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Assessment of Multi-mycotoxin Adsorption Efficacy of Grape Pomace

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ABSTRACT: Grape pomace (pulp and skins) was investigated as a new biosorbent for removing mycotoxins from liquid media. In vitro adsorption experiments showed that the pomace obtained from Primitivo grapes is able to sequester rapidly and simultaneously different mycotoxins. Aflatoxin B_1 (AFB_1) was the most adsorbed mycotoxin followed by zearalenone (ZEA), ochratoxin A (OTA), and fumonisin B_1 (FB_1), whereas the adsorption of deoxynivalenol (DON) was negligible. AFB_1 and ZEA adsorptions were not affected by changing pH values in the pH 3−8 range, whereas OTA and FB_1 adsorptions were significantly affected by pH. Equilibrium adsorption isotherms obtained at different temperatures (5−70 °C) and pH values (3 and 7) were modeled and evaluated using the Freundlich, Langmuir, Sips, and Hill models. The goodness of the fits and the parameters involved in the adsorption mechanism were calculated by the nonlinear regression analysis method. The best-fitting models to describe AFB_1, ZEA, and OTA adsorption by grape pomace were the Sips, Langmuir, and Freundlich models, respectively. The Langmuir and Sips models were the best models for FB_1 adsorption at pH 7 and 3, respectively. The theoretical maximum adsorption capacities (mmol/kg dried pomace) calculated at pH 7 and 3 decreased in the following order: AFB_1 (15.0 and 15.1) > ZEA (8.6 and 8.3) > OTA (6.3−6.9) > FB_1 (2.2 and 0.4). Single- and multi-mycotoxin adsorption isotherms showed that toxin adsorption is not affected by the simultaneous presence of different mycotoxins in the liquid medium. The profiles of adsorption isotherms obtained at different temperatures and pH and the thermodynamic parameters ($\Delta G°$, $\Delta H°$, $\Delta S°$) suggest that mycotoxin adsorption is an exothermic and spontaneous process, which involves physisorption weak associations. Hydrophobic interactions may be associated with AFB_1 and ZEA adsorption, whereas polar noncovalent interactions may be associated with OTA and FB_1 adsorption. In conclusion, this study suggests that biosorption of mycotoxins onto grape pomace may be a reasonably low-cost decontamination method.

KEYWORDS: *biosorption, mycotoxins, grape pomace, adsorption isotherm models*

INTRODUCTION

Winemaking generates waste in the form of grape pomace that remains after grape has been pressed. On the basis of some estimates, approximately 20% of the grape total weight is waste, which translates to >8 million tons worldwide of grape pomace.[1] Removal of grape pomace is costly, and if it is not treated effectively, it poses a serious environmental problem.[2] Several processes have been suggested for utilization of pomace, including extraction of anthocyanins, citric acid, ethanol, and grape seed oil.[3] At present, grape pomace is mainly used for soil conditioning or as low-cost raw material for animal feed, especially for herbivores and in the dry season when pastures are scarce.[4,5] Grape pomace represents a potentially valuable source of phenolic antioxidants, which can have technological applications as feed additives and possible nutritional and health benefits.[6] The content of tannins in grape pomace reduces ruminal crude protein degradability, which may prevent bloat when cows are fed high-concentrate diets.[7] Dietary polyphenol-rich grape products were found effective in broiler chicks and piglets in improving the physiology and biochemistry of the gut, thus leading to better nutrient absorption and increased disease resistance.[8,9] The supplementation of human food with grape pomace and its use during some food-processing processes have been also attempted. In the form of dried, milled flour, grape pomace has been incorporated into bakery products to make cookies, cakes, or specialty breads, which showed higher contents of dietary fiber and phenolic compounds and enhanced antioxidant properties.[10,11]

Recently, an innovative winemaking procedure involving the use of grape pomace has been suggested as a corrective measure to reduce ochratoxin A (OTA) levels in must and wine.[12] The procedure proposed the repassage of contaminated musts or wines over grape pomaces having no or little OTA contamination, and it removed up to 65% of OTA within 24 h.[12] These findings supported the hypothesis that grape pomace has affinity for OTA adsorption.[12] The work did not study the mechanism for OTA adsorption by grape pomace or explore its effectiveness in adsorbing mycotoxins other than OTA, such as aflatoxin B_1 (AFB_1), zearalenone (ZEA), fumonisin B_1 (FB_1), and deoxynivalenol (DON). These fungal metabolites are the most commonly occurring mycotoxins, which can be found worldwide in food and feed. They can cause a variety of diseases (mycotoxicoses) in a wide range of susceptible animal species including humans.[13,14] They often co-occur, depending on the environmental and substrate conditions, and can lead to toxicological interactions (synergistic effects).[15] So far, only activated carbon has been found effective in adsorbing in vitro these mycotoxins.[16−19] However,

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it is probably more suited to be used as an antidote for severe intoxication. The great variability in the results of long-term exposure experiments and its potential for also sequestering important nutrients diminishes its overall practical effectiveness for routine dietary inclusion.[18] Except for activated carbon, most substances (clays) used as mycotoxin binders to decontaminate mycotoxin-contaminated feeds fail in sequestering structurally different mycotoxins.[16,18] They appear to bind to only a limited group of mycotoxins (mainly aflatoxins) while showing very little or no binding to others. Therefore, feed additives acting as multi-mycotoxin binders are sought.

Due to the potential ability of grape pomace in binding OTA and its well-known health benefits, the objective of the present work was to explore the feasibility of using grape pomace as a low-cost biosorbent for mycotoxin decontamination. For the first time, this study examined the ability of grape pomace to adsorb simultaneously different mycotoxins from liquid media at physiological pH. The pomace obtained from a red grape variety (cv. Primitivo) was used for the adsorption study.

■ MATERIALS AND METHODS

Reagents and Samples. Mycotoxin standards (purity > 99%) were supplied by Sigma-Aldrich (Milan, Italy). All chemicals used were of analytical grade unless otherwise stated. All solvents (HPLC grade) were purchased from J. T. Baker (Deventer, The Netherlands). Water was of Milli-Q quality (Millipore, Bedford, MA, USA). Mycotoxin adsorption was studied at different pH values using different media (1 mmol/L), such as citrate buffers at pH 3, 4, and 5 and phosphate buffers at pH 6, 7, 8, and 9. Mycotoxin stock solutions of AFB_1, OTA, ZEA, and DON (1 mg/mL each) were prepared by dissolving solid commercial toxins in acetonitrile (HPLC grade). FB_1 stock solution (1 mg/mL) was prepared in acetonitrile/water (50:50, v/v). Stock solutions were stored in the dark at 4 °C. A multi-mycotoxin standard solution containing 200 μg/mL of each toxin (AFB_1, ZEA, FB_1, OTA, and DON) was prepared by mixing equal volumes of mycotoxin stock solutions. This solution or the mycotoxin stock solutions were properly diluted with buffers at different pH values to prepare the mycotoxin working solutions for adsorption experiments and calibrants.

Fermented grape pomace was obtained by manually processing grapes of a red grape variety (Primitivo) grown and harvested in Conversano (Apulia, Italy). Seeds and stems were manually separated and discarded, and the peel and pulp were oven-dried at 50 °C until constant weight. The dried material was ground into fine powder by a microfine grinder drive, MF10 Basic model (IKA-Werke GmbH & Co., KG/Germany), provided with a 0.5 mm interchangeable sieve (MF 0.5 model) for particle size filtering. OTA analysis of grape pomace was performed according to the method of Solfrizzo et al.[20] and showed the absence of toxin.

LC Analysis of Mycotoxins. DON, AFB_1, ZEA, and OTA were analyzed according to a UPLC method, which allowed the simultaneous determination of the toxins. The UPLC apparatus consisted of a Waters Acquity UPLC system (Milford, MA, USA). Data acquisition and instrument control were performed by Empower 2 software (Waters). The column used was a 100 mm × 2.1 mm i.d., 1.7 μm, Acquity UPLC BEH RP-18, with an Acquity UPLC column in-line filter (0.2 μm). Chromatographic separation of DON, AFB_1, ZEA, and OTA was achieved through a 13.5 min gradient delivery of a mixture of A (water/acetonitrile 85:15 v/v) and B (methanol/acetonitrile 50:50 v/v, containing 0.5% acetic acid) at a flow rate of 0.4 mL/min. The temperatures of the column and sample compartment were maintained at 40 and 15 °C, respectively. The injected volume was 5 μL in a partial loop with needle overfill mode. The UV absorption spectra of DON and AFB_1 were recorded in the range of 190−400 nm. UV absorbance data were collected with a bandwidth of 1.2 nm and without digital filtering, at wavelengths of 220 nm for DON and 350 nm for AFB_1. A LC UV chromatogram was acquired at 220 nm absorbance wavelength for the first 3 min and then at 350 nm. Fluorescence detection of AFB_1, ZEA, and OTA was carried out using a wavelength program with, respectively, excitation and emission wavelengths of 333 and 460 nm until 7.5 min for AFB_1 detection, then of 274 and 440 nm from 7.5 to 8.5 min for ZEA, and of 333 and 460 nm from 8.5 to 13.5 min for OTA. AFB_1 was detected by both UV and fluorometric detectors without postcolumn derivatization.

