Journal of Food Protection, Vol. 61, No. 4, 1998, Pages 469–475

Activated Carbons: In Vitro Affinity for Ochratoxin A and Deoxynivalenol and Relation of Adsorption Ability to Physicochemical Parameters

FABIO GALVANO,[1]* AMEDEO PIETRI,[2] TERENZIO BERTUZZI,[2] ANDREA PIVA,[3] LUIGI CHIES,[1] AND MARCO GALVANO[4]

[1]*Dipartimento di Scienze e Tecnologie Agroforestali ed Ambientali, Università di Reggio Calabria, P.zza S. Francesco 7, 89061 Reggio Calabria;* [2]*Istituto di Scienze degli Alimenti e della Nutrizione, Facoltà di Agraria, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza;* [3]*Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Bologna; and* [4]*Istituto di Scienze Biochimiche e Farmacologiche, Facoltà di Scienze Biologiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy*

MS 97-61: Received 21 March 1997/Accepted 24 June 1997

ABSTRACT

In vitro affinity tests were conducted to test the effectiveness of 19 activated carbons (ACs), hydrated sodium calcium aluminosilicate (HSCAS) and sepiolite (S) in binding ochratoxin A (OA) and deoxynivalenol (DON) from solution. Relationships between adsorption ability and physicochemical parameters of ACs (surface area, iodine number, methylene blue index) were tested. When 5 ml of a 4-μg/ml aqueous solution of OA was treated with 2 mg of AC, the ACs adsorbed 0.80 to 99.86% of the OA. HSCAS and S were not effective in binding OA. In two saturation tests carried out with increased amounts of OA (5 ml of 10- and 50-μg/ml aqueous solutions of OA, respectively), three ACs also showed high adsorption ability (adsorbing 92.23 to 96.57% of the OA). When 5 ml of a 4-μg/ml aqueous solution of DON was treated with 10 mg of AC, ACs adsored 1.83 to 98.93% of the DON. HSCAS and S were not effective in binding DON. An overall relation of adsorption ability to the physicochemical parameters of ACs was observed. The methylene blue index was more reliable than iodine number and surface area in predicting ability of ACs to adsorb OA and DON. Based on the data observed on the present study as well as on aflatoxin B_1 and fumonisin B_1 from previous studies, it is concluded that ACs have high in vitro affinity for chemically different mycotoxins, and can be considered as potential multi-mycotoxin-sequestering agents. However, the ability to bind the main mycotoxins singly or in combination should be confirmed by in vivo investigations. Moreover, information on the amounts of AC to be added to feeds, and on the possible long-term effect on absorption of essential nutrients are needed.

Ochratoxin A (OA) is a mycotoxin primarily produced by some strains of *Aspergillus ochraceus* and, more commonly, by *Penicillium viricatum,* which frequently occurs on cereal grains, different kinds of beans (coffee, soy, cocoa), mixed feeds, animal tissues *(17),* and has been found in human blood *(10)* and milk *(3, 17).* In recent years OA has received growing attention because of its carcinogenic, nephrotoxic, and teratogenic properties in animals *(10).* Furthermore, OA has been implicated in a fatal human disease referred to as Balkan endemic nephropathy *(3).*

Deoxynivalenol (DON) (also referred to as vomitoxin) is a trichothecene mycotoxin primarily produced by *Fusarium graminearum* that naturally occurs in feeds, grains, and grain-derived product such as beer *(11).* DON has been demonstrated to produce several toxic effects in both animals and humans *(1, 15).* The occurrence of DON in feed causes feed refusal, vomiting, diarrhea, and severe intestinal hemorrhage. In addition, DON has been implicated in the outbreaks of severe human toxicosis reported in China *(9)* and India *(2).*

Because of the diversity of chemical structures and physicochemical properties of mycotoxins, approaches to their detoxification vary considerably. In a review on the effects of food processing on mycotoxins, Scott *(16)* reported widely variable effects of food processing and cooking in removing or detoxifying OA. With regard to the addition of sorbents as a detoxification strategy for OA, Ramos et al. *(13)* reported that, although hydrated sodium calcium aluminosilicate (HSCAS) was demonstrated to be very effective in detoxifying aflatoxins, its efficacy against OA was very low. Rotter et al. *(14)* tested an activated charcoal and reported that the results of the in vitro test were contrary to those of the in vivo test: the activated charcoal was ineffective in reducing OA toxicity in chickens despite the fact that it showed a high adsorption ability in vitro.

With regard to the detoxification of DON, Scott *(16)* emphasized the need of studies on the effects of food processing on DON. Ramos et al. *(13)* reported that HSCAS had no efficacy in detoxifying DON. Although some studies have shown the efficacy of activated charcoals in detoxifying other trichothecenes (e.g., T-2 toxin) *(13),* we are aware of no studies on the efficacy of activated carbons (ACs) in detoxifying DON.

