J. Agric. Sci. Mansoura Univ., 28(12): 8257 - 8268, 2003

REMOVAL OF AFLATOXIN B_1 AND FUMONISIN B_1 FROM MALT EXTRACT USING ADSORPTION AGENTS TECHNOLOGY

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ABSTRACT

Malt extract is widely used in beverages, food and pharmaceutical industries. The use of mycotoxin-contaminated barely in the production of malt resulted in the contamination with mycotoxins and frequently the presence of mycotoxins in the final product. The aim of the present work was twofold: (1) testing of two adsorbent agents including commercially hydrated sodium calcium aluminosilicate (HSCAS) and an Egyptian montmorillonite (EM) to adsorb aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) in aqueous solution, and (2) the application of these adsorbent agents in the removal of AFB_1 and FB_1 from malt extract. In one experiment, four level of each sorbent e.g. 0.5, 1, 2 and 4% (w/v) and three level of each mycotoxins e.g 5, 10 and 50 ppm were tested. Results revealed that the adsorbent agents had an excellent capability of adsorbing AFB_1 and FB_1 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 AFB_1 and FB_1 respectively in aqueous solutions. EM showed an adsorption ratio ranged from 95.4 to 99.2 and 78.2 to 92.2% for AFB_1 and FB_1 respectively. Both adsorbent agents were effective at 0.5% level in the adsorption of AFB_1 and FB_1. A second experiment was conducted to evaluate the ability of these adsorbent agents at level of 0.5% (w/v) to adsorb AFB_1 and FB_1 in malt extract spiked with 50, 100 and 200 ppb. Our results indicated that the capability of adsorbing of HSCAS ranged from 98.5 to 98.9 and 88.2 to 91.9% for AFB_1 and FB_1 respectively. Whereas, the capability of adsorbing of EM ranged from 98.1 to 98.7 and 88.2 to 92.5% for AFB_1 and FB_1 respectively. These data concluded that sorbent technology is effective in the removal of AFB_1 or FB_1 in malt extract used in beverages and other industries, and importantly, EM is as effective as HSCAS at a dose as low as 0.5% (w/v).

Keywords: Malt, aflatoxin B_1, fumonisin B_1, sorbent materials, HSCAS and montmorillonite

INTRODUCTION

Malt is the dried product of barley germinated under controlled conditions. It is widely used in beverages and food industry as well as pharmaceuticals. Hickenbottom (1996) estimated that over 100 million bushels are malted in USA, most of which is used in beer production. In Egypt, malt is used in the production of bread, beverages, food flavoring, optional ingredients in bakery products and color additives in the preparation of caramel.

The use of mold –contaminated barley in the production of malt resulted in the contamination with mycotoxins and consequently the presence of mycotoxions in the consumer product (Scott *et al.*, 1993; Shin *et al.*, 1997; Scott and Kanhere 1995; Scott and Lawrence, 1995). The incidence of toxigenic *Aspergillus* and *Fusarium spp.* on barley crop was studied by

Abornson *et al* (2002). *Aspergillus flavus* and *A. parasiticus* are known to have the ability to produce aflatoxins under favorite conditions (Gourama and Bullerman, 1995), whereas, *Fusarium molinoforme* produce fumonisins (Marasas *et al.*,1984).

Aflatoxins are carcinogenic, mutagenic, and teratogenic compounds (Abdel-Wahhab *et al.*, 1998 and 1999 and Abdel-Wahhab and Aly, 2003). Seventeen aflatoxins have been isolated, but only four, called B_1, B_2, G_1 and G_2, are significant contaminants of foods and is the most acutely toxic of the aflatoxins (Park *et al.*,2002). Aflatoxin B_1 is usually bound in the greatest concentration in foods.

