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Activated Carbons: In Vitro Affinity for Fumonisin B_1 and Relation of Adsorption Ability to Physicochemical Parameters

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ABSTRACT

In vitro affinity tests were conducted to assess the effectiveness of 19 activated carbons (ACs), hydrated sodium calcium aluminosilicate (HSCAS), and sepiolite (S) in binding fumonisin B_1 (FB_1) from solution. Relationships between adsorption ability and physicochemical parameters of ACs (specific surface area, iodine value, and methylene blue index) were tested. When 5 ml of a 4-μg/ml aqueous solution of FB_1 was treated with 10 mg of AC, ACs adsorbed 0.46 to 100% of the FB_1. HSCAS and S were not effective in binding FB_1. In two saturation tests carried out with decreased amounts of sorbent (5 and 2 mg, respectively), three ACs also showed high adsorption ability (adsorbing 96.48 to 99.20% of the FB_1). A general relationship between adsorption ability and the physicochemical parameters of the ACs was observed, supporting the inference of a close relationship between molecule trapping and surface physicochemical adsorption processes. The methylene blue index was more reliable than iodine number and surface area for predicting ability of ACs to adsorb FB_1. In tests of simultaneous adsorption ability carried out using 5 ml of a solution containing 10 μg/ml FB_1 plus 50 μg/ml aflatoxin B_1 (AFB_1) and 2 or 5 mg of sorbent, ACs showed a higher affinity for AFB_1 than for FB_1. However, two ACs bound ca. 100% of the two mycotoxins. When 5 ml of an aqueous extract solution obtained from naturally contaminated corn containing 1.84 μg/ml FB_1 and 0.042 μg/ml AFB_1 was treated with 10 mg of sorbent, one AC adsorbed ca. 95% and 99% of FB_1 and AFB_1, respectively. It is concluded that certain ACs have high in vitro affinity for FB_1 and AFB_1 singly or in combination, and may hold promise as multi-mycotoxin sequestering agents. However, further in vivo investigations are needed to confirm the abilities of ACs to sequester the most important mycotoxins singly or in combinations, establish the amounts to be added to feeds, and determine any long-term effects they may have on gastrointestinal absorption of essential nutrients.

Key words: Activated carbons, fumonisin B_1, binding, detoxification

Fumonisins are a class of recently discovered mycotoxins produced by *Fusarium moniliforme, Fusarium proliferatum,* and other *Fusarium* species that naturally occur on corn throughout the world. Fumonisins include A_1, A_2, B_1, B_2, B_3, and B_4, although Norred *(16)* has argued that other fumonisins are likely to be discovered. Fumonisin B_1 (FB_1), considered the most important and most active of the fumonisins, has been associated with severe animal diseases such as equine leukoencephalomalacia *(23),* porcine pulmonary edema, and immunosuppression in poultry *(16, 21).* Moreover, additional effects such as nephrosis in sheep *(16),* toxicity in fish *(15),* cardiac thrombosis in baboons *(13),* atherosclerosis in nonhuman primates *(8),* and liver toxicity and carcinogenicity in rats and mice *(17)* have been reported. Epidemiological investigations carried out in some areas of South Africa and China have led researchers to suspect strongly that FB_1 may be carcinogenic to humans. In these areas high frequency and high levels of contamination of corn with FB_1 have been found to be correlated with high incidence of esophageal cancer in people consuming corn as a staple *(30).*

Hazardous levels of human and animal exposure to FB_1 have been reported in surveys carried out in Europe *(20, 25, 34),* the USA *(4),* China *(40),* Thailand *(39),* Southeast Asia *(38),* and South Africa *(30).*

Although some information is currently available on methods for detoxification in cases of FB_1 contamination, Norred and Voss *(17)* have pointed out the need for efficacious methods for detoxification of fumonisin-contaminated foods and feeds. Physical removal of fine particulate material from corn reduces FB_1 contamination by about 26 to 69%, and can be considered as a preliminary decontamination procedure *(33).* Contrasting data on the thermostability of FB_1 have been reported. According to Alberts et al. *(1)* FB_1 is heat stable, insofar as no reduction in the FB_1 concentration was observed after boiling *F. moniliforme* culture material in water for 30 min followed by drying at 60°C for 24 h. Dupuy et al. *(6)* confirmed the overall resistance of FB_1 on corn to heat treatments and drying processes. On the other hand, Scott and Lawrence *(27)* observed that about 40% of the FB_1 was recovered when it