Recently, we reported that ACs are very highly effective in binding aflatoxin B_1 (AFB_1) both in vitro *(6)* and in vivo *(5),* and fumonisin B_1 (FB_1) in vitro *(4)* and also simultaneously with AFB_1. Due to these promising results, we

* Author for correspondence. Tel: 0039.95.351201; Fax: 0039.95.354487-0039.965.682453; E-mail: fgalvano@mbox.fagr.unict.it

decided to evaluate the affinity in vitro of ACs, HSCAS and sepiolite (S) also for OA and DON. An overall relation of adsorption ability to the physicochemical parameters of ACs reported in previous tests on aflatoxin B_1 and fumonisin B_1 *(6)* suggested we investigate this relationship also for OA and DON.

MATERIALS AND METHODS

Sorbents. The sorbents tested were 15 experimental activated carbons (EACs) from exhausted olive residues (AF), peach stones (PEP2), and almond shells (MAP2) obtained with laboratory equipment of the Chemistry Department of the University of Catania by several experimental activation processes appropriately selected to obtain the desired PCPs, as follows: four commercial ACs (CACs) produced in industrial processing equipment, an HSCAS demonstrated to have a high affinity in vitro for AFB_1 *(6)*, and sepiolite (S), a clay commonly used in animal feed as a pellet binder and demonstrated to have a good affinity for AFB_1 *(13)*. All the sorbents were finely pulverized.

Activated carbons: measurement of physicochemical parameters. The physicochemical parameters of the ACs determined were surface area (SA), iodine number (IN), and methylene blue index (MBI). The meaning of the physicochemical parameters, the methods, and the equipment used for their determination have been described in detail in a previous study by Galvano et al. *(6)*. Briefly, the surface area (SA, in square meters per gram), measured by adsorption of a very small molecule such as nitrogen, is roughly correlated with the adsorption properties of the ACs. Iodine number (IN, milligrams of I_2 adsorbed by 1 g of AC) is a relative indicator of AC microporosity; it is often used as an approximation of SA because of the simple equipment required. However, it must be realized that any relationship between SA and IN cannot be generalized. MBI (milligrams of methylene blue adsorbed by 1 g of AC), measured by adsorption tests carried out at 298°K in aqueous solution followed by UV-VIS analysis, is a test which covers predominantly the medium-size pore (mesopore) range and is an important indicator in practice of AC ability to adsorb organic molecules of medium-large size from a solution.

Methods of analysis. Samples of each sorbent were individually weighed into glass tubes (three replicates per sample) and amounts of OA and DON (Sigma Chemical Co., St. Louis, MO, USA, purity >99%) in aqueous solution were separately added. After a reaction time of 1 h at 25°C, with mixing at 15-min intervals, all the tubes were centrifuged for 10 min at 1,500.

Three adsorption tests for OA were carried out, varying the amount of OA. In test 1, 5 ml of a solution containing 4 μg of OA per ml were added to 2 mg of each sorbent. In this test, relationships between adsorption abilities and the physicochemical parameters of the ACs were investigated. To differentiate AC adsorption ability and determine the saturation limit, in tests 2 and 3, respectively, 5 ml of two solutions containing 10 and 50 μg of OA per ml were added to 2 mg of each of the ACs that did not reach saturation in test 1.

Two adsorption tests were carried out for DON, varying the amount of sorbents. In tests 4 and 5, 5 ml of a solution containing 4 μg of DON per ml were added to 2 mg and 10 mg of each sorbent, respectively.

HPLC apparatus and procedure. OA and DON were detected according to the methods of Howell and Taylor *(7)*, and Visconti and Bottalico *(18)*, respectively. The high-performance liquid chromatography (HPLC) instrument used was a Perkin-Elmer LC 200 (Perkin-Elmer Corp., Norwalk, CT, USA) equipped with the ISS 200 sampling system. The system was governed by Perkin-Elmer Turbochrom 4 PC software. The stationary phase was 5-μm LiChrospher RP-18 in a 150 by 4 mm (i.d.) column (Merck, Darmstadt, Germany). For OA, a Perkin Elmer LS 30 fluorescence detector set at excitation and emission wavelengths of 337 and 470 nm was used. The mobile phase was isocratic acetonitrile-aqueous solution (1,000 ml of water plus 20 ml of acetic acid) 53:47, with a flow rate of 1.2 ml/min. Retention time was 4.5 min. For DON, a Perkin Elmer LC 235 diode array detector set at 220 nm was used. The mobile phase was isocratic acetonitrile-water 10:90, with a flow rate of 1.0 ml/min. Retention time was 2.5 min.

Statistics. Adsorption data are presented as means ± SEM of three replicates per sample; $P < 0.05$ values were considered to be significant (Student Newman Keuls multiple range test). Relationships between FB_1 adsorption and PCPs were tested by the best fit to linear and non linear function models (Fig.P®, Biosoft®, Ferguson, MO, USA).

RESULTS

Physicochemical parameters. The parameters SA, IN, and MBI for each AC are shown in Table 1.

Ochratoxin A. In test 1, the adsorption abilities of the ACs (Figure 1) ranged from 0.80 to 99.86% adsorption of the available OA. AF13 showed high adsorption ability

TABLE 1. *Physicochemical parameters of experimental (EAC) and commercial (CAC) activated carbons*

Activated carbon[a]	Physicochemical parameter		
	Surface area (m^2/g)	Iodine number (mg/g)	Methylene blue index (mg/g)
EAC			
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	1,802.0	256.0
CAC			
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 by sulfuric acid.

