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RESEARCH ARTICLE

First report on the presence of aflatoxins in fig seed oil and the efficacy of adsorbents in reducing aflatoxin levels in aqueous and oily media

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ABSTRACT

Aflatoxin contamination of dried figs has been a chronic problem for decades but aflatoxin distribution within the fruit has not yet been revealed. In this study, we conducted aflatoxin analyses separately in the seedless part of dried figs and in fig seed oil. The results showed that both seedless part and seed oil were contaminated with aflatoxins at levels close to the regulatory limits set for products for direct consumption. Effectiveness of various adsorbents in removing aflatoxins from aqueous and oily compartments of the fruit and the effects of these treatments on bioactive compounds and physicochemical characteristics were also investigated.

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Dried fig; mycotoxin distribution; adsorption; decontamination; aflatoxin

1. Introduction

Mycotoxins are metabolites of toxigenic mold species and aflatoxins are the most known group of mycotoxins due to their mutagenic, teratogenic and carcinogenic effects. The International Agency for Research on Cancer (IARC) classified aflatoxins as "Group 1, carcinogenic to humans" based on experimental evidences (IARC 1993). Various types of food and feed were reported to be contaminated with aflatoxins. Among these, dried figs draw attention with both high incidence of contamination and high contamination levels (RASFF 2018, 2019).

In fig processing plants, dried figs coming from the orchards are examined under ultraviolet light and figs showing bright greenish yellow fluorescence are removed. Fluorescent figs are regarded as "contaminated fruits" due to the relationship between the fluorescence and aflatoxin contamination (Steiner *et al*. 1988). It was shown that aflatoxin content was effectively lowered after removing fluorescent figs from the batch. Aflatoxin-contaminated figs not only raise health concerns among consumers but also cause economic problems and environmental hazards. It would be very useful if the aflatoxins could be removed from the product without forming any toxic compounds originated from the toxins themselves. Then, the decontaminated figs or maybe some parts of the fruits could be used by the industry and various problems stemmed from the contaminated products would be solved.

Of course, prevention of mold contamination and toxin production is the best solution to aflatoxin problem (Luo *et al*. 2018). However, it is not that easy in practice due to moderate temperature, high humidity, sudden rains, etc. and developing decontamination strategies are inevitable. Among these, adsorption is regarded as superior to other processes such as heat treatment, oxidation etc. since the former does not change the chemical structure of the toxin and does not cause the formation of toxic by- and degradation-products (Olopade *et al*. 2019). Adsorbents are currently used in a wide range of applications including pollution control, purification, separation, and others across a large number of industrial sectors (Jenkins 2015). Moreover, the effectiveness of the adsorbents for reducing various mycotoxins from different food products have been examined in scientific studies (Var *et al*. 2008, Liu *et al*. 2021, Muaz and Riaz 2021) and successful results have been obtained. On the other hand, causing loss of some bioactive components contributing to nutritional value of the product was reported as the main disadvantage of adsorption process (Gokmen *et al*. 2001). A number of authors investigated different techniques to decontaminate dried figs (Altug *et al*. 1990, Zorlugenc *et al*. 2008, Karaca and Nas 2009); however, to our knowledge, there is

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no information regarding the employment of adsorption for aflatoxin removal from figs.

Recently, a new product "fig seed oil", mainly produced from low quality figs has become very popular. It can be consumed as a food additive or a phytochemical product or used for pharmaceutical and cosmetic purposes (Icyer *et al*. 2017). Several studies concerning the composition and bioactive components of fig seed oil have been conducted in the last few years (Duman and Yazici 2018, Guven *et al*. 2019, Hssaini *et al*. 2020). However, although fig fruit is quite susceptible to mold contamination and toxin production, aflatoxin contamination in fig seed oil has not yet been examined. Therefore, the lack of scientific information on contamination level of fig seed oil and on the effectiveness of widely used adsorbents on reducing aflatoxin levels in seedless part and seed oil of dried figs as well as on the impact of this treatment on important characteristics were our justifications for conducting the present research.

The aim of this study was to determine the aflatoxin contamination levels separately in the seedless part of dried figs and in fig seed oil and thus to have an idea on the aflatoxin contamination status of fig seed oil. In addition, the efficacies of activated carbon, bentonite, marl, polyvinylpyrrolidone and gelatin in adsorbing aflatoxins in aqueous extracts of dried figs and in fig seed oil were evaluated. The effects of adsorbent treatments on important physicochemical properties of fig extracts and fig seed oils were also investigated.

2. Materials and methods

2.1. Materials

The study was carried out on dried figs of Sarilop cultivar grown in the Aegean Region (Aydın province) of Turkey. Dried fig samples showing bright greenish-yellow fluorescence when examined under UV light were used in the experiments. These figs were discarded from the lot in fig processing plants, collected by Aegean Exporters' Association in November 2018 and kindly supplied to us. Activated carbon (Akticol FA), polyvinylpyrrolidone (PVP, granular) and bentonite (Aktivit) were obtained from Erbsloeh (Geisenheim, Germany). Marl (containing approximately 90% calcium carbonate) and gelatin (gel strength 300, Type A) were purchased from Odemis Pazari (Izmir, Turkey) and Sigma-Aldrich (Steinheim, Germany), respectively. A mixed standard solution of aflatoxins (containing 1.00, 0.29, 0.99 and 0.27 μg of aflatoxins B_1, B_2, G_1 and G_2, respectively, in 1 ml of methanol) was obtained from Supelco (Bellefonte, PA, USA). Other chemicals were at least reagent grade and purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Separation of the seeds from dried figs

Dried fig samples were divided into three groups (lots I, II and III) of approximately 2 kg of each. Fig samples from all groups were cut into small pieces with a sharp knife. A portion of these fig pieces (about 250 g) was transferred to a Waring blender jar and 150 ml of distilled water was added. After homogenization for 1 min, the content of the jar was poured onto a coarse filter paper placed on top of a funnel. Fig seeds on the filter paper were taken with a tweezer and transferred to a petri dish. Seeds were washed under running tap water to remove any flesh residues and transferred onto a clean coarse filter paper to dry at room temperature for 24 h. Dried fig seeds were used for oil extraction while the seedless part of the fruit was directly subjected to the aflatoxin analysis.