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was heated with corn meal at 190°C and 20 to 30% when it was heated with moist corn meal at 190°C; FB_1 was found to be completely unstable in corn meal at 220°C. Therefore, neither drying nor normal food processing and cooking are effective in destroying FB_1. In vitro and in vivo tests performed by Norred et al. *(18)* and Voss et al. *(35)* have shown that treatment of FB_1-contaminated corn with ammonia at atmospheric pressure is ineffective for detoxification, whereas Park et al. *(19)* observed a 79% reduction of FB_1 following an ammonia treatment at high pressure and temperature. Sydenham et al. *(31)* reported calcium hydroxide to be highly effective for removing FB_1 from contaminated corn. Other chemical treatments based on potassium hydroxide *(2, 11, 23)* and hydrochloric acid *(30, 32)* have been found to hydrolyze FB_1, whereas sodium hypochlorite destroys it *(27)*. Small decreases in FB_1 concentrations following fermentation of wort by *Saccharomyces cerevisiae* and fermentation of corn by yeast have been reported by Scott et al. *(26)* and by Bothast et al. *(3)*, respectively.

No data on the efficacy of adding sorbents to FB_1-contaminated feeds and foods are currently available, although this detoxification strategy has been shown to be very effective for other carcinogenic mycotoxins, such as aflatoxin B_1 (AFB_1) *(22, 24)*. Recently, we reported that activated carbons (ACs) have a very high effectiveness in binding aflatoxin B_1 in vitro *(10)* and in vivo *(9)*. The objective of the present investigation was to evaluate the FB_1 affinity of ACs, hydrated sodium aluminosilicate (HSCAS), and sepiolite (S) in vitro. An overall relationship between adsorption ability and the physicochemical parameters (PCPs) of ACs reported in a previous test on AFB_1 *(10)* suggested we should also look for such a relationship in tests on FB_1.

MATERIALS AND METHODS

Sorbents

The following sorbents were tested: 15 experimental activated carbons (EACs) from exhausted olive residues (AF), peach stones (PEP2), and almond shells (MAP2) prepared with laboratory equipment of the Chemistry Department of the University of Catania by several experimental activation processes appropriately selected to obtain the desired PCPs; four commercial ACs (CACs) produced in industrial processing equipment; an HSCAS demonstrated to have a high affinity in vitro for AFB_1 *(10)*; and sepiolite (S), a clay commonly used in animal feed as a pellet binder and demonstrated to have a good affinity for AFB_1 *(22, 24)*. All of the sorbents were finely pulverized.

Activated carbons: physicochemical parameters and measurement equipment

The following PCPs of ACs (Table 1) were determined: number surface area (SA), iodine (IN) and methylene blue index (MBI). The meaning of the PCPs and the methods and equipment used for their determination have been described more extensively in a previous study by Galvano et al. *(10)* on the in vitro affinity of ACs for AFB_1.

Samples of each sorbent were individually weighed into glass tubes (three replicates per sample) and the requisite amounts of FB_1 (Sigma Chemical Co., St. Louis, MO, USA, purity >99%) in aqueous solution were added. After a treatment time of 1 h at 25°C, with mixing at 15-min intervals, all the tubes were centrifuged (centrifuge model 972R, ALC, Milan, Italy) for 10 min at 1,500 rpm. Three adsorption tests were carried out with varied amounts of AC. In test 1, 5 ml of a working solution containing 4 μg/ml of FB_1 was added to 10 mg of each sorbent. In this test, relationships between FB_1 adsorption and PCPs of ACs were investigated. To differentiate the adsorption ability of ACs and determine their saturation limits, in tests 2 and 3, 5 ml of the working solution was added to 5 mg and 2 mg of the AC, respectively, for each AC which did not reach the saturation condition in the previous test.

Five tests of simultaneous affinity for FB_1 and AFB_1 were conducted on the five ACs (AF32, AF48, CAC1, CAC2, and CAC4) that showed the highest adsorptive properties for the two mycotoxins. Five milliliters of a solution containing 10 μg/ml of FB_1 and 50 μg/ml of AFB_1 was added to 2 and 10 mg of the ACs in tests 4 and 5, respectively. For tests 6 through 8, FB_1 and AFB_1 were extracted from naturally contaminated corn according to the method of Shephard et al. *(28)*, since a 3:1 (vol/vol) mixture of methanol and water is capable of extracting both FB_1 and AFB_1; the methanol was evaporated at reduced pressure, and the volume was replaced with water; 5 ml of the aqueous extract solution, containing 1.84 μg/ml FB_1 and 0.042 μg/ml AFB_1, was added to 2, 5, and 10 mg of each of the five ACs, in tests 6, 7, and 8, respectively.