(93.77%). However, CAC1, CAC2, CAC4, AF48, and AF32 showed very high adsorption abilities, in that they adsorbed over 99% of the available OA from the solutions containing 4 μg (test 1) and 10 μg of OA per ml (test 2) (Table 2). In test 3, 5 ml was added of a solution containing 50 μg of OA per ml to ACs; AF48 (96.57%), CAC2 (95.53%) and CAC1 (92.23%), also showed high adsorption abilities (Table 2), whereas the adsorption abilities of CAC4 (72.97%) and AF32 (64.03%) decreased. HSCAS (13.27%) and S (10.50%) showed poor adsorption abilities (Figure 1). OA adsorption was correlated with SA and IN respectively according to two linear equations: OA adsorption = 0.09 SA − 12.05

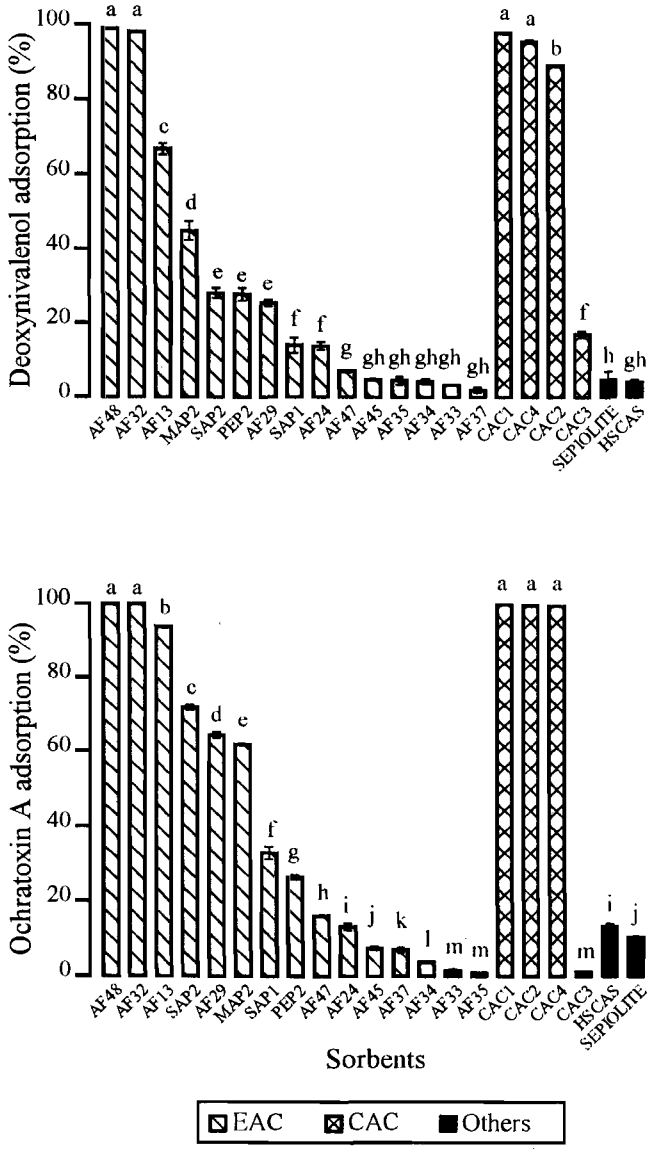

FIGURE 1. *Adsorption of ochratoxin A (4 μg/ml) and deoxynivalenol (4 μg/ml) from aqueous solution by 2 mg (for ochratoxin A) and 10 mg (for deoxynivalenol) of the following sorbents: experimental activated carbons (EAC) obtained from olive residues (AF), peach stones (PEP), and almond shells (MAP); commercial activated carbons (CAC), hydrated sodium calcium aluminosilicate (HSCAS) and sepiolite (S). Each bar represents the mean, n = 3; error bars denote standard error of the mean; different letters on bar tops mean significantly different (P < 0.05).*

TABLE 2. *Adsorption ability of experimental (EAC) and commercial (CAC) activated carbons at increasing ochratoxin A (OA) concentrations*

	Adsorption of available OA (%)[a]		
	OA (μg/ml)[b]		
Sorbents[c]	4	10	50
CAC1	99.78 ± 0.03A	99.71 ± 0.01B	92.23 ± 0.32A
CAC2	99.50 ± 0.01A	99.54 ± 0.06C	98.10 ± 0.38A
CAC4	99.46 ± 0.05A	99.14 ± 0.06D	99.50 ± 0.01A
AF32	99.86 ± 0.03A	99.66 ± 0.03BC	89.79 ± 0.73B
AF48	99.85 ± 0.01A	99.84 ± 0.02A	99.44 ± 0.10A
AF13	93.77 ± 0.32B	NA[d]	NA

[a] Values indicate group mean ± SEM: $n = 3$. Different letters in columns represent significant differences ($P < 0.05$).
[b] Five ml of solution added to 2 mg of sorbent.
[c] CAC, commercial AC; AF, olive residue EAC.
[d] NA, not assayed.

