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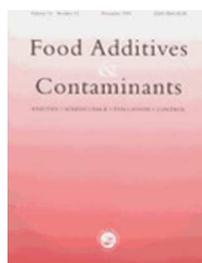
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**Yeast cell based feed additives: Studies on aflatoxin B1 and zearalenone**

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Methods/Techniques:	Chromatography - HPLC, Chromatography - LC/MS
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Food Types:	Animal feed
Abstract:	Thirty commercially available yeast cell wall products and two reference bentonites were tested for their ability to bind aflatoxin B1 (AFB1) and zearalenone (ZON) in buffer solutions at pH 3 and pH 6.5 as well as in real gastric juice. For most products, the binding efficacy of AFB1 correlated with the ash content which was between 2.6 and 89% and constituted the inorganic non-volatile components, like mineral clays, of the samples. Samples with smectite as main ash component showed highest binding efficacy. Yet, correlation with the content of mannanooligosaccharides (MOS) and beta-glucanes from yeast cell walls was not observed. Products containing > 30% ash showed AFB1 adsorption values > 90% at least in one of the investigated media whereas most products with < 10% ash did not exceed adsorption rates of 20%. In the case of ZON, adsorption efficiency ranged between 10 and 60%. It tended to be lowest for products with MOS and beta-glucan contents <10% and greatest for products with MOS and beta-glucan contents > 50%. However, there was no general correlation between the adsorption of ZON and the concentration of MOS

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	and beta-glucans. Different products of one brand sold in different countries were observed to bind AFB1 to different extent which was explained by different ash contents and mineral composition. In the case of ZON, differences in adsorption between products of the same brand were less pronounced.

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## Yeast cell based feed additives: Studies on aflatoxin B<sub>1</sub> and zearalenone

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### Abstract

Thirty commercially available yeast cell wall products and two reference bentonites were tested for their ability to bind aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and zearalenone (ZON) in buffer solutions at pH 3 and pH 6.5 as well as in real gastric juice. For most products, the binding efficacy of AFB<sub>1</sub> correlated with the ash content which was between 2.6 and 89% and constituted the inorganic non-volatile components, like mineral clays, of the samples. Samples with smectite as main ash component showed highest binding efficacy. Yet, correlation with the content of mannanooligosaccharides (MOS) and  $\beta$ -glucanes from yeast cell walls was not observed. Products containing > 30% ash showed AFB<sub>1</sub> adsorption values > 90% at least in one of the investigated media whereas most products with < 10% ash did not exceed adsorption rates of 20%. In the case of ZON, adsorption efficiency ranged between 10 and 60%. It tended to be lowest for products with MOS and  $\beta$ -glucan contents <10% and greatest for products with MOS and  $\beta$ -glucan contents > 50%. However, there was no general correlation between the adsorption of ZON and the concentration of MOS and  $\beta$ -glucans. Different products of one

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3 brand sold in different countries were observed to bind AFB1 to different extent which was  
4 explained by different ash contents and mineral composition. In the case of ZON, differences  
5 in adsorption between products of the same brand were less pronounced.  
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9 **Keywords:** Aflatoxin B<sub>1</sub>, zearalenone, adsorption, binding, yeast cell products,  
10 glucomannans, β-glucans, smectite  
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## 12 13 **Introduction**

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15 Mycotoxins are an inherent problem in feed production and animal nutrition. In warm and  
16 moist countries, aflatoxins are among the main representatives. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a  
17 potent carcinogen and can be metabolized *in vivo* to AFM<sub>1</sub> which, in turn, can be found in  
18 milk and other animal products (Prandini et al. 2009). Zearalenone (ZON) is a frequently  
19 occurring mycoestrogen produced by several *Fusarium* species. Due to its structural  
20 similarity to estrogens like 17-β-estradiol, ZON can cause reproductive problems in  
21 mammals, e.g. (Bennett et al. 2003).  
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28 Primary strategies to reduce the risk of mycotoxin contamination include good agricultural  
29 practices in the field (crop rotation, soil cultivation, weed and insect control, careful use of  
30 fungicides) and upon harvest as well as transportation and storage under dry and cool  
31 conditions (Jouany 2007). However, complete avoidance of mycotoxins is not possible.  
32 There are several approaches to cope with the problem: To lower the mycotoxin  
33 concentration in contaminated crops by physical or chemical treatment (Jouany, 2007) or to  
34 reduce the animals' mycotoxin uptake by addition of feed additives working on the basis of  
35 biological decontamination through microbes or enzymes, e.g. (Fuchs et al. 2002; Molnar et  
36 al. 2004) or on the basis of adsorption of mycotoxins to binding agents in the animals'  
37 digestive system. Binders should be non-toxic, have a high adsorption capacity (so that their  
38 inclusion level into feed may be low) and bind mycotoxins selectively and irreversibly at  
39 different pH-values and in complex systems, like the digestive tract. Several different types  
40 of binders have been tested: Bentonites like NovaSil plus (NSP, formerly called hydrated  
41 sodium calcium aluminosilicate, HSCAS, but structurally a calcium montmorillonite clay);  
42 zeolites; organoclays; charcoal; diatomaceous earth; and yeast cell wall derived products  
43 (YCP). Unlike charcoal, NSP and other bentonites have been found to selectively bind  
44 aflatoxins through chemisorption, e.g. (Grant et al. 1998; Phillips 1995; Vekiru et al. 2007).  
45 However, the adsorptive performance (adsorption capacity, selectivity etc.) of the binders  
46 can be very different even if they belong to the same mineralogical group. Limitations of  
47 clays are that they accumulate in manure, may be contaminated with toxic metals and  
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3 dioxins which requires rigorous testing before use and that they bind other mycotoxins than  
4 aflatoxins only to a limited extent (Yiannikouris et al. 2004a).  
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8 Glucans can be found in a wide variety of cereals, plant algae, bacteria, fungi and yeast  
9 sources (like *Saccharomyces cerevisiae*). Yeast glucans are major cell wall components  
10 often present as an inner wall layer and associated with other cell wall components such as  
11 chitin (together they represent about 50-60% of the wall dry weight). Strongly glycosylated  
12 mannoproteins form the outer cell wall layer. The glucan-associated activity is affected by  
13 the glucan characterizing parameters like: primary structure, solubility, degree of branching,  
14 molecular weight, as well as polymer charge and/or solution conformation (e.g. triple or  
15 single helix) (Zekovic et al. 2005). Mannan oligosaccharides (MOS) are also constituents of  
16 yeast cell walls. They are widely used in animal nutrition to enhance growth performance  
17 and gastrointestinal health. However, they have not been reported to bind mycotoxins.  
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25 Some manufacturers of yeast cell wall derived products claim that preparations containing  
26 yeast cell wall glucans, besides being nutritional aids and growth promoters, bind a variety of  
27 mycotoxins *in vitro* and *in vivo*, thereof especially ZON, without changing the nutritional value  
28 (regarding mineral and vitamin profile) of the feed. The mechanism of binding by  $\beta$ -D-  
29 glucans has been investigated by Yiannikouris and co-workers and has been discussed in  
30 several publications, e.g. (Yiannikouris et al. 2004a; Yiannikouris et al. 2006; Yiannikouris et  
31 al. 2004b; Yiannikouris et al. 2004c). It was shown that it is important to differentiate  
32 between the adsorption affinity of whole yeast cell wall and processed cell wall. A further  
33 factor influencing the binding ability is the presence of chitin which stiffens the cell wall  
34 structure and restricts access of e.g. ZON to the binding sites of  $\beta$ -D-glucans (Yiannikouris  
35 et al. 2004c). Therefore, the preparation of the cell wall material prior to its application as a  
36 mycotoxin binder is of major importance and greatly influences the adsorption ability of the  
37 final product. Hence, in order to describe the precise properties of  $\beta$ -D-glucan preparations,  
38 origin, molecular structure and purity are important as they are known to influence the  
39 activity of  $\beta$ -D-glucan preparations. It would be very useful and provide transparency for the  
40 end-user if cell wall derived products were accompanied by a certificate of analysis reporting  
41 parameters like moisture, glucan, MOS and ash content as well as other parameters like  
42 protein and fat content.  
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54 Low percentages of clays (1-6%) are commonly added to feed as technological anti-caking  
55 feed additives. Greater percentages indicate targeted addition of clays for mycotoxin  
56 removal by binding. Possible ways to examine clay addition in products based on yeast cell  
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3 wall ingredients are powder X-ray diffraction analysis or determination of the ash content of  
4 the product, which indicates the non-volatile inorganic matter therein.  
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7 The aim of this work was to test a great number of yeast cell wall based products for their  
8 ability to bind AFB1 and ZON and to correlate the respective adsorption rates with the  
9 glucomannan- and ash content in the samples. In addition, variations in binding efficiency  
10 within products of the same brand, but marketed in different countries and stemming from  
11 different lots, should be assessed.  
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## 15 **Materials and methods**