Fumonisins suspected to cause oesophageal cancer in Transkei region of South Africa (Rheeder *et al.*,1992) and fumonisin B_1 has recently been declared to be a class 2B carcinogen, i.e., possibly carcinogenic to humans, by the International Agency for Research on Cancer (IARC). Among the fumonisins, FB_1 is the most abundant in food and is known to be the most potent (Martins *et al.*, 2001, Omarttag, 2001 and Petersen and Thorup, 2001). The risk of these toxins appeared when we know that fumonisin B_1 is water soluble compound (Seo et al, 1996) while 48% of aflatoxin B_1 was recovered from corn steep liquour during starch process (Aly, 2002).

Park *et al*; (2002) noted 16% of the roasted barley and corn samples were contaminated with aflatoxin and fumonisin. These products are commonly used beverage and sold in tea bags in Korea. Scott and Lawrence (1995) and Scott *et al.*,(1997) detecting fumonisins in commercial beers in Canada. Moreover, Hlywka and Bullerman (1999) found detectable quantities of FB_1 in 21of 25 samples of beer. From this point of view, the removal of these mycotoxins from malt used in beer and other beverages industry is of great demand. Several reports indicated that phyllosilicates clay have the ability to chemisorbs aflatoxin from aqueous solutions (Phillips *et al.*, 1988). Some aluminosilicates bind AFB_1 *in vitro* to varying degrees and form complexes of varying strength with AFB_1. The hydrated sodium calcium aluminosilicate (HSCAS) formed a more stable complex with AFB_1 than many of the other compounds tested *in vitro* (Phillips *et al.*,1988). The HSCAS, bentonite and montmorillonite were found to protect the laboratory animals from the toxic and teratogenic effects of aflatoxins (Abdel-Wahhab *et al.*,1998,1999 and 2002).

The aim of the present study was to evaluate the ability of HSCAS and the Egyptian monmorillanite to adsorb AFB_1 and FB_1 from aqueous solution during the extraction of malt in food and beverages industry.

MATERIALS AND METHODS

Materials:

Malts were purchased from El-Ahram company for beverages, Cairo, Egypt. Malt samples had no detectable levels of AFB_1 or FB_1.

Chemicals:

Aflatoxin B_1 and fumonisin B_1 standards were purchased from Sigma Chemical Co. (St. Luis Mo.) All other chemicals were HPLC grade. A stock

solution of AFB_1 was dissolved in acetonitrile: methanol (1:1), while the stock solution of FB_1 was dissolved in acetonitrile: water (1:1)

Sorbents:

HSCAS was purchased from Engelhard Corporation (Cleveland, OH), whereas monmorillanite was provided by Ceramic Dept, NR.C, Cairo, Egypt. Four concentration of each sorbent (i.e. 0.5, 1, 2, and 4 % w/v) were individually weighed into glass tubes (three replicates per sample) and the amount of each mycotoxin (5, 10 and 50 ppm) in aqueous solution were separately added. After a reaction time of 1 hr at 25°C, with mixing at 15-min intervals, all the tubes were centrifuged for 10 min at 1500 rpm. Three adsorption tests for each mycotoxin were carried out, varying the amount of the mycotoxin.

Preparation of mycotoxins – contaminated malt:

Malt samples were mixed with either AFB_1 dissolved in chloroform or FB_1 dissolved in methanol at three concentration levels (i.e., 50, 100 and 200 ppb) in an amber glass jar . Three replicates of each contamination level for each mycotoxin were used.

The solvents were allowed to evaporate by placing the open gar in the flow of a fame hood overnight.

Preparation of malt extract:

Spiked malt samples (25 gm) were steeped in 100 ml distilled water for 6 hr, the steep water were collected and adjusted to 100 ml. Sorbent materials (HSCAS or montmorillonite) were added to the malt extract at a level of 0.5% (w/v) and shaking for 30 min at room temperature . All extracts were centrifuged for 10 min at 1500 rpm, then filtrated through whatman # 4 filter paper and the filtrate extracts were used for the determination of AFB_1 or FB_1.