($r^2 = 0.58$), (Figure 2); OA adsorption = 0.09 IN − 15.98 ($r^2 = 0.56$) (Figure 3). The best-fitting equation model of regression for the MBI of OA adsorption was monoexponential (1st-order) decay with residual (Figure 4) ($Y = a^{(-kX)} + R$, where the parameters were: $a = -114.43$; $k = 0.48$; $R = 100.00$).

Deoxynivalenol. In test 4 (with 2 mg of sorbents), very poor adsorption was observed (data not shown). In test 5 (with 10 mg of sorbents), adsorption ability ranged from 1.83 (AF37) to 98.93% (AF48). However, CAC1 (97.87%), CAC2 (89.13%), CAC4 (95.63%), and AF32 (98.13%) also showed high adsorption abilities. HSCAS (3.90%) and S (4.53%) showed poor adsorption ability (Figure 1). DON adsorption was correlated with SA and IN respectively according to the linear equations: DON adsorption = 0.08 SA − 17.95 ($r^2 = 0.51$) (Figure 2); DON adsorption = 0.08 IN − 16.67 ($r^2 = 0.47$) (Figure 3).

The best-fitting equation model of regression for the MBI of DON adsorption was monoexponential (1st-order) decay with residual (Figure 4) ($Y = a^{(-kX)} + R$, where the parameters were $a = -109.05$; $k = 0.03$; $R = 100.00$).

DISCUSSION

In the present study the same five ACs that showed high in vitro affinity for AFB_1 *(6)* and FB_1 *(4)* (CAC1, CAC2, CAC4, AF32, and AF48) also showed high adsorption abilities for OA and DON.

As mentioned earlier, Rotter et al. *(14)* demonstrated the in vitro ability of a charcoal to bind OA. The authors used 3 μg of OA/mg of charcoal, and reported 90% adsorption ability, corresponding to a saturation limit of ca. 2.7 μg of OA/mg of charcoal. Under the condition of the present study, using an OA-to-sorbent ratio of 125 μg of OA/mg of AC (test 3), saturation limits ranged from ca. 80 (AF32) to 121 (AF48) μg of OA/mg of AC. Thus, five of the ACs selected in our study (AF32, CAC4, CAC1, CAC2, and AF48) showed an adsorption ability from ca. 30- to 45-fold