FB_1 was analyzed according to a HPLC method. The HPLC-FLD apparatus was an Agilent 1100 series (Agilent, Waldbronn, Germany) equipped with a binary pump, autosampler, column thermostat set at 30 °C, and spectrofluorometric detector with excitation and emission wavelengths set at 335 and 440 nm, respectively. The column was a 100 × 4.6 mm i.d., 2.6 μm, Kinetex core−shell particle with pentafluorophenyl stationary phase (Phenomenex, Torrance, CA, USA). The isocratic mobile phase consisted of the mixture water/methanol/acetonitrile (50:25:25, v/v/v) containing acetic acid (1%) and eluted at an 0.8 mL/min flow rate for 20 min. Prior to HPLC analysis, FB_1 samples were precolumn derivatized with *o*-phthaldialdehyde reagent, according to the method described by De Girolamo et al.[21]

LC methods were linear in the concentration range of 0.07−1.0 μg/mL (five mycotoxin levels) for DON; 0.007−1.0 μg/mL (seven mycotoxin levels) for AFB_1, ZEA, and OTA; and 0.05−5.0 μg/mL (eight mycotoxin levels) for FB_1. Calibrants were prepared in buffer at pH 3 and 7 and analyzed in triplicate. The coefficients of determination (R^2) were ≥0.996. The limits of quantitation were 70 ng/mL for DON, 7 ng/mL for ZEA, 0.7 ng/mL for OTA and AFB_1, and 50 ng/mL for FB_1 (S/N ratio = 10). These limits were 2−4 orders of magnitude below toxin concentration of the working solutions used for adsorption tests and safeguarded the ability to perform accurate LC measurements even when strong mycotoxin adsorption occurred (>90%).

Mycotoxin Adsorption Experiments. Grape pomace (pulp and skins) was weighed in a 4 mL silanized amber glass vial and suspended with an appropriate volume of (multi-)mycotoxin working solution in buffer. The suspension was vigorously mixed by vortex for a few seconds and then shaken in a thermostatically controlled shaker at constant temperature and 250 rpm speed. Adsorption studies were carried out for 90 min to ensure complete adsorption of mycotoxins. After the incubation period, the sample was allowed to settle, and 1 mL of suspension was transferred into an Eppendorf tube and centrifuged for 20 min at 18000*g* and 25 °C. Supernatant sample was split into two aliquots and analyzed for the residual mycotoxin content by HPLC for FB_1 and by UPLC for AFB_1, ZEA, OTA, and DON. Samples analyzed by UPLC were properly diluted by mixing 700 μL of the sample (in citrate or phosphate buffer solution) with 300 μL of a mixture of acetonitrile/methanol (1:2, v/v) containing acetic acid 1% and then filtered by microspin filter tubes 0.2 μm, RC/G (Grace Davison Discovery Science, Deerfield, IL, USA). A blank control was prepared using the mycotoxin working solution in buffer without grape pomace. This was subjected to the same test procedure and served as background control during the analysis to investigate the stability of toxins in the buffer solutions or any possible nonspecific adsorption. Chemical precipitation and losses of mycotoxins due to nonspecific adsorption were not detected.

Preliminary adsorption experiments were performed at constant temperature (37 °C) to determine the effect of various parameters (particle size, contact time, medium pH, and pomace dosage) on the adsorption of mycotoxins. Mycotoxin concentration was 1 μg/mL in all cases.

To study the effect of particle size on mycotoxin adsorption, five fractions of grape pomace were tested by quintuplicate independent adsorption experiments, at pH 7 and 0.5% w/v (5 mg/mL) dosage. The fractions (>500, 500−300, 300−100, 100−60, <60 μm) were obtained by sieving the dried ground material (>500 μm) and using woven wire test sieves (Endecotts, London, UK) with different nominal sizes of apertures placed in sequence.

To investigate the effect of contact time on the adsorption process, the grape pomace was tested at pH 7 and 0.1% w/v (1 mg/mL) dosage (triplicate independent experiments). Samples were withdrawn

Table 1. Isotherm Models and Equations Used To Analyze Experimental Adsorption Data

eq 2	Freundlich	$Q_{eq} = K_f C_{eq}^{1/n}$	K_f = constant related to capacity of the adsorbent for the mycotoxin n = adsorption intensity
eq 3	Langmuir	$Q_{eq} = Q_{max}[(K_L C_{eq})/(1 + K_L C_{eq})]$	Q_{max} = constant related to maximum mycotoxin uptake K_L = constant related to the energy of adsorption and affinity of the adsorbent
eq 4	Langmuir separation factor (R_L)[a]	$R_L = 1/(1 + K_L C_0)$	K_L = Langmuir constant C_0 = adsorbate initial concentration
eq 5	Sips	$Q_{eq} = (q_m a_s C_{eq}^{1/n})/(1 + a_s C_{eq}^{1/n})$	q_m = constant related to maximum mycotoxin uptake a_s = constant related to energy (affinity) of adsorption $1/n$ = index for the heterogeneity of binding sites[b]
eq 6	Hill	$Q_{eq} = (Q_{max} C_{eq}^{nH})/(K_D + C_{eq}^{nH})$	Q_{max} = constant related to maximum mycotoxin uptake K_D = Hill constant nH = Hill cooperativity coefficient of the binding interaction[c]

[a] R_L value indicates the adsorption nature: $R_L > 1$, unfavorable; $R_L = 1$, linear; $0 < R_L < 1$, favorable; $R_L = 0$, irreversible. [b] $1/n$ value indicates the heterogeneity of the adsorption sites ($0 < 1/n < 1$): $1/n < 1$, heterogeneous system; $1/n = 1$ material with relatively homogeneous binding sites. [c] $nH > 1$, positive cooperativity; $nH = 1$, no cooperativity; $nH < 1$, negative cooperativity.

at appropriate time intervals (1−120 min). Supernatant liquid portions were filtered by 0.2 μm syringe filter tubes (Sartorius Stedim Biotech, Goettingen, Germany) and analyzed for residual mycotoxin content.

To study the effect of pH on toxin adsorption, quintuplicate independent experiments were performed at different pH values (3−9), using 0.5% w/v (5 mg/mL) dosage. To investigate the desorption capacity of mycotoxins from grape pomace due to pH change, 10 mg of pomace was weighed into a 2 mL screw-cap test tube and mixed with 1 mL of mycotoxin working solution (pH 3), containing 1 μg/mL of each toxin (AFB_1, ZEA, OTA, FB_1, and DON) (1% w/v adsorbent dosage). Samples were incubated at 37 °C for 90 min in a rotary shaker (250 rpm). Then, they were centrifuged, and the supernatants were completely removed and analyzed for residual mycotoxin content to calculate mycotoxin adsorption. The adsorbent pellets were washed with 1 mL of buffer at pH 7 (30 min shaking), and then they were centrifuged and the supernatants analyzed to assess mycotoxin desorption. Desorption studies were performed in triplicate. Values for mycotoxin adsorption (pH 3) and desorption (pH 7) were calculated for each toxin and expressed in percent.

The effect of pomace dosage on toxin adsorption was investigated by equilibrium isotherms. Adsorption experiments were performed in triplicate, at constant pH (7 and 3), testing a fixed amount of toxins with different pomace dosages (0.05−3% w/v corresponding to 0.5−30 mg/mL). Adsorption data were expressed as percentage of mycotoxin adsorbed and plotted as a function of grape pomace dosage. Mycotoxin adsorption plots were fitted by the Langmuir model (Table 1).