TABLE 1. *Physicochemical parameters (PCPs) of experimental and commercial activated carbons (EACs and CACs)*

Activated carbon[a]	Surface area (m^2/g)	Iodine number (mg/g)	Methylene blue index (mg/g)
EACs			
AF13	561.1	728.0	34.0
AF24	291.0	437.0	16.2
AF29	433.7	566.0	14.0
AF32	787.2	931.0	109.6
AF33	260.3	237.0	1.41
AF34	309.0	292.0	3.8
AF35	309.9	162.0	4.8
AF37	403.0	450.0	NA[b]
AF45	493.1	563.0	NA
AF47	482.0	575.0	NA
AF48	865.0	966.0	239.4
AF50[c]	811.0	943.0	89.0
AF52[c]	950.0	1,100.0	170.0
PEP2	943.0	811.0	206.0
MAP2	1,254.0	1,082.0	256.0
CACs			
CAC1	1,122.0	1,100.0	200.0
CAC2	1,116.0	1,250.0	200.0
CAC3	905.0	1,350.0	260.0
CAC4	1,059.0	1,100.0	283.0

[a] Sources of ACs: AF, olive residues; PEP, peach stones; MAP, almond shells.
[b] NA, not assayed.
[c] Activated with sulfuric acid.

Methods of analysis

FB_1 was determined according to the method of Shephard et al. *(28)*; in tests 6 through 8, 4 ml of each solution was purified by the method of Shephard et al. *(28)* before the determination; the remaining 1 ml was used for AFB_1 analysis according to the official method of the European Communities *(7)*.

HPLC apparatus and procedure

The instrument used for HPLC was a Perkin-Elmer LC 200 (Perkin-Elmer Corp., Norwalk, CT, USA) equipped with an ISS 200 sampling system and a Perkin Elmer LS 30 fluorescence detector set at excitation and emission wavelengths of 335 nm and 440 nm, respectively. The system was controlled by Perkin-Elmer Turbochrom 4 PC software. The stationary phase for the column (125 mm by 4 mm i.d.) was LiChrospher 5-μm RP-18 (Merck, Darmstadt, Germany). The mobile phase for FB_1 determinations was an isocratic 46:54 (vol/vol) mixture of acetonitrile and an aqueous solution prepared from 1 liter of water plus 20 ml of acetic acid plus 1 ml of triethylamine; the flow rate was 1.2 ml/min. The mean retention time for FB_1 was 5.4 min. The HPLC apparatus and procedure for AFB_1 determination in the adsorption tests were as described by Galvano et al. *(10)*.

Statistics

Adsorption data are presented as means ± SEM for three replicates per sample. Differences at $P < 0.05$ (Student-Newman-Keuls multiple range test) were considered to be significant. Relationships between FB_1 adsorption and PCPs were tested by best fitting to linear and nonlinear function models (Fig. P, Biosoft, Ferguson, MO, USA).

RESULTS

In test 1, the adsorption abilities of ACs (Figure 1) ranged from 0.46 to 100% adsorption of the available FB_1. CAC1, CAC2, CAC4, AF48, and AF32 showed particularly high adsorption abilities, since they adsorbed from 96.48 to 100% of the available FB_1 from the working solution. In tests 2 and 3 (Table 2) these five ACs also showed high

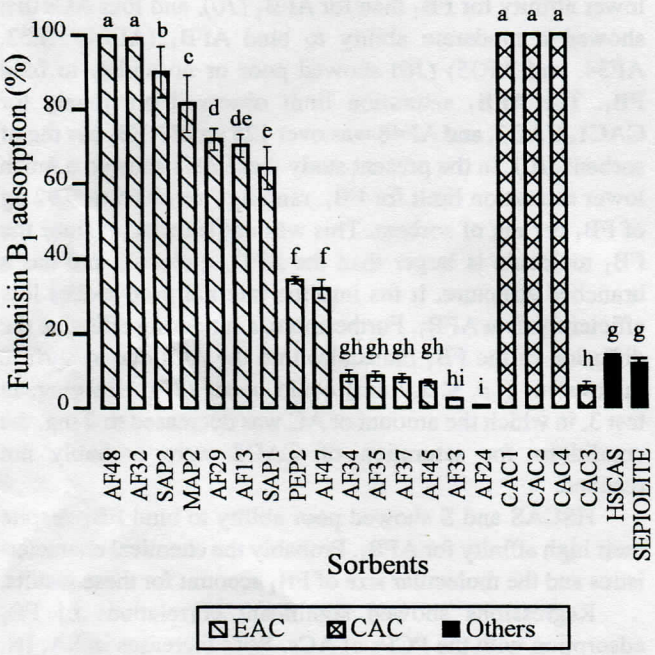

FIGURE 1. *Fumonisin B_1 adsorption from aqueous solution (4 μg/ml) by 10 mg of experimental activated carbons (EAC), commercial activated carbons (CAC), hydrated sodium calcium aluminosilicate (HSCAS), and sepiolite (S). Each bar represents the mean, n = 3; error bars denote the standard error of the mean; different letters on the tops of bars indicate a significant difference of means (P < 0.05).*

