JOURNAL OF SCIENTIFIC PERSPECTIVES

E-ISSN: 2587-3008

July 2018, Vol:3, Issue:3

http://ratingacademy.com.tr/journals/index.php/jsp/

REMOVAL OF OCHRATOXIN A FROM RED WINE BY USING RED KIDNEY BEAN PEEL

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ARTICLE INFO

Article History:
Received: *18 June 2018*
Accepted: *05 July 2018*

Keywords*: Ochratoxin A, red wine, adsorption, red kidney bean peel.*

DOI: *10.26900/jsp.2018.8*

ABSTRACT

Present study was performed to reduce the OTA (ochratoxin A) levels of red wine by using red kidney bean peel as adsorbent. The adsorption studies were evaluated by adsorption isotherm, kinetic models. The adsorption data conformed to the Freundlich isotherm and pseudo-second-order kinetic model with respect to the correlation coefficients. The adsorption equilibria of OTA for real wine sample and OTA synthetic solution were almost established within 240 and 300 min, respectively. The removal efficiency was decreased by increasing temperature. Thermodynamic parameters indicated that adsorption process was spontaneous, exothermic, chemical and the randomness was decreased. The removal percent of polyphenols and anthocyans were lower at lower adsorbent masses. This data was useful to develop an environmentally friendly process to remove OTA from red wine at low amount of OTA, without affecting wine quality parameters.

1. INTRODUCTION

Ochratoxins are a group of mycotoxins produced mainly by strains of some *Aspergillus* and *Penicillium* species. Their structures imply the linking between two moieties; a substituted dihydroisocoumarin and L-phenylalanine. The family of ochratoxins consists of three members, A, B, and C, which differ slightly from each other in chemical structures. Ochratoxin A (OTA) was first reported in South Africa as a secondary metabolite produced by a strain of Aspergillus ochraceus (Van der Merwe et al., 1965). This mycotoxin has been shown to be nephrotoxic, hepatotoxic, teratogenic and carcinogenic to animals and has been classified as a possible carcinogen to humans (Castegnaro et al., 1998, Pfohl-Leszkowicz et al., 1998, Bacha et al., 1993, Nikolov et al., 1996, Radic et al., 1997). The chemical structure of Ochratoxin A is (R-N-[(5-chloro-3, 4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl) carbonyl] phenylalanine) (Figure 1).

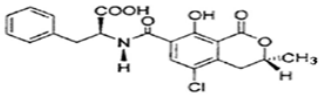

Figure 1. The chemical structure of OTA.

OTA occurs in a variety of foods, including beer, wine, vinegar, grape juice, coffee, cocoa, pulses, spices, dried fruits and meats. The presence of OTA, especially in wine, is believed to be a risk factor for human health. Since 2001, the European Union has established a regulation, which was revised some times, including maximum levels for commercial wines and grape juice (2 μg/L), and dried vine fruits (10 μg/kg) among others (Commission Regulation, 2006). Due to the high toxicity of OTA, many methods to control their effects have been proposed. One of the methods is based on the adsorption of OTA by several organic and inorganic materials. In this study, red kidney bean peel was used as adsorbent for reducing OTA level in a commercial red wine. It is a promising method for removing OTA by using household wastes. Red kidney bean peel was interacted with OTA by Van der Walls interaction, hydrogen bonding and electrostatic forces. These attractions led up to a high removal OTA capacity. Adsorption by using household waste as adsorbent have advantages according to attainability, inexpensiveness and simple proceeding.

The target of this work was performing OTA adsorption onto red kidney bean peel, evaluated the data by using adsorption isotherms, kinetics and comparing adsorption data obtained from synthetic solution and red wine.

2. MATERIAL AND METHODS

2.1. Reagents and Materials

The OTA standard was purchased from Sigma-Aldrich (St. Louis, MO). All solvents were high-performance liquid chromatography (HPLC) grade. Methanol was from Panreac (Barcelona, Spain), acetic acid was from Carlo-Erba (Milano, Italy), acetonitrile was from Sigma-Aldrich, and ultrapure water was obtained from a Milli-Q apparatus (Millipore, Molsheim, France). OTA stock solution (1000 ng/mL) was prepared in methanol.

