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ADSORPTION OF AFLATOXIN B1 IN CORN ON NATURAL ZEOLITE AND BENTONITE

**Nuryono^{1,*}, Ali Agus², Sri Wedhastri³, Y.M.S. Maryudhani³,
Deni Pranowo¹, Yuniarto⁴, and Ebrahim Razzazi-Fazeli⁵**

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Jl. Sekip Utara, Yogyakarta 55281

²Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

³Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

⁴Animal Husbandry Academy Brahma Putra, Yogyakarta, Indonesia

⁵Proteomic Unit, VetCore Facility for Research, University of Veterinary Medicine, Vienna, Austria

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ABSTRACT

A study on adsorption of AFB1 in corn (kernel and grained) on natural zeolite and bentonite has been investigated. The first work was adsorption in a batch system of standard AFB1 solution on adsorbents. Some factors such as contact time, concentration of AFB1 and particle size of adsorbent were evaluated. The amount of AFB1 adsorbed was calculated based on the difference of AFB1 concentration before and after adsorption determined by high performance liquid chromatography (HPLC) method. Adsorption of AFB1 in corn sample was emphasized by mixing aqueous suspension of sample with adsorbent. Concentration of AFB1 in suspension was analyzed by enzyme-linked immuno-sorbent assay (ELISA) method. Result shows that adsorption of AFB1 on adsorbents of natural zeolite and bentonite is very fast. Within 15 min 99% of AFB1 (200 ng/mL) has been adsorbed by 25 mg of bentonite and 96% by zeolite. The particle size higher than 200 mesh did not give significant effect on the AFB1 adsorption capability. Effectiveness of zeolite in adsorbing AFB1 is lower than that of bentonite. Capability in reducing AFB1 contamination in corn samples (kernel and meal) for both adsorbents is lower than that in standard solution.

Keywords: aflatoxin; corn; adsorption; bentonite; zeolite

ABSTRAK

Kajian penurunan kadar aflatoksin B1 (AFB1) dalam jagung (kernel dan butiran) melalui teknik adsorpsi dengan zeolit dan bentonit alam sebagai adsorben telah dilakukan. Langkah pertama adalah adsorpsi AFB1 standar pada adsorben dalam sistem batch. Beberapa faktor, yaitu waktu kontak, konsentrasi AFB1 dan ukuran partikel adsorben telah dievaluasi. Jumlah AFB1 yang teradsorpsi dihitung berdasarkan selisih konsentrasi AFB1 sebelum dan sesudah adsorpsi dan ditentukan dengan kromatografi cair kinerja tinggi (KCKT). Adsorpsi AFB1 dalam sampel jagung dikerjakan dengan mencampur suspensi sampel jagung dalam air dan adsorben. Konsentrasi AFB1 dalam suspensi dianalisis dengan metode enzyme-linked immuno sorbent assay (ELISA). Hasil menunjukkan bahwa adsorpsi AFB1 pada adsorben zeolit dan bentonit alam sangat cepat. Dalam waktu 15 menit 99% dari AFB1 (200 ng/mL) telah terserap oleh 25 mg bentonit dan 96% oleh zeolit. Ukuran partikel lebih tinggi dari 200 mesh tidak memberikan dampak signifikan terhadap kemampuan adsorpsi AFB1. Efektivitas adsorpsi zeolit terhadap AFB1 lebih rendah daripada bentonit. Kedua adsorben memiliki kemampuan mengadsorpsi AFB1 dalam sampel jagung (kernel dan butiran) lebih rendah dibandingkan dalam larutan standar.

Kata Kunci: aflatoksin; jagung; adsorpsi; bentonit; zeolit

INTRODUCTION

Aflatoxins are a mycotoxin group possessing highly carcinogenic, mutagenic, and teratogenic properties [1-2]. There are seventeen aflatoxins that have been isolated, however, only four, so called B1, B2, G1 and

G2, are significant contaminants of foods and feeds, and aflatoxin B1 is the most acutely toxic of the aflatoxins [3].

In Indonesia, aflatoxins are frequently found in agriculture commodities and their derivatives [4-5]. In addition, residue and its metabolites are also obtained

* Corresponding author.