### 16 *Reagents and solutions*

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19 Solid standards of AFB1 and ZON were provided by Romer Labs Diagnostic GmbH (Tulln,  
20 Austria). Acetonitrile (ACN, HPLC gradient grade) was purchased from VWR (Vienna,  
21 Austria) and trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich (Vienna, Austria).  
22 Reagents for preparation of buffer solutions were purchased from Merck (Darmstadt,  
23 Germany) and Riedel-deHaën (Seelze, Germany). Milli-Q water prepared using an ultra-pure  
24 water system (Millipore, Molsheim, France) was used in all experiments.  
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31 Stock solutions of AFB1 (85 mg/L) and ZON (100 mg/L) were prepared in ACN. The  
32 preparation of buffer solutions (citrate buffer, pH 3.0, and phosphate buffer, pH 6.5) has  
33 been described earlier (Vekiru et al. 2007). Real gastric juice (from swine) was cleaned from  
34 suspended particles by centrifugation and filtration.  
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### 38 *Tested products*

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40 Thirty products containing different percentages of yeast cell wall constituents, mineral  
41 components and other ingredients were purchased worldwide. In addition, two mineral  
42 binders (products 1 and 2) were used as reference materials and for quality control purposes  
43 during our experimental work. Activated carbon and cholestyramine (Sigma-Aldrich and  
44 Fluka, Vienna, Austria) were chosen as positive control for binding of ZON. Product details,  
45 including manufacturers' information and our experimentally determined data like ash-  
46 content and percentage of MOS and  $\beta$ -glucans are listed in Table 1, headed by the two  
47 reference bentonites and then sorted by decreasing ash content. In the case of 4 products  
48 (Mycosorb, Microbond, Integral, MTB-100), different batches – in part sold in different  
49 countries – were analyzed in order to investigate the product variations in ash, MOS and  $\beta$ -  
50 glucan content as well as in binding efficacy of AFB1 and ZON.  
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#### *Adsorption tests at different pH-values and in real gastric juice*

Adsorption tests were carried out at pH 3 (citrate buffer), pH 6.5 (phosphate buffer) and in real gastric juice from swine (pH 5): 10 mg of adsorbent were weighed accurately ( $\pm 0.3$  mg) into 15 mL polypropylene test tubes (Sarstedt, Nuembrecht, Germany), 5 mL of citrate buffer, phosphate buffer or gastric juice, each containing 0.2 mg/L of AFB1 or 0.5 mg/L of ZON, were added corresponding to 0.2% (w/v) of binder and the tubes were vortexed and incubated at 37°C for 1 h under shaking in vertical position in a rack on a microtiter plate shaker (1000/min). For each medium, 5 mL of a reference solution containing toxin, but no binder were treated like the samples. Immediately after incubation, the vials were centrifuged (3700 rpm, 15 min) and aliquots of the buffer solutions were transferred into autosampler vials. In the case of gastric juice, 0.5 mL of supernatant were mixed with 0.5 mL of ACN, centrifuged to precipitate proteins and an aliquot of the clear solution was used for HPLC-analysis. As AFB1 can transform into AFB2a in acidic solutions (Vekiru et al. 2007), chromatograms were checked for AFB2a which was found to elute earlier in previous experiments. However, formation of AFB2a was not detected in any of the investigated media. Each adsorption test was carried out in triplicate for each medium, product and reference solution. Each replicate was analysed by HPLC once. The two reference bentonites were worked-up and analyzed in every experimental run for quality control purposes.

The percentage of adsorption was calculated by dividing the difference of the concentrations in the reference solution without binder (maximum concentration) and in the test solution after adsorption by the concentration in the reference solution and multiplying by 100.

#### *Chemisorption tests*

Adsorption tests were carried out with selected products separately for AFB1 (0.2 mg/L) and ZON (0.5 mg/L) at a binder concentration of 0.5% (w/v) and at pH 6.5 (phosphate buffer) as described above. After shaking for 1 h at 37°C and centrifugation, the supernatant was removed for HPLC analysis and the pellet was re-suspended by vortexing in 1 mL of buffer. The re-suspended pellet was washed twice with 1 mL of the buffer used for the preceding adsorption experiment and subsequently extracted 3 times with 1 mL each of methanol by shaking at 37°C (600/min) for 0.5 h. Supernatants of each washing and extraction cycle were collected and analysed separately.

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3 The chemisorption index ( $C\alpha$ ) was calculated by dividing the difference of toxin adsorbed  
4 during the adsorption assay and of toxin desorbed in the course of the desorption steps by  
5 the total amount of toxin used for the adsorption assay. The percentage of desorption was  
6 determined by dividing the amount of toxin desorbed by the amount of toxin originally  
7 adsorbed and multiplication by 100.  
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#### 10 11 12 *Adsorption rate at increasing amounts of AFB1*

13 A constant quantity (0.2% w/v) of selected products (which showed high AFB1 binding  
14 efficiency in the previous test) was mixed with increasing amounts of AFB1 at 37°C and the  
15 amount of toxin bound was evaluated. Adsorption tests were performed in gastric juice as  
16 described above using 0.2, 2.0, 4.0, and 8.0 mg/L AFB1 with the exception that the samples  
17 were incubated under shaking for 24 h (instead of 1 h).  
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#### 22 23 *HPLC-analysis*

24 The HPLC system used consisted of an Agilent G1311A quaternary pump, a G1322A  
25 vacuum degasser, a G1313A autosampler, a G1316A column compartment and a G1315A  
26 UV-diode array detector. AFB1 was analyzed on a Zorbax SB Aq column (150 x 4.6 mm, 5  
27  $\mu\text{m}$  particle size) equipped with a pre-column at 35°C at a flow rate of 0.7 mL/min using 50%  
28 aqueous ACN adjusted to pH 2.9 with TFA as mobile phase. The injection volume was 100  
29  $\mu\text{l}$  and the total run time was 7 min. UV-absorbance was monitored at 365 nm. Calibration  
30 was performed in the range of 10-4500  $\mu\text{g/L}$   
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36 For HPLC analysis of ZON 25  $\mu\text{l}$  of sample or standard solutions were injected and  
37 separated isocratically on an Eclipse XDB-C8 column (150 x 4.6 mm, 5  $\mu\text{m}$ ) equipped with a  
38 pre-column at 25°C and at a flow rate of 0.8 mL/min. The mobile phase consisted of 54%  
39 aqueous methanol containing 1% of acetic acid (v/v) and the UV absorbance was recorded  
40 at 275 nm. The total runtime was 8 min. Calibration was carried out in the range of 10-1000  
41  $\mu\text{g/L}$ .  
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#### 46 47 *Ash content*

48 The ash content was determined based on the AOAC Official Method 942.05. In short, 5 g of  
49 sample were dried over night at 105°C and subsequently subjected to a temperature  
50 gradient with 550°C as maximum- and 105°C as final temperature prior to cooling in a  
51 dessicator.  
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#### 55 56 57 *Mineral composition*

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4 The mineral composition of the products was determined by powder X-ray diffraction (Moore  
5 et al. 1997). In addition, the content of carbonates was determined by Scheibler method  
6 (ÖNORM L1084) and simultaneous thermal analysis (STA, Smykatz-Kloss 1974).  
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#### 9 10 *Content of MOS and $\beta$ -glucans*

11 The total content of MOS and glucans was determined as described at  
12 <http://www.eurasyp.org/public.technique.home.screen> with minor modifications. The method  
13 involves pre-solubilization of the yeast cell wall (YCW) with concentrated sulfuric acid,  
14 subsequent acid hydrolysis, neutralization and determination of the released glucose and  
15 mannose (which are formed in proportional quantities from the glucans and mannans) by ion  
16 chromatography.  
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### 22 **Results and discussion**