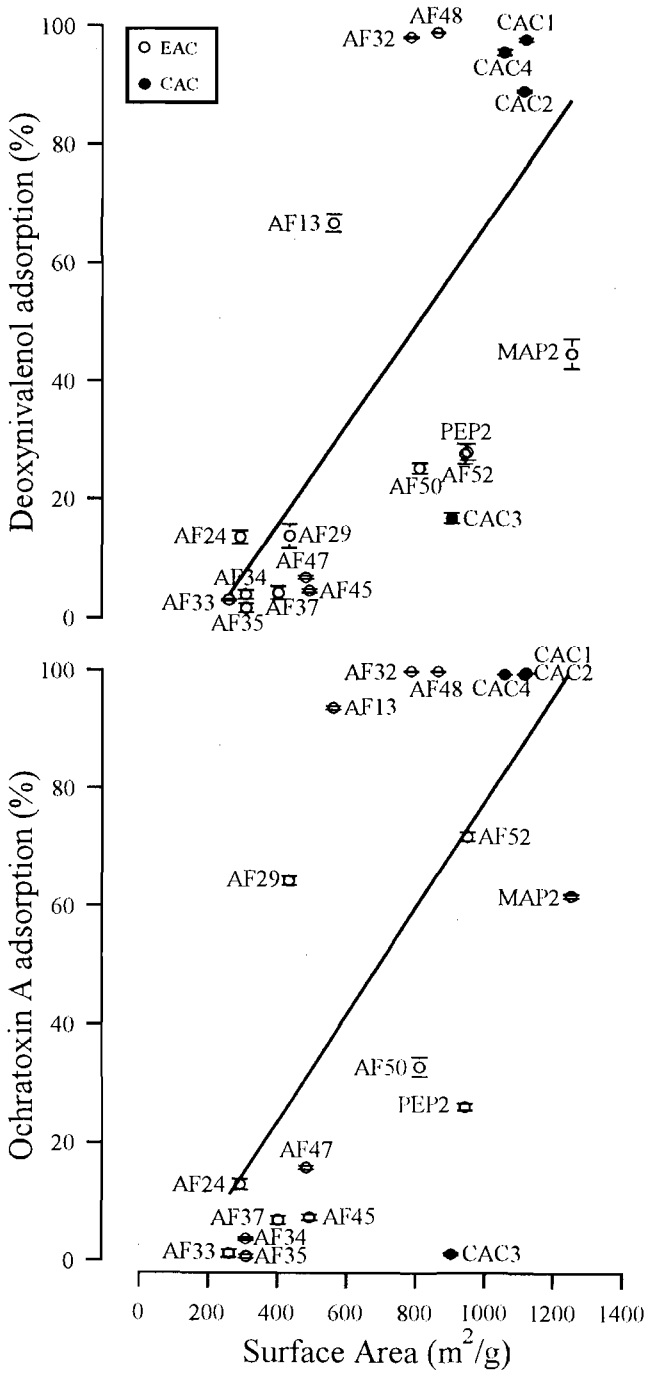

FIGURE 2. *Relationship between the surface area and the ability to adsorb ochratoxin A (4 μg/ml) and deoxynivalenol (4 μg/ml) from aqueous solution by 2 mg (for ochratoxin A) and 10 mg (for deoxynivalenol) of the following sorbents: experimental activated carbons (EAC) obtained from olive residues (AF), peach stones (PEP), and almond shells (MAP), and commercial activated carbons (CAC). Each point represents the mean, n = 3; error bars denote standard error of the mean. Values of the linear equations are: ochratoxin A adsorption = 0.09 surface area − 12.05 (r^2 = 0.58); deoxynivalenol adsorption = 0.08 surface area − 17.95 (r^2 = 0.51). CAC3 was not considered as a data point of the regressions.*

higher than the charcoal tested by these latter authors *(14)*. These observations confirm that the adsorption abilities of ACs vary widely, depending on the typology of carbonaceous substances and activation processes. Hence, it cannot be excluded that the ACs tested in this study could be also effective in in vivo tests.

The saturation limits for OA are near to those observed for AFB_1 *(6)* (Table 3). This was not surprising, since the

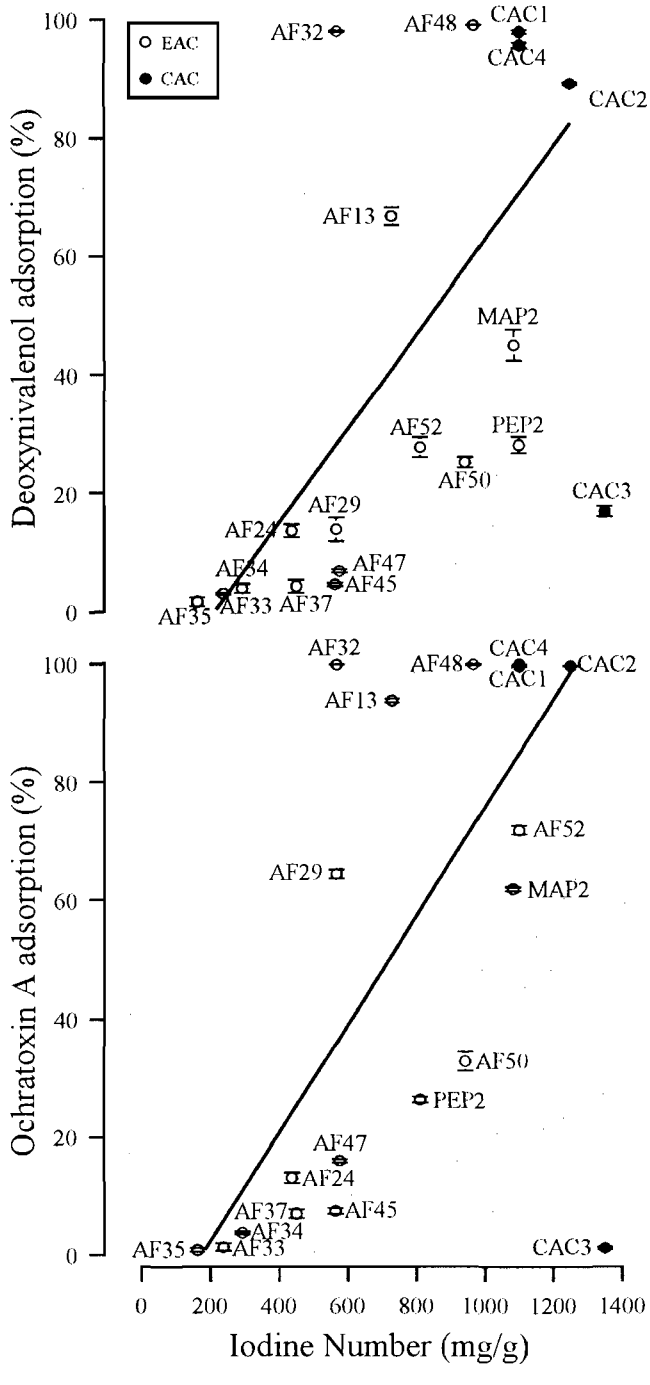

FIGURE 3. *Relationship between iodine number and the ability to adsorb ochratoxin A (4 μg/ml) and deoxynivalenol (4 μg/ml) from 5 ml of aqueous solution by 2 mg (for ochratoxin A) and 10 mg (for deoxynivalenol) of the following sorbents: experimental activated carbons (EAC) obtained from olive residues (AF), peach stones (PEP), and almond shells (MAP), and commercial activated carbons (CAC). Each point represents the mean, n = 3; error bars denote standard error of the mean. Values of the linear equations are: ochratoxin A adsorption = 0.09 iodine number − 15.98 (r^2 = 0.56). Deoxynivalenol adsorption = 0.08 iodine number − 16.67 (r^2 = 0.47). CAC3 was not considered a data point of the regressions.*