Email address : nuryono_mipa@ugm.ac.id

in husbandry products such as milk [6-7], eggs [8], and chicken meat [9]. Contamination of maize and other food commodities with aflatoxins is a public health concern because of the ability of aflatoxins to cause human and animal diseases. Aflatoxins have been implicated with acute and chronic aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of the immune system, aggravation of kwashiorkor and impaired childhood growth [10]. Outbreaks of acute human aflatoxicosis occur frequently especially with respect to maize, the dietary. Sudjadi [11] reported that 80 among 81 patients (66 males and 15 females) experience liver cancer and it was suspected because they consumed soya fermented, fried peanut, peanut spices and soya sauce. Mycotoxins of AFB₁, AFG₁, and AFM₁ were detected in the samples of liver collected from 58% of patients with the concentration more than 400 µg/kg.

Numerous strategies, such as physical separation, thermal inactivation, irradiation, microbial degradation and treatment with a variety of chemicals have been used for the detoxification or inactivation of mycotoxin-contaminated feedstuff [12]. One strategy is to bind the aflatoxin molecule to a compound that cannot be absorbed from the animal's digestive tract. The bound aflatoxins are then excreted in the faeces [13].

It was also reported that phyllosilicates clay has the ability to chemisorb aflatoxin from aqueous solutions [14]. Some aluminosilicates bind AFB₁ *in vitro* to varying degrees and form complexes of varying strength with AFB₁. The hydrated sodium calcium aluminosilicate (HSCAS) formed a more stable complex with AFB₁ than many of the other compounds tested *in vitro*. The HSCAS, bentonite and montmorillonite were found to protect the laboratory animals from the toxic and teratogenic effects of aflatoxins [15].

Aly et al. [16] reported result of study on adsorption of AFB₁ and FB₁ onto a commercially hydrated sodium calcium aluminosilicate (HSCAS) and Egyptian montmorillonite (EM) in an aqueous solution at different tested levels. The adsorption ratio of HSCAS ranged from 95.3 to 99.1% and 84.7 to 92.4% of the available AFB₁ and FB₁ respectively. EM showed an adsorption ratio ranged from 95.4 to 99.2% and 78.2 to 92.2% for AFB₁ and FB₁ respectively. Both adsorbents were effective at 0.5% level. Results of the ability of these adsorbents at level of 0.5% (w/v) to adsorb AFB₁ and FB₁ in malt extract spiked with 50, 100 and 200 ppb indicated that the capability of adsorbing of HSCAS ranged from 98.5 to 98.9% and 88.2 to 91.9% for AFB₁ and FB₁ respectively. Whereas, the capability of adsorbing of EM ranged from 98.1 to 98.7% and 88.2 to 92.5% for AFB₁ and FB₁, respectively.

The isothermal adsorption and the adsorptive mechanism of aflatoxin B₁ (AFB₁) on calcium montmorillonite (Mont) were studied *in vitro* trials [17].

The maximum amounts of AFB₁ on Mont in aqueous solution at 2 and pH 8 were 613.5 and 628.9 µg of AFB₁/g of Mont, respectively. The Mont, when added to the diet of broiler chicken at 0.5%, significantly diminished the adverse effects of feeding 200 µg of AFB₁/kg of feed. And the concentrations of Ca, P, Cu, Fe, Zn in the broiler bones were not affected by AFB₁ and Mont, but the concentrations of Mn, Pb, and F were decreased by Mont.

Thieu and Pettersson [18] reported results of an *in vitro* study using single concentration and isotherm adsorption to evaluate the capacity of Vietnamese produced zeolite and bentonite to adsorb aflatoxin B₁ (AFB₁) in simulated gastrointestinal fluids (SGFs), and a commercial sorbent hydrated sodium calcium aluminosilicate (HSCAS) was used as reference. Adsorption of AFB₁ onto zeolite and bentonite varied according to the pH of SGFs and was lower than HSCAS. Linearity between the increased amount of AFB₁ adsorbed on sorbents and the decrease of sorbent concentration was observed for bentonite and HSCAS, except for zeolite in SGFs at pH 7. The observed maximum amounts of AFB₁ adsorbed on bentonite and HSCAS were 1.54 and 1.56 mg/g, respectively. The adsorption capacities of bentonite and HSCAS for AFB₁ were 12.7 and 13.1 mg/g, respectively, from fitting the data to the Freundlich isotherm equation.