2.2.2. Oil extraction from fig seeds

About 25 g of fig seeds and 100 ml of n-hexane were homogenized in a plastic beaker at 10 000 rpm for 1 min using a homogenizer (IKA T18 Ultra-Turrax, Staufen, Germany). The beaker content was shaken at 120 rpm for 2 h on an orbital shaker (OS-20, Boeco, Hamburg, Germany) and then filtered through a Whatman filter paper. Seed residues on the filter paper were re-extracted with another portion of n-hexane following the steps explained above. The filtrates were combined and dried over anhydrous sodium carbonate. The hexane was evaporated using a rotary evaporator (Scilogex RE100-Pro, Rocky Hill, CT, USA) and the residue (fig seed oil) in the rotary bottle was transferred to an amber vial for further analyses.

2.2.3. Preparation of aqueous fig extracts

Preliminary experiments were conducted to determine the dilution ratio between dried figs and water to be used in the preparation of the extracts. Accordingly, 100 g of dried figs and 175 g of distilled water were blended in Waring blender. This ratio resulted in a brix value of 22 ± 2 in the extract which is very close to that of fresh fig fruit (Aytekin Polat and Caliskan 2008). The homogenizate was filtered through a filter paper and this filtrate was used as the aqueous extracts of dried figs.

2.2.4. Aflatoxin analysis

Aflatoxin analysis was consisted of an extraction procedure and a quantification step performed by high performance liquid chromatography (HPLC). Aflatoxins were extracted from the fruit samples according to the method of Stroka *et al*. (2000) and a procedure suggested by Bao *et al*. (2013) was used for oil samples. Both methods comprise a homogenization (with methanol-water mixture) and an immunoaffinity clean-up steps. The extracts were injected to an HPLC apparatus (Shimadzu LC-10AD, Kyoto, Japan) equipped with a fluorescence detector (Shimadzu RF-20A, Kyoto, Japan). The separation of the analytes was performed on an ODS 2 Hypersil (3 μm, 150 × 4.6 mm I.D., Thermo Scientific, Waltham, MA, USA) analytical column maintained at 25 °C. The excitation and emission wavelengths of fluorescence detection were at 362 and 455 nm, respectively. A post-column derivatization was performed using an electrochemical derivatization apparatus (Coring System Diagnostix GmbH, Gernsheim, Germany). The mobile phase was a mixture of methanol and water (40:60, v/v) containing 216 mg potassium bromide and 636 μL 4 M nitric acid at a flow rate of 1 ml/min.

A series of standard solutions (with concentrations of 5–250, 1.5–72.5, 5–247.5 and 1.4–67.5 μg/L aflatoxins B_1, B_2, G_1 and G_2, respectively) were prepared from the standard solution of aflatoxins by appropriate dilutions in methanol. These solutions were used to prepare calibration curves and aflatoxins in the samples were quantified using these curves. Calibration curves with low aflatoxin concentrations (0.05–0.5, 0.03–0.3, 0.03–0.3 and 0.02–0.2 μg/L aflatoxins B_1, B_2, G_1 and G_2, respectively) were also drawn to determine limit of detection (LOD) and limit of quantification (LOQ) values. LOD and LOQ values were calculated from these curves by multiplying the standard deviation of the response by 3.3 and 10, respectively, and dividing by the slope of the calibration curve. LOD values were determined as 0.03, 0.02, 0.12 and 0.05 μg/L and LOQ values were determined as 0.10, 0.05, 0.47 and 0.16 μg/L for aflatoxins B_1, B_2, G_1 and G_2, respectively. Recovery tests were conducted by spiking the samples with aflatoxins standard solutions to obtain a known final concentration (1 ng/g) of aflatoxin B_1. After letting the sample for 15 min at room temperature, the extraction and the injection procedures were done as explained above. Triple injections were conducted and the mean recovery values were calculated as 87, 60, 120 and 85% for aflatoxins B_1, B_2, G_1 and G_2, respectively.