Equilibrium Adsorption Isotherms. Three sets of adsorption isotherms were carried out at constant temperature and pH by testing a fixed amount of grape pomace with buffered solutions of mycotoxins at different toxin concentrations (0.05−5 μg/mL). Equilibrium isotherms were set up according to the results of preliminary adsorption experiments, using 90 min of contact time, a <500 μm particle size fraction, a 0.1% w/v (1 mg/mL) pomace dosage for AFB_1 and ZEA adsorption, and a 0.2% w/v (2 mg/mL) pomace dosage for OTA and FB_1 adsorption. Due to the inefficacy of grape pomace in adsorbing DON from liquid media, DON was excluded from this study. Isotherms consisted of 10 or more experimental points and were carried out in triplicate. The first set of isotherms (single-mycotoxin system) was conducted at constant temperature (37.0 ± 0.5 °C) and different pH values (3 and 7). These isotherms were used to calculate the parameters (maximum adsorption capacity, adsorption affinity, and heterogeneity of the adsorption process) related to the adsorptions of AFB_1, ZEA, OTA, and FB_1 onto grape pomace and the effect of pH on the adsorption. The second set of isotherms was conducted at constant pH (7) and different temperatures (5.0 ± 0.5, 37.0 ± 0.5, 50.0 ± 0.5, and 70.0 ± 0.5 °C). These isotherms allowed investigation of the influence of temperature change on the uptake of mycotoxins and calculation of the thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $\Delta S°$) related to the mycotoxin adsorption. The third set of isotherms (multi-mycotoxin system) was carried out at constant temperature (37.0 ± 0.5 °C) and pH 7 using mycotoxin working solutions containing a pool of five mycotoxins, in the 0.05−5 μg/mL concentration range and in the 1:1 ratio. These multiple adsorption isotherms were performed to verify if the simultaneous presence of AFB_1, ZEA, OTA, FB_1, and DON can interfere with the adsorption process related to each toxin. The change for adsorption values due to the simultaneous presence of mycotoxins was evaluated by comparing adsorption isotherms and related parameters obtained by single- and multi-mycotoxin adsorption experiments carried out in the same experimental conditions.

Data Calculation and Curve Fitting. The amount of adsorbed mycotoxin was calculated as the difference between the amount of mycotoxin in the supernatant of the blank tubes with no grape pomace and the amount found in the supernatant of the experimental tubes with the pomace. This amount was related then to the quantity present in the supernatant of the blank tubes and expressed in percent. The amount of bound mycotoxin per unit mass of pomace was calculated using eq 1:

$$Q_{eq} = [(C_0 - C_{eq})V]/m \qquad (1)$$

Q_{eq} = quantity of mycotoxin adsorbed per milligram of grape pomace (μg/mg); C_0 = concentration of mycotoxin in the supernatants of the blank tubes with no grape pomace (μg/mL); C_{eq} = residual mycotoxin concentration in the supernatant of the experimental tubes with grape pomace at equilibrium (μg/mL); V = volume of solution (mL); and m = mass of grape pomace (mg).

Adsorption isotherms were obtained by plotting the amount of mycotoxin adsorbed per unit of mass of adsorbent (Q_{eq}) against the concentration of the toxin in the external phase (C_{eq}), under equilibrium conditions ($Q_{eq} = f(C_{eq})$). These data were transferred to SigmaPlot (Systat.com, version 12.3) and fitted by the Langmuir, Freundlich, Sips, and Hill isotherm models (Table 1).[22−27] A dimensionless constant known as the separation factor (R_L) derived from the Langmuir equation (Table 1) was used to assess the favorability of adsorption.[25] The Gibbs free energy change ($\Delta G°$, kJ/mol), the standard enthalpy ($\Delta H°$, kJ/mol), and the standard entropy ($\Delta S°$, kJ/mol·K) were calculated according to the study performed by Ringot et al.[28] The SigmaPlot nonlinear regression method, which uses the Marquardt−Levenberg algorithm, was used as a viable tool to define the best-fitting relationship between a set of experimental data and the proposed isotherm models. Statistical analysis was performed using a factorial ANOVA with concentration (C_{eq}) or pomace dosage as categorical predictor variable and quantity of mycotoxin adsorbed (Q_{eq}) as dependent variable. The normality test (Kolmogorov−Smirnov test), the constant variance test (Spearman rank correlation), and the Durbin−Watson statistic test were used to test, respectively, for normally distributed population, constant variance assumption, and correlation between residuals. The threshold for significance level for normality and constant variance test was set at $p < 0.05$. The expected

Table 2. Effect of Particle Size on AFB_1, ZEA, OTA, and FB_1 Adsorption by Grape Pomace

particle size (μm)	mycotoxin adsorption[a] (%), mean ± SD (n = 5)			
	AFB_1	ZEA	OTA	FB_1
>500	74.8 ± 0.7 a	63.1 ± 0.3 a	50.3 ± 1.8 a	26.3 ± 2.9 a
300−500	79.1 ± 1.2 b	69.0 ± 1.6 b	54.8 ± 2.8 b	26.6 ± 1.1 a
100−300	80.0 ± 1.4 b	69.2 ± 1.9 b	56.6 ± 1.6 b	27.5 ± 3.3 a
60−100	81.9 ± 0.3 c	69.5 ± 1.6 b	60.5 ± 0.7 c	27.5 ± 2.4 a
<60	80.2 ± 0.7 b	68.9 ± 0.8 b	58.9 ± 1.2 c	27.8 ± 1.6 a

[a]Values labeled with the same letters in a column are not significantly different ($p < 0.05$).

value of the Durbin−Watson statistic for random, independent, normally distributed residuals was 2. The coefficient of determination (R^2), the standard errors of the estimate ($s_{y|x}$), the residual sum of squares (SS_{res}), and the predicted residual error sum of squares (PRESS) were calculated to assess the fitness/suitability of the regression models. The isotherm models that provided the lowest SS_{res}, $s_{y|x}$, and PRESS and the highest R^2 were considered to give the closest fit.

RESULTS AND DISCUSSION

Effect of Particle Size. The effect of particle size on the mycotoxin adsorption process was studied using fractions with particle size ranging from >500 to <60 μm (Table 2). All fractions adsorbed significant amounts of AFB_1, ZEA, OTA, and FB_1, whereas DON adsorption was negligible. For most toxins, the coarse fraction yielded significantly lower adsorption values (75% AFB_1, 63% ZEA, 50% OTA, and 26% FB_1). These values slightly increased by decreasing particle size (<500 μm) and were constant in the range from 500 to <60 μm. Maximum adsorptions recorded in this range were 82% AFB_1, 70% ZEA, 61% OTA, and 28% FB_1. Therefore, the grape pomace fraction <500 μm was used for subsequent adsorption experiments.

Effect of Contact Time. The effect of contact time for the adsorption of mycotoxins by grape pomace was studied for a period of 2 h (Figure 1). From the kinetic experiments, the plot of mycotoxin adsorption versus time showed that the rate of AFB_1, ZEA, OTA, and FB_1 adsorption by grape pomace is fast at the initial stages of the contact period and then becomes slower near equilibrium. Half of the total adsorption occurred in <3 min, and maximum adsorption was reached in 15 min. No further changes in the adsorption were noted beyond the 15 min period up to 2 h (Figure 1). This finding is of significant importance in the toxin reduction by adsorption. A rapid uptake of toxins and establishment of equilibrium in a short period imply the efficacy of pomace for its use in reduction of toxin bioaccessibility in the gastrointestinal (GI) tract.

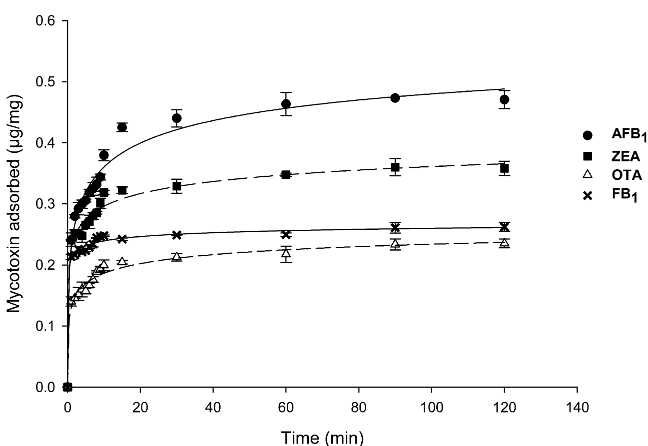

Figure 1. Effect of contact time on adsorption rate of AFB_1, ZEA, OTA, and FB_1 by grape pomace. Adsorption experiments were performed at constant pH (7) and temperature (37 °C), using 0.1% w/v pomace dosage and 1 μg/mL mycotoxin concentration.