Mycotoxins analysis:

Aflatoxin analysis

Aflatoxin B_1 was extracted according to AOAC (1995), samples (10 ml) of malt extract were mixed twice with 15 ml chloroform in separating funnel and shaking for 3 min The lower face was dried over sodium sulfate anhydrous. Chloroform was over evaporated under nitrogen, the dry film was dissolved in acetonitrile HPLC grade. The concentration of AFB_1 was determined using HPLC on waters apparatus with delivery system model 600, and scanning fluorescence detector (Ex. 365. Em 450 nm). The millennium software program was used for calculations. A Nova pak C_{18} column (3.4 X150 mm, 4*u*) was used. Mobile phase A; acetonitrile: H_2O 15: 85 v/v; mobile phase B. 100 % methanol.

Fumonisin analysis

Fumonisin immunoaffinity HPLC clean up columns. (Viacom, Watertown, MA) were used for extract fumonisin B_1 from the samples. Column were fitted with 10 ml reservoirs and 5ml volume of an aqueous wash

solution (2.5% NaCl, w/v; 0.5 % $NaHCO_3$ w/v; 0.01 % tween 20 v/v) was added. A 5 ml volume of filtered was added and the total volume allowed to drained through the column via gravity. The column was washed with 1 ml of the aqueous solution followed by 1 ml of HPLC grade water. Both washes were passed through the column via gravity and the elute was discarded. FB_1 was eluted from the column via gravity with 1.5 ml 80 % methanol (v/v) and collected in glass vials. Samples were dried under nitrogen at 55 C^0 and stored at –20 C until analysis (Canela *et al.*, 1996). The concentration of FB_1 was determined using HPLC on waters apparatus with delivery system model 600, and scanning fluorescence detector (Ex. 335. Em 440 nm). The millennium software program was used for calculations. A Nova pak C_{18} column (3.4 X150 mm, 4*u*) was used. Mobile phase was acetonitrile: H_2O 80: 20 v/v, and the flow rate was 1 ml/min.

Statistical analysis:

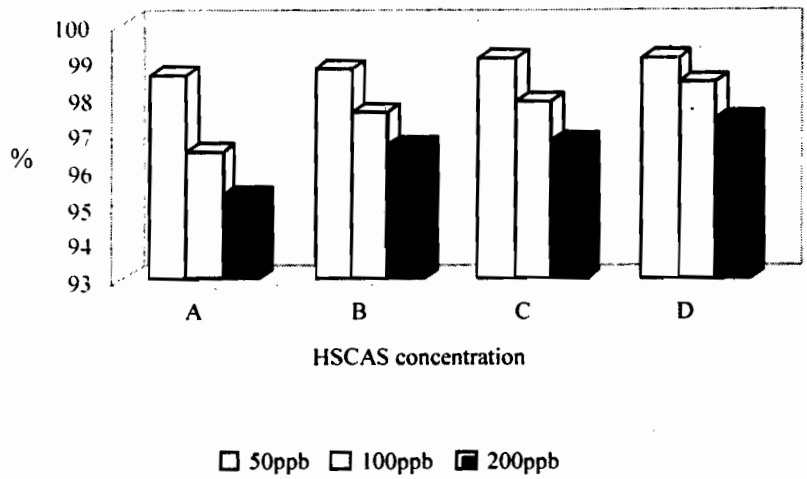
All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

The removal ability of aflatoxin and fumonisin using adsorbents agent were studied on aqueous solution as model system of any liquid contaminated with these toxins. HSCAS and EM in four concentrations and three levels of each mycotoxin (i.e., 50, 100 and 200 ppb) were used. The adsorption capacity (Figs 1, 2, 3 and 4) did not significantly affected by the adsorbent agents or with the levels tested (0.5 to 4% w/v) at all contaminated levels of mycotoxins used. Whereas, the binding capacity was dependent on the mycotoxin type. The present results clearly indicated that the adsorption capacity of HSCAS at different concentrations was very high. It ranged from 95.3 - 99.1% for AFB_1, whereas it ranged from 85.1 – 92.4% for FB_1 in aqueous solution (Figs. 1 and 2). On the other hand, the adsorption capacity of EM was very high for AFB_1 and ranged from 95.4- 99.2%, meanwhile it was high for FB_1 and ranged from 78.2 – 92.2%. The adsorbtion ability is not signif icantly differed by increasing the concentration of adsorbents agents. So addition of sorbents at level as low as 0.5% (w/v) resulted in a higher adsorption of both mycotoxins (Figs. 3 and 4).