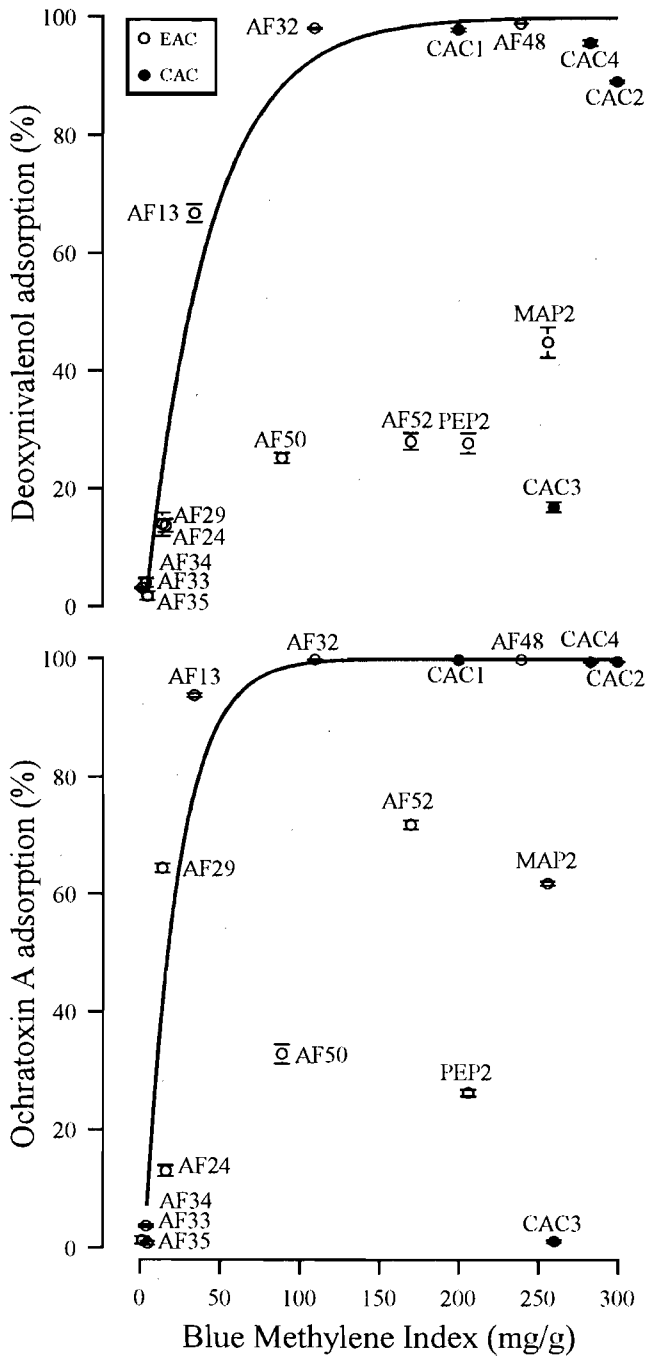

FIGURE 4. *Relationship between methylene blue index and the ability to adsorb ochratoxin A (4 μg/ml) and deoxynivalenol (4 μg/ml) from 5 ml of aqueous solution by 2 mg (for ochratoxin A) and 10 mg (for deoxynivalenol) of the following sorbents: experimental activated carbons (EAC) obtained from olive residues (AF), peach stones (PEP), and almond shells (MAP), and commercial activated carbons (CAC). Each point represents the mean, n = 3; error bars denote standard error of the mean. The equation model of the regressions for the methylene blue index of ochratoxin A and deoxynivalenol adsorption is monoexponential (1st-order) decay with residual* ($Y = a^{(-kX)} + R$, *where the parameters are:* $a = -114.43$; $k = 0.48$; $R = 100.00$), *and* $a = -109.05$; $k = 0.03$; $R = 100.00$, *for ochratoxin A and deoxynivalenol, respectively. CAC3, PEP2, AF50, AF52, and MAP2 were not considered as data points for the regressions.*

molecular size and the hydrophobicity of AFB_1 and OA are similar, even though their ionic properties are different.

With regard to DON, as indicated earlier, in test 4 (with 2 mg of ACs) very poor adsorption was observed. For this reason in test 5 we tested a fivefold higher amount (10 mg) of each sorbent, observing adsorption abilities ranging from 1.83 (AF37) to 98.93% (AF48). Since except for AF48 all of the ACs reached saturation no further tests were conducted. However, CAC1, CAC2, CAC4, AF32, and AF48 also showed good adsorption abilities toward DON.

The saturation limit of AF48 was ca. 2 μg of DON/mg of AC (Table 3). The overall saturation limits of ACs for DON are the lowest we observed for a single mycotoxin (Table 3). Since the DON molecule is smaller than the AFB_1, OA, and particularly, FB_1 molecules, the low adsorptions are likely related to its physicochemical properties. Further studies should verify this latter fact. In any case, since this is the first report on the in vitro affinity of sorbents for DON, no comparison with studies of other authors is possible.