Effect of pH. The removal of mycotoxins from aqueous medium through an adsorption process is, in most cases, highly dependent on the pH. Medium pH can affect the surface charge of adsorbents as well as the degree of ionization of toxins, and subsequently it can lead to a shift in reaction kinetics and equilibrium characteristics of the adsorption process. This is more important when the adsorption process involves electrostatic interactions. In general, the charge of the toxin depends on its pK_a. The toxin is mainly in protonated form at pH < pK_a and in deprotonated form at pH > pK_a. The effect of pH on AFB_1, ZEA, OTA, and FB_1 adsorption by grape pomace was different according to the degree of ionization of molecules (Table 3). AFB_1 is a non-ionizable molecule (Figure 2), and the pK_a in water is not applicable. Therefore, a change of pH should not affect AFB_1 adsorption. Accordingly, grape pomace sequestered AFB_1 to the same extent in all pH ranges assayed in the study (Table 3). ZEA is a diphenolic compound with an estimated pK_a of 7.6,[29] so some amount of phenolate anion can be present in water near pH 7. ZEA adsorption was unaffected by pH in the range of 3−8 and slightly decreased at pH 9 ($p < 0.05$). OTA consists of a dihydroisocoumarin moiety linked through its 7-carboxyl group by an amide linkage to L-phenylalanine (Figure 2). The pK_a values are in the ranges of 4.2−4.4 and 7.0−7.3, respectively, for the carboxyl group of the phenylalanine moiety and the phenolic hydroxyl group of the isocoumarin part.[30] This indicates that, in aqueous solutions near pH 7, both the monoanion (OTA^-) and the dianion (OTA^{2-}) are present, whereas the protonated and uncharged toxin is present in acid solutions (pH <4). OTA adsorptions recorded at pH ≤4 were constant and significantly higher than those recorded at pH ≥5 ($p < 0.05$) (Table 3). This result

Table 3. Effect of pH on AFB_1, ZEA, OTA, and FB_1 Adsorption by Grape Pomace

pH	mycotoxin adsorption[a] (%), mean ± SD (n = 5)			
	AFB_1	ZEA	OTA	FB_1
3	83.2 ± 1.1 a	67.9 ± 1.3 a	67.6 ± 0.9 a	26.7 ± 2.1 a
4	83.0 ± 0.2 a	68.0 ± 0.8 a	65.4 ± 1.3 a	25.8 ± 2.9 a
5	83.4 ± 0.8 a	68.2 ± 1.4 a	62.2 ± 1.6 b	26.4 ± 2.4 a
6	84.6 ± 0.9 a	69.0 ± 1.3 a	62.3 ± 1.0 b	33.8 ± 5.2 b
7	82.0 ± 0.8 a	67.0 ± 0.9 a	61.3 ± 0.7 b	34.7 ± 2.8 b
8	82.6 ± 0.4 a	68.3 ± 1.0 a	62.4 ± 1.1 b	35.4 ± 0.6 b
9	83.0 ± 1.1 a	64.1 ± 1.8 b	60.7 ± 1.0 b	33.7 ± 1.1 b

[a]Values labeled with the same letters in a column are not significantly different ($p < 0.05$).

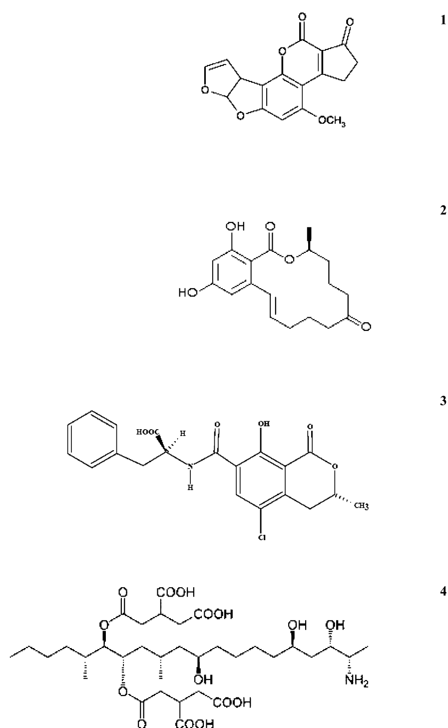

Figure 2. Structures of mycotoxins: **1**, AFB_1; **2**, ZEA; **3**, OTA; **4**, FB_1.

suggests that OTA adsorption is higher when it is in the uncharged form and slightly decreases when it is in the anionic form. Contrary to AFB_1, ZEA, and OTA, FB_1 is a more polar and water-soluble toxin, containing carboxylic, hydroxyl, and amino functional groups (Figure 2). No report concerning the pK_a of FB_1 is available in the literature.[31] The pK_a values for tricarballylic acid are 3.5, 4.6, and 5.8, and the aliphatic amine group should have a pK_a >9. Therefore, at pH between 6 and 9, FB_1 could be in the anionic form because of deprotonation of carboxylic groups, whereas it could be in the cationic form at pH <6 due to protonation of the amine group. FB_1 adsorption by grape pomace recorded at pH ≥6 was significantly higher than that recorded at pH ≤5 ($p < 0.05$) (Table 3). It seems that the anionic form of FB_1 contributes to adsorption by grape pomace, whereas the cationic form decreases such adsorption. During digestion, the pH of the food bolus is not constant but, depending on GI compartments, changes to a large extent. In monogastric animals, it ranges from 1.5−2.5 in the stomach to 5.5−6.0 in the intestinal lumen. Taking into account the findings of this study, it can be supposed that AFB_1 and ZEA adsorptions by grape pomace are stable within the pH range that can be found in the GI tract of monogastric animals, whereas pH change could affect FB_1 and OTA adsorptions to some extent. Hence, a desorption study was performed to assess if change of pH can cause release of the sequestered toxins. Mycotoxins were first adsorbed onto grape pomace at pH 3, and then the pellet containing the adsorbed mycotoxins was washed with a medium at pH 7. Adsorption and desorption values were, respectively, 94 and 4% for AFB_1; 87 and 10% for ZEA; 84 and 14% for OTA; and 16 and 45% for FB_1. These results suggest that grape pomace has high efficacy in adsorbing AFB_1, ZEA, and OTA at acid pH, and it is able to retain the adsorbed toxins when the pH rises to neutral values. FB_1 is sequestered poorly at pH 3, and almost half of the adsorbed toxin can be released when the pH of the medium changes from 3 to 7.

Effect of Adsorbent Dosage. AFB_1, ZEA, OTA, and FB_1 adsorption was significantly affected by the adsorbent dosage, and the percentage of mycotoxins removed from neutral or acid buffer solutions increased with increasing dosages of grape pomace (Figure 3). The Langmuir model provided good correlations for isotherm adsorption plots (R^2 > 0.990). AFB_1, ZEA, and OTA isotherms obtained at different pH values were comparable (Figure 3). Experimental values for AFB_1, ZEA, and OTA adsorption were in the ranges of 28−98, 21−94, and 6−94%, respectively. FB_1 adsorptions were significantly higher at pH 7 (17−44%) than at pH 3 (11−31%) (Figure 3). The Langmuir model allowed the calculation of the theoretically estimated maximum adsorption (Ads_{max}) and the C_{50},[32] which is the theoretically estimated adsorbent dosage to achieve a 50% reduction of the absorbable toxin (Table 4). The C_{50} could not be calculated for FB_1 at pH 3. For all toxins, Ads_{max} and C_{50} values were in accordance with the experimental values for mycotoxin adsorption shown in Figure 3. With these findings taken into account, it is not useful to increase the dosage of grape pomace beyond 4 mg/mL to sequester AFB_1, ZEA, and OTA from a 1 μg/mL or higher mycotoxin solutions. Therefore, for all of the subsequent isotherm experiments, grape pomace dosage was fixed at 1 mg/mL for AFB_1 and ZEA and at 2 mg/mL for OTA and FB_1.

Adsorption Isotherms (Single-Mycotoxin System). The adsorption isotherm is an invaluable curve describing the phenomenon governing the retention (or release) or mobility of a substance from the aquatic environments to a solid phase at a constant temperature and pH.[33] Its physicochemical parameters together with the underlying thermodynamic assumptions provide insight into the adsorption mechanism and surface properties as well as the degree of affinity of the adsorbents.[33] Several equations have been published to study the isotherm adsorption of mycotoxins to organic and inorganic adsorbent materials.[22,34] In this study, four adsorption models often reported in the literature were used to provide the best description of mycotoxin adsorption by grape pomace (Table 1). The nonlinear regression analysis method,[35] instead of the linear regression with transformed variables, was applied to assess the goodness of the fits and to calculate the parameters involved in the adsorption mechanism (Table 5). All adsorption models provided a good fit line to experimental adsorption data (small variance and $R^2 \geq 0.979$) (Table 5). The amount of mycotoxin adsorbed per unit mass of grape pomace increased gradually by increasing mycotoxin molecules in the working solution; thus, isotherms showed an exponential relationship and a typical L-1 (Langmuir) shape (Figure 4). This suggests that, in the experimental conditions of this study, the plateau was not reached and part of the capacity of the absorbent was occupied by the mycotoxins.