TABLE 2. *Adsorption ability with decreasing amounts of activated carbons*

Activated carbons[c]	Adsorption (%)[a] of available FB_1[b]		
	10 mg	5 mg	2 mg
CAC1	100.00 ± 0.00[A]	95.08 ± 0.15[B]	NA[d]
CAC2	100.00 ± 0.00[A]	99.69 ± 0.10[A]	99.20 ± 0.11[A]
CAC4	100.00 ± 0.00[A]	99.06 ± 0.38[A]	96.48 ± 1.10[B]
AF32	99.04 ± 0.05[A]	85.65 ± 2.05[C]	NA
AF48	99.88 ± 0.12[A]	99.13 ± 0.21[A]	98.12 ± 0.23[A]

[a] Values indicate group mean ± SEM; $n = 3$. Different letters in columns indicate a significant difference of the means ($P < 0.05$).
[b] Sorbents were combined with 5 ml of a 4-μg/ml FB_1 solution.
[c] CAC, commercial AC; AF, olive residue AC.
[d] NA, not assayed.

adsorption abilities with decreased amounts of sorbent. In test 2 (with 5 mg of AC), CAC2, CAC4, and AF48 still adsorbed more than 99% of the available FB_1 from the working solution, whereas the adsorption abilities of CAC1 (95.08%) and AF32 (85.65%) clearly decreased. In test 3 (with 2 mg of AC), CAC2 and CAC4 retained high adsorption abilities (99.20 and 98.12%, respectively) whereas AF48 had a slightly decreased adsorption ability (96.48%).

HSCAS and S showed poor adsorption abilities (13.32 and 11.90%, respectively; Figure 1).

FB_1 adsorption was correlated with SA and IN, respectively, according to the following linear equations: FB_1 adsorption = 0.10SA − 14.26 ($r^2 = 0.66$) (Figure 2); FB_1 adsorption = 0.11IN − 24.51 ($r^2 = 0.79$) (Figure 3).

The best fitting regression equation model for the relationship between FB_1 adsorption and MBI was one of monoexponential (first-order) decay with residual (Figure 4), of the form $Y = a^{(-kX)} + R$, with the parameters $a = -94.36$, $k = 0.02$, and $R = 100.00$.

In tests 4 and 5 (with 2 mg and 10 mg of ACs, respectively), adsorption abilities ranged from 74.13 to 99.94% and from 8.00 to 100.00% for AFB_1 and FB_1, respectively (Figure 5).

In tests 6, 7, and 8 (with 2, 5, and 10 mg of ACs, respectively) adsorption abilities ranged from 4.03 to 99.56% and from 3.53 to 95.93% for AFB_1 and FB_1, respectively (Figure 6).

DISCUSSION

In the present study some of the ACs exhibited a high in vitro affinity for FB_1. CAC1, CAC2, CAC4, AF32, and AF48 showed particularly high adsorption abilities, even at decreased AC/FB_1 ratios. On the other hand, the adsorption abilities observed for CAC3 and PEP2 were medium to low. These data are generally consistent with the results of a previous test on in vitro affinity of ACs for AFB_1 *(10)*, but some differences have also been observed. For instance, in the AFB_1 study AF48 showed the highest affinity, whereas in the present study CAC2 was the most effective. Additionally the saturation tests showed that CAC1 and AF32 have a