2.2.5. Adsorbent treatments

Contaminated fig extracts and fig seed oil samples were treated with activated carbon, bentonite, marl, polyvinylpyrrolidone and gelatin in order to determine the effectiveness of these adsorbents in reducing the levels of aflatoxins. Before treatments, aflatoxin B_1 and total aflatoxins levels of contaminated dried figs extracts were 71.46 ± 6.09 and 143.66 ± 10.40 μg/kg, respectively. These values were 11.15 ± 0.56 and 43.55 ± 1.84 μg/L, respectively, for contaminated fig seed oil samples. A portion of contaminated sample (40 g of the extract or 7.5 g of the oil) was put into a polypropylene centrifuge tube and the required amount of one of the adsorbents corresponding to 0.1–5.0% of the sample was added. The tube was shaken at 200 rpm for 2 h on the orbital shaker and then centrifuged at +4 °C for 10 min at 7000 rpm (NF 800 R, Nuve, Ankara, Turkey). The supernatant was filtered through a microfilter (0.45 μm) and then injected to the HPLC. The adsorbent doses and treatment time chosen in the study was based on the data in the literature (Bueno *et al*. 2005, Carraro *et al*. 2014, Carrasco-Sanchez *et al*. 2017). During treatments, a gelling problem was observed in case of direct addition of gelatin. For this reason, a 12.5% solution of gelatin was prepared in water at around 50 °C. The required amount of this solution giving a gelatin concentration of 0.1–1.0% in the final solution was added.

2.2.6. Determination of the effect of adsorbents on physicochemical properties of dried fig extracts and fig seed oil

Effects of the adsorbent treatments on soluble solid content (Brix), pH and titratable acidity values of dried fig extracts were determined. A bench-top refractometer (2WAJ, Abbe refractometer, Bluewave Industry Co., Ltd., Shanghai, China) and a digital pH-meter (Hanna Instruments, 2211 pH/ORP meter, Woonsocket, RI, USA) were used for Brix and pH measurements, respectively. Titratable acidity was determined by titrating the sample with sodium hydroxide solution (0.1 mol/l) using phenolphthalein as an indicator.

Effects of the adsorbents on the antioxidant activities and total phenolic contents of dried fig extracts and fig seed oils were determined. Twenty-five mL of methanol:water mixture (80:20, v/v) and a certain amount of sample (2.625 ml of extract or 2.5 g of oil) were transferred to a polypropylene tube. The tube was shaken at 200 rpm on the orbital shaker for 1 h and centrifuged at 7000 rpm for 10 min at 4 °C. Then, the supernatant was filtered through a Whatman filter paper into an amber vial and this filtrate was used for

both antioxidant activity and total phenolic content determination. Antioxidant activity of the samples was determined using the method described by Brand-Williams *et al.* (1995). Two and a half mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) were dissolved in 100 ml of methanol and the absorbance of this solution at 515 nm was approximately 0.800. The required amount of this solution was mixed with the sample (300 μL extract or 150 μL oil) to get a final volume of 3 ml. This mixture was vortexed for 2 min and held for 1 h in the dark. The absorbance of the mixture was measured at 515 nm using a spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan) and the decrease in absorbance was calculated according to the initial absorbance of the DPPH solution. Trolox was used as standard and the antioxidant activity was expressed as mmol trolox equivalent per g extract or mL oil. Stock (0.5%, w/v) and standard solutions (0–25 mM) of trolox were prepared in methanol. Standard solutions were treated with DPPH solution as described above, a calibration curve was constructed and the antioxidant activities of the samples were determined using this curve. Total phenolic content of the samples was determined according to the method of Singleton *et al.* (1999). Two hundred microliters of the sample (extract or oil) was put into a test tube and 1500 μL of distilled water and 100 μL of Folin-Ciocalteu reagent were added. The tube content was vortexed for 2 min and held for 3 min at ambient temperature. After adding 1200 μL of sodium carbonate solution (7.5%, w/v) and holding tubes for 2 h in the dark, the absorbance was measured at 765 nm. Gallic acid was used as standard and total phenolic content was expressed as g gallic acid equivalent (GAE) per kg extract or oil. Stock (0.05%, w/v) and standard solutions (0–100 mg/L) of gallic acid were prepared in distilled water. Standard solutions were treated with Folin–Ciocalteu and sodium carbonate solution as described above, a calibration curve was constructed and the phenolic contents of the samples were determined using this curve.

The absorbance spectra of the untreated oil sample were taken over the range of 360–800 nm using the spectrophotometer and the absorption maxima were determined as 459.5 ve 435.5 nm. The absorbance values of all treated oil samples were measured at these wavelengths and thus the effect of adsorbent treatments on oil color was determined.

Tocopherols were determined according to the AOCS Official Method Ce 8–89 (AOCS 2009) with a slight modification. Briefly, 0.25 g of fig seed oil sample was diluted with 1 ml of 2-propanol, filtered through a microfilter with a pore size of 0.45 μm (Chromafil Xtra PTFE-45/25, Macherey-Nagel, Duren, Germany) and injected to the HPLC. Analytical column was Zorbax Eclipse XDB-C18 column (Agilent Technologies, 250 mm × 4.6 mm I.D., 5 μm particle diameter, Santa Clara, USA), column temperature was 25 °C and the injection volume was 20 μL. The mobile phase was HPLC grade methanol with a flow rate of 1 ml/min. Detection was carried out at 289 nm for alpha-tocopherol and 297 nm for delta-tocopherol and gamma-tocopherol using a photodiode array detector (Shimadzu SPD-M20A, Kyoto, Japan). Standard solutions of alpha-, delta- and gamma-tocopherols were separately prepared in ethanol using analytical standards of these compounds (Supelco, Sigma-Aldrich, Bellefonte, CA, USA) and 6-point calibration curves (0.25–25 mg/L for alpha-tocopherol, 1–50 mg/L for delta-tocopherol and 5–500 mg/L for gamma-tocopherol) were drawn. Quantification of the tocopherols in the samples was performed using these curves.