Red kidney bean peel used as adsorbent was obtained from agricultural land in Izmir-Turkey. Firstly, the peels were washed with distilled water and then preheated in an oven at 100 - 110 °C for about 24 h to reduce the moisture content. The dried slices were ground by using grinder and sieved to obtain a particle size range of 0.5–1mm and stored in plastic bottle for further use. No other chemical or physical treatments were used prior to adsorption experiments.

2.2. FTIR Analysis of red kidney bean peel

To clarify the functional groups of adsorbent, the infrared spectrum was obtained with a FTIR (Perkin Elmer Spectrum BX-II) spectrophotometer with a pellet of powdered potassium bromide and adsorbent. Infrared spectra were carried out in the region of 4000–400 cm-1. The FTIR spectrum of adsorbent was shown in Figure 2. It was shown that the bands corresponding to the functional groups of O-H stretching vibration (3430 cm−1), C-H aromatic and aliphatic stretching vibration (2925 cm−1), C=O stretching vibration (1730-1630 cm−1), C=C aromatic stretching vibration (1600 and 1500 cm−1), C-H deformation vibration (1465 and 1378 cm−1) and C-O stretching vibration (1040 cm−1) were observed (Estevin et al., 2006). The bands of O-H groups, C =O stretching and -C-C group (880cm-1)

in the spectrum indicated the possible involvement of that functional group on the surface of red kidney bean peel in OTA adsorption process. These groups helped to form the interaction between the red kidney bean peel and OTA.

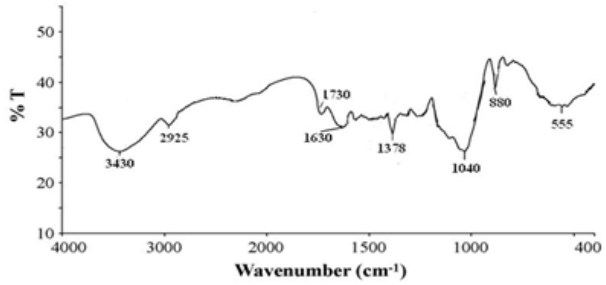

Figure 2. FTIR spectrum of red kidney bean peel.

2.3. Adsorption Studies for Synthetic OTA Solutions

The effect of adsorbent dose on the amount of OTA adsorbed from synthetic solution was obtained by contacting 25mL of OTA solution of initial concentration of 100 ng/L with different amount of red kidney bean peel (0.005-0.100 g) into a number of 50 mL glass bottles at pH 3.48. The bottles were placed in a temperature controlled shaker at 298 K until reaching equilibrium. The agitation was provided at 150 rpm. Then, each test solution was centrifuged at 4000 rpm for 15 min. One milliliter of each supernatant was used for OTA analysis.

The contact time of adsorbents, the adsorption experiments were performed with 25 mL of 100 ng/mL OTA synthetic solutions at various time intervals for 540 min with 0.25 g of red kidney bean peel (RKB) at pH of 3.50. These data were used to clarify the adsorption kinetics. Equilibrium studies were performed by contacting fixed amount of red kidney bean peel (0.25 g) with 25 mL of synthetic OTA solution of different initial concentrations(1.5, 3, 5, 10, 25, 50, 100, 125, 250, and 500 ng/L) in 50mL glass bottles at a temperature of 298 K and pH of 3.50. The initial and equilibrium concentrations of OTA were analyzed using Agilent Technologies 1100 (Heilbron, Germany) series liquid chromatographic system equipped with a fluorescence at 333 (excitation) and 458 nm (emission), controlled by Chemstation 3D software. The amount of adsorption at equilibrium, qe (mg/g), was calculated by Eq. (1).

$$q_e = (C_0 - C_e)\frac{V}{M}$$

where C_o and C_e (mgL^{-1}) are the liquid-phase concentrations of OTA at initial and equilibrium, respectively. V is the volume of the solution (L) and M is the mass of dry sorbent used (g). The percentage removal of synthetic OTA solution (R %) was calculated by using Eq.(2).

$$R\% = (C_0 - C_e)/C_0 x100 \qquad (2)$$

The effect of temperature on the adsorption of OTA onto 0.25g of RKB was investigated at six different temperatures (298, 303, 308, 313 and 318K) using 25mL initial concentration of 100 ng/mL OTA synthetic solutions.