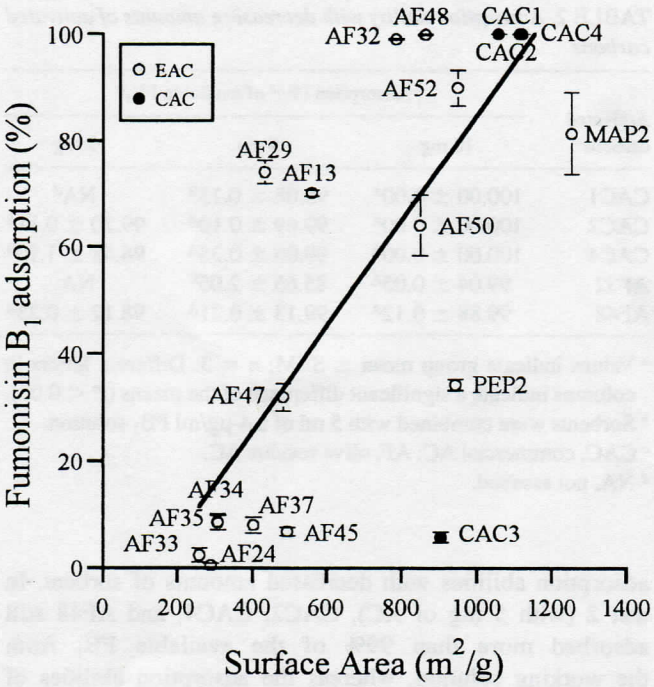

FIGURE 2. *Relationship between surface area of experimental (EAC) and commercial (CAC) activated carbons and ability to adsorb fumonisin B_1. Each point represents the mean, n = 3; error bars denote the standard error of the mean. CAC3 was not considered as a data point of the regression.*

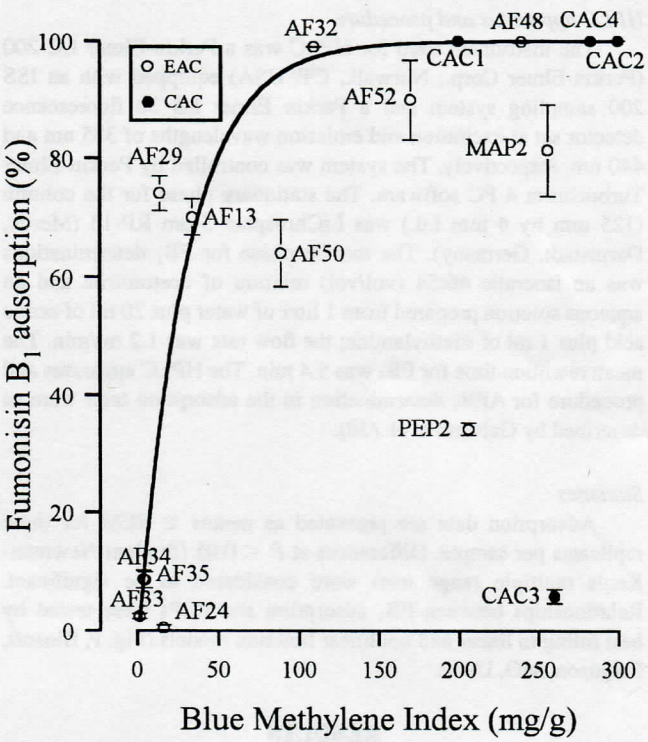

FIGURE 4. *Relationship between methylene blue index of experimental (EAC) and commercial (CAC) activated carbons and ability to adsorb fumonisin B_1. Each point represents the mean, n = 3; error bars denote the standard error of the mean. CAC3, PEP2, AF50, AF52, and MAP2 were not considered as data points of the regression.*

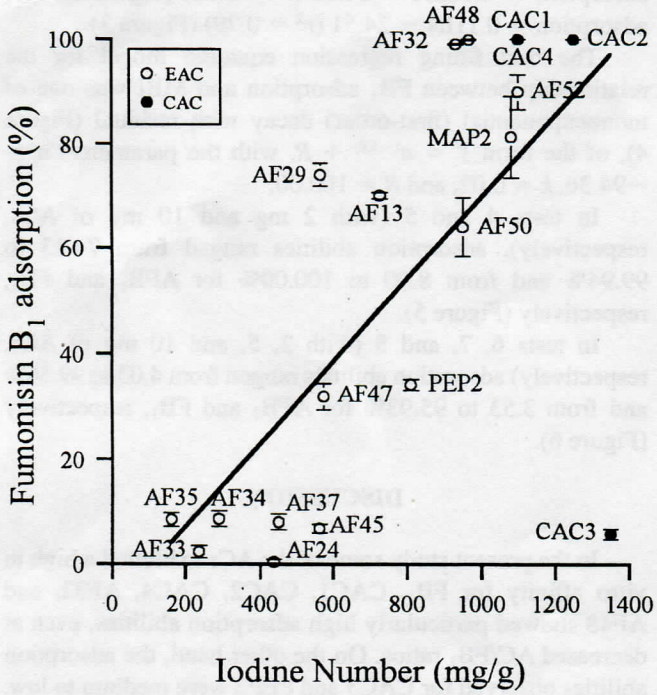

FIGURE 3. *Relationship between iodine number of experimental (EAC) and commercial (CAC) activated carbons and ability to adsorb fumonisin B_1. Each point represents the mean, n = 3; error bars denote the standard error of the mean. CAC3 was not considered as a data point of the regression.*