Fatty acid methyl esters (FAMEs) were prepared according to the AOCS Official Method Ce 2–66 (AOCS 1997). Accordingly, 0.2 g of fig seed oil sample was dissolved in 2 ml of n-hexane and treated with 0.2 ml of methanolic potassium hydroxide solution. After mixing vigorously and waiting for phase separation (30 min), clear upper layer was taken with a micro-syringe and injected to the injection port of the gas chromatography apparatus (Agilent 7820 A, Santa Clara, USA) equipped with flame ionization detector. FAMEs were separated on a capillary column (Agilent Technologies, DB-FATWAX UI, 30 m × 0.25 mm i. d., 0.25 μm film thickness, Santa Clara, USA). The injection volume was 1 μL with a split ratio of 1:100 and the carrier gas was hydrogen at a flow rate of 1.4 ml/min. The column temperature was programmed to 50 °C for 2 min and increased to 174 °C for 14 min at 50 °C/min and then increased to 215 for 25 min at 2 °C/min. The temperatures of the injector and the detector were 250 and 280 °C, respectively. Peaks in the chromatogram were identified by comparison of their retention times with those of standard methyl esters (Supelco 37-component FAME Mix, Bellefonte, PA, USA).

2.2.7. Statistical analysis

All the adsorption experiments were performed in triplicate and the mean values (aflatoxin reduction rates, physicochemical characteristics of aqueous fig extracts and fig seed oil) obtained for each experimental group were analyzed using Minitab (v. 13, Minitab Inc., State College, PA, USA) statistical software. A two-way

Table 1. The concentrations of aflatoxin B_1 and total aflatoxins determined in the seedless part of dried figs and fig seed oil.

	Lot I		Lot II		Lot III	
	Seedless part	Fig seed oil	Seedless part	Fig seed oil	Seedless part	Fig seed oil
Aflatoxin B_1 (μg/kg)	198.13	5.01	47.23	3.17	135.00	1.31
Total aflatoxins (μg/kg)	308.18	11.88	94.84	10.52	272.68	6.91

analysis of variance was run to determine whether there was an interaction between the type and the dose of the adsorbent on reduction rates of the aflatoxins. Then, the means were compared using Duncan's Multiple Range Tests ($P \leq 0.05$) using MSTAT-C statistical software (MSTAT 1991, Michigan State University, MI, USA).

3. Results and discussion

3.1. Aflatoxin distribution within dried figs

The concentrations of aflatoxin B_1 and total aflatoxins determined in the seedless part of dried figs and fig seed oil are shown in Table 1. Total aflatoxin concentrations in the fig lots I, II and III were 320.1, 105.4 and 279.6 μg/kg, respectively. The amount of aflatoxins determined in the seedless part were higher than that determined in the seed oil. It was determined that 2.5%, 6.3% and 1.0% of aflatoxin B_1 detected in the lots I, II and III, respectively, were found in the seed oil. These values were determined as 3.7%, 10.0% and 2.5% for total aflatoxins. The results showed that fig seed oil as well as seedless part of the fruit could be contaminated with aflatoxins. The oils of some other crops such as corn, peanut, coconut, sunflower seed, olive, sesame and palm were also reported to be contaminated with aflatoxins in the past (Samarajeewa and Arseculeratne 1983, Ghitakou *et al*. 2006, Banu and Muthuray 2010, Elzupir *et al*. 2010, Shephard 2018). It is known that fungal spores can transfer to fig fruit by any means (wind, insects, fig wasp, etc.) and enter the fruit through its ostiole (Doster *et al*. 1996). It means that fungal colonization and aflatoxin formation mainly occur in the internal cavity of the fruit in which the seeds are located. The oil inside the seed might be contaminated with aflatoxins due to any physical damage (falling to the ground, bird-insect attack, etc.) that can occur in the seeds before and/or during drying of the fruit. A similar situation can occur when the seeds crack during homogenization of the fruit in the seed oil production. It should be also noted that the factors such as polarities of the toxins and their solubilities in oil are very critical for the transfer of the aflatoxins to the oil of the seed (Table 1).

3.2. Effects of adsorbent treatments on removal of aflatoxins from aqueous extracts of dried figs

The effects of different adsorbent treatments on the levels of aflatoxin B_1 and total aflatoxins in aqueous extracts of dried figs are shown in Figure 1. The treatments resulted in 61.53–97.31% and 55.11–95.77% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively. Activated carbon was the most effective agent in reducing aflatoxin levels in dried fig extracts. Over 95% reductions in the levels of aflatoxin B_1 and total aflatoxins were achieved with the use of this adsorbent at all tested concentrations and no significant differences were found among the concentrations ($p > 0.05$). Likewise, after activated carbon treatments, about 99% reductions were recorded for aflatoxins (Diaz *et al*. 2003) and also for other mycotoxins such as zearalenone (Bueno *et al*. 2005) and ochratoxin A (Fernandes *et al*. 2019). No doubt the effectiveness of activated carbon in binding mycotoxins is due to its extremely large surface area and highly porous structure (Galvano *et al*. 2001). Lemke *et al*. (2001) claimed that activated carbon adsorbs many compounds including aflatoxins primarily by hydrogen bonding.

