsorption process occurred in the application of *S. cerevisiae* in maize with AFB_1. Low adsorption percentages (16 to 66%) were obtained for high concentrations of aflatoxin 800 µg/kg when compared as percentages obtained at a concentration below 150 µg/kg (40 to 93%); being justified by the saturation of the yeast binding sites in a nonlinearity line in the absorptive process.

Brewer's yeast and cell wall yeast, in general, had the highest amount of aflatoxin adsorbed. It is important to note that β-D-glucans located in the yeast cell wall are responsible for the adsorption of mycotoxin, as well as the presence of weak intermolecular bonds in yeast, such as Van der Waals [37].

For autolyzed yeast, there is cell wall breakdown and release of β-D-glucans not previously available in the medium; however, studies indicate that there is a reduction in the adsorptive capacity when there is lysis of the cell wall [38]. Inactivated yeast is obtained through drying, which can result in the reduction of carbohydrate content by cell autolysis; and therefore, reduction in the amount of β-D-glucans [39].

Conclusion

The application of autolyzed yeast, inactivated yeast, cell wall and brewer's yeast for adsorption of aflatoxins is feasible. Among the incubation times, the yeast showed the best behavior was that of 20 mins, since all showed an increase in the amount of AFB_1 adsorbed. In addition, it was observed that, in general, the application of the extended Langmuir model was the most adequate for cell wall and brewer's yeasts, since it had high determination coefficients and Qmax values, being considered more efficient in the adsorption of AFB_1. However, the autolyzed and inactivated yeast did not show the expected behavior in the time of 10 and 30 min, and may be justified by the reduction of the adsorption capacity when there are changes in the cell membrane and occupation of the binding sites. These fit better into the Freundlich model, which predicts the formation of a multilayer, which may be justified by the reversibility of adsorption and interaction between aflatoxin itself.

Conflict of Interest

The authors declare that the research was conducted in the absence of any interests.

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References

1. Bok JW, Keller NP, Lae A. 2004. A regulator of secondary metabolism in *Aspergillus spp. Eukaryot Cell* 3(2): 527-535. https://doi.org/10.1128/EC.3.2.527-535.2004
2. Diniz SSS. 2002. Micotoxinas. Editora Rural, Campinas, Brazil.
3. Jaiaranam P, Kalyanasundaram I. 1990. Natural occurrence of toxigenic fungi and mycotoxins in rice bran. *Mycopathologia* 110(2): 81-85. https://doi.org/10.1007/BF00446995
4. Spinosa HS, Górniak SL, Palermo-Neto J. 2008. Toxicologia aplicada à medicina veterinária. Manole, Barueri, Brazil.
5. Golblatt LA. 1969. Aflatoxin scientific background, control and implications. Academic Press, New York, USA.
6. Devegowda G, Raju MVLN, Swamy HVLN. 1998. Mycotoxins: novel solutions for their counteraction. *Feedstuffs* 70(50): 12-15.
7. Ritter AC, Hoeltz M, Noll IB. 2011. Toxigenic potential of *Aspergillus flavus* tested in different culture conditions. *Ciência e Tecnologia de Alimentos* 31(3): 623-628. https://doi.org/10.1590/S0101-20612011000300011
8. Moss MO. 2002. Risk assessment for aflatoxins in foodstuffs. *Int Biodeterior Biodegradation* 50(3-4): 137-142. https://doi.org/10.1016/S0964-8305(02)00078-1
9. Organización panamericana de la salud. 1983. Micotoxinas. Critérios de Salud Ambiental, 11. Washington: OMS.
10. Oliveira CA, Germano PM. 1997. Aflatoxins: current concepts on mechanisms of toxicity and their involvement in the etiology of hepatocellular carcinoma. *Rev Saude Publica* 31(4): 417-424. https://doi.org/10.1590/S0034-89101997000400011
11. Zain ME. 2011. Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society* 15(2): 129-144. https://doi.org/10.1016/j.jscs.2010.06.006
12. Creppy EE. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett* 127(1-3): 19-28. https://doi.org/10.1016/S0378-4274(01)00479-9
13. International Agency for Research on Cancer - World Health Organization. 2002. IARC monographs on the evaluation of carcionogenic risk to humans. Lyon, WHO.
14. Oathey JT, Rarick MD, Ji GE, Linz JE. 2000. Blinding of aflatoxin B_1 to *Bifidobacterium in vitro*. *J Food Prot* 63(8): 1133-1136. https://doi.org/10.4315/0362-028X-63.8.1133
15. Peltonem K, El-Nezami H, Haskard C, Ahokas J, Salminen S. 2001. Aflatoxin B_1 binding by dairy strains of lactic acid bacteria and bifidobacteria. *J Dairy Sci* 84(10): 2152-2156. https://doi.org/10.3168/jds.S0022-0302(01)74660-7
16. Murphy PA, Hendrinch S, Landyren C, Bryant CM. 2006. Food mycotoxins: an update. *J Food Sci* 71(5): 51-65. https://doi.org/10.1111/j.1750-3841.2006.00052.x
17. Bata A, Lasztity R. 1999. Detoxification of mycotoxin- contaminated food and feed by microorganisms. *Trends Food Sci Technol* 10(6-7): 223-228. https://doi.org/10.1016/S0924-2244(99)00050-3
18. Magan N, Olsen M. 2006. Mycotoxins in food- detection and control. CRC Press, Boca Raton, FL, USA.
19. Di Gregorio MC, de Neeff DV, Jager AV, Corassin CH, de Pinho Carão AC, et al. 2014. Mineral adsorbents for prevention of mycotoxins in animal feeds. *Toxin Reviews* 33(3): 125-135. https://doi.org/10.3109/15569543.2014.905604
20. Oliveira CAF, Corassin CH. 2014. Aflatoxins. In: Duarte SC, Pena ALS, Lino CM (eds). Mycotoxins and their implications in food safety. Future Science Ltd, London, UK, pp: 6-19.
21. Alberts JF, Gelderblomb WCA, Botha A, van Zyl WH. 2009. Degradation of aflatoxin B1 by fungal lactase enzymes. *Int J Food Microbiol* 135(1): 47-52. https://doi.org/10.1016/j.ijfoodmicro.2009.07.022