lower affinity for FB_1 than for AFB_1 *(10)*, and four ACs that showed a moderate ability to bind AFB_1 (AF24, AF33, AF34, and AF35) *(10)* showed poor or no ability to bind FB_1. The AFB_1 saturation limit observed previously for CAC1, CAC2, and AF48 was over 125 μg of AFB_1 per mg of sorbent *(10)*. In the present study these ACs showed a much lower saturation limit for FB_1, ranging from 9.65 to 9.92 μg of FB_1 per mg of sorbent. This was not surprising: since the FB_1 molecule is larger than the AFB_1 molecule and has a branched structure, it fits into the internal pore spaces less efficiently than AFB_1. Furthermore, a hindering effect on the diffusion of the FB_1 molecules into the ACs due to surface micropores (i.d. < 2.0 nm) could occur *(29)*. However, in test 3, in which the amount of AC was decreased to 2 mg, the conditions for saturation of CAC2 were probably not reached.

HSCAS and S showed poor ability to bind FB_1 despite their high affinity for AFB_1. Probably the chemical characteristics and the molecular size of FB_1 account for these results.

Regressions showed significant correlations of FB_1 adsorption with the PCPs of ACs. With increases in SA, IN, and MBI a corresponding increase in FB_1 adsorption was observed. The trends of the regressions were similar to those observed for AFB_1 *(10)*, despite major differences between the molecular structures and properties of FB_1 and those of AFB_1. Linear models for SA and IN and an exponential model for MBI were found to describe appropriately the

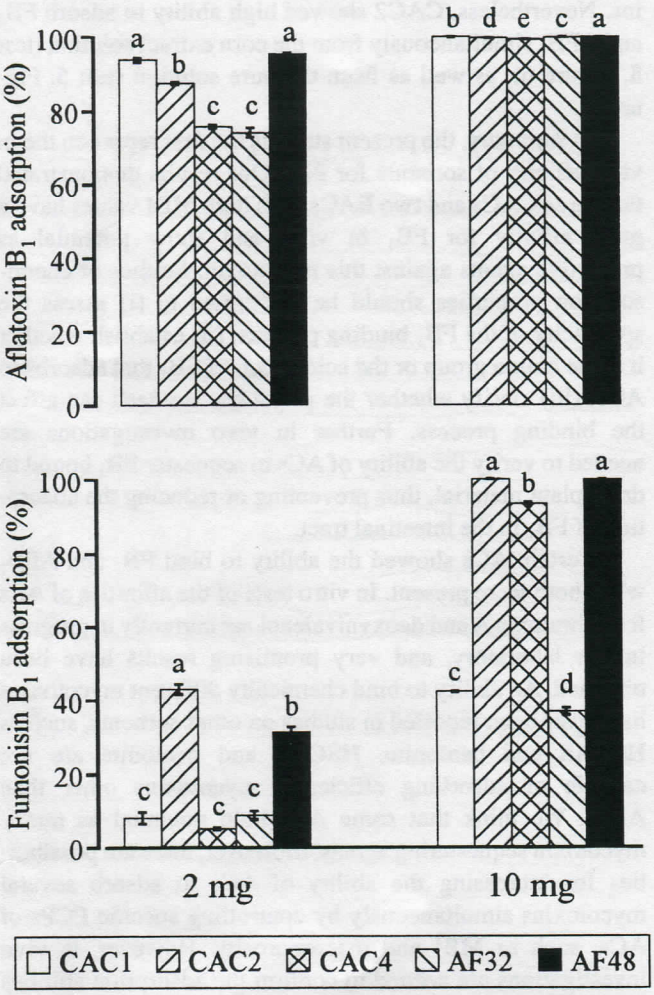

FIGURE 5. *Simultaneous adsorption of fumonisin B_1 (10 μg/ml) and aflatoxin B_1 (50 μg/ml) from aqueous solution by 2 mg and 10 mg of five activated carbons. Each bar represents the mean, n = 3; error bars denote the standard error of the mean; different letters on the tops of bars indicate a significant difference of means (P < 0.05).*