AFB_1 Adsorption Isotherms. AFB_1 adsorption by grape pomace yielded similar equilibrium isotherms at pH 3 and 7 (Figure 4). Experimental values for AFB_1 adsorption in percent varied in the range of 57−30%. The experimental values for maximum adsorption capacity recorded at pH 3 and 7 were, respectively, 1.53 ± 0.04 μg/mg (4.89 ± 0.12 mmol/kg) and 1.54 ± 0.05 μg/mg (4.94 ± 0.16 mmol/kg) (Figure 4). Among the tested isotherm equations, the better representation of the experimental results of the AFB_1 adsorption isotherms was obtained using the Sips and Hill models (R^2 > 0.998 and low error values) (Table 5). These equations are often found to be

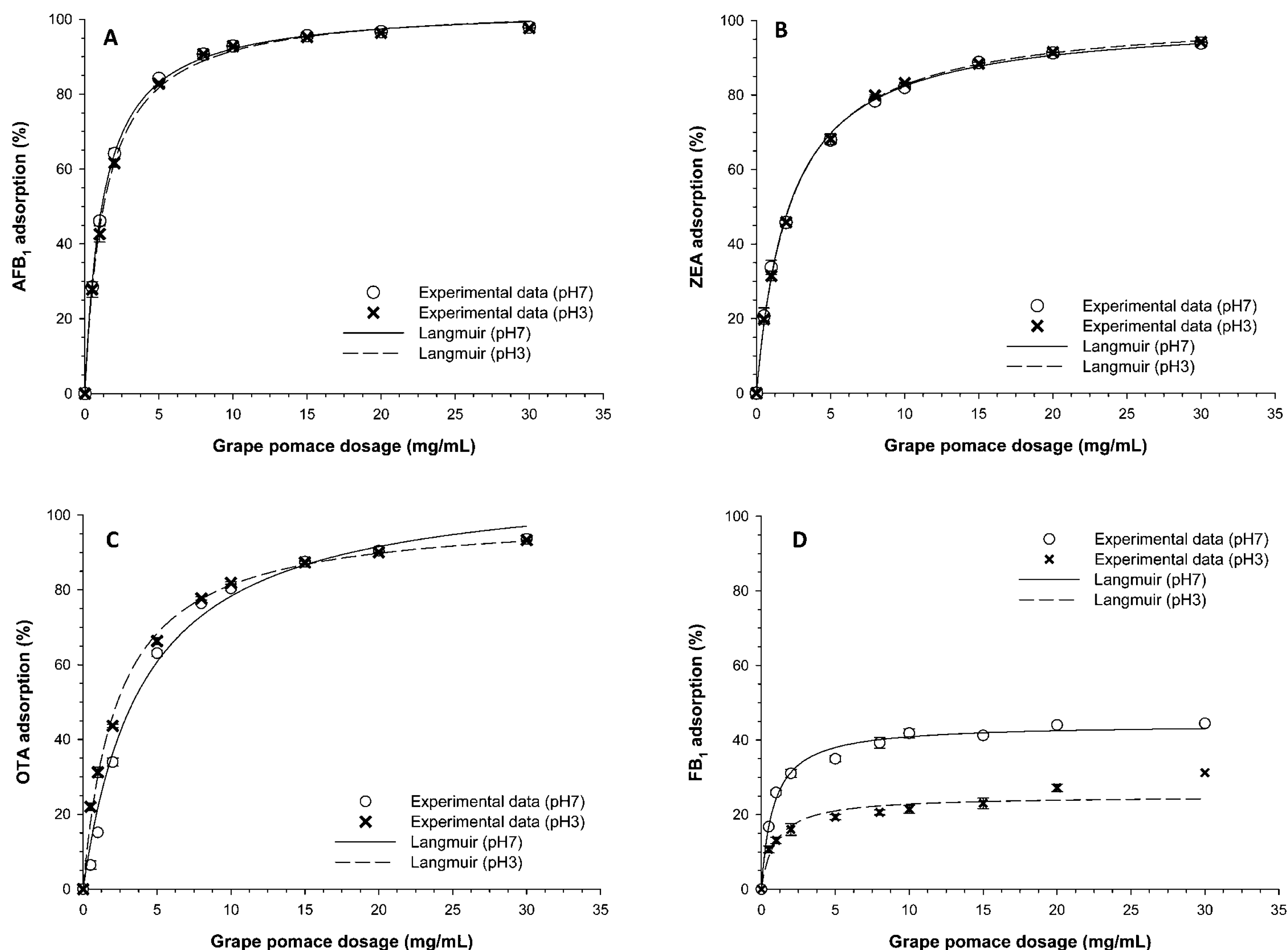

Figure 3. Effect of adsorbent dosage on (A) AFB_1, (B) ZEA, (C) OTA, and (D) FB_1 adsorption by grape pomace. Equilibrium adsorption isotherms were obtained at constant temperature (37 °C) and pH (3 and 7) by testing a fixed amount of toxins (1 μg/mL) with increasing adsorbent dosages (0.05−3% w/v).

Table 4. Theoretically Estimated Values for Maximum Adsorption (Ads_{max}) and Inclusion Rate of Grape Pomace To Obtain a 50% Reduction of the Absorbable Toxin (C_{50})[a]

	Ads_{max} (%)		C_{50} (mg/mL)	
toxin	pH 3	pH 7	pH 3	pH 7
AFB_1	103.9 ± 0.5	103.2 ± 0.3	1.4	1.2
ZEA	101.9 ± 0.5	100.7 ± 0.7	2.5	2.5
OTA	100.3 ± 0.9	100.2 ± 2.2	2.5	4.0
FB_1	22.8 ± 0.5	45.5 ± 0.4	nd	30.0

[a] Ads_{max} and C_{50} were calculated by fitting the adsorption isotherms in Figure 3 with the Langmuir isotherm model.

superior to either the Freundlich or Langmuir equation in correlations, as they were developed for adsorption on heterogeneous solids. The Sips isotherm (Table 1) is a combined form of the Langmuir and Freundlich expressions deduced for predicting the heterogeneous adsorption systems and circumventing the limitation of the rising adsorbate concentration associated with the Freundlich isotherm model.[26] At low adsorbate concentrations, it reduces to the Freundlich isotherm, whereas at high concentrations, it predicts a monolayer adsorption capacity characteristic of the Langmuir isotherm. The Hill model (Table 1) assumes that adsorption is a cooperative phenomenon, with the ligand binding ability at one site on the macromolecule, and may influence different binding sites on the same macromolecule.[27] The Sips and Hill models are mathematically equivalent[36] and provide same error values and coefficients of determination (Table 5). The $1/n$ exponent in the Sips model is analogous to the nH exponent in the Hill model. However, they have different meanings, $1/n$ being the heterogeneity factor of the adsorption and nH the cooperativity coefficient of the binding interaction. According to the Sips and Hill models, the theoretical maximum AFB_1 adsorption capacity was 4.73 ± 0.77 μg/mg (15.15 ± 2.47 mmol/kg) and 4.69 ± 0.55 μg/mg (15.02 ± 1.76 mmol/kg) at pH 3 and 7, respectively (Table 5). The constant a_s in the Sips equation is related to the energy and affinity of adsorption. The values for the a_s parameter in the Sips model were 0.17 ± 0.03 and 0.16 ± 0.02 mL/μg at pH 3 and 7, respectively (Table 5). The Sips exponent ($1/n$) was <1 in all cases, suggesting heterogeneous binding sites. Similarly, the Hill cooperativity coefficient (nH) was <1, indicating a negative cooperativity of the binding interaction. In conclusion, these findings suggest that the AFB_1 adsorption mechanism is heterogeneous and occurs at a finite (fixed) number of definite localized sites, which are nonequivalent, with lateral interaction (negative interaction) and steric hindrance between the AFB_1 adsorbed molecules.