Regressions showed a clear correlation of OA and DON adsorption with the physicochemical parameters of the ACs. However, the linear regressions of OA and DON adsorption with SA and IN (Figures 2 and 3) showed r^2 values lower than those of AFB_1 *(6)* and FB_1 *(4)*. This can be ascribed to the differences between the molecular structures and properties of the molecules. In contrast, the exponential equation model described appropriately the relationships between the adsorption trend and the MBI of ACs. These results are consistent with those observed in our previous tests on AFB_1 *(6)* and FB_1 *(4)*, and confirm MBI as the most reliable indicator of ACs adsorptive properties toward mycotoxins.

Since in agreement with the previous tests *(4, 6)* the performance of AF50, AF52, PEP2, MAP2, and CAC3 were low despite their high MBIs, surface acidity (for AF50 and AF52) and source of ACs (for PEP2 and MAP2) are confirmed to affect the adsorption properties of ACs. In any

TABLE 3. *In vitro adsorption of mycotoxins from standard solution: saturation limit of activated carbons and HSCAS for aflatoxin B_1, fumonisin B_1, aflatoxin B_1 plus fumonisin B_1 (simultaneously), ochratoxin A, and deoxynivalenol*

	Saturation limit[a] (μg of mycotoxin/mg of sorbent)				
Sorbents[b]	Aflatoxin B_1 *(6)*	Fumonisin B_1 *(4)*	Aflatoxin B_1 + fumonisin B_1 *(4)*	Ochratoxin A	Deoxynivalenol
CAC1	123.0	3.8	25.0 + 2.1	115.0	2.0
CAC2	123.0	9.9	25.0 + 5.0	119.0	1.8
CAC4	95.0	9.6	25.0 + 4.7	91.0	1.9
AF32	112.0	3.4	25.0 + 1.8	80.0	2.0
AF48	>125.0	9.8	25.0 + 5.0	121.0	>2.0
HSCAS	79.0	0.3	NA[c]	1.3	0.8

[a] The data for the saturation limits of aflatoxin B_1, fumonisin B_1, and the simultaneous application of these are respectively from references *(6)*, *(4)*, and *(4)*.

[b] CAC, commercial AC; AF; olive residue EAC; HSCAS, Hydrated sodium aluminosilicate.

[c] Reference; [e] NA, not assayed;

event, CAC3 was confirmed to be ineffective as a mycotoxin sequestering agent.

With S and HSCAS, very low adsorption abilities were observed. Particularly, the low efficacy of HSCAS in binding mycotoxins other than aflatoxins was confirmed. Our results are in agreement with those reported in some studies on the efficacy of HSCAS in reducing the in vivo toxicity of OA *(8)* and DON *(12)*.

We performed the present tests with the aim of concluding a preliminary in vitro screening of the ACs affinity for the main mycotoxins. Even though further tests on other hazardous mycotoxins (e.g., T-2 toxin and sterigmatocystin) could be performed, it is possible to point out some topics for consideration, as follows. Evidence of the high ability of ACs in binding mycotoxins in vitro arise from our studies. The highest abilities have been observed in the adsorption of AFB_1 *(4)* and OA, whereas the lowest was in the adsorption of DON (Table 3). Besides, ACs have been demonstrated to adsorb FB_1 efficiently and also simultaneously AFB_1 *(4)*. Thus, ACs are capable of binding several mycotoxins in vitro and it is reasonable to consider their potential use as multi-mycotoxin-sequestering agents, unlike other extensively studied sorbents, such as HSCAS and bentonite, which are not capable of efficiently adsorbing mycotoxins other than AFB_1 *(13)*.

The molecular size and physicochemical properties of the mycotoxins clearly affect the efficiency of the binding action. However, further studies on the mechanism of the binding action (e.g., performing studies on chemisorption indices) are needed to clarify the mechanism of the binding process and improve adsorption performance.

Although in vitro tests are strongly encouraging, we realize that further in vivo studies are required to confirm the effectiveness of ACs in detoxifying mycotoxins. Indeed, the in vivo efficacy of ACs will likely be lower, since the binding sites of ACs can be occupied by many other compounds. Up to date we performed only one in vivo test on ACs, observing up to 50% reduction of carryover of AFB_1 from dairy cows' feed to milk *(5)*. Hence further in vivo studies are needed. In any case, assuming that in vivo studies would confirm the effectiveness of ACs in detoxifying mycotoxins, three questions concerning whether ACs could be added to feeds remain open. The first is the possible long-term undesired adsorption of essential nutrients (i.e., vitamins and minerals). Long-term in vivo studies should verify that possibility, and, eventually, two strategies could be adopted: (i) addition of supplemental essential nutrients demonstrated to be insufficiently available, and (ii) increase of the selectivity of AC toward mycotoxins by modulating the activation process and the physicochemical properties.