Eight potential aflatoxin-sequestering agents (SAs) were tested for their ability to adsorb aflatoxin B₁ (Afb₁) and aflatoxin G₁ (Afg₁) *in vitro*. They belong to main SA classes: silicate minerals (calcium, magnesium and sodium bentonites, kaolinite, zeolite and clinoptinolite), activated carbon and yeast cell wall-derived [19]. The Ca bentonite and clinoptinolite were able to bind available Afb₁ in MM and RM methods, while they appeared inefficient (available Afb₁ sequestered less than 80%) when W was used. The adsorption ability of zeolite was confirmed only with the W method. Ineffective or limited sequestering activity was obtained with kaolinite and yeast cell wall-derived products with each method. The AFB₁ and AFG₁ sequestering efficiencies observed in the present work resulted very similar showing strong and positive correlation ($P < 0.001$) within methods ($r = 0.79$, $r = 0.96$ and $r = 0.99$, respectively for W, MM and RM methods). The two simulated gastrointestinal methods (MM and RM, respectively) gave similar results and could be considered useful for *in vitro* pre-screening of potential sequestering agents.

The present paper reports a research studying capability of Indonesian natural bentonite and zeolite to adsorb aflatoxin B₁ in aqueous solution, and the application of this technique for the reduction of FB₁

from aqueous solution during the extraction of kernel and grain corn samples.

EXPERIMENTAL SECTION

Materials

Standard solution of AFB1 in toluene : acetonitrile (9:1) 25.92 ng/mL was prepared by dissolving powder of AFB1 standard purchased from Sigma Chemical Co., St. Louis, MO. Working standard solutions used for HPLC analysis were 25, 50, 100 and 200 ng/mL. HPLC eluent consisted of double distilled water, methanol (JT Baker, USA) and acetonitrile (Fisher Scientific, USA) in HPLC grade used without pre-treatment. ELISA kit of AFB1 (Ridascreen®) was used for analysis of AFB1 in corn suspension.

Kernel Corn sample (P 21 hybrid) 5 kg was collected from Klaten district (Central Java) with water content of 15-18%. Apart of the sample was grounded in order to get the particle size similar to that for cattle ransom. To prepare AFB1 contaminated corn, the sample was grown up with fungi producing aflatoxin (*Aspergillus Flavus*) for two weeks.

Bentonite sample was collected from a source location in Pacitan district (East Java) and zeolite was sampled from Wonosari district, Yogyakarta. The samples were dried in an oven at 110 °C for 2 h and grounded in order to give various particle sizes (80, 100, 200 and 400 mesh).

Instrumentation

A shaking (GFL 3015) and centrifuge (Eppendorf, 5810R) apparatus were used for adsorption and separation, respectively. For analysis of AFB1 was used a set of HPLC system consisting of a delivery pump (LDC Analytical, constametricR 4110, USA), an auto-sampler (Hitachi, AS 2000, Japan), a fluorescence detector (Shimadzu RF 535, Japan) and a HPLC column (25 x 4.6 mm, Keystone Scientific, INC). Analysis of AFB1 in corn sample was carried out by using ELISA (Enzyme-linked immunosorbent assay) method using and a Micro-plate Reader Type 550 122 (Bio-Rad Laboratories, Richmond, CA, USA).

Characterization of zeolite and bentonite was carried out with infrared spectrophotometer and x-ray diffraction method for identifying functional groups and crystalline level of adsorbent, respectively.