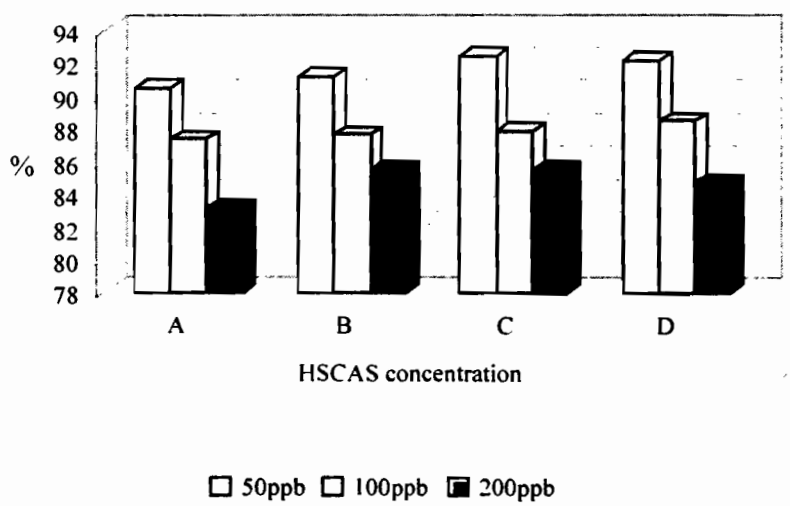
Application on malt extracts: Each adsorption agents at level of 0.5% w/v individually used to remove aflatoxin or fumonisin from contaminated malt extract. Table (1) showed that malt extract was contaminated with 23, 49.53 and 101.3 ppb of aflatoxin B_1 as a result of steeping in water. These amounts formed 46%, 49.53 and 50.65% of the initial contaminated levels in malt. Malt extract was contaminated with fumonisin at levels higher than aflatoxin B_1.

Fig.(1): Adsorption ability of HSCAS for AFB_1



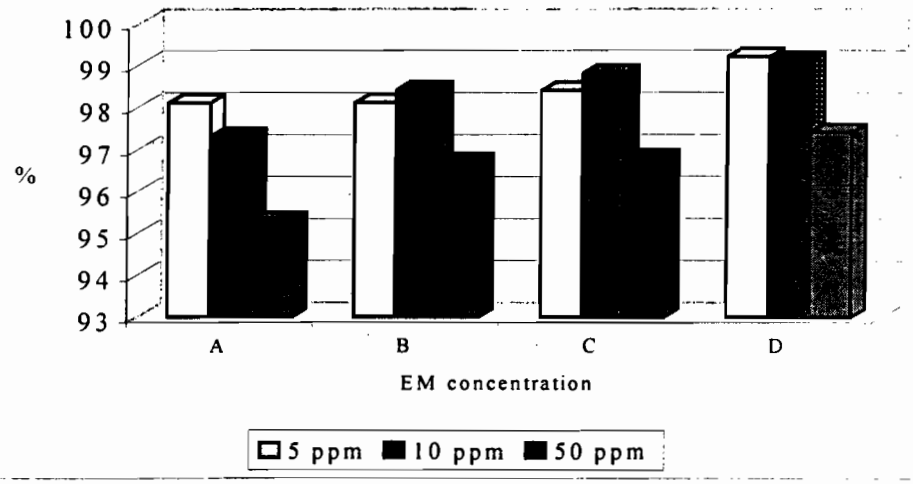
.. B. C and D different concentrations of sorbents (i.e. A= 0.5, B =1, C =2 and D = 4 % w/v)