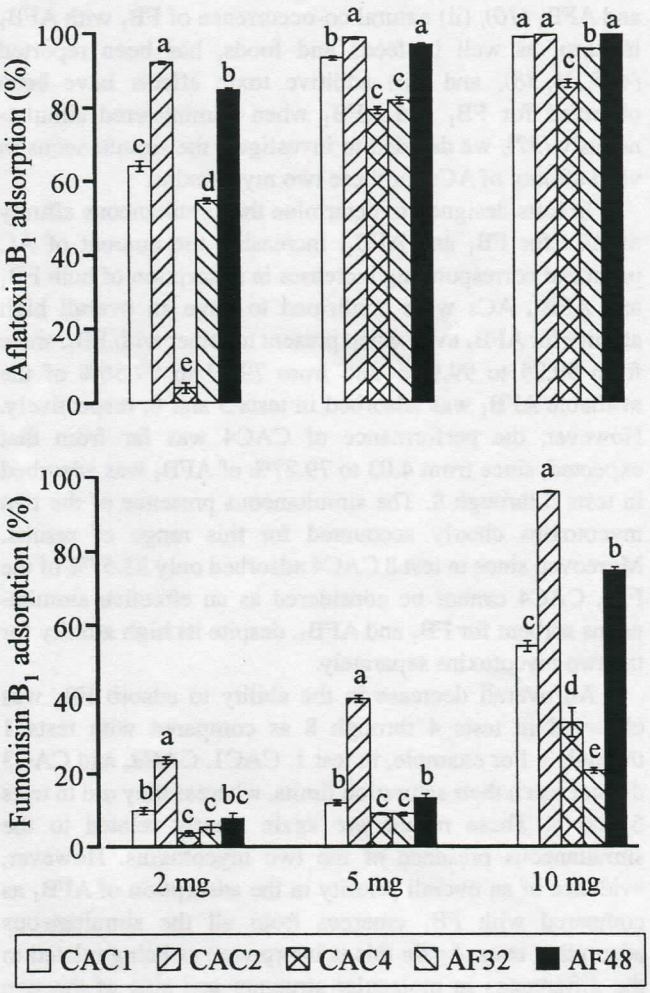

FIGURE 6. *Simultaneous adsorption of fumonisin B_1 (1.84 μg/ml) and aflatoxin B_1 (0.042 μg/ml) from extract solution, obtained from naturally contaminated corn, by 2 mg, 5 mg, and 10 mg of five activated carbons. Each bar represents the mean, n = 3; error bars denote the standard error of the mean; different letters on the tops of bars indicate a significant difference of means (P < 0.05).*

relationships between the adsorption trends and the PCPs of ACs. Furthermore, as was true for AFB_1 *(10)*, MBI was more reliable than SA and IN in predicting ability to adsorb FB_1. These results confirm that MBI is the most reliable indicator of the adsorptive properties of ACs toward mycotoxins with moderately large molecules because of the close correlation between the MBI and the mesopores, whose pore diameters of 2.0 to 50.0 nm should fit such molecules better than micropores and macropores *(10)*. However, since the FB_1 molecule is larger than the AFB_1 molecule, it is possible that a higher degree of macroporosity could improve ACs' adsorptive properties with respect to FB_1. Studies in progress in our laboratory may confirm this conjecture. The adsorptive performance of CAC3, PEP2, and MAP2 was low despite their high MBI values. These results are in agreement with data obtained in tests of affinity for AFB_1 *(10)*. In that study we attributed the low performance of these three ACs to their surface acidity. However, in the present study MAP2 showed an affinity for FB_1 higher than its affinity for AFB_1 observed previously *(10)*. Since MAP2 has a higher surface acidity than CAC3 and PEP2 *(10)*, the present data seem to exclude an involvement of surface acidity in the FB_1 adsorption process. This interpretation is also supported by the performance of AF50 and AF52, which showed quite high adsorption ability even though they were obtained by sulfuric acid activation (Table 1). However, given that MAP2 and PEP2 were obtained, respectively, from almond shells and peach stones by impregnation with H_3PO_4 followed by activation *(10)*, our results confirmed an overall influence of both source material and chemical treatment in affecting the adsorption ability of ACs *(12, 14)*. In any case, CAC3 is confirmed to be ineffective for binding mycotoxins.

In agreement with the conclusions reported by Ramos et al. *(24)* in a recent review on the prevention of mycotoxicosis by means of adsorbent compounds, we think that a suitably effective sorbent must be able to bind as many chemically different mycotoxins as possible. Since (i) the same five ACs showed an overall high affinity for both FB_1

and AFB_1 *(10),* (ii) natural co-occurrence of FB_1 with AFB_1 in corn, as well in feeds and foods, has been reported *(4, 5, 36, 38),* and (iii) additive toxic effects have been observed for FB_1 and AFB_1 when administered simultaneously *(37),* we decided to investigate the simultaneous in vitro affinity of ACs for these two mycotoxins.