#### 23 *Products*

24 The ash-, MOS- and  $\beta$ -glucan contents and the mineral composition determined in this work  
25 are given in Table 1. Regarding the ash and MOS- plus  $\beta$ -glucan content of the tested  
26 products there are 3 groups to distinguish: Pure yeast or yeast cell wall products without  
27 inorganic binders added, having an ash content below 10% and generally highest  
28 percentages of MOS and glucans (41-72%); mixtures of yeast or yeast cell wall products  
29 (30-50%) and inorganic binding agents, containing between 10 and 30% ash; and products  
30 with ash contents > 30% composed mainly of inorganic binder with smaller percentages of  
31 yeast (0-16%).  
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40 Ten of the investigated products contained between 3.8 and 9.8% ash. Low percentages of  
41 ash are common in yeast cell-based products because some mineral components are  
42 necessarily added during manufacturing for anti-caking purposes. Five of the products  
43 containing < 10% ash were intended for welfare of animals by offering benefits to the GI  
44 system and by stimulating the immune system, whereas the 4 Mycosorb products were  
45 claimed to have mycotoxin binding properties. Mycotoxin binding is also the main aim of the  
46 majority of products containing 10-30% ash and 30-50% MOS and glucans. Three further  
47 products of this group were intended for well-being of animals by protecting against  
48 pathogens and by lessening the adverse effects of mycotoxins. One further product claimed  
49 to detoxify various mycotoxins by biological degradation. Finally, 7 of the 8 products (5 of the  
50 6 yeast cell wall based products) containing more than 30% ash and less than 16% of MOS  
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3 and glucans aimed at mycotoxin removal by binding and only one of these products was  
4 intended mainly to help maintain a healthy GI system.  
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7 *Adsorption tests at different pH-values and in real gastric juice at pH 5*

8 Homogenous products with small particle size can easily be tested at small scale (5 ml) for  
9 their binding efficacy by indirect pipetting technique, which includes preparation of a binder  
10 slurry and addition of slurry-aliquots to test tubes containing a certain concentration of the  
11 toxin. Using this technique, binder concentrations as low as 0.002% can be added  
12 reproducibly so that differentiation of high capacity binders (which all bind 100% of AFB1 at  
13 0.2% of binder concentration) becomes possible. Yeast cell wall preparations, however, are  
14 not homogenous and product particles are rather coarse, so that indirect pipetting technique  
15 is not applicable. In order minimize the volume of toxin spiked solution required for the test  
16 we used 0.2% of the product (10 mg / 5 mL) for performing the adsorption tests. This  
17 resulted in 100% binding of AFB1 by the reference bentonites, but allowed differentiation of  
18 the other products.  
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27 **AFB1:** The results indicated a trend that products with high ash content showed high binding  
28 efficiency of AFB1 (Figure 1A). For instance, all of the 8 products containing > 30% ash  
29 showed adsorption values > 90% at least at pH 6.5. Likewise, 8 of the 10 products  
30 containing < 10% ash did not exceed adsorption values of 25% under any of the tested  
31 conditions (pH 3, pH 6.5, gastric juice). Of the 14 products containing between 10 and 30%  
32 ash, five reached adsorption efficiencies of 100%, 3 further products showed at least 50%  
33 adsorption in buffer solutions, 5 had adsorption rates between 30 and 50% and one was a  
34 very poor AFB1 binder, binding < 15%. Hence, not only the amount, but also the type of  
35 mineral constituting the ash is a key factor in adsorption of AFB1. Therefore, the mineral  
36 composition of the individual products was determined by powder X-ray diffraction. Smectite,  
37 reported as the active ingredient in additives used as aflatoxin binders (Kannevischer et al.  
38 2006; Marroquin-Cardona et al. 2009), was the main ingredient in 11 and a minor  
39 component in 2 further products containing > 20% ash. With the exception of the three  
40 products of the Microbond product line (13, 14, 16), all of these products showed > 90%  
41 AFB1 adsorption in buffer and > 70% adsorption in gastric juice. Products 3 and 4, in which  
42 smectite was not detected, showed < 50% adsorption in gastric juice. One further indication  
43 that the binding capacity of yeast cell wall derived products depends strongly on the amount  
44 and type of mineral component added is the poor binding efficacy for AFB1 of several  
45 products (e.g. products 28-32) exhibiting high MOS and  $\beta$ -glucan content. This observation  
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3 is in accordance with the finding by Baptista et al. (2004) that manno-oligosaccharides and  
4 thermolysed yeast are not able to inactivate aflatoxins *in vivo*.  
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8 Of the 3 media tested, highest adsorption values were obtained in phosphate buffer at pH  
9 6.5, in most cases followed by citrate buffer at pH 3. Adsorption rates in gastric juice were  
10 lowest, possibly due to adsorption of gastric juice components to the binder, reducing the  
11 binder's capacity for AFB1 (see later for further explanations).  
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15 **ZON:** In the case of ZON, most products showed adsorption rates between 20 and 40%  
16 (Figure 1B). Two products (No. 3, 4) were very poor binders, binding less than 15% under all  
17 of the tested conditions (pH 3, pH 6.5, gastric juice). Only 10 products showed adsorption  
18 capabilities greater than 40% in at least one of the investigated media. Interestingly, the two  
19 reference bentonites NSP and GB7 and one further product with high ash content (No. 5,  
20 88.9% ash) were among the best adsorbents for ZON at pH 3 and 6.5 whereas 2 further  
21 products containing about 90% ash bound less than 10%. This result indicates that the type  
22 and composition of mineral binder greatly affects the product's efficiency for adsorption of  
23 ZON in buffer solutions whereas the ash content itself does not seem to be a factor.  
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31 Contrary to the results in buffer solutions, adsorption in gastric juice was low (<15%) for all  
32 products with ash contents of about 90% and MOS and  $\beta$ -glucan contents below 10%. This  
33 suggests that these kinds of product are hardly qualified for an *in vivo* application. The other  
34 7 products showing adsorption capacities greater than 40% in at least one of the  
35 investigated media also contained considerable amounts of MOS or  $\beta$ -glucans, which  
36 seemed to improve the ZON adsorption rates especially in real gastric juice. Moreover,  
37 several products rich in  $\beta$ -glucans showed greater adsorption in gastric juice than in buffer  
38 solutions.  
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45 In our study, all products containing > 10% MOS and  $\beta$ -glucans showed  $\geq 20\%$  adsorption in  
46 all tested media. However, although the products with greatest MOS and  $\beta$ -glucan content  
47 were among those with greatest adsorption, adsorption rates did not generally increase with  
48 greater MOS and  $\beta$ -glucan content. The type of polysaccharide (MOS or  $\beta$ -glucan) was not a  
49 determinant factor, either.  
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54 Activated carbon and cholestyramine, tested as positive control samples for ZON adsorption,  
55 showed adsorption rates of 100 and 74.5% at pH 3, of 100 and 87.6% at pH 6.5, and of 100  
56 and 78.2% in gastric juice.  
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**Influence of the adsorption medium:** Although the adsorption rate of AFB1 was different to that of ZON for each individual product, the ratio of adsorption of ZON to adsorption of AFB1 was similar at pH 3 and at pH 6 for most products. However, the ratio of adsorption of ZON to adsorption of AFB1 was greater in gastric juice than in buffer solutions for 14 products of all ash and MOS plus  $\beta$ -glucan contents. On the other hand, the ZON to AFB1 adsorption ratio was significantly smaller in gastric juice than in buffer solutions for the 3 products with high ash content which showed great adsorption of ZON in buffer solutions, but not in gastric juice (products 1, 2 and 5). One explanation might be that gastric juice, although subjected to filtration and centrifugation prior to use, still contains components which interfere with adsorption. For instance, adsorption of ZON to gastric juice particles occurred up to 31% in the absence of binder. Hence, adsorption rates in gastric juice were corrected by the adsorption of AFB1 and ZON, respectively, in "blank" solutions of the respective toxin prepared without binder in gastric juice. Still, adsorption rates in gastric juice were increased compared to adsorption rates in buffer solutions for 14 products (see above). However, gastric juice components can also adsorb to the binder and thereby reduce its capacity for binding the target mycotoxins as observed for products 1, 2 and 5 in the case of ZON. In the case of AFB1, the capacity of the reference bentonites NSP and GB7 is great enough so that this effect was not observed.

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Translation of this finding into *in vivo* behaviour is tricky. Adsorption to gastric juice particles constitutes only a temporary mycotoxin binding as gastric juice particles are digested later in the intestinal system. Hence, the released mycotoxins should then be able to bind to mineral or MOS and  $\beta$ -glucan components or to other indigestible diet constituents in the intestinal tract before absorption. However, due to the complexity of the involved matrices differentiation of the different adsorption processes in the GI tract is hardly possible.