Procedure

Adsorption of standard AFB1 on adsorbents

Adsorption was emphasized in a batch system. Adsorbent 25 mg was mixed with 5.0 mL of AFB1

aqueous solution in plastic container. The mixture was shaken mechanically and centrifuged. Concentration of AFB1 in supernatant was determined using HPLC method with mobile phase of water : methanol : acetonitrile 62:32:16 (% v/v) at a flow rate of 1.0 mL/min. The amount of AFB1 adsorbed (ng/mg) was calculated with the following equation:

$$m = \frac{(C_0 - C)V}{M} \quad (1)$$

where C_0 and C are initial and final concentration, respectively, (ng/mL) of AFB1. V is volume of solution (mL) and M is adsorbent weight (g). The above work was done for both adsorbents (zeolite and bentonite). Three variables including contact time, initial concentration of AFB1 and particle size of adsorbents were investigated. The variable of contact time was varied from 15 to 180 min, the initial concentration was from 200 to 2000 ng/mL and four different particle sizes (80, 100, 200 and 400 mesh) were examined.

Reduction of AFB1 in corn suspension

Reduction was carried out similar to the adsorption of AFB1 in standard solution. Corn samples (kernel and grounded form) 5 g and adsorbent were suspended with 15 mL and shaken mechanically for 2 h. The weight of adsorbent was varied from 25 to 1000 mg. The suspension was filtered and AFB1 in the filtrate was analyzed with ELISA method at 450 nm. The amount of AFB1 adsorbed was calculated by subtracting initial concentration of AFB1 in filtrate (without addition of adsorbent) with concentration of filtrate after adsorption.

RESULT AND DISCUSSION

Adsorbent Characters

Characterization of adsorbents was carried out by two techniques, namely x-ray diffraction and infrared spectroscopic methods for identification of crystalline level and functional groups, respectively. Infrared spectra were expressed in Fig. 1.

Absorbance pattern in infrared spectra of bentonite (Fig. 1(a)) may be interpreted as follows: absorbance band at 3448.5 cm^{-1} indicates vibration of hydroxyl groups from silanol (Si-OH) or/and aluminol (Al-OH). Strong and sharp absorbance at 1049.3 cm^{-1} is asymmetric stretching vibration from T-O groups (T=Si/Al) in silica (Si-O-Si) and/or alumina (Al-O-Al). Band at 956.6 cm^{-1} is predicted as stretching vibration from Si-O in Si-OH or Al-O in Al-OH. Stretching vibration of Si-O-Si is showed at 794.5 cm^{-1} and bending vibration from Si-O-Si or Al-O-Al gives absorbance at 474.5 cm^{-1} . The band at 1635.5 cm^{-1} is resulted from bending vibration of -OH groups from

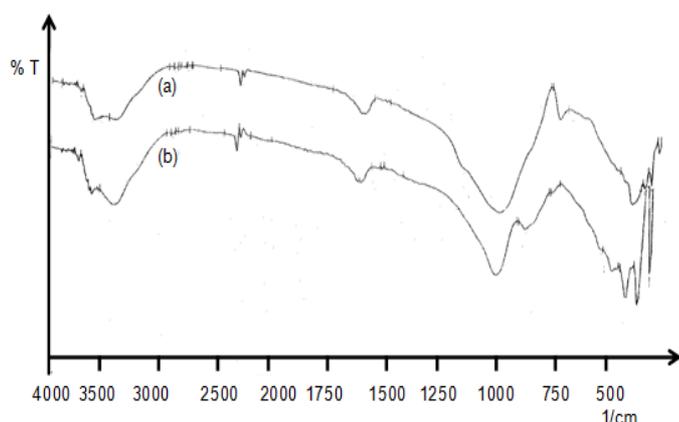


Fig 1. Infrared spectra of bentonite (a) and zeolite (b)