Bentonite is another effective agent in reducing aflatoxin levels in aqueous dried fig extracts (Figure 1). Treating with this agent resulted in minimum 89% reductions in levels of both aflatoxin B_1 and total aflatoxins in aqueous dried fig extracts. Aflatoxin reduction rates obtained with bentonite and activated carbon treatments were not significantly different in most cases ($p > 0.05$). Similarly, Diaz *et al*. (2003) and Fernandes *et al*. (2019) indicated that both bentonite and activated carbon were quite effective in aflatoxin removal from model systems yielding close reduction rates. Bentonite, a naturally occurring clay, has a layered structure which mainly consists of montmorillonite (Senturk *et al*. 2009). It was reported that this clay was effective in reducing aflatoxin M_1 levels in milk (Carraro *et al*. 2014) and aflatoxin B_1 levels in model systems (Tabari *et al*. 2018). Wang *et al*. (2020) claimed that bentonite has active sites within its interlamellar region for aflatoxin sorption. According to the authors, the carbonyl moiety in the aflatoxin molecule is important for binding. On the other hand, Phillips *et al*. (2019) claimed that other mechanisms such as

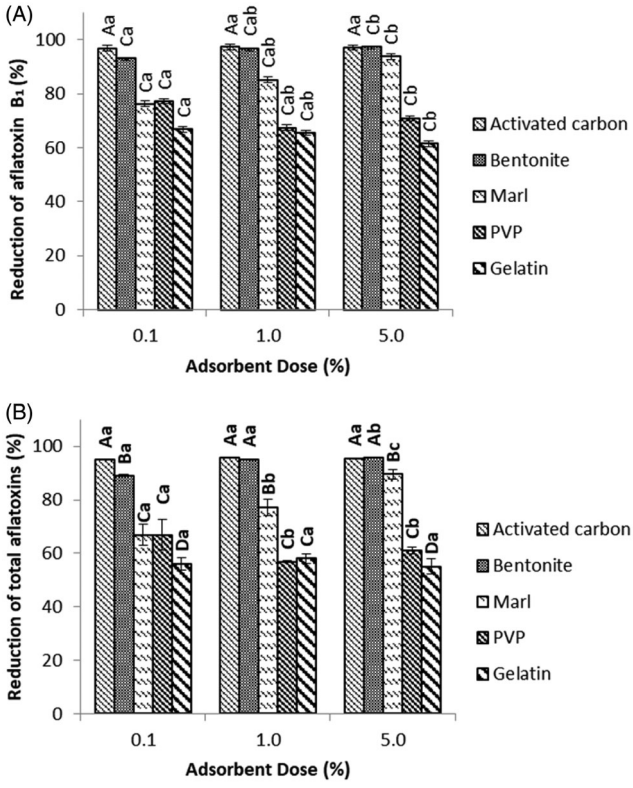

Figure 1. The effects of treating aqueous extracts of dried figs with various adsorbents on (A) aflatoxin B_1 levels and (B) total aflatoxins levels. All the error bars were calculated based on the standard deviation of three measurements. Different letters shown with lower case indicate significant differences among the concentrations of a specific adsorbent ($p < 0.05$). Different letters shown with upper case indicate significant differences among the adsorbents at a specific concentration ($p < 0.05$). The gelatin doses tested were 0.1, 0.5 and 1.0% due to the gelling problem at higher doses.

electron donor acceptor complexation, ion-dipole interaction, and coordination between exhange cations and the carbonyl oxygens can also lead to the aflatoxin binding. It was shown that the adsorption strength of bentonite strongly depends on its mineral content (Galvano *et al.* 2001) and the pH of the media (Bueno *et al.* 2005).

According to our results, aflatoxins in fig extracts could also be effectively reduced by marl treatment (Figure 1). Reductions of 76.23–93.79% and 66.94–89.60% in the levels of aflatoxin B_1 and total aflatoxins, respectively, were recorded after treating the extracts with this agent. Reduction rates increased with increasing the concentration of the agent ($p < 0.05$). At the treatment concentration of 5.0%, the aflatoxin reduction rates obtained with marl were not significantly different than those obtained with activated carbon and bentonite ($p < 0.05$). Marl, a calcareous clay containing a high amount of calcium carbonate, is conventionally used for deacidification of grape must (Karababa and Develi Isikli 2005). It lowers the acidity by precipitating tartaric acid and malic acid as calcium tartrate and calcium malate, respectively. This causes an increase in the pH of the must from around 3.5 to 5.0–6.0 (Rezaei *et al.* 2020). In the past, reductions in aflatoxin levels were observed during processing of the must, especially after treating with marl (Bahar and Altug 2009, Heshmati *et al.* 2019). However, it was not clarified that if the reduction was caused as a result of an adsorption effect or due to structural change of the toxin caused by alkalization of the medium.

PVP was the only synthetic compound tested in this study. PVP and its cross-linked form polyvinylpolypyrrolidone (PVPP) are efficient adsorbents which are widely used in fruit juice industry as fining agents. As can be seen from Figure 1, PVP treatments of fig extracts resulted in 67.36–77.23% and 56.92–66.89% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively. Previously, it was reported that PVPP and PVP were effective in reducing fumonisin levels in red wines (Carrasco-Sanchez *et al.* 2017) and zearalenone levels in model systems (Alegakis 1999), respectively. Moreover, these compound were regarded as "promising" due to their high performance in reducing aflatoxin toxicity when used as feed additives (Celik *et al.* 2000, Stroud 2007).

Our results showed that gelatin treatment had the most limited effect on reducing aflatoxins in fig extracts (Figure 1). This treatment resulted in 61.53–66.78% and 55.11–58.07% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively. Close results (57.6–78.0% reductions) were reported by Heshmati *et al.* (2019) who treated grape musts with gelatin. Lasram *et al.* (2008) reported that ochratoxin A levels were reduced by approximately 58% after treating red wines with a gelatin solution of 0.1 ml/L. Gelatin is a water-soluble and gel-forming hydrocolloid. It was assumed that mycotoxins could be trapped and/or adsorbed within the gel network formed by the gelatin (Heshmati *et al.* 2019).