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*Natural variation in composition and binding efficacy between different products of one brand*

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Frequently, products of the same brand and manufacturer, but at least in part produced in different countries are manufactured with mineral components (e.g. anti-caking agents) from local mines to avoid raw material transportation. Hence, products of the same brand but sold in different countries can differ in the type, content and composition of clay which may result in different mycotoxin adsorption efficacy. In this work, the natural variation of composition and binding efficacy of products of the same brand but of different lots and / or sold in different countries was investigated by analyzing 10 products of Mycosorb sold in 6 different

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3 countries; 3 products of Microbond, each sold in a different country; 2 products of Integral  
4 sold in the same country; and 2 products of MTB-100, each sold in two different countries.  
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6 in 4 different countries (CN 2, CY, IE, BR, No. 24, 25, 26, 27) was  $7.3 \pm 0.7\%$ . Two further  
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8 products (AU, CN 1, No. 18, 20) were in between, containing  $21.9 \pm 2.4\%$  ash, with calcite  
9 as the main inorganic component. Likewise for **AFB1**: The greatest variations were  
10 observed within the Mycosorb products (Figure 2). The 4 products sold in Japan (JP 1-4,  
11 products 9, 10, 12, 15) contained  $27.7 \pm 1.6\%$  ash with smectite as the main component,  
12 whereas the average ash content of 4 other products sold adsorption of AFB1 was  
13 significantly higher in the Japanese products where it amounted to  $86.0 \pm 5.2\%$  in gastric  
14 juice,  $92.3 \pm 8.3\%$  at pH 3 and  $100 \pm 0.0\%$  at pH 6.5. In the other 6 investigated products (of  
15 low and medium ash content, no smectite) the adsorption of AFB1 was below 30% at pH 6.5  
16 and even below 20% at pH 3 and in gastric juice. Similarly, the 2 products of MTB-100 sold  
17 in Vietnam and Denmark (products 21, 30) differed widely in their ash contents (5.6 and  
18 19.2%) and in their adsorption efficacy for AFB1 (differences by a factor of 1.4 – 2.2 in the  
19 individual media, see Figure 1A), even though both contained calcite as main component. In  
20 contrast to Mycosorb and MTB-100, products of Microbond sold in the USA, South Africa  
21 and Japan (products 13, 14, 16) had similar ash contents ( $25.9 \pm 0.9\%$ ), similar mineral  
22 composition and showed similar adsorption under different conditions ( $56.3 \pm 1.4\%$  at pH  
23 3.0,  $58.9 \pm 13.2\%$  at pH 6.5,  $32.1 \pm 2.9\%$  in gastric juice). The same is true for the two  
24 Integral products sold in Canada (products 11, 17, relative standard deviations (RSDs) of  
25 ash content 11.7%, RSDs of adsorption under different conditions < 4.5%).  
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37 **ZON**: The MOS and  $\beta$ -glucan contents in the 10 different products of Mycosorb varied  
38 between 32.7 and 48.6% with an average value of  $41.1 \pm 5.4\%$  (Figure 2B). The differences  
39 in adsorption of ZON between the different products were also much lower compared to the  
40 differences in adsorption of AFB1. Adsorption rates were between 24.1 and 38.4% at pH 3,  
41 26.6 and 38.0% at pH 6.5 and between 25.9 and 46.0% in gastric juice. The two MTB-  
42 products (21, 30) differed both in the MOS and  $\beta$ -glucan content (by a factor of 1.3) and in  
43 the adsorption of ZON in different media (by a factor of 1.07, 1.25 and 1.34 in gastric juice,  
44 at pH 6.5 and at pH 3, respectively). The two Integral products from Canada (11, 17) had  
45 similar MOS and  $\beta$ -glucan contents, but different adsorption values (between factor 1.23 in  
46 gastric juice and factor 1.39 at pH 3). On the other hand, the three Microbond products (13,  
47 14, 16) from different countries had a relative standard deviation (RSD) of MOS and  $\beta$ -  
48 glucan contents of 12.2% and RSDs of adsorption in different media between 5.9 (pH 3) and  
49 16.6% (gastric juice).  
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### *Chemisorption tests*

In Figure 3, the chemisorption indices ( $C\alpha$ ) of selected products are plotted together with the percentages of adsorption and desorption determined in the chemisorption experiments. In the case of AFB<sub>1</sub>, there was a strong correlation between the ash content and  $C\alpha$ . This is in part explained by the definition of  $C\alpha$  which reflects both the initial capacity of adsorption and the strength of the interactions between mycotoxin and binder. Hence, products with low adsorption under the test conditions cannot reach a  $C\alpha$  of 1, even if no desorption takes place. In our experiments,  $C\alpha$  was greatest for the mineral binders and lowest for products with low ash and high MOS and  $\beta$ -glucan content. In addition, product 11, despite having an ash content of 28%, showed adsorption of only 33% and thereof desorption of 75% which is in contrast to other products of medium ash content. In general, products with low adsorption had greatest desorption values and vice versa.

In the case of ZON, the increased ratio of product used for this test (0.5% w/v) resulted in significantly greater adsorption rates than the standard test using 0.2% w/v (see above). Yet, none of the tested products exceeded a chemisorption index of 0.2. This is explained by high desorption of all products (65-85% of the adsorbed amount) rather than by low adsorption values as adsorption under test conditions with 0.5% binder was between 45 and 80% for all products but one (product 3). Although products with greatest MOS and  $\beta$ -glucan content had greatest chemisorption indices, there was no direct correlation between  $C\alpha$  and the MOS plus  $\beta$ -glucan content. The ash content was not a factor, either. The low chemisorption indices and the high desorption values of all products indicate that ZON is adsorbed by physisorption, i.e. weak interactions with the adsorbent material and that no strong chemical bonds are formed.

### *Adsorption rate at increasing amount of AFB<sub>1</sub>*

In this test the adsorption rate at increasing amount of the toxin was evaluated in order to compare the performance of different products which yielded similar results in the screening adsorption tests. As binder saturation during the screening test was not obtained for any of the products in the case of ZON, this test was only performed for AFB<sub>1</sub>.

On the one hand, establishment of adsorption isotherms for calculation of affinity and maximum binding capacity requires work in the range of binder saturation. On the other hand, different products should be compared under the same experimental conditions. Hence, the same percentage of binder as used in adsorption tests was chosen (0.2% w/v) which resulted in an almost vertical line in the isotherm plot for the reference bentonite (No.

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3 1) up to an initial concentration of 4 mg/L AFB1 (before addition of binder) and in significantly  
4 flatter lines for the yeast cell based products No. 7 and 10 (Figure 4). Therefore, although  
5 calculation of affinity and maximum binding capacity was not possible from the obtained  
6 data, different products with similar binding efficiency in adsorption tests could well be  
7 differentiated by means of their adsorption rates at increasing amount of AFB1.  
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12 *Points to consider when comparing experimental data with other published articles*