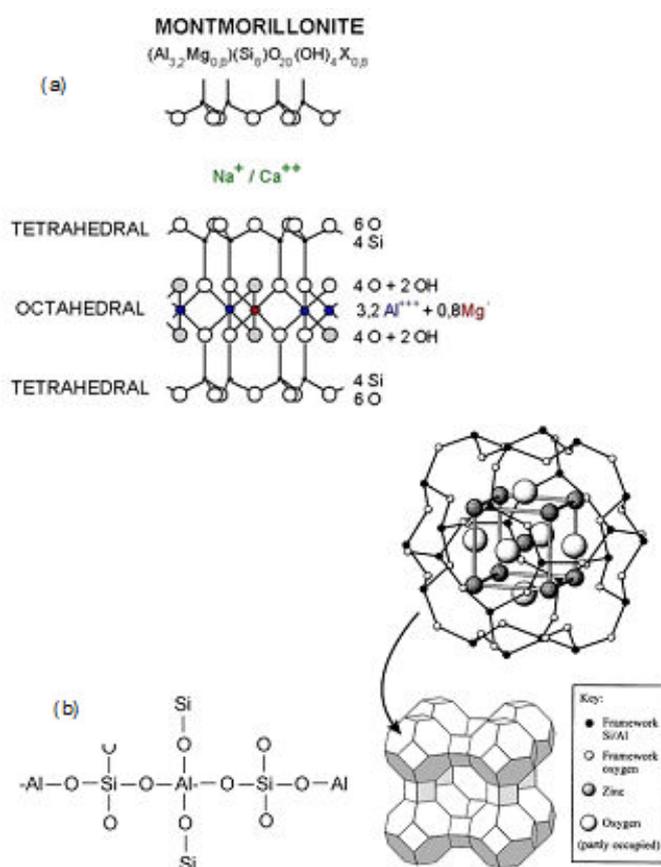


Fig 2. Structure Model of bentonite (a) and zeolite (b)

T-OH. In general, absorbance band in infrared spectra of bentonite informs that functional groups present are silanol/aluminol (T-OH) and T-O-T groups. It is observed that infrared spectra of zeolite (Fig. 1(b)) give similar spectra of bentonite indicating similarity of the presence of functional groups. The difference lies on the absorbance intensity.

Although the functional group in bentonite and zeolite are similar, the structure of both materials is

Table 1. XRD patent of adsorbents

| Adsorbent | 2θ (°) | d (°A) | Minerals |
|-----------|--------|--------|--------------------------------------|
| Bentonite | 22.45 | 3.957 | Montmorillonite |
| | 27.91 | 3.194 | |
| | 25.88 | 3.440 | |
| Zeolite | 27.95 | 3.190 | modernite, levynite and ansime |
| | 5.82 | 15.170 | |
| | 5.54 | 15.939 | |
| | | | |

Table 2. Freundlich parameters for adsorption of AFB1 on adsorbents

| Adsorbent | K_f | n | R^2 |
|-----------|-------|------|--------|
| Zeolite | 1.39 | 0.63 | 0.9713 |
| Bentonite | 5.33 | 0.68 | 0.9939 |

different. From Table 1 can be seen that XRD data gives three big peaks at 2θ 22.45, 27.91 and 25.88 with d 3.957, 3.194 and 3.440 °A. Those indicate characteristic values for the structure of montmorillonite and as the main component of the bentonite. The structure model of montmorillonite is expressed in Fig. 2(a). From data of XRD for zeolite summarized in Table 2 can be stated that the main components of zeolite are modernite, levynite and ansime.

Adsorption of Standard AFB1 in Aqueous Solution

Analysis of AFB1 used for the adsorption process was HPLC and by using optimum condition, it was found that AFB1 gave chromatographic peak at retention time of 19.15 min (Fig. 3). Evaluation of standard working solution expressed with standard curve shows higher linearity with coefficient correlation (R^2) of 0.9998.

Effect of Contact Time and Adsorbent Particle Size

Experiment to examine the effect of contact time on the adsorption of standard AFB1 was emphasized by varying contact time from 15 to 180 min at concentration of AFB1, adsorbent weight and particle size constant, namely 2 µg/mL, 25 mg and 200 mesh, respectively. The analogue step was done for other variables. Result is expressed in Fig. 4 for the effect of contact time and Fig. 5 for particle size effect. From Fig. 4 can be seen that adsorption of AFB1 on both zeolite and bentonite is very fast. Within 15 min, the process reaches equilibrium. Due to the technical reason, process less than 15 min is not possible to be carried out; hence adsorption kinetics could not be quantitatively evaluated.