Fig.(2): Adsorption ability of HSCAS for FB_1



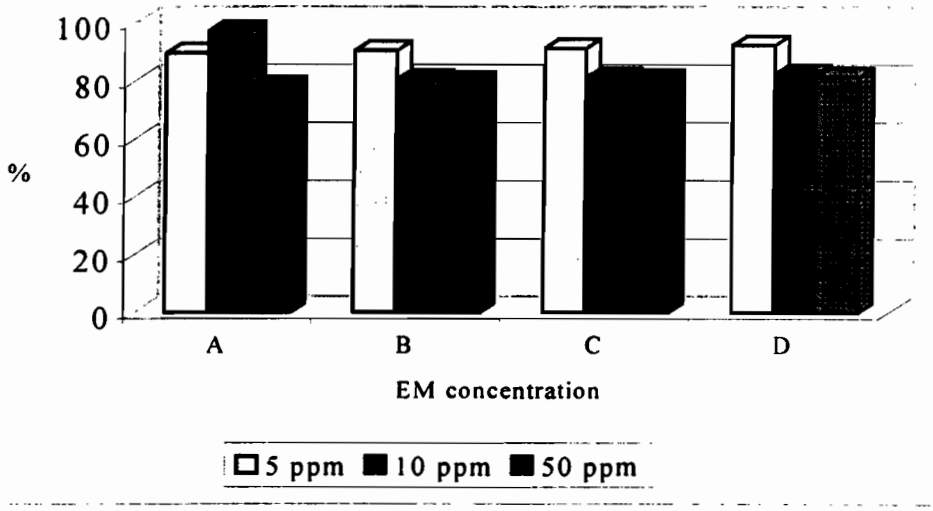
A. B. C and D different concentrations of sorbents (i.e. A= 0.5, B =1, C =2 and D = 4 % w/v)

Fig. (3): Adsorption ability of EM for AFB1



A, B, C and D different concentrations of sorbents (i.e. A= 0.5, B =1, C =2 and D = 4 % w/v)

Fig. (4): Adsorption ability of EM for FB1



A, B, C and D different concentrations of sorbents (i.e. A= 0.5, B =1, C =2 and D = 4 % w/v)

Fig. (5) % Reduction of AFB_1 in spiked malt extracts treated with 0.5% (w/v) of HSCAS or EM

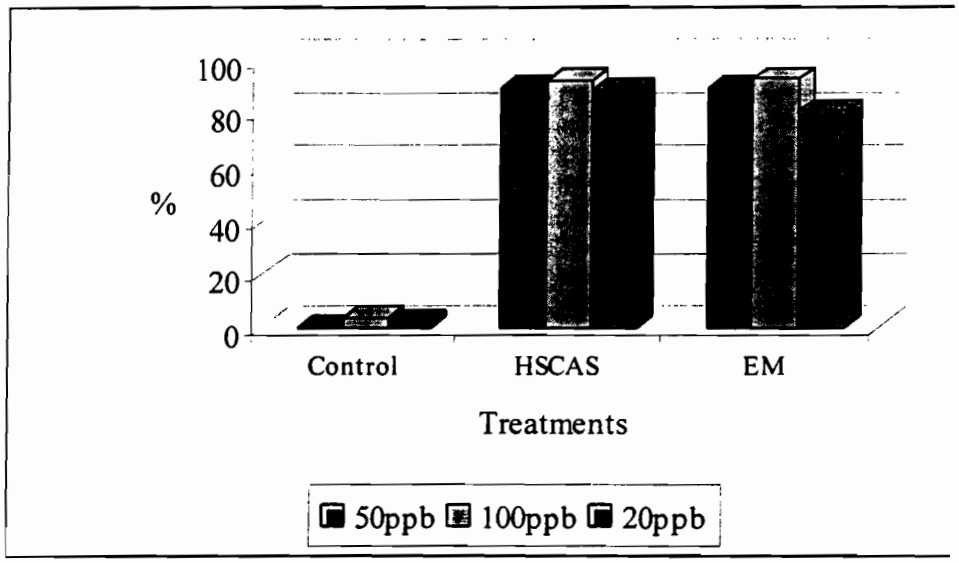
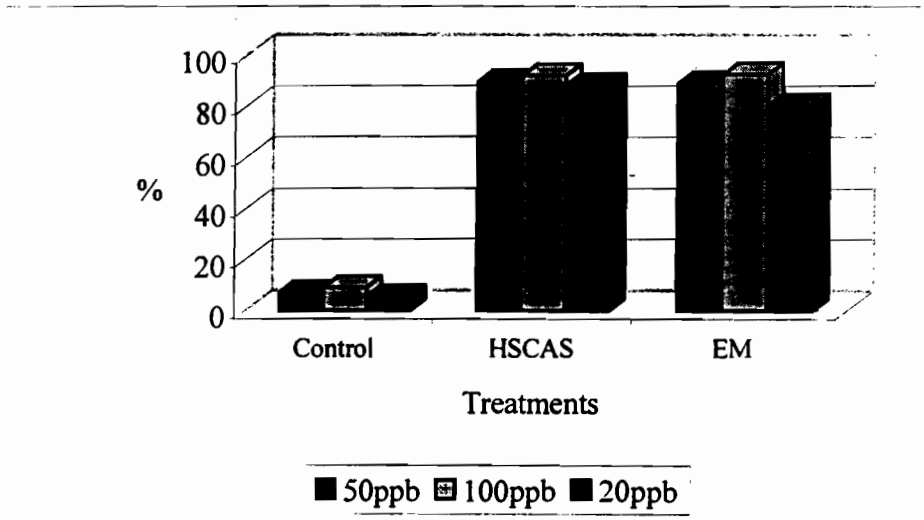