To different amounts (0.1g, 0.25g, 0.5g, 0.75g and 1.0g) of each adsorbent, 10mL of red wine sample was added to reveal the adsorbent amount effect for real wine sample. The batch adsorption studies were performed as procedure for synthetic OTA solution for 0.25 g of RKB. Total polyphenol and total anthocyan contents were determined for wine before and after batch adsorption process by using Shimadzu UV 1601 (Kyoto, Japan) series spectrophotometric system at 280 and 520 nm, respectively (Gonzalez-Rodriguez et al., 2002).

2.4. OTA Analysis

HPLC analyses were performed on an Agilent Technologies 1100 (Heilbron, Germany) series liquid chromatographic system equipped with a fluorescence at 333 (excitation) and 458 nm (emission) controlled by Chemstation 3D software. The chromatographic conditions were follows: C18 reverse phase (250 mmx4mm, 5 μm) HPLC analytical column; volume of injection, 20 μL loop; isocratic elution (water/acetonitrile/acetic acid, 48.5:50.5:1, v/v/v); flow rate, 1.5 mL/min; and temperature, 50 ℃. An aliquot of the original OTA test solution was used as the HPLC standard.

The calibration curve of OTA ranged from 1.0 to 10.0 ng/mL with an equation of y=0.1985x+0.0812 with the regression coefficient R2=0.9993. The direct injection method was applied to the analysis of the red wine sample (Dall'Asta et al., 2004). The supernatant containing OTA in adsorption experiments was collected in a vial, evaporated to dryness under an N2 stream, and redissolved in the HPLC mobile phase. The limit of detection for OTA was 0.20 ng/mL; the limit of quantification was 1.15 ng/mL; the reproducibility of interday and intraday for 2.0 ng/mL was 2.68 and 3.75%, respectively.

3. RESULTS AND DISCUSSION

3.1. Effect of Contact Time

Figure 3 indicated the effect of contact time onto adsorption of OTA by using synthetic OTA solution and real wine sample. It was concluded that the adsorption behavior was similar for adsorption by using synthetic OTA solution and real red wine. Furthermore, equilibrium removal efficiency for real red wine (91%) was lower than the synthetic one (94%). Additionally, adsorption onto RKB was rapid for the first 120 min and thereafter it proceeded at a slower rate and finally reached saturation. The contact time was nearly 240 min for synthetic OTA solution and 300 min for real red wine. The batch adsorption studies were carried out for 300 min for synthetic and real sample. The adsorption for real red wine took relatively longer contact time to attain equilibrium due to interference of the polyphenols with the adsorption of OTA.

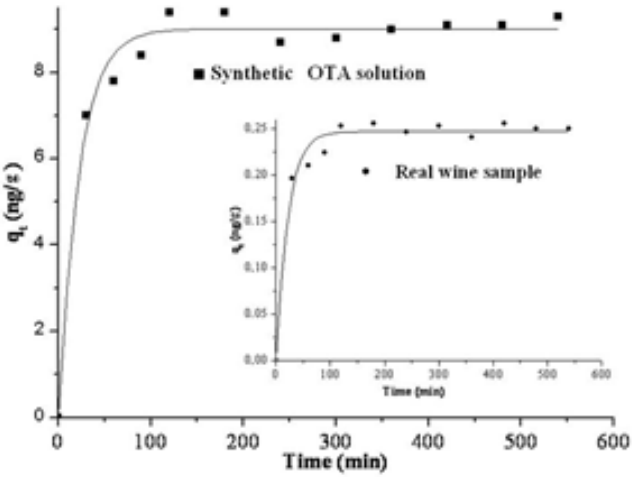

Figure 3. Effect of contact time for the adsorption of OTA in synthetic solution at pH 3.5 (C0 =100 ngmL-1) and in real red wine (C0 =2.8 ngmL-1) at 298K.