Fig. 5 shows that adsorption capability increases with smaller particle size. For four different particle sizes may be classified into two groups' namely big size

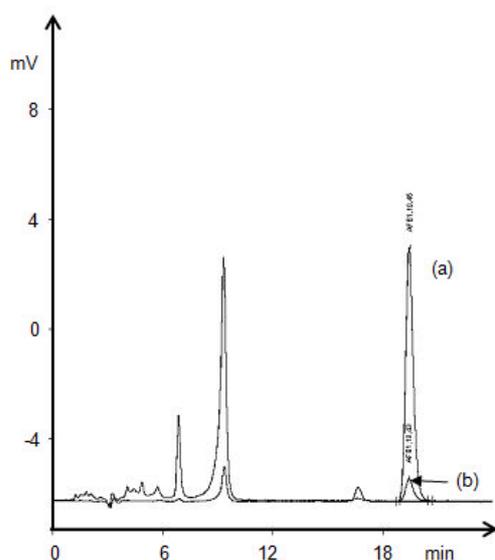


Fig 3. Chromatogram of standard AFB1 in aqueous solution with concentration of 200 ng/mL (a), and 2000 ng/mL after being adsorbed with 25 mg zeolite for 3 h (b)

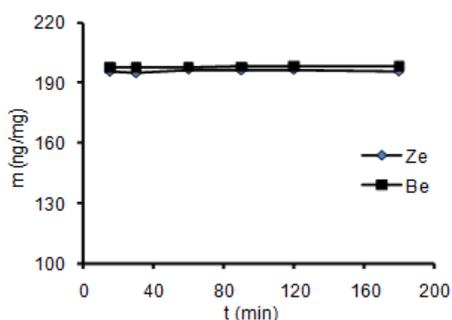


Fig 4. Correlation curve between the amount AFB1 adsorbed (m) versus contact time (t) on adsorbent zeolite (Ze) and bentonite (Be)

(80 and 100 mesh) and small size (200 and 400 mesh). Capability of adsorption in a group of particle size is not significant different, especially for zeolite.

Effect of AFB1 Concentration

As mentioned in the experimental section, the effect of concentration on adsorption was carried out by mixing standard AFB1 solution with adsorbent (constant weight) in a batch system at various initial concentration of AFB1 and other variables (particle size, contact time and weight of adsorbent) constant. An example of standard AFB1 chromatogram before and after adsorption can be seen in Fig. 6 and data of adsorption is expressed in Fig. 7.

From Fig. 6 can be observed that initial concentration up to 2000 ng/mL the adsorption is still increased indicating very highly capacity of the

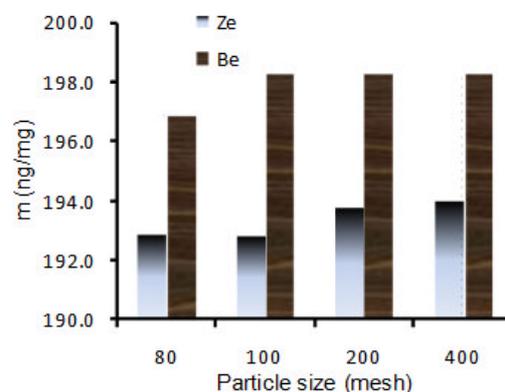


Fig 5. Diagram of correlation between the amount AFB1 adsorbed (m) versus particle size on adsorbent zeolite (Ze) and bentonite (Be)

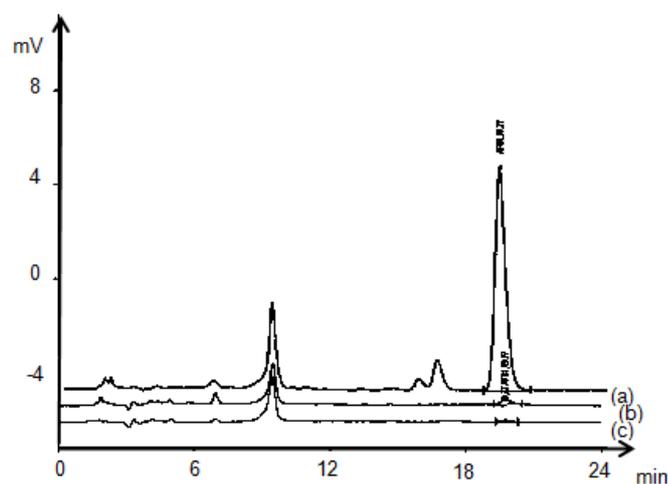


Fig 6. Chromatogram of standard AFB1 200 ng/mL (a), 2000 ng/mL after being adsorbed with zeolite (b) and bentonite (c)