22. Kolossova AJ, Stroka J, Breidbach A, Kroeger K, Ambrosio M, et al. 2009. Evaluation of the effect of mycotoxins binders in animal feed on the analytical performance of standardized methods for determination of mycotoxins in feed. *JRC Scientific and Technical Reports*. European Commission, Belgium.

23. Yannikouris A, Jouany JP. 2002. Mycotoxins in feeds and their fate in animals: a reviews. *Anim Res* 51(2): 81-99. https://doi.org/10.1051/animres:2002012

24. Joannis-Cassan C, Tozlovanu M, Hadjeba-Medjdoub K, Ballet N, Pfohl-Leszkowicz A. 2011. Binding of zearalenone, aflatoxin B_1, ochratoxin A by yeast- based products: a method for quantification of adsorption performance. *J Food Prot* 74(7): 1175-1185. https://doi.org/10.4315/0362-028X.JFP-11-023

25. European Food Safety Authority. 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety.

26. Ramos AJ, Hernández E. 1996. *In vitro* aflatoxin adsorption by means of montmorillonite silicate. A study of adsorption isotherms. *Anim Feed Sci Technol* 62(2-4): 263-269. https://doi.org/10.1016/S0377-8401(96)00968-6

27. SAS Institute. 2004. SAS® user's guide: statistics. Cary, North Carolina, USA.

28. Neff DV, Ledoux DR, Rottinghaus GE, Bermudez AJ, Dakovic A, et al. 2012. *In vitro* and *in vivo* efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B_1. *Poult Sci* 92(1): 131-137. https://doi.org/10.3382/ps.2012-02510

29. Alleoni LRF, Camargo AO, Casagrande JC. 1998. Isotermas de Langmuir e de Freundlich na descrição da adsorção de boro em solos altamente intemperizados. *Scientia Agricola* 55(3): 379-387. https://doi.org/10.1590/S0103-90161998000300005

30. LeVan MD, Vermeulen T. 1981. Binary Langmuir and Freundlich isotherms for ideal adsorbed solutions. *J Phys Chem* 85(22): 3247-3250. https://doi.org/10.1021/j150622a009

31. Giles CH, Macewans TH, Nakhwwa N, Smith D. 1960. Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface areas of solids. *J Chem Soc* 1960(0): 3973-3993. https://doi.org/10.1039/JR9600003973

32. Oliveira CAF, Bovo FC, Corassin CH, Jager AV, Reddy KR. 2013. Recent trends in microbiological decontamination of aflatoxins in foodstuffs. In: Razzaghi-Abyaneh M (ed) Aflatoxins - Recent Advances and Future Prospects. Intech Open, Croatia. https://doi.org/10.5772/51120

33. Santos RR, Vermeulen S, Haritova A, Fink-Gremmels J. 2011. Isotherm modeling of organic activated bentonite and humic acid polymer used as mycotoxin adsorbents. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 28(11): 1578-1589. https://doi.org/10.1080/19440049.2011

34. Dawson KA, Evans J, Kudupoje M. 2001. Understanding the adsorption characteristics of yeast cell wall preparations. In: Lyons TP, Jacques KA (eds.) Biotechnology in the Feed Industry. Nottingham University Press, Nottingham, UK, pp 169–181.

35. Diaz DE. 2005. The Mycotoxin Blue Book. Nottingham University Press, Nottingham, UK.

36. Madrigal-Santillán E, Madrigal-Bujaidar E, Márquez-Márquez R, Reyes R. 2006. Antigenotoxic effect of *Saccharomyces cerevisiae* on the damage produced in mice fed with aflatoxin B_1 contaminated corn. *Food Chem Toxicol* 44(12): 2058-2063. https://doi.org/10.1016/j.fct.2006.07.006

37. Bovo F, Franco LT, Rosim RE, Barbalho R, Oliveira CAF. 2015. *In vitro* ability of beer fermentation residue and yeast-based products to bind aflatoxin B_1. *Braz J Microbiol* 46(2): 577-581. https://doi.org/10.1590/S1517-838246220130400

38. Hernandez-Mendoza A, Garcia HS, Steele JL. 2009. Screening of *Lactobacillus casei* strains for their ability to bind aflatoxin B_1. *Food Chem Toxicol* 47(6): 1064-1068. https://doi.org/10.1016/j.fct.2009.01.042

39. Santin AP. 1996. Estudo da secagem e da inativação de leveduras: *Saccharomyces cerevisiae*. Universidade Federal de Santa Catarina, Florianópolis, Brazil.