Table 5. Isotherm Model Parameters for the Adsorption of Mycotoxins by Grape Pomace at Different pH Values

		AFB_1		ZEA		OTA		FB_1	
model	parameter	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3
Freundlich	K_f (±SE)	**0.65 ± 0.01**	**0.66 ± 0.01**	**0.42 ± 0.01**	**0.43 ± 0.01**	**0.18 ± 0.01**	**0.43 ± 0.01**	**0.16 ± 0.01**	**0.06 ± 0.01**
	$1/n$ (±SE)	**0.70 ± 0.01**	**0.70 ± 0.01**	**0.77 ± 0.02**	**0.76 ± 0.01**	**0.87 ± 0.02**	**0.77 ± 0.01**	**0.84 ± 0.03**	**0.45 ± 0.09**
	R^2	0.9974	0.9971	0.9888	0.9973	0.9939	0.9963	0.9910	0.9791
	SS_{res}	0.024	0.023	0.051	0.010	0.003	0.013	0.003	0.0004
	$s_{y/x}$	0.023	0.028	0.036	0.019	0.013	0.022	0.014	0.005
	PRESS	0.027	0.027	0.071	0.012	0.004	0.018	0.008	0.0006
Langmuir	Q_{max} (±SE)	**2.86 ± 0.07**	**3.02 ± 0.11**	**2.73 ± 0.20**	**2.65 ± 0.14**	**2.54 ± 0.47**	**2.79 ± 0.21**	**1.60 ± 0.19**	**0.13 ± 0.01**
	K_L (±SE)	**0.31 ± 0.01**	**0.29 ± 0.02**	**0.19 ± 0.02**	**0.20 ± 0.01**	**0.08 ± 0.02**	**0.19 ± 0.02**	**0.11 ± 0.02**	**0.99 ± 0.15**
	R^2	0.9976	0.9976	0.9916	0.9972	0.9935	0.9953	0.9941	0.9791
	SS_{res}	0.022	0.018	0.038	0.010	0.003	0.017	0.002	0.0008
	$s_{y/x}$	0.022	0.026	0.032	0.019	0.013	0.025	0.011	0.007
	PRESS	0.026	0.023	0.054	0.013	0.004	0.025	0.005	0.0010
Sips/Hill[a]	q_m (±SE)	**4.69 ± 0.55**	**4.73 ± 0.77**	**2.93 ± 0.70**	**4.78 ± 1.45**	**nc**[b]	**nc**	**1.17 ± 0.28**	**0.28 ± 0.11**
	a_s (±SE)	**0.16 ± 0.02**	**0.17 ± 0.03**	**0.17 ± 0.05**	**0.10 ± 0.03**			**0.16 ± 0.04**	**0.29 ± 0.17**
	$1/n = nH$ (±SE)	**0.85 ± 0.02**	**0.86 ± 0.03**	**0.98 ± 0.07**	**0.97 ± 0.04**			**1.09 ± 0.08**	**0.59 ± 0.09**
	R^2	0.9988	0.9985	0.9916	0.9980			0.9948	0.9829
	SS_{res}	0.011	0.012	0.038	0.008			0.002	0.0004
	$s_{y/x}$	0.016	0.020	0.032	0.017			0.011	0.005
	PRESS	0.013	0.014	0.061	0.010			0.006	0.0005

[a]The Sips and Hill models are mathematically equivalent and provide the same error values and adsorption parameters. [b]Does not converge.

ZEA Adsorption Isotherms. Isothermal adsorption of ZEA by grape pomace was not affected by pH (Figure 4). The experimental values for ZEA adsorption recorded at pH 3 and 7 were comparable and ranged from 38 to 20%. The experimental values for maximum adsorption capacity were 1.25 ± 0.03 μg/mg (3.91 ± 0.09 mmol/kg) and 1.12 ± 0.09 μg/mg (3.51 ± 0.27 mmol/kg) at pH 3 and 7, respectively. The Langmuir equation was found to be the best fitting isotherm model for ZEA adsorption (Table 5). At both pH values, the correlations obtained by this model were in excellent agreement with the experimental adsorption data and yielded comparable adsorption parameters (Table 5). The predicted maximum ZEA adsorption capacity was 2.65 ± 0.14 μg/mg (8.32 ± 0.44 mmol/kg) and 2.73 ± 0.20 μg/mg (8.58 ± 0.63 mmol/kg) at pH 3 and 7, respectively. The Langmuir K_L parameter, which is related to the free energy of adsorption, was 0.20 ± 0.01 and 0.19 ± 0.02 mL/μg at pH 3 and 7, respectively. These values suggest favorable ZEA adsorption by grape pomace. The favorability of ZEA adsorption was confirmed by the essential features of the Langmuir isotherm model, called the separation factor, R_L (Table 1).[25] At both pH values, this dimensionless factor ranged from 1.00 to 0.50 for the initial ZEA concentration values of 0.05−5.00 μg/mL. At all ZEA concentrations, R_L values were in the range of 0−1, which is indicative of favorable adsorption. In addition, the R^2 and error values demonstrate that the Sips and Hill models can also adequately fit ZEA adsorption data (Table 5). The Hill cooperativity coefficient (nH) was approximately 1, indicating no cooperativity of the binding interaction. Similarly, the heterogeneity factor ($1/n$) of the Sips isotherm equation was almost equal to 1. This implies that the Sips isotherm equation reduces to the Langmuir equation, and ZEA adsorption data are more of the Langmuir form rather than of the Freundlich form. In conclusion, these results are all indicative of a homogeneous adsorption process for ZEA by grape pomace.

OTA Adsorption Isotherms. Unlike AFB_1 and ZEA adsorptions, OTA adsorption was slightly affected by pH. The experimental values for OTA adsorption were 54−33% at pH 3 and 33−26% at pH 7. The related experimental values for maximum adsorption capacity were, respectively, 0.82 ± 0.08 μg/mg (2.04 ± 0.20 mmol/kg) and 0.64 ± 0.09 μg/mg (1.58 ± 0.22 mmol/kg) (Figure 4). At both pH values tested in the study, the Freundlich and Langmuir models provided a good fit line to experimental data (small variance and R^2 values >0.993), whereas the Hill and Sips models did not converge (Table 5). The latter models, exceeding the maximum number of repeated fit attempts before failure (200 iterations), provided inaccurate parameters. Maximum adsorption capacities were calculated by the Langmuir model and comparable at pH 3 and 7, being 2.79 ± 0.21 μg/mg (6.91 ± 0.52 mmol/kg) and 2.54 ± 0.47 μg/mg (6.29 ± 1.16 mmol/kg), respectively. Between the biparametric models, the Freundlich model was superior to the Langmuir model in fitting adsorption data. This may be explained by the binding process occurring under conditions outside the assumptions of the Langmuir model, and it suggests a heterogeneous binding mechanism. The Freundlich isotherm is the earliest known relationship describing the nonideal and reversible adsorption, not restricted to the formation of monolayer.[23] This empirical model can be applied to multilayer adsorption, with nonuniform distribution of adsorption heat and affinities over the heterogeneous surface. The Freundlich model provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model. The heterogeneity index in the Freundlich model, $1/n$, gives information on the population of the binding sites and the adsorption intensity and is associated with the favorability of the binding process. A value of $1/n = 1$ suggests that the population of binding sites is homogeneous, whereas a value below unity implies favorability of the adsorption. At acid and neutral pH, the values computed for the Freundlich parameter $1/n$ were 0.77 ± 0.01 and 0.87 ± 0.02, respectively. These values were <1 and indicate