13 Table 2 summarizes results of published *in vitro* studies for the commercially available  
14 products investigated in this study. Unfortunately, for most of the products it was not possible  
15 to obtain independent experimental results (provided by someone other than the  
16 manufacturer of the product) regarding *in vitro* binding action. It was even more difficult to  
17 find results which refer to the product as it is sold on the market and not to formulations used  
18 for research work, which do not exactly represent the content of the commercially available  
19 product. Formulations used for research work (e.g. model compounds) are usually used in  
20 order to investigate the individual differences of various product ingredients in their mode of  
21 action and to determine factors that influence their efficacy (e.g. models of  $\beta$ -glucans as  
22 used by Yiannikouris et. al. (Yiannikouris et al. 2004d) or purified  $\beta$ -glucan fractions of yeast  
23 cell walls isolated from different strains of *S. cerevisiae* (Yiannikouris et al. 2004c)).  
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32 Further inconveniences are different experimental conditions used by the individual research  
33 groups (percentage of binder, concentration of the toxin, type and pH of the medium,  
34 incubation parameters), which renders comparison of the presented data difficult. One also  
35 has to distinguish between adsorption isotherms allowing the calculation of the maximum  
36 binding capacity and single adsorption studies giving just the percentage of adsorption and  
37 the amount of mycotoxin bound/kg of binder under the chosen conditions.  
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42 For instance, the amount of AFB1 bound/kg of the reference binders GB7 and NSP  
43 (products 1 and 2) was 0.32 mmol in the present work (use of 10 mg of product, 5 mL of  
44 solution containing 0.2 mg/L AFB1). The same value was obtained for several other products  
45 investigated (No. 5, 7, 8, 9, 10, 12, 15, 19) in at least one of the tested media. The difference  
46 in the adsorption capacity of the products became only visible when tests at increasing  
47 amount of AFB1 were performed (see above and Figure 4). Comparison of our data  
48 obtained in single adsorption studies for the reference binders with literature values for  
49 Mycosorb and MTB-100 tested in single adsorption studies (Table 2) points out the impact of  
50 the experimental conditions on the amount of AFB1 bound/kg of binder. Use of 10 mg of  
51 binder (0.2%) and 5 mL of 0.2 mg/L AFB1 solution leads to 0.32 mmol AFB1 bound/kg of  
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3 binder for 100% adsorption whereas use of 0.1% of binder in a solution containing 0.5 mg/L  
4 AFB1 gives 1 mmol AFB1 bound/kg of binder for an adsorption value of just 62%. Hence,  
5 comparison of values obtained in single adsorption studies does not necessarily give a  
6 ranking of the best binders.  
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10 Determination of maximum adsorption capacities, which are a more useful tool for  
11 comparisons, demonstrates the superiority of mineral binders compared to yeast cell  
12 products in the adsorption of AFB1 (maximum adsorption capacities between 9 and 18 mmol  
13 AFB1/kg binder for YCP and between 260 and 420 mmol/kg binder for mineral binders,  
14 Table 2). This efficiency of YCP may be too low for effective binding under more complex  
15 matrix conditions (as in the intestinal tract of animals).  
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21 In the case of ZON, the best performing Mycosorb products in our investigations were able  
22 to bind 0.31 mmol ZON/kg yeast cell based product. This is in the lower range of the already  
23 reported data for single adsorption studies (see Table 2B). Again, serious comparison with  
24 literature values was hampered by different experimental conditions. Isothermal analysis has  
25 only been reported for ZON and organoclays, not for ZON and Mycosorb. The maximum  
26 adsorption capacity of organoclays is up to 390 mmol ZON/kg binder (Lemke et al. 1998), a  
27 value which cannot be reached by YCP considering the low adsorption efficiencies at 0.2%  
28 of binder and 0.5 mg/L ZON in solution (see Figure 1B).  
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### 34 **Conclusion**

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36 This is the first study comparing the AFB1 and ZON adsorption capabilities of a great  
37 number of commercially available yeast cell products and correlating these with the ash- and  
38 MOS- as well as  $\beta$ -glucan contents of the products. Commercially available yeast cell based  
39 products, even of the same brand, were shown to differ in type and content of MOS and  $\beta$ -  
40 glucan as well as in ash content and mineral composition. Differences in the content and  
41 type of mineral clay components account for different binding capabilities of AFB1. The  
42 adsorption rate at increasing amount of the toxin (determined in order to differentiate  
43 between products with similar AFB1 binding efficiency in adsorption tests) revealed big  
44 differences between “pure” mineral binders and yeast cell based products with mineral  
45 components added.  
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53 Whereas AFB1 adsorption did not correlate with the MOS and  $\beta$ -glucan content of the  
54 investigated products, the ZON adsorption rate tended to increase with increasing MOS and  
55  $\beta$ -glucan content. However, the adsorption capability was very low for the applied high  
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3 inclusion rate of 0.2% w/v of the products and based on weak interactions. *In vivo* trials  
4 using mycotoxin biomarkers could be used to clarify whether the beneficial effect of yeast  
5 cell products against mycotoxicosis is based on effective binding of the toxin or on the  
6 general beneficial biological action of MOS and  $\beta$ -glucans to animals. In our study, ZON-  
7 adsorption was independent of the type of yeast cell based product – claimed toxin binders  
8 (see Table 1) had similar or even lower binding capacities than yeast cell based additives  
9 designed for improvement of gastro intestinal health. Of the tested pure clay mineral  
10 products (>90% ash), some showed surprisingly great adsorption of ZON in buffer solutions  
11 (ca. 40%), but none of them adsorbed more than 15% of ZON in gastric juice.  
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18 The impact of the product composition on the adsorption properties and the influence of  
19 experimental conditions on the amount of mycotoxin bound / kg of binder stresses how  
20 important it is that manufacturers present detailed product information (moisture,  $\beta$ -glucan  
21 and MOS as well as ash content, and also other parameters like protein, fat and content)  
22 and that authors pass on this information and add experimental details (percentage of binder  
23 used, concentration of toxin, medium and pH of the binding study) in their articles for better  
24 comparability of literature results.  
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15 **Figures :**

- 16 Figure 1A, B: Adsorption efficiency, ash, beta-glucan and MOS content of 32 products for  
17 AFB1 and ZON at pH 3, 6.5 and in gastric juice  
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19 Figure 2A, B: Variability of 10 different batches of Mycosorb concerning ash content, MOS  
20 and  $\beta$ -glucan content and adsorption in different media. Error bars denote the  
21 analytical standard deviation  
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24 Figure 3:  $C_{\alpha}$  and percentages of adsorption and desorption of selected products  
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26 Figure 4: Adsorption rate at increasing amounts of AFB1 (products No. 1, 7 and 10). Cw:  
27 Concentration of AFB1 left in the test solution supernatant after adsorption (mg/L), q:  
28 Amount of AFB1 (g) adsorbed to 1 kg of product  
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34 **Tables:**

- 35 Table 1: Product data. Sacc. cer.: *Saccharomyces cerevisiae*, MT: mycotoxin, YCW: yeast  
36 cell wall, BHT: butylated hydroxytoluene  
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38 <sup>1)</sup> daughter company of Trouw Nutrition  
39 <sup>2)</sup> manufacturer's information  
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41 \*\*: Main component (> 25% of mineral content)  
42 \*: Minor component (between ~1% and 25% of mineral content)  
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44 Percentage of carbonates refers to the total composition of the product  
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47 Table 2A: Overview of articles investigating adsorption of AFB1 onto yeast cell based  
48 products also included in our study  
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50 Table 2B: Overview of articles investigating adsorption of ZON onto yeast cell based  
51 products (single adsorption)  
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55 <sup>1)</sup> as stated in the original literature  
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Table 1:

Product No.	Product name	Provider; Marketed in	Intended use	Manufacturer's information	Mineral composition	Ash	$\beta$ -Glucan	MOS	$\beta$ -Glucan +MOS
1	Bentonite GB7	directly from a mine in Greece; not available for the end user	anti-caking and adsorption of aflatoxins	untreated bentonite	smectite**, calcite 3%	87.5	0.0	0.0	0.0
2	NovaSil plus	product of Trouw Nutrition Int., USA, manufactured by Engelhard, USA; USA	anti-caking and adsorption of aflatoxins	HSCAS	smectite**, quartz*, calcite 2%	85.4	0.0	0.0	0.0
3	Select Biocycle	Product of Select Sires, USA, manufactured by Agrarian Marketing Corporation, USA; Canada	positive effects on gut microflora and rumen microorganism	distillers dried grains with solubles, CaCO <sub>3</sub> , Sacc. cer. active dry yeast, potassium iodide, dried egg, lactic acid, calcium lactate, malic acid, acetic acid, tartaric acid, fumaric acid and sodium benzoate	quartz*, mica*, calcite 1%	89.0	5.0	1.4	6.5
4	Captex FUSA	Dox-al; Bulgaria	against fusarium toxins	4.2-4.6% moisture, 608 mg/kg clinoptilolite, 116 mg/kg Resindox™, 207 mg/kg esterified glucomannans (Sacc. cer.) and 185 mg/kg CaCO <sub>3</sub>	dolomite**, zeolite*, feldspars*, calcite 1%, dolomite 60%	89.0	5.2	3.4	8.6
5	Finale	Marsyt, USA; USA	MT removal by adsorption	montmorillonite clay, diatomaceous earth, Sacc. cer., magnesium silicate, calcium propionate	quartz**, mica*, smectite*, calcite 3%	88.9	2.8	1.4	4.2
6	MT.X Plus®	Olmix, France; UK	MT removal by adsorption	amadéite, montmorillonite, diatomaceous earth, yeast cell walls	quartz*, mica*, calcite 13%, dolomite 2%	75.0	5.9	4.3	10.2
7	Mastersorb Gold®	Grasp, Brasil; Brasil	MT (fumonisins, ZON, T2, DON, AFB1) removal by adsorption, protection against pathogens	mineral, phycolytic and phytogetic components, details unknown	quartz*, smectite*, kaolinite*, mica*, calcite 2%	65.2	10.0	5.9	15.8
8	Tonily	Nordos, France <sup>1)</sup> ; France	MT removal (AFB1, ZON, DON) by adsorption, stimulation of liver function, immune stimulation due to MOS and	mineral clay (sodium bentonite), dried yeast extract, antioxidants (BHT)	smectite**, quartz*, gibbsite*, calcite 3%	59.1	8.5	6.1	14.6