Fig (6). % Reduction of FB_1 in spiked malt extracts treated with 0.5% (w/v) of HSCAS or EM



These levels ranged between 92 and 94.7% of the initial levels of fumonisin. Addition of either HSCAS or EM to the malt extracts contaminated with 50, 100 and 200 ppb resulted in a significant reduction of AFB_1. HPLC analysis revealed that only 0.57, 1.47 and 2.13 ppb could be detected in the samples spiked with the three levels respectively and treated with 0.5% w/v HSCAS. Whereas, analysis of the samples spiked with the same levels and treated with EM showed residual levels of 0.8, 1.87 and 2.53 ppb respectively for the three contamination levels (Table 1). Results in Fig. (5) showed that the adsorption ability of HSCAS or EM at level of 0.5% w/v ranged from 98.5-98.9% for HSCAS and 98.2-98.7% for EM of the available AFB_1 in malt extract at different contamination levels.

It is of interest to mention that both sorbents had a high affinity to sorb FB_1 at different contamination levels. Addition of HSCAS to the spiked malt extracts resulted in the adsorption of FB_1 ranged from 85.25-91.97% for HSCAS and 88.4-92.47% for EM (Fig 6). The residual FB_1 that could be detected by HPLC analysis was 5.3, 8.03 and 23.5 ppb in the three contamination levels respectively for HSCAS, whereas the residual FB_1 in the samples spiked with the same levels and treated with EM were 0.43, 1.22 and 1.48 ppb respectively (Table 2).

Table (1): AFB_1 residual in malt extract spiked with 50, 100 and 200 ppb and treated with 0.5% (w/v) HSCAS or EM

Treatments	Control			HSCAS			EM		
AFB_1 (ppb) in spiked malt	50	100	200	50	100	200	50	100	200
AFB_1 in malt extract (Mean ± SE)	23.1 ± 0.37	49.53 ± 1.68	101.3 ± 1.61	0.57 ± 0.12	1.47 ± 0.12	2.13 ± 0.21	0.8 ± 0.08	1.87 ± 0.05	2.53 ± 0.17
Percentage	46.2	49.53	50.65	1.14	1.47	1.07	1.6	1.87	1.27

Table (2): FB_1 residual in malt extract spiked with 50, 100 and 200 ppb and treated with 0.5% (w/v) HSCAS or EM

Treatments	Control			HSCAS			EM		
FB_1 (ppb) in spiked malt	50	100	200	50	100	200	50	100	200
FB_1 in malt extract (mean ± SE)	46.5 ± 1.06	91.03± 1.45	189.4 ± 1.52	5.3 ± 0.86	8.03 ± 0.54	23.5 ± 0.79	5.5 ± 0.43	7.5 ± 1.22	23.6 ± 1.48
Percentage	93.0	91.03	94.7	10.6	8.03	11.75	11.0	7.5	11.8

DISCUSSION

The newest concept for mycotoxin detoxification is in the area of sorbent technology. In the present study HSCAS was found to have a high affinity for AFB_1. It causes a reduction percentage of AFB_1 in malt extract ranged from 98.5- 98.9 %.