In tests designed to determine the simultaneous affinity of ACs for FB_1 and AFB_1, increasing the amount of AC produced corresponding increases in adsorption of both FB_1 and AFB_1. ACs were confirmed to have an overall high affinity for AFB_1 even when present together with FB_1, since from 99.25 to 99.94% and from 79.27 to 99.56% of the available AFB_1 was adsorbed in tests 5 and 8, respectively. However, the performance of CAC4 was far from that expected, since from 4.03 to 79.27% of AFB_1 was adsorbed in tests 6 through 8. The simultaneous presence of the two mycotoxins clearly accounted for this range of results. Moreover, since in test 8 CAC4 adsorbed only 33.57% of the FB_1, CAC4 cannot be considered as an effective simultaneous sorbent for FB_1 and AFB_1, despite its high affinity for the two mycotoxins separately.

An overall decrease in the ability to adsorb FB_1 was observed in tests 4 through 8 as compared with tests 1 through 3. For example, in test 1, CAC1, CAC2, and CAC3 did not reach their saturation limits, whereas they did in tests 5 and 8. These results are again clearly related to the simultaneous presence of the two mycotoxins. However, evidence of an overall priority in the adsorption of AFB_1 as compared with FB_1 emerges from all the simultaneous adsorption tests. Again this is interpreted as being related to the differences in molecular structure and size of the two mycotoxins. Since the AFB_1 molecule is smaller and simpler in shape than the FB_1 molecule, it probably enters the internal pore spaces more readily than FB_1 and partially saturates the pores. If enough internal pore spaces are available, as in tests 4 and 8 (with 10 mg of AC), FB_1 adsorption can occur; if not, there will be little or no FB_1 adsorption. This particular behavior should not be a practical limitation on the effectiveness of ACs since the studies on the co-occurrence of the two mycotoxins in corn have reported that FB_1 generally predominates over AFB_1 *(4, 36, 38),* in contrast with the situation in tests 4 and 5 where the AFB_1/FB_1 ratio was 5:1. We carried out tests 7 through 9 with conditions more similar to the natural conditions (i.e., feed and water mixing in the rumen or in the stomach). In tests 6 through 8 the adsorption abilities of ACs were lower if compared with those of tests 4 and 5, particularly in regard to FB_1. In test 5, CAC2 and AF48 did not reach their saturation limits (thus indicating them to be higher than 5 μg of FB_1 per mg of AC), whereas in test 8 their saturation limits were 0.88 and 0.69 μg of FB_1 per mg of AC, respectively. This was not surprising, since mycotoxins are obviously expected to be adsorbed more efficiently from pure solutions than from a solution extracted from corn. Furthermore, in tests 6 through 8 we noted an undesired but expected decolorization process: most of the typical yellow corn pigments contained in the extract solution were adsorbed by ACs, therefore partially saturating their pore volume and hindering adsorption of mycotoxins. Nevertheless, CAC2 showed high ability to adsorb FB_1 and AFB_1 simultaneously from the corn extract solution (test 8, Figure 6), as well as from the pure solution (test 5, Figure 5).

In summary, the present study is the first report on the in vitro affinity of sorbents for FB_1. Our results demonstrated that three CACs and two EACs with high MBI values have a great affinity for FB_1 in vitro and show potential as protective agents against this mycotoxin. Studies of chemisorption properties should be performed to (i) assess the specificity of the FB_1 binding process; (ii) establish whether it is the amino group or the acid group of FB_1 that adsorbs to ACs; (iii) clarify whether the pH of the stomach can affect the binding process. Further in vivo investigations are needed to verify the ability of ACs to sequester FB_1 bound to dried plant material, thus preventing or reducing the absorption of FB_1 in the intestinal tract.

Certain ACs showed the ability to bind FB_1 and AFB_1 when both were present. In vitro tests of the affinities of ACs for ochratoxin A and deoxynivalenol are currently in progress in our laboratory, and very promising results have been obtained. An ability to bind chemically different mycotoxins has never been reported in studies on other sorbents, such as HSCAS and bentonite. HSCAS and bentonite are not capable of adsorbing efficiently mycotoxins other than AFB_1. We think that some ACs hold potential as multimycotoxin sequestering agents. Moreover, there are possibilities for increasing the ability of ACs to adsorb several mycotoxins simultaneously by controlling specific PCPs of ACs, such as MBI and macroporosity. However, in vivo investigations are needed to confirm the adsorption abilities of ACs for the most important mycotoxins singly and in combinations, establish the amounts to be added to feeds or foods, and determine any long-term effects they may have on gastrointestinal absorption of essential nutrients.

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