			vitamin E, anti-oxidative effect							
9	Mycosorb_Japan_1	Alltech, USA; Japan	MT removal by adsorption	Sacc. cer. extract > 60%, HSCAS, CaCO <sub>3</sub>	smectite**, quartz*, feldspars*, calcite 3%, dolomite 2%	29.7	19.7	13.0	32.7	
10	Mycosorb_Japan_2	Alltech, USA; Japan	see product No. 9	see product No. 9	smectite**, quartz*, calcite 3%, dolomite 1%	28.1	25.9	12.3	38.1	
11	Integral_Canada_1	Alltech, USA; Canada	MT removal by adsorption	yeast by-product	calcite 17%, dolomite 7%	28.1	22.5	11.4	33.9	
12	Mycosorb_Japan_3	Alltech, USA; Japan	see product No. 9	see product No. 9	smectite**, quartz*, calcite 2%, dolomite 1%	27.1	27.9	11.2	39.0	
13	Microbond_USA	Cenzzone, USA; USA	immunostimulation, protection against pathogens, improvement of digestion and nutrient adsorption, diminishing of adverse effects of MTs	β-glucans, MOS, digestive enzymes	smectite**, quartz*, sodium sulfate*, aluminium sulfate hydrate*, calcite ~0.5%	26.7	22.8	9.6	32.4	
14	Microbond_Southafrica	Cenzzone, USA; South Africa	see product No. 13	see product No. 13	smectite**, quartz*, sodium sulfate*, aluminium sulfate hydrate*, calcite ~0.5%	26.2	21.1	9.0	30.1	
15	Mycosorb_Japan_4	Alltech, USA; Japan	see product No. 9	see product No. 9	smectite**, quartz*, calcite 2%, dolomite ~0.5%	26.0	26.9	11.9	38.8	
16	Microbond_Japan	Cenzzone, USA; Japan	see product No. 13	see product No. 13	smectite**, quartz*, sodium sulfate*, aluminium sulfate hydrate*, calcite ~0.5%	24.9	26.4	11.6	38.0	
17	Integral_Canada_2	Alltech, USA; Canada	see product No. 11	see product No. 11	calcite 15%, dolomite 7%	23.8	24.3	11.1	35.4	

18	Mycosorb_Australia	Alltech, USA; Australia	see product No. 9	see product No. 9	calcite 14%, dolomite 6%	23.6	22.5	11.8	34.3
19	Nutricell® Polysorb	Biorigin, Brasil; Brasil	MT (AFB1, ZON, T2-toxin) removal by adsorption (produced from YCW by hydrolysis, extraction and further treatment)	max. 8% moisture, 17-25% ash, > 45% carbohydrates (β-glucans + MOS) 20 ± 5 proteins, 5-12% fat	smectite**, quartz*, feldspars*, cristobalite*	22.4	22.3	25.3	47.6
20	Mycosorb_China_1	Alltech, USA; China	see product No. 9	see product No. 9	calcite 13%, dolomite 6%	20.2	30.5	19.0	49.5
21	MTB-100_Vietnam	Alltech, USA; Vietnam	U.S. Patent No 6045834, MT removal by adsorption	molasses distillers dried solubles, maize condensad distillers solubles, HSCAS, CaCO <sub>3</sub> min. crude protein 22%, max. crude fat 0.5%, max. crude fiber 11%, max. moisture 15%	calcite 12%, dolomite 5%	19.2	27.9	14.9	42.8
22	DETOXA PLUS 168	Alinat, Argentine; China	biological degradation of ZON, FB1, OTA, T2 by <i>Sacc. telluris</i> strains and YCW for adsorption of aflatoxins	min. crude protein 32%, max. fiber 1%, max. moisture 8%, max. ash 18%, min. β-glucans 14%	quartz*, zeolite*, K-feldspar*	16.4	25.0	13.8	38.7
23	Safmannan	Saf Agri (Lesaffre), Mexico; Brasil	improvement of GI and immune system (obtained by autolysis of <i>Sacc. cer.</i> yeast)	moisture 2-3%, proteins 14-17%, β-glucans 24-26%, MOS 22-24%, fat 20-22%, ash 3-5%	calcite ~0.5%	9.8	25.0	21.3	46.3
24	Mycosorb_China_2	Alltech, USA; China	see product No. 9	see product No. 9	quartz*, zeolite*, calcite 2%	8.1	27.1	17.0	44.0
25	Mycosorb_Cyprus	Alltech, USA; Cyprus	see product No. 9	see product No. 9	not determined	7.8	25.8	14.9	40.7
26	Mycosorb_Irland	Alltech, USA; Ireland	see product No. 9	see product No. 9	quartz*, zeolite*, calcite 1%	6.8	26.1	18.9	45.0
27	Mycosorb_Brasil	Sanphar; Brasil	see product No. 9	see product No. 9	not determined	6.7	28.6	20.0	48.6

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6	28	ActiveMOS	Biorigin, Brasil; Brasil	protection against pathogens, positive effects on gut microflora (prebiotic rich in MOS, produced from cell wall of Sacc. cer)	max. protein 30%, max. moisture 8%, MOS 25±3%, β-glucans 30±3%, ash 6%	not determined	6 <sup>2)</sup>	37.6	19.9	57.5
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10	29	Betamune	Biorigin, Brasil; Brasil	modulation of cellular and humoral immune response, positive effects on animal growth; action based on cell wall of Sacc. cer.	min. β-glucan 70%, max. moisture 10%, ash 5.7%	not determined	5.7 <sup>2)</sup>	67.0	0.8	67.8
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13	30	MTB-100_Denmark	Alltech, USA; Denmark	see product No. 21	see product No. 21	calcite 4%	5.6	36.9	18.6	55.5
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16	31	Nutricell MOS 55	Biorigin, Brasil; Brasil	protection against pathogens, benefits for the GI system	details unknown	not determined	4.4	70.0	2.0	72.0
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19	32	Biolex <sup>®</sup> MB40	Leiber GmbH, Germany; Brasil	removal of MTs and pathogenic bacteria by adsorption, stimulation of immune system and prebiotic properties (contains extracted Sacc. cer.)	crude protein 25%, crude fat 7.5%, β-glucan 35-30%, MOS 20-25%, 4% ash	undefined mineral traces	3.8	26.1	27.5	53.5
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Table 2A: Overview of articles investigating adsorption of AFB1 onto YCP also included in our study.

AFB1-Adsorption							
Product name	Percentage of binder used (%)	solvent	pH	Toxin conc [mg/L]	Type of study	mmol AFB1 bound/kg binder	Literature
Bentonite GB7	0.002% (w/v)	buffer	7.0	0.4-8	adsorption isotherm	Max 420	(Vekiru et al. 2007)
NovaSil plus	0.02 mg/mL $\triangleq$ 0.002% (w/v)	buffer	2.0 & 6.5	0.4-8	adsorption isotherm	Max 396	(Marroquin-Cardona et al. 2009)
NovaSil plus	0.002% (w/v)	buffer	7.0	0.4-8	adsorption isotherm	Max 390	(Vekiru et al. 2007)
HSCAS	0.002% (w/v)	water	unadjusted	0.4-8	adsorption isotherm	Max 260	(Lemke et al. 2001)
HSCAS (NovaSil)	0.001% (w/v)	water	unadjusted	0.4-8	adsorption isotherm	Max 336	(Grant et al. 1998)
Mycosorb	0.02 mg/mL $\triangleq$ 0.002% (w/v)	water	2.0 & 6.5	0.4-8	adsorption isotherm	Max 9 & 15	(Marroquin-Cardona et al. 2009)
Mycosorb	1 mg/mL $\triangleq$ 0.1% (w/v)	aqueous solution	not given	2-10	adsorption isotherm	Max 17.6	(Dawson et al. 2001)
Mycosorb	0.1% (w/v)	unknown	not given	0.5	single adsorption (62% adsorption)	1	(Newman 2000)
MTB-100	1% (w/v)	water/MeOH (9:1)	3, 7, 10 and unadjusted	5.0	single adsorption (97% adsorption)	1.55	(Diaz et al. 2002)