Montmorillonite is commonly the main constituent of the clay know as Bentonite. It has the properties of adsorbing organic substances either on its external surfaces or within its inter laminar spaces by the interaction with or substitution for the exchange cations present in their spaces (Latif and Quisenberry, 1968 and Abdel-Wahhab *et al.*, 2002). EM used in the present

study showed a high capability to bind both AFB_1 and FB_1 from malt extract. The binding capacity ranged from 98.2– 98.7 % and 88.4- 92.4 % for AFB_1 and FB_1 respectively. This may be due to the large molecular structure of EM which increase the adsorption of organic compounds in each of the layers (Fushiwaki and Urano, 2001). Moreover, Sharom *et al.* (1980) pointed out that the adsorption of the organic compounds (i.e., pesticides) is dependent on their solubility in water. In this regards EM was expected to have a high adsorption ratio for FB_1. Similar results were found by Phillips *et al.* (1988), Abdel-Wahhab *et al.* (1998, 1999 and 2002). On the other hand, Galvano *et al.* (1998) reported that HSCAS has a very low adsorption abilities with mycotoxin other than AFB_1. Carroll (1969) reported that phyllosilicates are composed of layers-lattice silicates and chain silicates. These silicates are essentially comprised of repeating layers of (1) divalent or trivalent cations (e,g., aluminas) held in octahedral coordination with oxygens and hydroxyls, and (2) silicas that are tetrahedrally coordinated with oxygens and hydroxyls.

Malt extracts are used to produce the various specially bears and other beverages such as Birell and Fayrouz. The aflatoxin and fumonisin contaminated malt resulted in the presence of these mycotoxins in the final products. The differences in contaminated levels of the two mycotoxins reported in the present study may be due to the differences in its solubility according to polarity and other characteristics affected on the toxin migrate in steep aquous solution (Canela et al., 1996 and Pujol et al., 1999) According to Aly (2002) reported that although aflatoxin is water insoluble, steeping of contaminated corn with high concentration of AF_S caused a significant loss of AF_S in steeping water, this may probably due to the binding of AF_S with water-soluble component. Regarding to FB_1 is known as water-soluble (Park *et al.*, 2002). Hence, the occurrence of both mycotoxins in malt extracts is possible if the mycotoxins contaminated barley is used. In the light of these facts, it is clear that viable strategies to detoxify and remediate mycotoxins in malt extract are critically needed. These results also were supported by the findings reported by Canela et al. (1996) and Abdalla et al. (2003). These authors reported that FB1 is migrate from contaminated macaroni or corn to water during boiling in water and this effect is due to the solubility of FB_1 not to thermal process.

Generally phyllosilicates possess three types of active binding sites: (1) those located at basal planes within interlayer channels, (2) those located on the surface, and (3) those located at the edges of clay particles. It is possoble that AFB_1 and FB_1 could be binding within interlayers, at the surface, at edges, or at a combination of sites (Phillips *et al.* 2002). In the context of our results, two points are worth discussing. The first one concerns the ability of HSCAS to bind FB_1. The other aspect refers to evaluate the ability of EM for AFB_1 and FB_1 in malt extract used in certain beverages and food industry and in pharmaceuticals as well. In this regard, our results indicated that both tested sorbent materials have a high affinity for AFB_1 and FB_1 and importantly, EM succeeded to sorb more than 98% of AFB_1 and 92.2% of FB_1 in malt extract.

In conclusion, Both HSCAS and EM have the high affinity to adsorb AFB_1 and FB_1 from malt extracts used in beverages and beer industry. Also it

can conclude that EM is a promise as effective and economically application in the carry over of AFB_1 and FB_1 in certain aqueous solutions used in food or pharmaceutical industry.

