REMOVAL OF AFLATOXIN B₁ AND FUMONISIN B₁ FROM MALTEXTRACT USING ADSORPTION AGENTS TECHNOLOGY
Aly, Soher, E.; M. A. Abdel-Wahhab and Mona M. Abdel-Galil
Food Toxicology and Contaminants Dept. National Research Center
Dokki, Cairo, Egypt

ABSTRACT

Malt extract is widely used in beverages, food and pharmaceutical industries. The use of mycotoxin-contaminated barley 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 B₁ (AFB₁) and fumonisin B₁ (FB₁) in aqueous solution, and (2) the application of these adsorbent agents in the removal of AFB₁ and FB₁ 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 AFB₁ and FB₁ 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 in aqueous solutions. EM showed an adsorption ratio ranged from 95.4 to 99.2 and 78.2 to 92.2% for AFB₁ and FB₁ respectively. Both adsorbent agents were effective at 0.5% level in the adsorption of AFB₁ and FB₁. A second experiment was conducted to evaluate the ability of these adsorbent agents at level of 0.5% (w/v) to adsorb AFB₁ and FB₁ in malt extract spiked with 50, 100 and 200 ppb. Our results indicated that the capability of adsorbing of HSCAS ranged from 96.5 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. These data concluded that sorbent technology is effective in the removal of AFB₁ or FB₁ 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 B₁, fumonisin B₁, 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 mycotoxins 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
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Abornson et al (2002). Aspergillus flavus and A. parasiticus are known to have the ability to produce aflatoxins under favorable conditions (Gourama and Bullerman, 1995), whereas, Fusarium moniliforme 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 B1, B2, G1, and G2, are significant contaminants of foods and is the most acutely toxic of the aflatoxins (Park et al., 2002). Aflatoxin B1 is usually bound in the greatest concentration in foods.

Fumonisins suspected to cause oesophageal cancer in Transkei region of South Africa (Rheeber et al., 1992) and fumonisin B1 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, FB1, 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 B1 is water soluble compound (Seo et al, 1996) while 48% of aflatoxin B1 was recovered from corn steep liquor 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 FB1, in 21 of 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 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 (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 montmorillonite to adsorb AFB1 and FB1 from aqueous solution during the extraction of malt in food and beverages industry.

MATERIALS AND METHODS

Materials:

Malt were purchased from El-Ahram company for beverages, Cairo, Egypt. Malt samples had no detectable levels of AFB1 or FB1.

Chemicals:

Aflatoxin B1 and fumonisin B1 standards were purchased from Sigma Chemical Co. (St. Louis Mo.) All other chemicals were HPLC grade. A stock

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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 montmorillonite was provided by Ceramic Dept, NR.C, Cairo, Egypt. Four concentrations 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 fume 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 355 Em 450 nm). The millennium software program was used for calculations. A Nova pak C$_18$ column (3.4 X150 mm, 4u) was used. Mobile phase A; acetonitrile: H$_2$O 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% NaHCO₃ w/v; 0.01% tween 20 w/v) was added. A 5 ml volume of filtered water was added and the total volume allowed to drain 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. FB₁ 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 and stored at -20°C until analysis (Caneta et al., 1998). The concentration of FB₁ 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 C₁₈ column (3.4 X150 mm, 4μ) was used. Mobile phase was acetonitrile: H₂O 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 in the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller and Duncan, 1959). All statements of significance were based on probability of P ≤ 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 affect 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 AFB₁, whereas it ranged from 85.1 - 92.4% for FB₁ in aqueous solution (Figs. 1 and 2). On the other hand, the adsorption capacity of EM was very high for AFB₁ and ranged from 95.4 - 99.2%, meanwhile it was high for FB₁ and ranged from 78.2 - 92.2%. The adsorption ability is not significantly affected 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 fumonisins from contaminated malt extract. Table (1) showed that malt extract was contaminated with 23, 49.53 and 101.3 ppb of aflatoxin B₁ as a result of steeping in water. These amounts formed 48%, 49.53 and 50.65% of the initial contaminated levels in malt. Malt extract was contaminated with fumonisins at levels higher than aflatoxin B₁.
Fig. (1): Adsorption ability of HSCAS 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. (2): Adsorption ability of HSCAS 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. (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)

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Fig. (5) % Reduction of AFB₁ in spiked malt extracts treated with 0.5% (w/v) of HSCAS or EM

Fig (6). % Reduction of FB₁ 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 AFB1. 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 AFB1 in malt extract at different contamination levels.