3.2. Effect of adsorbent dose on OTA adsorption

From the Figure 4, it was observed that uptake per unit mass of red kidney bean peel decreased for both real red wine and synthetic OTA solution by increasing mass of adsorbent. Furthermore, the percentage of OTA removal increased from 86.0 to 94.0% with an increase in adsorbent mass from 0.005 to 0.100 g for synthetic OTA solution. When the percentage of the removal increased with the dosage increase of RKB, the uptake per unit mass decreased. This result was attributed to the availability of more adsorption sites by increasing the mass of adsorbent and some of the adsorption sites remain unsaturated during the adsorption process. The batch adsorption data were collected by using 0.25g of RKB.

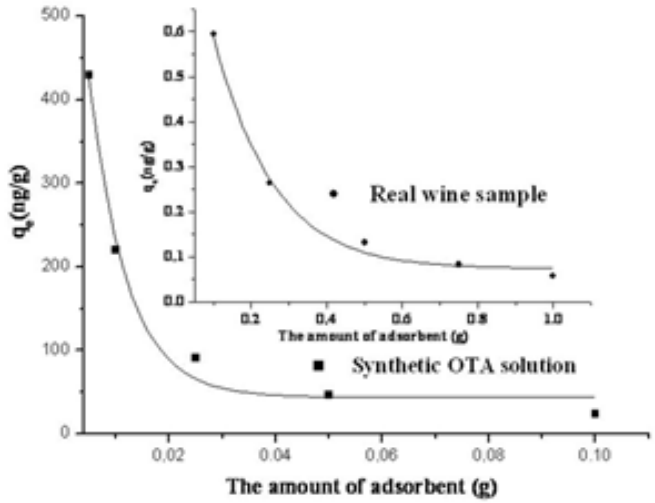

Figure 4. Effect of adsorbent mass for the adsorption of OTA in synthetic solution at pH 3.5 (C0 =100 ngmL-1) and in real red wine (C0 =2.8 ngmL-1) at 298K.

3.3. Adsorption Isotherms

The adsorption isotherms clarified the specific relation between the concentration of the adsorbate and its adsorption degree onto adsorbent surface at a constant temperature. To reveal the adsorption capacity of red kidney bean peel for the removal of OTA in synthetic OTA solution, the Langmuir and Freundlich isotherm models were used and the isotherms were shown in Figure 5. The Langmuir isotherm theory assumes monolayer coverage of adsorbate over a homogenous adsorbent surface (Langmuir, 1916). A basic assumption is that sorption takes place at specific homogeneous sites within the adsorbent. Once an OTA molecule occupies a site, no further adsorption can take place at that site. The linear form of Langmuir isotherm is (Langmuir, 1916):

$$\frac{C_e}{q_e} = \frac{1}{C_m a_L} + \frac{C_e}{C_m} \tag{3}$$

where q_e is the amount adsorbed at equilibrium (ng/g), C_e is the equilibrium concentration of the adsorbate (ng/L), and C_m (ng/g) and a_L are the Langmuir constants related to the maximum adsorption capacity and the energy of adsorption, respectively. These constants can be evaluated from the intercept and the slope of the linear plot of experimental data of C_e/q_e versus C_e. The values of constants C_m and a_L were -24.5 ng/g and -0.02, respectively. A further analysis of the Langmuir equation can be made on the basis of a dimensionless equilibrium parameter, R_L (Hall et al., 1966), also known as the separation factor, given by:

$$R_L = \frac{1}{\left(1 + a_L C_0\right)} \tag{4}$$

The value of R_L lies between 0 and 1 for favourable adsorption, while $R_L > 1$ represents unfavourable adsorption, and $R_L = 1$ represents linear adsorption while the adsorption process is irreversible if $R_L = 0$. C_0 (the initial OTA concentration mmol L^{-1}) and a_L were used to calculate the dimensionless constant separation factor. The dimensionless parameter R_L remained among 1.03 to 2.10 ($R_L>1$) for initial concentration 1.5 -25ng/L indicated the unfavorable adsorption process. Favorability of adsorption for high initial concentration (C_0=50-500ng/L) was not revealed out because of $R_L <0$. The applicability of the isotherm models to the adsorption data was inferred by the correlation coefficient, R^2 value of Langmuir and Freundlich plots. The higher the R^2 value, the better the fit. The correlation coefficient of Langmuir isotherm plot ($R_l^2=0.79$) was lower than the correlation coefficient of Freundlich isotherm plot ($R_f^2=1.0$). These results indicated that adsorption data did not correspond the Langmuir isotherm model.