3.3. Effects of adsorbent treatments on physicochemical characteristics of aqueous extracts of dried figs

The effects of various adsorbent treatments on the physicochemical characteristics of aqueous extracts of dried figs are given in Table S1. The Brix values of dried fig extracts were 21.7 ± 0.5 at the beginning and varied between $21.1 \pm 0.0 - 22.3 \pm 0.3$ after adsorbent treatments. Statistical analysis showed that adsorbent

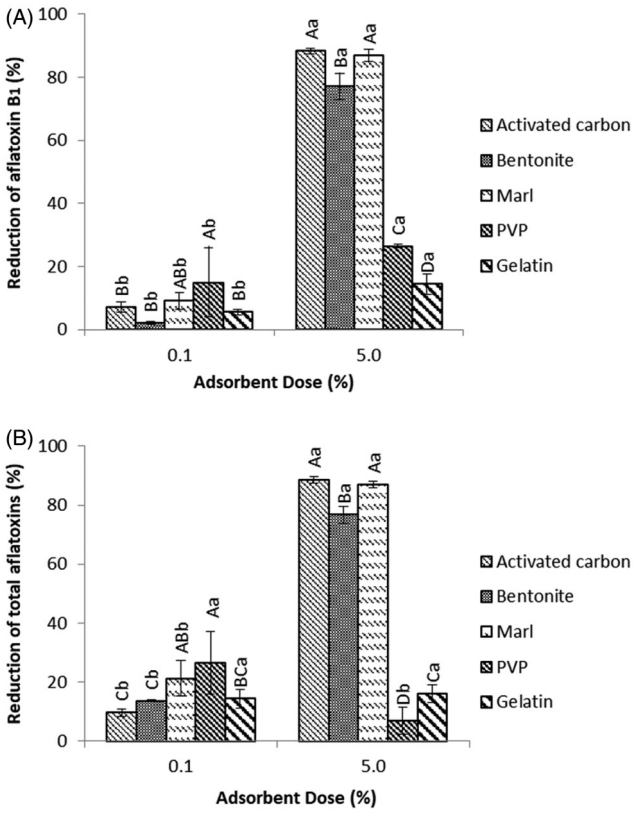

Figure 2. The effects of treating fig seed oil with various adsorbents on (A) aflatoxin B_1 levels and (B) total aflatoxins levels. All the error bars were calculated based on the standard deviation of three measurements. Different letters shown with lower case indicate significant differences among the concentrations of a specific adsorbent ($p < 0.05$). Different letters shown with upper case indicate significant differences among the adsorbents at a specific concentration ($p < 0.05$). The gelatin doses tested were 0.1, 0.5 and 1.0% due to the gelling problem at higher doses.

treatments did not have any significant effect on the Brix values of the extracts ($p < 0.05$). It shows that the adsorbents we tested did not adsorb sugars which constitute a substantial proportion of the total soluble material in the extract. There are contradictory results in the literature about the effect of adsorbents on Brix values of various juice samples. For instance, the Brix values decreased after treating apple juice with activated carbon (Gokmen *et al*. 2001, Coklar and Akbulut 2010) and increased after treating sour cherry juice with calcium and potassium carbonates (Yesiloren and Eksi 2015). Gulcu (2008) reported that the Brix values of grape juice decreased after bentonite treatment and did not change after gelatin treatment. We observed that pH and titratable acidity values generally reduced after adsorbent treatments. These values were most affected by marl treatment. A drastic increase in pH (from 4.39 to 5.97) and a sharp decrease in titratable acidity (from 1.39 to 0.38 g/100 g) were observed after marl treatment of the extracts.

The initial antioxidant activity and total phenolic content of dried fig extracts were 3.06 ± 0.07 g trolox/kg and 1.89 ± 0.36 g GAE/kg, respectively (Table S1). Activated carbon and bentonite treatments at concentrations of 1.0% and 5.0% significantly reduced antioxidant activity and total phenolic content of the extracts. Likewise, significant decreases were observed in total phenolic contents of activated carbon-treated apple juice (Gokmen *et al*. 2001) and bentonite-treated grape juice (Gulcu 2008). Coklar and Akbulut (2010) recorded 20–75% reductions in total phenolic contents of apple juice after treating with activated carbon at concentrations of 0.5–3.0 g/L. In the present study, gelatin treatments significantly reduced the antioxidant activity of dried fig extracts. Similar results were also observed in grape juice (Gulcu 2008) and pomegranate juice (Erkan-Koc *et al*. 2015) after gelatin treatments.