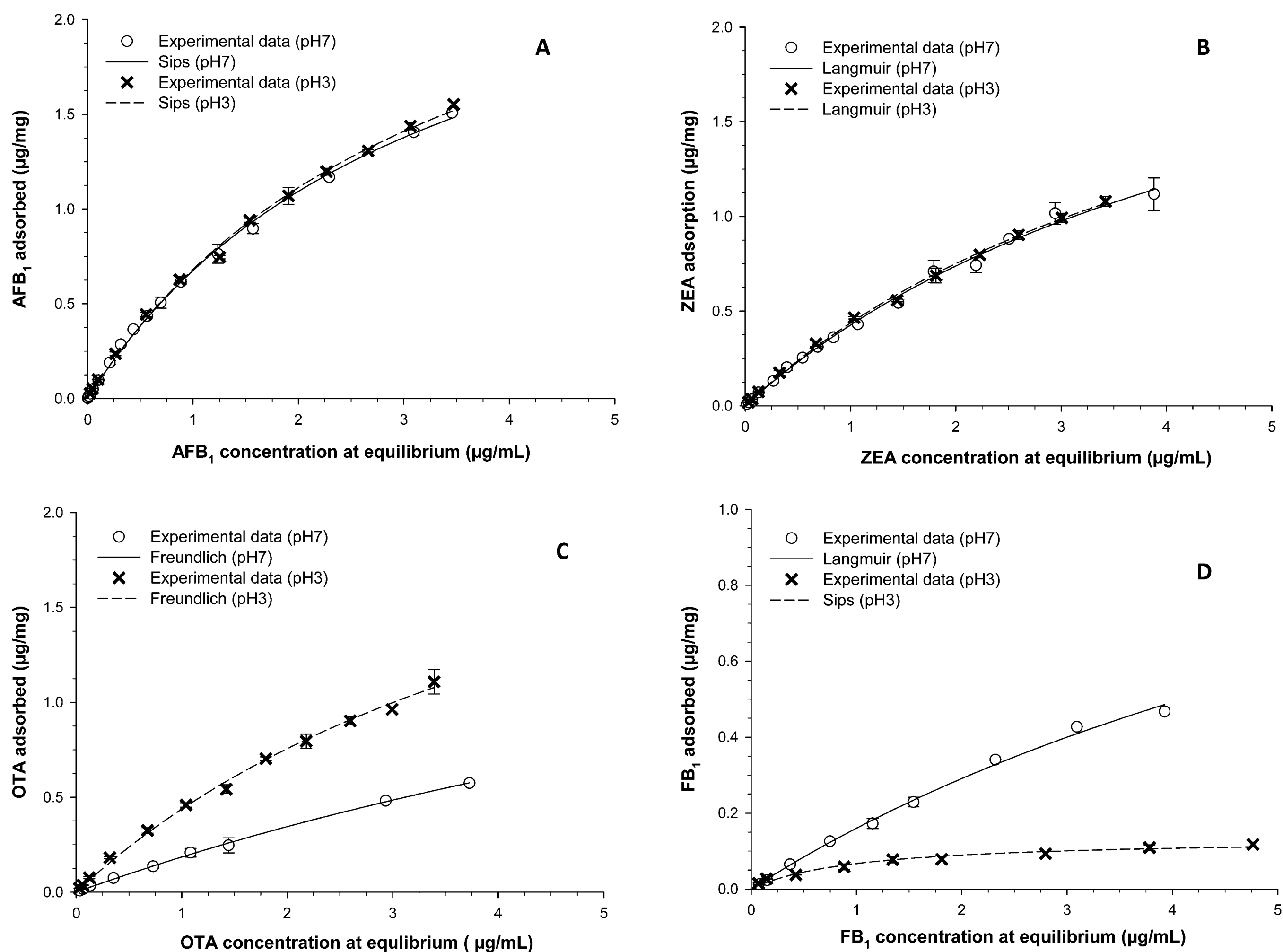

Figure 4. Effect of mycotoxin concentration on (A) AFB_1, (B) ZEA, (C) OTA, and (D) FB_1 adsorption by grape pomace. Equilibrium adsorption isotherms were obtained at constant temperature (37 °C) and pH (3 and 7) by testing a fixed amount of grape pomace with increasing toxin concentrations (0.05−5 μg/mL).

favorability for the OTA adsorption process. However, the $1/n$ parameter being estimated at pH 3 lower than at pH 7, it can be assumed that more binding sites or higher adsorption intensity can be found at acid pH than at neutral pH. Accordingly, the K_f Freundlich constant, which is attributed to affinity of the adsorbent, was 2-fold higher at pH 3 than at pH 7 (Table 5). In conclusion, increase of medium pH from 3 to 7 reduces the affinity of adsorption. The decreased affinity for OTA adsorption by grape pomace at pH 7 may be induced by the presence of an anionic form of the toxin, which probably leads to the repulsion between OTA molecules and negative charges that could be found on the adsorption surface. In agreement with these findings, OTA adsorption by negative charged surfaces (such as zeolites) has been found significantly higher at pH 3 than at pH 7 and 9.[30] The existence of a heterogeneous adsorption in the case of OTA suggests that different types of interaction can occur, such as hydrophobic and ionic forces.

FB_1 Adsorption Isotherms. Similarly to OTA adsorption, different mechanisms may be involved in FB_1 adsorption depending on pH and then on the degree of ionization of molecules. Highest adsorption was achieved at pH 7 (Figure 4). The experimental values for FB_1 adsorption were 30−5% at pH 3 and 25−22% at pH 7. The experimental values for maximum adsorption capacity recorded at these pH values were, respectively, 0.12 ± 0.01 μg/mg (0.16 ± 0.01 mmol/kg) and 0.54 ± 0.06 μg/mg (0.74 ± 0.08 mmol/kg) (Figure 4). With error and R^2 values reported in Table 5 taken into account, it was determined that the Langmuir model is the best isotherm model to fit the adsorption data at pH 7, whereas the Sips and Hill models produce better correlations at pH 3. The applicability of these isotherm models to the FB_1−grape pomace system implies that FB_1 adsorption is complex, and it confirms that more than one mechanism can be involved. In particular, monolayer adsorption (homogeneous adsorption) may exist at pH 7, whereas heterogeneous and lower adsorption can occur at pH 3. The predicted maximum adsorption capacities calculated by the Langmuir model at pH 7 and by the Sips/Hill models at pH 3 were 1.60 ± 0.19 μg/mg (2.22 ± 0.26 mmol/kg) and 0.28 ± 0.11 μg/mg (0.39 ± 0.15 mmol/kg), respectively (Table 5). In addition, at pH 7 the exponents in the Sips and Hill models were equal to 1. This suggests that the Sips model reduces to the Langmuir equation and the material holds relatively homogeneous binding sites. Moreover, no cooperativity in the binding interactions occurs when the adsorption system is at neutral pH, that is, when FB_1 has negative charges. At pH 3, FB_1 should be in the cationic form. Likely, the positive charge in the adsorbable molecules reduces the capacity and affinity of the adsorption and results in

negative cooperativity of the binding process. Thus, it can be concluded that in aqueous solutions near the physiological pH (pH 7), FB_1 adsorption onto grape pomace is favored and occurs mainly by polar noncovalent interactions, such as electrostatic interactions or hydrogen bonds involving the carboxylic functional groups.

Multi-mycotoxin Adsorption Isotherms. In general, adsorbate−surface interactions complicate the adsorption in multicomponent systems.[37] Multi-mycotoxin systems have received less attention than single-mycotoxin systems, and multi-mycotoxin adsorption isotherms have never been described in the literature. Therefore, the adsorption of AFB_1, ZEA, OTA, and FB_1 by grape pomace was also studied by equilibrium adsorption isotherms using a five-component system. This system contained simultaneously AFB_1, ZEA, OTA, FB_1, and DON in equal ratios. Of course, these ratios are not reflected in the real world of natural contamination in which aflatoxin and fumonisin occur orders of magnitude apart. Multi-mycotoxin adsorption isotherms were obtained as in the single-mycotoxin adsorption system, testing the toxins at pH 7 and 37 °C. The adsorption parameters calculated for each toxin in the presence (five-mycotoxin system) and absence (single-mycotoxin system) of other mycotoxins are presented in Table 6. AFB_1 adsorption isotherm plots were fitted by the Sips/Hill models, because they produced better goodness of fit. ZEA, OTA, and FB_1 adsorption isotherms were well fitted by the Langmuir equation. For all mycotoxins considered in the study, the experimental values for mycotoxin adsorption obtained in the single- and multi-mycotoxin adsorption systems were comparable. Similarly, the predicted isotherm adsorption parameters calculated by these systems did not differ (Table 6). The effect of mycotoxin interactions on adsorption may be represented by the ratio of the adsorption capacity for one mycotoxin in the presence of the other mycotoxins, Q^{mix}, to the adsorption capacity for the same mycotoxin when it is present alone in the solution, Q^0. When $(Q^{mix}/Q^0) > 1$, adsorption is promoted by the presence of other mycotoxins; when $(Q^{mix}/Q^0) = 1$, there is no net interaction; and when $(Q^{mix}/Q^0) < 1$, adsorption is suppressed by other mycotoxins. As shown in Table 6, the values of Q^{mix}/Q^0 were all almost equal to 1. Therefore, it can be supposed that mycotoxin adsorption by grape pomace is not suppressed or enhanced by the presence of other mycotoxins in the experimental conditions. In conclusion, for the first time, this study proves that grape pomace can adsorb simultaneously AFB_1, ZEA, OTA, and FB_1 and that these mycotoxins did not compete for adsorption. Grape pomace shows the potential to be used as a broad-spectrum adsorbent material.