The Freundlich isotherm (Freundlich, 1906) is an empirical equation assuming that the adsorption process takes place on heterogeneous surfaces and adsorption capacity is related to the concentration of OTA at equilibrium.

$$q_e = K_f C_e^{\,n_f} \tag{5}$$

$$\ln q_e = \ln K_f + n_f \ln C_e \quad (6)$$

where K_f is roughly an indicator of the adsorption capacity and n_f is the adsorption intensity. The magnitude of the exponent, n_f, gives an indication of the favorability of adsorption. Values of $n_f > 1$ represent unfavorable adsorption condition. The values of K_f and n_f for Freundlich isotherm were 0.534ng/g and 1.13, respectively. Freundlich isotherm model exhibited better fit compared to the Langmuir model. The isotherm parameters were summarized in Table 1. The fact that this occurred may be due to a heterogeneous surface with a non-uniform distribution of heat of adsorption over the surface. Furthermore, multilayer adsorption was occurred due to the presence of energetically heterogeneous adsorption sites. The heterogeneity arises from the presence of different functional groups on the surface, and the various adsorbent–adsorbate interactions (Hameed and El-Khaiary, 2008).

In fact, OTA is a weak acid partially dissociated at the pH of wine and carries a negative charge. Red kidney bean peel was protonated in acidic medium (pH=3.5) for that reason the negative moiety of OTA might be pulled by protanated regions. The carboxyl group of phenyalanine moiety onto OTA helped for higher removal.

Additionally, the adsorption data were collected for red wine sample. The polyphenols interfere with the adsorption of OTA by adsorbents (Sims et al., 1995, Aleixandre et al., 1996, Manfredini, 1989). The interference effect of the organic components of wine onto adsorption were clarified through performing polyphenols and anthocyans adsorption by using 0.10; 0.25; 0.50; 0.75 and 1.00 g of RKB. The removal percent of polyphenols and anthocyans were lower by comparing with the removal percent of OTA (Table 2). This was favorable result and the red kidney bean peel is an alternative adsorbent for removing OTA. The removal percentage was increased by using 0.10 to 0.25 g of adsorbent for red wine sample, after than it was remained at 94% for 0.50 g of adsorbent and the percentage was decreased by the amount of adsorbent increase to 1.00 g. At lower adsorbent dosage, the removal percentage of OTA was higher than the removal percentage of organic components. At higher adsorbent dosage, the adsorption behavior was changed and OTA and the other organic components competed to the surface of red kidney bean peel. Consequently, 1.00 g of RKB had disadvantage because of removing more organic components.

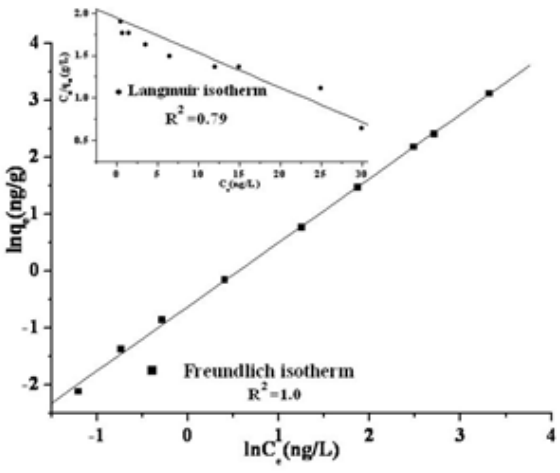

Figure 5. Langmuir and Freundlich isotherm plots for OTA adsorption onto red kidney bean peel at 298K (pH=3.5).