3.4. Effects of adsorbent treatments on removal of aflatoxins from fig seed oil

The effects of different adsorbent treatments on the levels of aflatoxin B_1 and total aflatoxins in fig seed oil are shown in Figure 2. At 0.1% concentration, PVP was the most effective adsorbent in reducing aflatoxin levels in fig seed oil (Figure 2). Treating contaminated oil samples with PVP at 0.1% resulted in 15.0% and 26.5% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively. On the other hand, when used at 5.0% concentration, activated carbon and marl were the most effective adsorbents in reducing aflatoxin levels in fig seed oil. These adsorbents resulted in 88% and 86% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively. Bentonite was another effective adsorbent with reduction rates of 77.1% and 76.7% in the levels of aflatoxin B_1 and total aflatoxins, respectively. PVP and gelatin had a relatively limited effect in reducing aflatoxin levels in fig seed oil. PVP and gelatin treatments caused 26.5% and 14.4% reductions, respectively, in aflatoxin B_1 levels in fig seed oil while total aflatoxin reductions observed after these treatments were below 20%. Chromatograms (overlapped) showing aflatoxin peaks in aqueous extracts of dried figs before and after treatment with various adsorbents at a concentration of 5% can be seen Figure 3.

It is known that coconut, olive, palm, peanut and maize are among the products that are risky for aflatoxin contamination. They all have high oil content

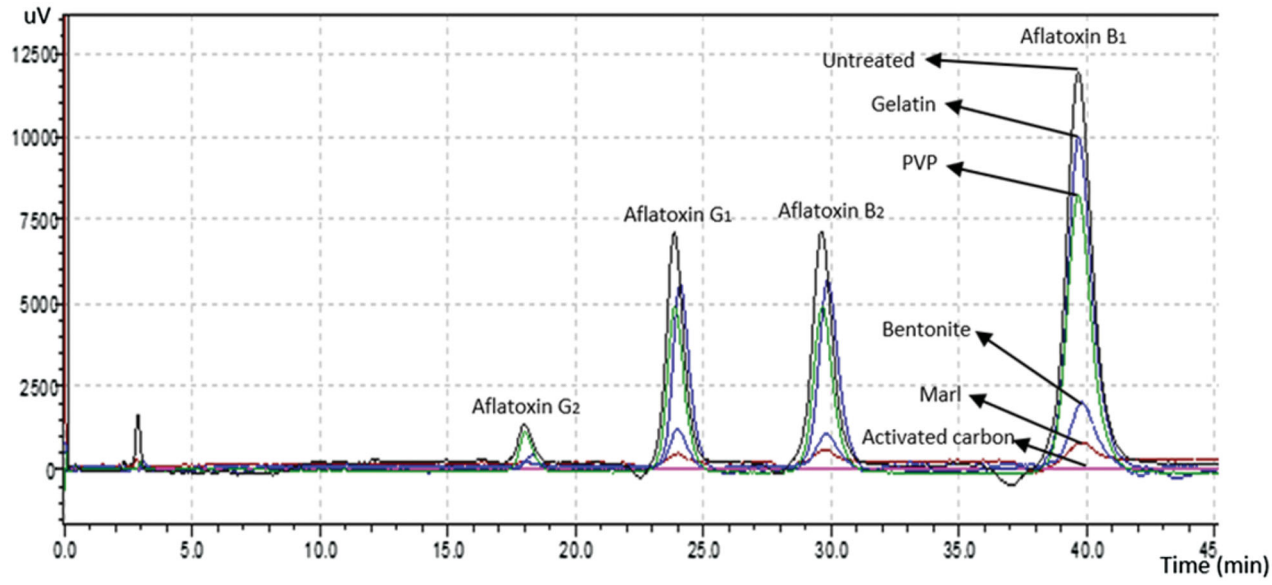

Figure 3. Chromatograms (overlapped) showing aflatoxin peaks in fig seed oil before and after treatment with various adsorbents at a concentration of 5.0%. The gelatin dose tested was 1.0% due to the gelling problem at higher doses.

and can be used as raw materials for edible oil industry. In this case, it is likely that the oils of these products can also be contaminated with aflatoxins. As a matter of fact, it was reported that coconut oil (Samarajeewa and Arseculeratne 1983), olive oil (Ghitakou *et al*. 2006) , sunflower oil, sesame oil, peanut oil (Elzupir *et al*. 2010), canola oil (Nabizadeh *et al*. 2018) and some other seed oils (Bhat and Reddy 2017) can be contaminated with high levels (above the legal limits) of aflatoxins. Kamimura *et al*. (1986) reported that the levels of aflatoxins can be reduced during edible oil production, especially due to oil-refining process. The adsorbents used for removal of undesired substances (trace elements, gossypol, pesticides, wax, gums, etc.) in the bleaching step during refining are likely effective on also aflatoxins and other mycotoxins. Although it has been known that many types of oilseeds can be contaminated with mycotoxins and these toxins can transfer to the oil of the seed, the number of studies focusing on the removal of mycotoxins by adsorbent treatments are very limited. However, in recent studies, newly functionalized adsorbents have been tested for removal of zearalenone from corn oil (Bai *et al*. 2018) and aflatoxin B_1 from rice bran oil (Ji and Xie 2020). In 2017, Ma et al. conducted a study with contaminated peanut oil samples and determined that montmorillonite was quite effective in reducing aflatoxin B_1 level (Ma *et al*. 2017). Compatible with this result, we observed that bentonit, of which the major clay mineral is montmorillonite, could reduce the aflatoxin B_1 level in fig seed oil by more than 75%.

It is obvious that the effectiveness of the adsorbents in aflatoxin removal from aqueous and oily media can be quite different. This can be easily seen from the results of the present study. For instance, activated carbon at 0.1% was quite effective in reducing aflatoxins causing 96.85% and 94.93% reductions in the levels of aflatoxin B_1 and total aflatoxins, respectively, in aqueous extracts of dried figs whereas these rates were only 7.11% and 9.67%, respectively, in fig seed oil. When this agent was used even at 5.0%, the aflatoxin reduction rates obtained in fig seed oil were below 90% which were lower than that obtained in the aqueous extracts.