It is of interest to mention that both sorbents had a high affinity to sorb FB1 at different contamination levels. Addition of HSCAS to the spiked malt extracts resulted in the adsorption of FB1 ranged from 86.25-91.97% for HSCAS and 88.4-92.47% for EM (Fig 6). The residual FB1 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 FB1 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: AFB1 residual in malt extract spiked with 50, 100 and 200 ppb and treated with 0.5% (w/v) HSCAS or EM

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>HSCAS</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1 (ppb) in spiked malt</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>AFB1 in malt extract (Mean ± SE)</td>
<td>23.1 ± 0.37</td>
<td>49.53 ± 1.68</td>
<td>101.3 ± 1.61</td>
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<tr>
<td>Percentage</td>
<td>46.2</td>
<td>49.53</td>
<td>50.85</td>
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Table 2: FB1 residual in malt extract spiked with 50, 100 and 200 ppb and treated with 0.5% (w/v) HSCAS or EM

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>HSCAS</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB1 (ppb) in spiked malt</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>FB1 in malt extract (mean ± SE)</td>
<td>46.5 ± 1.06</td>
<td>91.03 ± 1.45</td>
<td>189.4 ± 1.52</td>
</tr>
<tr>
<td>Percentage</td>
<td>93.0</td>
<td>91.03</td>
<td>84.7</td>
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**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 AFB1, it causes a reduction percentage of AFB1 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 AFB₁ and FB₁ from malt extract. The binding capacity ranged from 98.2–98.7% and 88.4–92.4% for AFB₁ and FB₁, 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 FB₁. 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 AFB₁. 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 fumonisn 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 aqueous solution (Canela et al., 1995 and Pujol et al., 1999). According to Aly (2002) reported that although aflatoxin is water insoluble, steeping of contaminated corn with high concentration of AF₅ caused a significant loss of AF₅ in steeping water, this may probably due to the binding of AF₅ with water-soluble component. Regarding to FB₁ 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. (1995) 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 FB₁ 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 possible that AFB₁ and FB₁ 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 FB₁. The other aspect refers to evaluate the ability of EM for AFB₁ and FB₁ 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 AFB₁ and FB₁, and importantly, EM succeeded to sorb more than 98% of AFB₁ and 92.2% of FB₁ in malt extract.

In conclusion, both HSCAS and EM have the high affinity to adsorb AFB₁ and FB₁ 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 AFB₁ and FB₁ in certain aqueous solutions used in food or pharmaceutical industry.

REFERENCES


استخدام المواد على نطاق واسع في تصنيع المشروبات والاغذية وبالتالي فإن السوموم الفطرية قد تكون ناجمة عن استخدام المواد الملوثة في إعداد المواد أو منتجاتها. يهدف البحث إلى استخدام مواد طبيعية لها خاصية مساهمة في تطشير الأغذية الكيميائية لازمنة الأفلاكن ولفيتنزين في المواد والنظرة التي تتحدد عليها كائنات الأفلاكن ومنها نوع مادة الأفلاكن وتوعية الذرتين أند التكاثر المستخدم من مواد الأفلاكن وكذلك التكاثر المالم، وتأثر الجزء الثاني من دراسة تطبيق هذه التكنولوجيا على مستخلص المواد الملوث بالسوموم الفطرية. استخدمت في هذه الدراسة مادة HSCAS ومواد ملوثة بالسوموم الفطرية. وفعالية من البذور المدة الفرعية من البيئات المحلية للمقارنة مع السواد المشردة. تم استخدام أزمة تركيزات من المواد المنصمة وثلاث مستويات من التلوث (0.05، 0.1، 0.15 جزء في المليون). أثبتت النتائج أن مادة HSCAS والفيتنزين ب (HSCAS) تتراوح بين 99.1-96.4% من التلوث. ونجد أن مادة فيتنزين B لها نسبة مرجعية على أن مصادر السموم الفطرية تصل إلى 95.4 99.1-96.4% من الأفلاكتين B والفيتنزين ب على التوالي. كما وجد أن هذه المواد ذات فعالية عالية عند تركيز 6.0% وتطبيقات هذه النتائج على مستخلص المواد الملوثة بتركيزات متفاوتة (0.05، 0.1، 0.15 جزء في المليون) لكل من الأفلاكتين والفيتنزين ومواد ملوثة بالسوموم الفطرية.

تراكب كلاً من الدخانات الأتم واللامركزية لمواد الأفلاكتين والفيتنزين ب على التوالي. أما بالنسبة لمستخلص مادة الفيتامين B، HSCAS والفيتنزين B، والفيتنزين B، 98.6% من الأفلاكتين B والفيتنزين B. 98.6% من الأفلاكتين A وفوتوترن B. وتكون ذات فعالية مرتفعة في المحاليل مثل المصابور والمتابرات ومسببات نقاء التصنيع الديكية مثل مكونات الألياف أو الفيتامينات. لذلك نتائج بناء على الاستخدام العملي في زينة السموم تحسب الدراسة حيث أن الأفلاكتين B ومواد الماء المستخدمة كانت غير متوفرة. ونحصل على نتائج تساعد على ضرورة استخدام السوموم الفطرية في البريح أو المواد، وكذلك مستخلص المواد قبل تضمينه، ومنعة الفيتامينات، B، والفيتنزين ب، والتخلص منه وتطبيق هذه التكنولوجيا في كل السواد، التي ربما تكون ملوثة بالسوموم الفطرية خاصية الأفلاكتين B والفيتنزين B. الآثار استخدمتها في التصميم الغذائي.