Table 1. Isotherm parameters for OTA adsorption onto red kidney bean peel

Langmuir parameters			Freundlich parameters		
C_m(ng/g)	a_L	R_l^2	K_f (ngmL^{-1}(Lng^{-1})^{nf})	n_f	R_f^2
-24.478	-0.021	0.79	0.5336	1.126	1.0

Table 2. Adsorption percent of polyphenols and anthocyans by using different adsorbent amount

Organic component	Adsorption (%)				
	Amount of adsorbent (g)				
	0.10	0.25	0.50	0.75	1.00
Total polyphenols, 280 nm	10	11	18	39	82
Total anthocyans, 320nm	12	18	35	58	100

3.4. Adsorption kinetics

In order to analyze the sorption kinetics of OTA on red kidney bean peel, the pseudo-first-order and pseudo-second-order kinetic models were applied to the experimental data. Lagergren proposed a method for adsorption analysis which is the pseudo-first-order kinetic equation of Lagergren (Lagergren, 1898) in the form:

$$\frac{1}{q_t} = \left(\frac{k_1}{q_1}\right)\left(\frac{1}{t}\right) + \left(\frac{1}{q_1}\right) \quad (7)$$

where q_t is the amount of OTA uptaken at different times t (ng g^{-1}), k_1 is rate constant for pseudo-first-order kinetics of OTA uptake (min^{-1}), t is the time (min), q_1 maximum adsorption capacity (ng g^{-1}). The linear plot of $1/q_t$ versus t was obtained. The values of q_1 and k_1 can be estimated from the slope and intercept of the plots for synthetic OTA solution and red kidney bean peel. In many cases the first-order equation of Lagergren does not fit well to the whole range of contact time and is generally applicable over the initial stage of the adsorption processes (McKay and Ho, 1999).

The pseudo-second-order equation based on equilibrium adsorption (Ho and McKay, 1978) is expressed as

$$\left(\frac{t}{q_t}\right) = \frac{1}{k_2 q_2^{\,2}} + \frac{t}{q_2} \qquad (8)$$

where k_2 is the rate constant (gng^{-1} min^{-1}), q_2 is the maximum adsorption capacity (ngg^{-1}) for the pseudo-second-order adsorption kinetic model. To calculate the values of k_2 and q_2 for both synthetic OTA solution and red wine sample, the intercept and the slope of the linear plots of (t/q_t) versus t were obtained. The first and second-order rate constants values were summarized in Table 3. It was concluded from Table 2 that the correlation coefficients for the pseudo-first-order kinetic model obtained for synthetic OTA solution and red wine were low. Additionally, the pseudo second kinetic model was appropriate for adsorption of OTA onto red kidney bean peel. For synthetic OTA solution the correlation coefficients for pseudo-first and second order kinetics models and q_l and q_2 values nearly same. Further more for real red wine, the correlation coefficient of pseudo-second-order kinetic model was higher than the correlation coefficients of pseudo-first-order kinetic model. This suggests that this sorption system was not a first-order. It was assumed that the adsorption onto RKB might be chemically with respect to pseudo-second- order kinetic model.

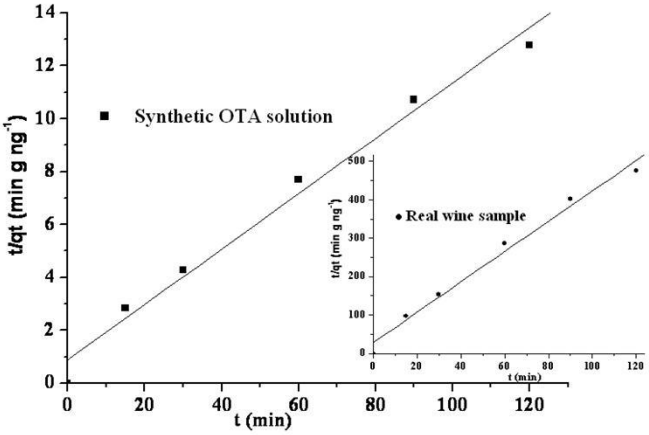

Figure 6. Pseudo-second order sorption kinetics of OTA onto red kidney bean peel at 298K (pH= 3.5).