3.5. Effects of adsorbent treatments on physicochemical characteristics of fig seed oil

The effects of various adsorbent treatments on some physicochemical characteristics of fig seed oil are given in Table S2. The absorbance values of fig seed oil at 435.5 and 459.5 nm were 0.81 ± 0.01 and 0.72 ± 0.01, respectively, before adsorbent treatments. It was determined that, in general, the adsorbent treatments did not have any significant effect on the absorbance values of the fig seed oil. The only exception to this situation was the result obtained with activated carbon treatment. Treating the fig seed oil with activated carbon at a concentration of 0.1% resulted in 20.9% and 22.2% reductions in the absorbance values at 459.5 and 435.5 nm, respectively. These rates were recorded as 32.1% and 22.2% after treating with activated carbon concentration of 5.0%. This result

shows that activated carbon could adsorb the compounds such as chlorophylls and carotenoids that contribute to the color of the fig seed oil. Similarly, decreases in the color values of fish oil (Monte *et al*. 2015) and soybean oil (Udomkun *et al*. 2018) were observed after activated carbon treatments.

Before treatments, antioxidant activity and total phenolic content of fig seed oil were 103.31 ± 2.97 g trolox/kg and 0.51 ± 0.09 g GAE/kg, respectively (Table S2). It was observed that the antioxidant activity of fig seed oil generally decreased after adsorbent treatments and these decreases were more pronounced when higher concentrations of adsorbents were treated. Activated carbon treatment at 0.1% and bentonit treatments at both 0.1% and 5.0% did not show a significant effect on antioxidant activity of fig seed oil while gelatin had a limited effect and caused a reduction of about 10%. Marl and PVP resulted in 19.3–26.8% and 36.0–39.3% reductions in the antioxidant activity of fig seed oil. These adsorbents had a significant effect on the phenolic content of the oil and caused 23.1–24.5% and 42.1–47.5% reductions. On the other hand, the highest reductions of antioxidant activity and total phenolic content of fig seed oil were observed after activated carbon treatment at 5%. This treatment resulted in 49.0% and 66.6% reductions of antioxidant activity and total phenolic content of fig seed oil, respectively. Likewise, Shi *et al*. (2017) treated sesame oil with three different types of activated carbon for removal of polycyclic aromatic hydrocarbons and recorded 17.4–29.2% reductions in polyphenol contents of the oil.

The effects of various adsorbent treatments on tocopherols contents of fig seed oil are given in Table S3. The initial contents of gamma-, delta- and alpha-tocopherols in fig seed oil were 4060.6 ± 156.6, 155.1 ± 4.8 and 40.4 ± 1.5 mg/L, respectively. The contents of any tocopherols did not change significantly after marl, PVP and gelatin treatments ($p > 0.05$). However, activated carbon and bentonite treatments at 5.0% resulted in significant reductions in the contents of all tocopherol isomers tested ($p < 0.05$). Reductions in the contents of gamma-, delta- and alpha-tocopherols were 61.8%, 27.7% and 44.1% after activated carbon treatment and 20.8%, 21.3% and 18.5% after bentonite treatment, respectively. Shi *et al*. (2017) reported that gamma-tocopherol content of sesame oil decreased after activated carbon treatment. Similar decreases were also observed in tocopherol contents of rice bran oil after treatments with magnetic graphene composite adsorbents (Ji and Xie 2020).

The effects of various adsorbent treatments on fatty acid composition of fig seed oil are given in Table S4. Our results showed that adsorbent treatments significantly affected fatty acids with 18 C-atoms. For instance, increases in polyunsaturated fatty acids (linoleic acid [18:2] and linolenic acid [18:3]) and decreases in monounsaturated (oleic acid [18:1]) and saturated (stearic acid [18:0]) fatty acids were observed. This finding shows that the adsorbents tested in the present study could adsorb saturated and monounsaturated fatty acids rather than polyunsaturated fatty acids. It might be due to the higher fluidity and thus better mobility of the latter compared to the former fatty acids. Remarkable changes in the fatty acid composition of various oil samples after different adsorbent treatments were also observed in previous studies (Shi *et al*. 2017, Ji and Xie 2020).

4. Conclusions

The aflatoxin contamination of dried fig samples used in the present study is quite higher than the international regulatory limits set for products for human and even animal consumption. There is a need for safe, practical, reliable and economic techniques to effectively reduce aflatoxins and lower their concentrations below regulatory limits. Among other treatments, adsorption seems as a promising technique for aflatoxin removal as it does not cause any change in the toxin structure and does not form any toxic by- or degradation-products. To the best of our knowledge, this is the first report demonstrating the presence of aflatoxin contamination in fig seed oil. Although the aflatoxin amounts determined in the oil are lower than those determined in the seedless part of the fruit, it can still pose a serious health hazard to the consumers. It was observed that the effectiveness of the adsorbents in reducing aflatoxin levels could substantially vary in aqueous and oily media. Activated carbon, bentonite and marl were the most effective adsorbents resulting in about 90% reductions in the aflatoxin levels. However, significant changes in physicochemical characteristics and antioxidant potentials were observed after adsorbent treatments. Optimization studies are required to determine the right concentration of the adsorbents to obtain maximum aflatoxin reduction and minimum loss in the quality of the product.

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Disclosure statement

The authors declare that there are no conflicts of interest relevant to this study.

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