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ازالة سموم الافلاتوكسين والفيومونزين من مستخلص المولت باستخدام تكنولوجيا الادمصاص

سهير السيد على – مسعد عطيه عبد الوهاب– منى عبد الجليل
قسم سموم وملوثات الغذاء– المركز القومى للبحوث

يستخدم المولت على نطاق واسع فى تصنيع المشروبات والاغذيه وبالتالى فان السموم الفطريه قد تتواجد فى تلك المنتجات عند استخدام الشعير الملوث فى اعداد المولت او منتجاته . يهدف البحث الى استخدام مواد طبيعيه لها خاصية ادمصاص للمركبات الكيميائيه لربط الافلاتوكسين والفيومونزين فى السوائل والظروف التى تعتمد عليها كفائة الادمصاص ومنها نوع مادة الادصاص ونوع التوكسين الملوث والتركيزات المستعمله من مواد الادمصاص وكذلك التركيزات الملوثه. وتناول الجزء الثانى من الدراسه تطبيق هذه التكنولوجيا على مستخلص المولت الملوث بالمسموم الفطريه. استخدمت فى هذة الدراسه مادة, HSCAS وهىمادة مستوردة ومادة المونتموريلليت وهى مادة طبيعيه من البيئه المحليه للمقارنه مع المادة المستورده . تم استخدام اربعة تركيزات من المواد المدمصه وثلاث مستويات من التلوث (٥٠،١٠،٥ جزء فى المليون). اثبتت النتائج أن مادة HSCASلها القدره على ربط كلا من الافلاتوكسين ب١ والفيومونزين ب ١بنسبه تتراوح بين ٩٣،٣- ٩٩،١ %و ٨٤،٧- ٩٢،٤ % على التوالى . وقد وجد أن مادة المونتموريلليت لها قدرة مرتفعه على أد مصاص السموم الفطرية تصل الى ٩٥،٤ -٩٩،٢ و ٧٨،٢_٩٢،٢ % من الافلاتوكسين ب١والفيومونزين ب١على التوالى. كما وجد أن هذه الواد ذات فعاليه عاليه عند تركيز ٥,% . وبتطبيق هذه النتائج على مستخلص المولت الملوث بتركيزات مختلفه (٥٠و١٠٠ و٢٠٠ جزء فى البليون)لكلا من والفيومونزين والافلاتوكسين . تراوحت كفاءه الادمصاص لمادة HSCAS بين ٩٨،٥_ ٩٨،٩ % و ٨٨،٢_ ٩١،٩ % للافلاتوكسين والفيومونزين على التوالى بينما بلغت نسبة ارتباط مادة المونتموريلليت ٩٨،١_٩٨،٧ و ٨٨،٢_٩٢،٥ % للافلاتوكسين والفيومونزين على التوالى. نستخلص من هذة الدراسه أن استخدام تكنولوجيا الادمصاص فى التخلص من السموم الفطريه الخطيرة مثل الافلاتوكسين ب١ والفيومونزين بحيث تكون ذات فعاليه مرتفعة فى المحاليل مثل العصائر والمشروبات ومشابهتها أثناء التصنيع الغذائي مثل محلول النقع للذرة المستخدم فى مصانع النشا أو الألبان . كذلك دلت الدراسة على إمكانية استخدام المادة المجليه فى إزالة السموم تحت الدراسة حيث أن الفروق بينها وبين المادة المستوردة كانت غير معنوية. وتوصى نتائج البحث على ضرورة الكشف عن السموم الفطرية فى الشعير او المولت وكذلك مستخلص المولت قبل تصنيعه ومعالجة المستخلص الملوث بمادة أد مصاص مثل المونتموريلليت ثم ترشيحه للتخلص منه وتطبيق هذه التكنولوجيا فى كل السوائل التى ربما تكون ملوثه بالسموم الفطرية خاصة الافلاتوكسين ب١ والفيومونزين ب ١قبل استخدامها فى التصنيع الغذائي.