Table 3. Kinetic parameters of OTA adsorption onto red kidney bean peel for synthetic OTA solution and real wine sample

	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	q_1(ng g^{-1})	k_1(min^{-1})	R_1^2	q_2(ng $g^{-1)}$	k_2(gng^{-1} min^{-1})	R_2^2
Synthetic OTA solution	9.883	12.999	0.982	9.611	0.012	0.984
Real wine sample	0.256	9.857	0.962	0.253	0.558	0.986

3.5. Adsorption thermodynamics

The amounts of sorption of OTA by RKB were measured at temperatures 298, 303, 308, 313 and 318 K to identify the adsorption thermodynamic. The thermodynamic parameters including Gibbs free energy change (ΔG°), enthalpy (ΔH°), and entropy change (ΔS°), have a significant role to determine the feasibility, spontaneity and heat change for the adsorption process. Thermodynamic parameters such as Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) change of adsorption can be evaluated by using following equations (Smith and Van Ness, 1987, Ho et al., 2002):

$$\Delta G^\circ = -RT \ln K_c \tag{9}$$

$$K_c = \frac{C_s}{C_e} \tag{10}$$

$$\ln K_c = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{11}$$

where C_s is the concentration of OTA adsorbed (ng/L); C_e is the equilibrium concentration (ng/L) at a given temperature; T is the solution temperature in K; K_c is the equilibrium constant; and R is the gas constant (8.314 J K^{-1} mol^{-1}). The enthalpy (ΔH°), and entropy change (ΔS°) of adsorption were estimated from van't Hoff equation (Eq. 11) plotting lnK_c versus 1/T. Thermodynamic parameters were summarized in Table 4.

Table 4. Thermodynamic parameters for OTA adsorption onto red kidney bean peel

***T*(K)**	***K_c***	**ΔG°(kJmol^{-1}K^{-1})**	**ΔH°(kJmol^{-1})**	**ΔS° (Jmol^{-1}K^{-1})**
298	2.75	-6.82		
303	2.59	-6.52		
308	1.39	-3.55	-121.7	-384.2
313	0.02	-1.16		
318	0.01	-0.03		

The negative values of ΔG° in the temperature range of 298–318K were due to the fact that the adsorption process was spontaneous and feasible thermodynamically. The negative value of ΔG° has increased from −6.82 to −0.03 kJmol^{-1}K^{-1} with an increase in temperature from 298 to 318K, indicated that the spontaneous nature was inversely proportional to the temperature. This result indicated that adsorption of OTA onto RKB became less favourable at higher temperature. The negative value of ΔH° (-121.7 kJ mol^{-1}) suggested that the exothermic nature of OTA sorption while the negative value of ΔS° (-384.2 J mol^{-1} K^{-1}) indicated that the decreasing randomness at the solid/solution interface during the adsorption of OTA onto red kidney bean peel for synthetic OTA solution. Furthermore, the magnitude of ΔH° gives an idea about the type of sorption. The heat of physical adsorption (van der Waals adsorption) of gases is relatively low, in the order of 1–5 kcal mol^{-1} (4–21 kJ mol^{-1}), whereas

that of chemisorption is much higher, of a magnitude of 24–120 kcal mol^{-1} (100–500 kJ mol^{-1}) comparable to that for a chemical union (Shaw, 1970; Giles et al., 1960). The adsorption of OTA onto RKB was almost a chemical process. This result was also supported with suitability of the adsorption kinetic to pseudo-second order kinetic model.

4. CONCLUSION

The results demonstrated that Ochratoxin A can be effectively removed from wine by using red kidney bean peel as adsorbent and this procedure was economical and environmentally friendly technique because of recycling of household wastes. The adsorption process was performed with high removal percentage of OTA and low removal percentage of total polyphenols and anthocyans at less dosage of red kidney bean peel. The adsorption behavior for real wine sample and synthetic OTA solution were similar. The components of red wine might not excessively affect the efficiency of OTA reduction. Consequently, this study enlightens the production process of wine for reducing the amount of OTA.

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