Table 6. Mycotoxin Adsorption Parameters Calculated for Mycotoxin Adsorption Isotherms Obtained by Single- and Multi-mycotoxin Adsorption Systems (37 °C, pH 7)

mycotoxin	parameter	single-mycotoxin system	multi-mycotoxin system	Q^{mix}/Q^{0a}
AFB_1	q_m^b	4.69 ± 0.55	4.71 ± 0.28	0.99
	a_s^c	0.16 ± 0.02	0.12 ± 0.07	
	$1/n^d$	0.85 ± 0.02	0.78 ± 0.06	
ZEA	Q_{max}^e	2.73 ± 0.20	2.72 ± 0.14	1.00
	K_L^f	0.19 ± 0.02	0.21 ± 0.02	
OTA	Q_{max}	2.54 ± 0.47	2.59 ± 0.17	0.98
	K_L	0.08 ± 0.02	0.14 ± 0.01	
FB_1	Q_{max}	1.60 ± 0.19	1.51 ± 0.27	1.06
	K_L	0.11 ± 0.02	0.12 ± 0.02	

[a] Q^{mix} = adsorption capacity for one mycotoxin in the presence of other mycotoxins; Q^0 = adsorption capacity for one mycotoxin when it is present alone in the solution. [b] Sips parameter related to maximum adsorption capacity (μg/mg). [c] Sips parameter related to energy (affinity) of adsorption. [d] Sips index for the heterogeneity of binding sites. [e] Langmuir parameter related to maximum adsorption capacity (μg/mg). [f] Langmuir parameter related to the energy of adsorption and affinity of the adsorbent.

Thermodynamic Studies. In adsorption studies, both energy and entropy factors must be considered to determine what processes will occur spontaneously.[28] The Gibbs free energy change, $\Delta G°$ (kJ/mol), is the fundamental criterion of spontaneity. Reactions occur spontaneously at a given temperature if ΔG is a negative quantity. Thermodynamic parameters for AFB_1, ZEA, OTA, and FB_1 adsorption by grape pomace were calculated at pH 7 and at different temperatures (5, 37, 50, and 70 °C). For all toxins, experimental adsorption data and predicted maximum adsorption capacities calculated according to isotherm models decreased with an increase in temperature, indicating an exothermic process. Accordingly, the values for the equilibrium constant K^0 for the adsorption reaction decreased as the temperature rose (Table 7). This may be due to a tendency for the mycotoxin molecules to escape from the solid phase with an increase in temperature of the solution. The K^0 was used to calculate the standard Gibbs free energy of adsorption, $\Delta G°$.[28] The negative values of $\Delta G°$ in Table 7 are indicative of a spontaneous adsorption process with a high affinity of the mycotoxins to the surface of the grape pomace. Thermodynamic parameters ($\Delta H°$ and $\Delta S°$) were calculated according to the van't Hoff equation and were determined from the slope and intercept of the plots of ln K^0 versus $1/T$.[28] The plots obtained for all mycotoxins at four different temperatures gave good correlation ($R^2 \geq 0.985$) (data not shown). The values of $\Delta H°$ and $\Delta S°$ are presented in Table 7. $\Delta H°$ values for mycotoxin adsorption are negative, confirming the exothermic nature of the phenomenon. The magnitude of the $\Delta H°$ value gives an indication of the type of adsorption, which can be either physical or chemical.[38] Physisorption involves weak associations, which include hydrogen bonds, van der Waals, dipole−dipole, and induced dipole. Chemisorption implies a chemical reaction or sharing of electrons between the adsorbent and the adsorbate, such as covalent bonds. Physisorption is described as having an enthalpy of <20 kJ/mol, whereas chemisorption is generally >20 kJ/mol.[34] For all mycotoxins the value of enthalpy was <20 kJ/mol, indicating a physical adsorption phenomenon (Table 7). Physical adsorption requiring small energy allows the equilibrium to be attained rapidly and the process to be easily reversible. Interestingly, $\Delta S°$ values were positive for AFB_1 and ZEA adsorptions, but negative for OTA and FB_1 adsorptions (Table 7). The negative values of $\Delta S°$ for OTA and FB_1 adsorption may be understood in terms of restriction of the movement of the molecules on the surface (two dimensions), as compared to the bulk solution (three dimensions). The binding of OTA and FB_1 on grape pomace is thus only enthalpically driven. Generally, this situation corresponds to polar noncovalent interactions (benefit in enthalpy associated to a cost in entropy), such as electrostatic interactions or hydrogen bonds. At the pH value of this study (7.0 ± 0.5), OTA and

Table 7. Thermodynamic Parameters for AFB_1, ZEA, OTA, and FB_1 Adsorption by Grape Pomace

toxin	temperature (K)	K^0	ln K^0	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	$\Delta S°$ (kJ/mol·K)
AFB_1	278	1270	7.15	−17.3	−7.2	34.0
	310	1124	7.02			
	323	775	6.65			
	343	731	6.59			
ZEA	278	673	6.51	−15.5	−8.94	22.2
	310	482	6.18			
	323	412	6.02			
	343	319	5.77			
OTA	278	394	5.98	−13.9	−14.7	−2.70
	310	238	5.47			
	323	170	5.14			
	343	118	5.77			
FB_1	278	431	6.07	−13.9	−17.4	−11.6
	310	160	5.07			
	323	294	5.68			
	343	79	4.37			

FB_1 are in the anionic form; thus, they may be involved in polar noncovalent interactions. On the contrary, positive $\Delta S°$ values for AFB_1 and ZEA adsorption process reflect an increase in the randomness at the solid/liquid interface and suggest hydrophobic interaction between the adsorbent and the adsorbate.[39−41] The positive entropy change may also be caused by the decrease in the number of water molecules surrounding both the adsorbent and the adsorbate molecules involved in the hydrophobic interaction. Generally, hydrophobic interactions are associated with a cost in enthalpy. In conclusion, the thermodynamic parameters calculated for adsorption of mycotoxins onto grape pomace give information about the type of the interaction involved in the adsorption process. At pH 7, hydrophobic interactions could be associated with AFB_1 and ZEA adsorption (small $\Delta H°$ and positive $\Delta S°$ values). Electrostatic interactions could explain the higher $\Delta H°$ and the negative $\Delta S°$ values calculated for OTA and FB_1 adsorption.

The present work shows that grape pomace can be used as a low-cost and healthy biosorbent for removing mycotoxins from liquid media. This is the first time that grape pomace has been tested as a multi-mycotoxin adsorbent. These findings show that a grape pomace prepared from a red grape variety (cv. Primitivo) is able to sequester, in different adsorption systems, AFB_1, ZEA, OTA, and FB_1. Contact time curves showed that the adsorption of mycotoxins by grape pomace is rapid, which is of significant importance in the toxin reduction by adsorption. Adsorption/desorption studies performed at physiological pH showed that mycotoxin adsorption by grape pomace is stable within the pH ranges that can be found in the GI tract of monogastric animals. For the first time, multi-mycotoxin adsorption isotherms were used to study the mechanism of mycotoxin adsorption by grape pomace and to calculate the adsorption and thermodynamic parameters. This work did not study the mycotoxin adsorption efficacy of pomaces obtained from different grape cultivars or different grape byproducts (such as stalks and seeds). Further studies are required to assess if the mycotoxin adsorption efficacy of grape pomace can be affected by the grape cultivar used to make wine, as well to understand which components are the active compounds for the adsorption process by grape pomace.

In conclusion, grape pomace is a sustainable source of bioactive compounds that may have a wide range of technological applications as biosorbent to decontaminate mycotoxin-contaminated foodstuffs. The use of grape pomace as a supplement in the formulation of food/feed products does not guarantee efficacy in sequestering mycotoxins, unless proper digestion or feeding experiments are performed. In addition to must, wine, and grape juice, grape pomace could be used to decontaminate mycotoxin-contaminated liquid foods, such as fruit juice. It is not expected to affect relevant quality parameters of the final products, apart from the color intensity and the enrichment in health-promoting phenolic content.

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■ ABBREVIATIONS USED

AFB_1, aflatoxin B_1; ZEA, zearalenone; OTA, ochratoxin A; FB_1, fumonisin B_1; DON, deoxynivalenol; UPLC, ultraperformance liquid chromatography; GI, gastrointestinal

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