Table 2B: Overview of articles investigating adsorption of ZON onto YCP also included in our study. (single adsorption)

ZON-Adsorption							
Product name	Percentage of binder used (%)	Solvent	pH	Toxin conc [mg/L]	% adsorption	mmol AFB1 bound/kg binder	Literature
Mycosorb	1mg/mL $\triangleq$ 0.1% (w/v)	buffer	3.0 & 8.0	2	38 & 42	2.4 & 2.6	(Avantaggiato et al. 2005)
Mycosorb	1mg/mL $\triangleq$ 0.1% (w/v)	buffer	3.0 & 8.0	20	23 & 18	1.4 & 1.1	(Avantaggiato et al. 2005)
Mycosorb Extra	10.2 mg/mL $\triangleq$ 1.02% (w/v)	buffer	changed during incubation from 3-7	0.334	88.7	0.09	(Doell et al. 2004)
Mycosorb	1 mg/g $\triangleq$ 0.1% (w/w) <sup>1)</sup>	not given	unknown	0.25-2	22-40	< 2.5	(Newman 2000)

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Mycosorb	1% (w/v)	buffer	4.5	40	68	8.5	(Völkl et al. 1999)
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Figure 1A:

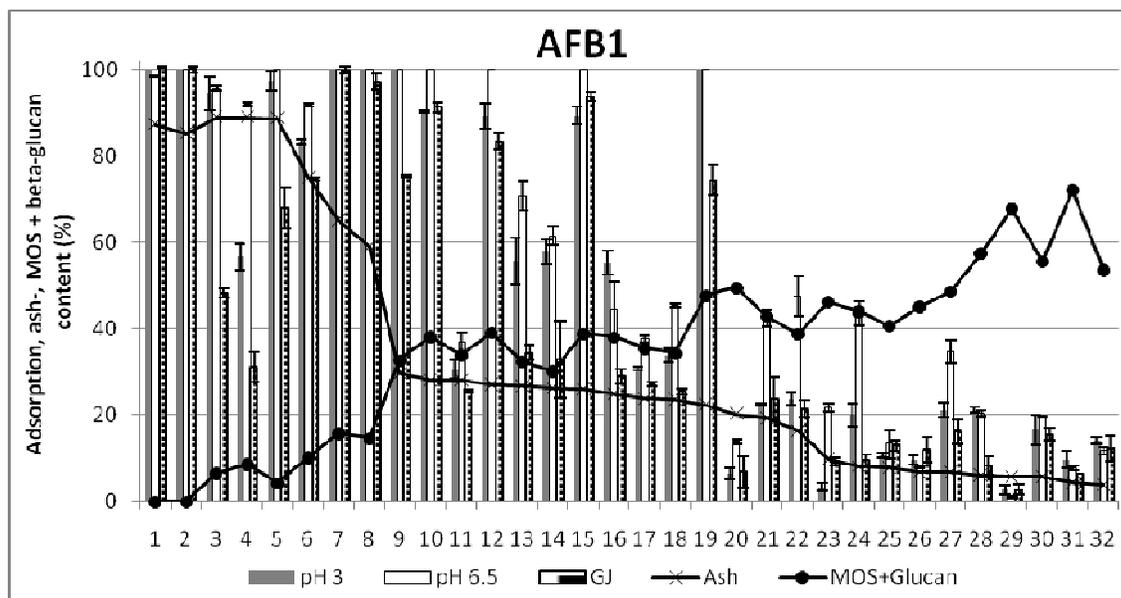


Figure 1B:

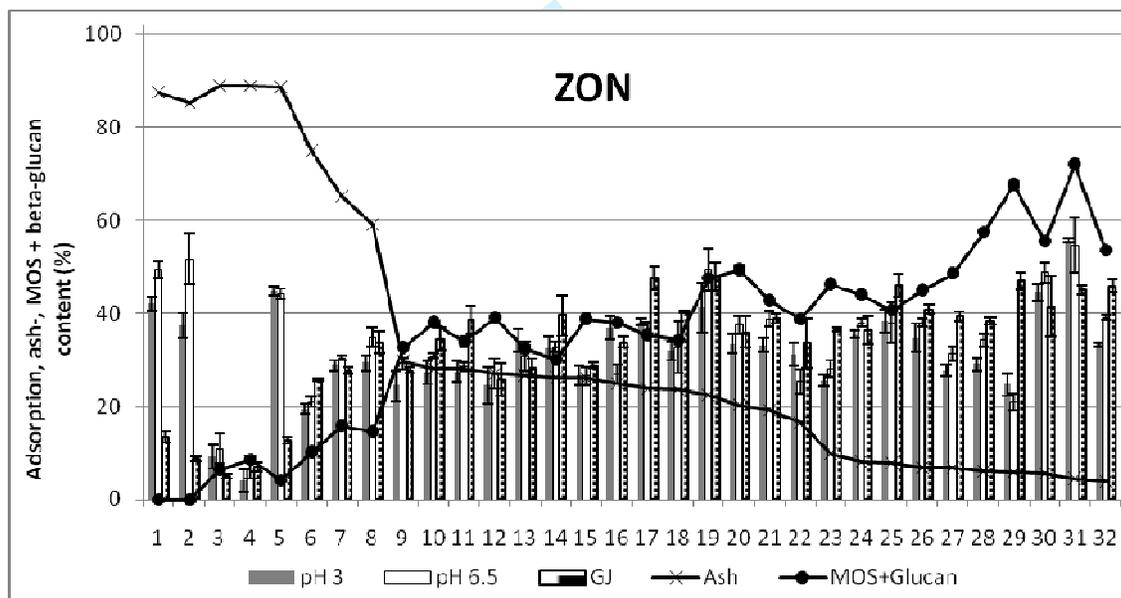


Figure 2A:

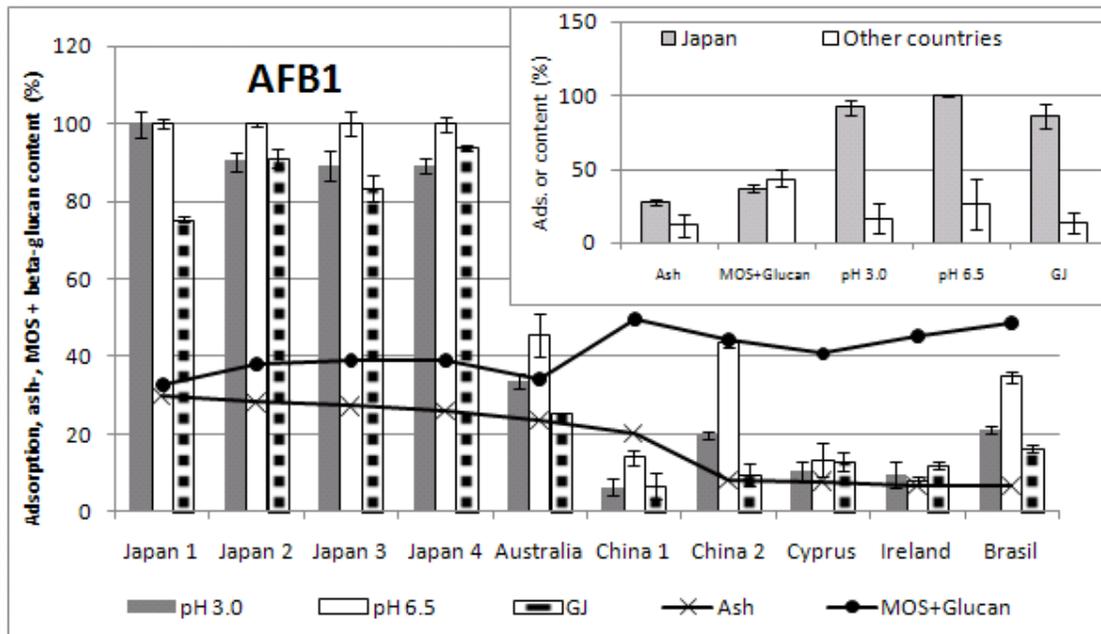


Figure 2B:

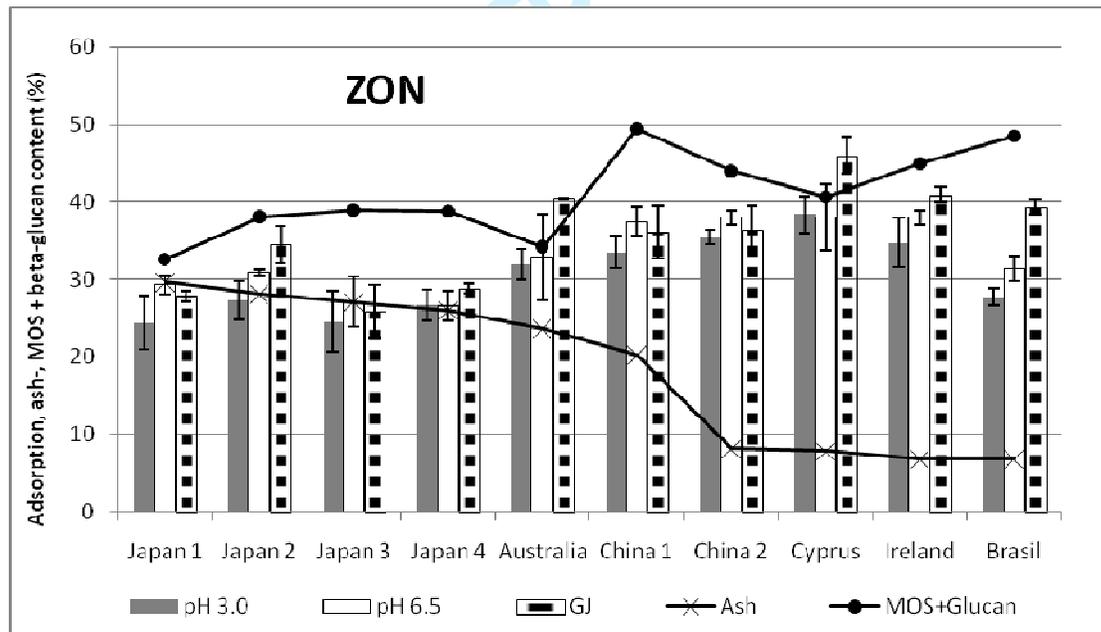


Figure 3:

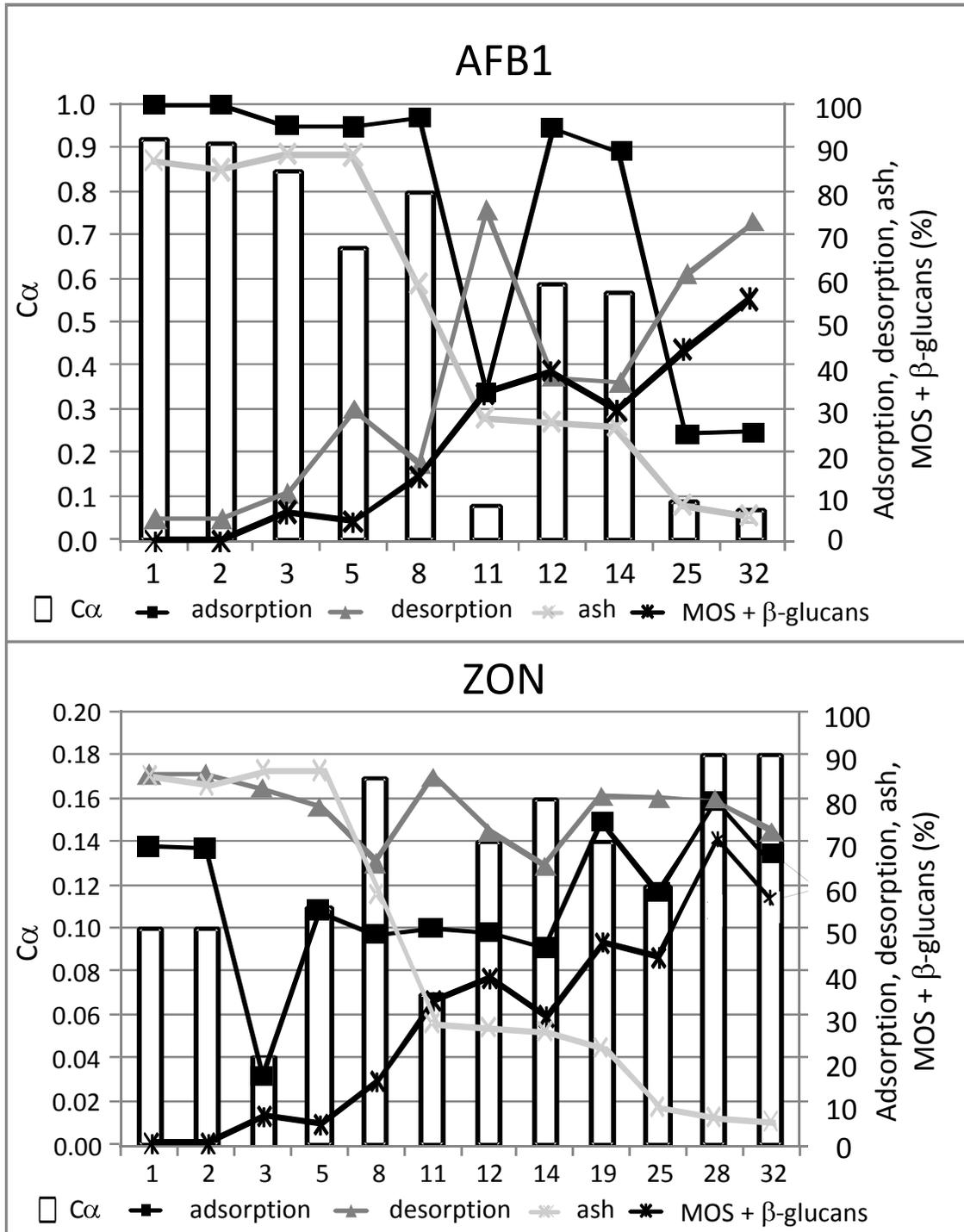
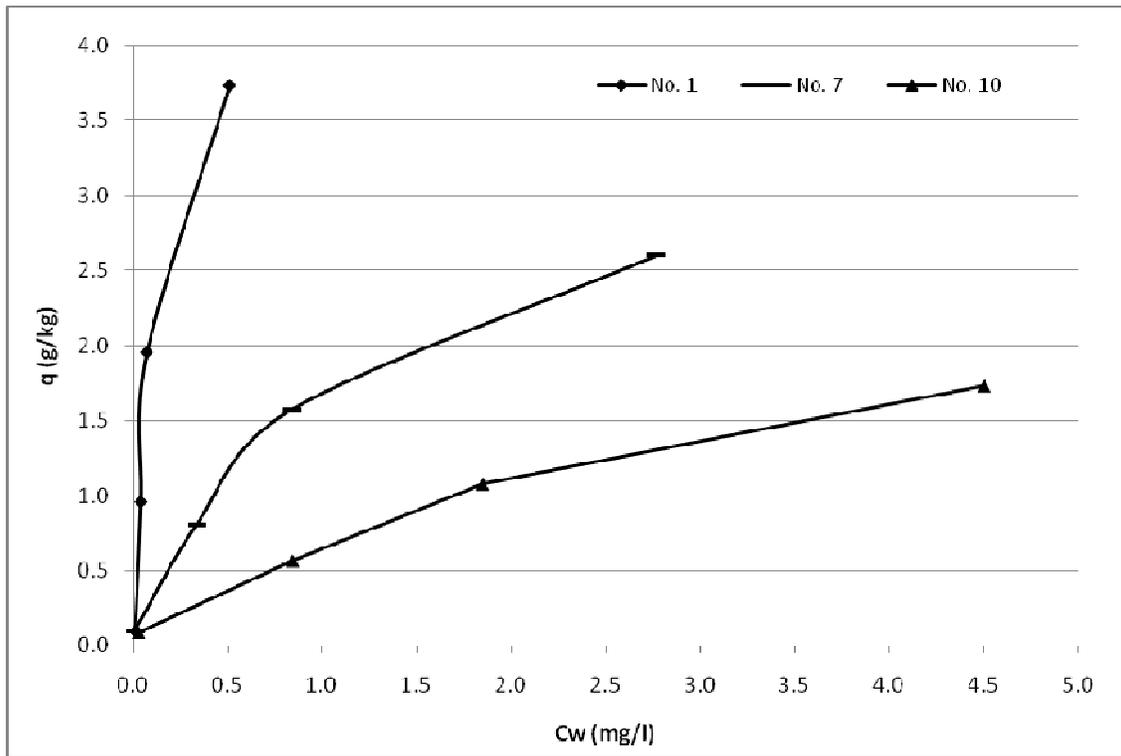


Figure 4:



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