adsorbents for AFB1. Adsorption at higher concentration was not carried out due to low solubility of AFB1 in water. However, if both adsorbents are compared, it can be observed that bentonite gives adsorption capability higher than zeolite does. It is agreed with quantitative evaluation of the experimental data using Freundlich model [20]. The Freundlich model assumes heterogeneous adsorption due to the diversity of the adsorption sites or the diverse nature of adsorbate. The Freundlich model is expressed as:

$$m = K_f C^{1/n}$$

where K_f is the Freundlich constant related to adsorption capacity of adsorbent and n is the Freundlich exponent related to adsorption intensity. K_f and n can be calculated from the slope and intercept of the linear plot of $\log m$ versus $\log C$. Table 2 displays the coefficients of the Freundlich model along with regression coefficients (R^2). From the table can be

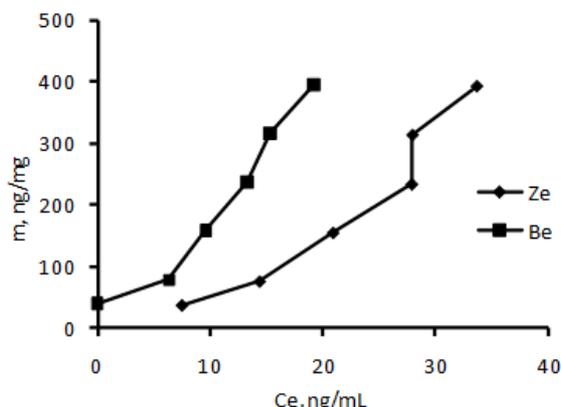


Fig 7. Correlation curve of the amount of AFB1 adsorbed (m) versus concentration of AFB1 in equilibrium (C_e)

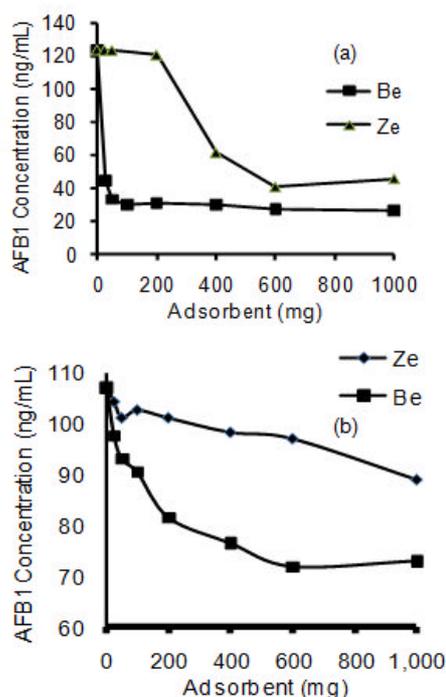


Fig 8. Reduction of AFB1 concentration in grounded (a) and kernel (b) corn by adsorption process

seen that capacity, represented by K_f , of bentonite is 3.8 fold higher than that of zeolite, even though the intensity, based on the value of n , is not so significant different. This result is different from that reported by Thieu and Pettersson [18]. The adsorption capacities of bentonite and HSCAS for AFB₁ were 12.7 and 13.1 mg/g, respectively, from fitting the data to the Freundlich isotherm equation and it is higher than resulted in this work. This supports Gallo and Masoero [19] that media influences the adsorption.

The structure of adsorbent seems to have more important role on the adsorption process rather than the

presence of the functional groups. Interlayer space and swelling level of bentonite are suspected as the main factor on higher adsorption capability of bentonite. If the adsorption related to functional groups, based on the infrared spectra, the capability of both adsorbents would be similar. In the other words, capability of adsorption is not depended significantly on the functional group present in the adsorbent and probable on the hydrophobic level. Zeolite, more hydrophobic than bentonite gives fewer tendencies to interact with AFB₁. It is consistent with results reported by Daković et al. [22]. Low polar AFB₁ had a high affinity for the unmodified zeolitic tuff and the adsorption of AFB₁ was greatly reduced for octadecyldimethylbenzyl ammonium modified zeolite. Desheng et al. [17] suggested that the mechanism of AFB₁ on montmorillonite was AFB₁ sorbed onto the edge of Mont by a double hydrogen bond, and AFB₁ molecules did not penetrate into the interlayer area of Mont. Bonding between AFB₁ and smectite clay was predicted to appear to be in the furan rings [22]. Other possible bonding is with the two oxygens in the coumarin ring of AFB₁ and interlayer cations or their associated water molecules.