The second question concerns the tendency of ACs to blacken the environment, the animals, and the feed, as indicated by Ramos et al. *(13)*. The same authors reported that some manufacturers have overcome this problem by producing ACs that contain up to 65% water and have the grainy consistency of brown sugar, thus eliminating the problem, which is associated with the use of the powder form. We suggest that some of the problems related to the powder form can be also eliminated by pelleting the feed. In addition we observed that, at least for AFB_1, the pelleting of the feed can increase the efficacy of the adsorbency of the ACs *(5)*.

The third question involves performing an economic evaluation of the addition of ACs to feed. Although today the price of ACs is perhaps prohibitive to the feed industry, the possibility of including ACs in feed should largely increase demand and, consequently, decrease the price. However, evaluation of the costs-benefits balance is needed.

In conclusion, although ACs are potentially high-efficiency multi-mycotoxin-sequestering agents, a great deal of work needs to be done. We hope that researchers involved in mycotoxin research will help to verify their in vivo efficacy and establish whether they can be added to feed.

ACKNOWLEDGMENTS

This work was financially supported by a grant from the MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica), ass. 60%, 1995, to Dr. Fabio Galvano, Università di Reggio Calabria, and from the European Commission Contract AIR3 94-1325.

REFERENCES

1. Atroshi, F., A. Rizzo, I. Biese, M. Salonen, L. A. Lindberg, and H. Saloniemi. 1995. Effects of feeding T-2 toxin and deoxynivalenol on DNA and GSH content of brain and spleen of rats supplemented with vitamin E and C and selenium combination. J. Anim. Physiol. Anim. Nutr. 74:157–164.
2. Bhat, R. V., Y. Ramakrishna, S. R. Beedu, and K. L. Munshi. 1989. Outbreaks of trichothecene mycotoxicosis associated with consumption of mold-damaged wheat products in Kashmir valley India. Lancet i:35–37.
3. Breitholtz-Emanuelsson, A., M. Olsen, A. Oskarsson, I. Palminger, and K. Hult. 1993. Ochratoxin A in cow's milk and in human milk with corresponding human blood samples. J. AOAC Int. 76:842–846.
4. Galvano, F., A. Pietri, T. Bertuzzi, M. Bognanno, L. Chies, A. De Angelis, and M. Galvano. 1997. Activated carbons: in vitro affinity for fumonisin B_1 and relation of adsorption ability to physicochemical parameters. J. Food Prot. 60:985–991.
5. Galvano, F., A. Pietri, T. Bertuzzi, G. Fusconi, M. Galvano, A. Piva, and G. Piva. 1996. Reduction of carryover of aflatoxin from cow feed to milk by addition of activated carbons. J. Food Prot. 59:551–554.
6. Galvano, F., A. Pietri, B. Fallico, T. Bertuzzi, S. Scirè, M. Galvano, and R. Maggiore. 1996. Activated carbons: in vitro affinity for aflatoxin B_1 and relation of adsorption ability to physicochemical parameters. J. Food Prot. 59:545–550.
7. Howell, M. V., and P. W. Taylor. 1981. Determination of aflatoxins, ochratoxin A, and zearalenone in mixed feeds, with detection by thin layer chromatography or high performance liquid chromatography. 1981. J. Assoc. Off. Anal. Chem. 64:1356–1363.
8. Huff, W. E., L. F. Kubena, R. B. Harvey, and T. D. Phillips. 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult. Sci. 71:64–69.
9. Luo, X. Y. 1988. Outbreaks of moldy cereals poisoning in China, p. 56–63. *In* Issues in food safety. Toxicology Forum Inc., Washington, D.C.
10. Marquardt, R. R., and A. A. Frohlich. 1992. A review of recent advances in understanding ochratoxicosis. J. Anim. Sci. 70:3968–3988.
11. Niessen L., M. Böhm-Schraml, H. Vogel, and S. Donhauser. 1993. Deoxynivalenol in commercial beer—screening for the toxin with an indirect competitive ELISA. Mycotoxin Res. 9:99–109.
12. Patterson, D. S. P., and L. G. Young. 1993. Efficacy of hydrated sodium calcium aluminosilicate, screening and dilution in reducing the effects of mold contaminated corn in pigs. Can. J. Anim. Sci. 73:615–624.
13. Ramos, A. J., J. Fink-Gremmels, and E. Hernández. 1996. Prevention

of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. J. Food Prot. 59:631–641.

14. Rotter, R. G., A. A. Frohlich, and R. R. Marquardt. 1989. Influence of dietary charcoal on ochratoxin A toxicity in Leghorn chicks. Can. J. Vet. Res. 53:449–453.
15. Rotter, B. A., B. K. Thompson, and M. Lessard. 1995. Effects of deoxynivalenol-contaminated diet on performance and blood parameters in growing swine. Can. J. Anim. Sci. 75:297–302.
16. Scott, P. M. 1983. Effects of food processing on mycotoxins. J. Food Prot. 47:489–499.
17. Van Egmond, H. P., and G. J. A. Speijers. 1994. Survey of data on the incidence and levels of ochratoxin A in food and animal feed worldwide. J. Nat. Toxins 3:125–144.
18. Visconti A., and A. Bottalico. 1983. Detection of Fusarium trichothecenes by high performance liquid chromatography. Chromatographia 17:97–100.