Reduction of AFB1 Contamination in Corn

In this work, AFB₁ contaminated corn samples were used and reduction was carried out by mixing aqueous suspension of corn sample with adsorbent in a batch system. Analysis of AFB₁ in suspension after and before adsorption process was analyzed using ELISA method and detected with visible spectrophotometer (ELISA reader). For standard AFB₁, in the range of 5 - 50 ng/mL gives curve represented as correlation between absorbance percentage versus concentration with correlation coefficient, R^2 , of 0.9971 and the equation: % ABS = -19.164 ln[AFB₁] + 93.06.

In the adsorption process, it was expected that AFB₁ in corn samples was extracted into aqueous suspension and then adsorbed by adsorbent. A variable examined was adsorbent weight, being varied from 25 to 1000 mg of corn sample at corn weight and water volume constant 5 g and 10 mL, respectively.

Consistent with adsorption of standard AFB₁ in aqueous solution, from Fig. 8 shows that capability of bentonite to adsorb AFB₁ in aqueous suspension of corn samples is higher than that of zeolite. However, the capacity of both adsorbents to adsorb AFB₁ in aqueous solution is much higher than in corn suspension. Zeolite and bentonite (25 mg) are able to adsorb completely AFB₁ 99 and 96%, respectively, of 200 ng/L (1.0 mg) in aqueous solution, but only 9.5 and 33.0%, respectively, of 107 ng/mL (1.6 mg) in suspension of kernel corn, and 67.3 and 78.1% in grain

corn suspension. It is not surprising because corn aqueous suspension contains various types of aflatoxins, even other mycotoxins, leading to competition among them in adsorption.

Evaluation of the capacity of cation type of different bentonites to adsorb aflatoxins in different media has been reported by Gallo and Masoero [19]. The AFB1 and AFG1 used were extracted from a contaminated corn meal (82.21 mg/kg of AFB1 and 97.20 mg/kg of AFG1). Three single-concentration adsorption tests, consisting of a simply-water (W), a gastro-intestinal simulating monogastric model (MM) and a ruminant model (RM) were used. The AFB1 and AFG1 recovered in controls were 92.3 and 104.9% in W and 89.5 and 101.5% in MM; while in RM were 65.2 and 81.9%, respectively. This supported that intrinsic rumen fluid factors could be involved in sequestering of aflatoxins. The sequestering agents (Mg bentonite and Na bentonite) were very efficient to sequester the available AFB1, with a sequestering activity of over 99.0% with each method.

CONCLUSION

The findings of this study indicated that result shows that adsorption of AFB1 on adsorbents of natural zeolite and bentonite is very fast. Within 15 min 99% of AFB1 (200 ng/mL) has been adsorbed by 25 mg of bentonite and 96% by zeolite. The particle size higher than 200 mesh did not give significant effect on the AFB1 adsorption capability. In comparison, effectiveness of zeolite in adsorbing AFB1 is lower than that of bentonite. The difference is probable caused by the structure rather than the present functional groups of the adsorbents. It was supported by data of infrared spectroscopy indicating similarity of spectra and x-ray diffraction (XRD) data showing different pattern. Capability in reducing AFB1 contamination in corn samples (kernel and meal) for both adsorbents is lower than that in standard solution. Adsorbent (1.0 g) of zeolite and bentonite reduce 17 and 37%, respectively, of AFB1 (107 ng/mL) in kernel corn suspension and 78 and 67%, respectively, of AFB1 (124 ng/mL) in grained corn suspension. Other mycotoxins contained in the corn samples are predicted as main factor of the decrease of adsorption capability.

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