

Adsorption and Desorption Characteristics and Purification of Isoflavones from Crude Soybean Extract Using Macroporous Resins

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Isoflavones in soybean have been well-known with many health-promoting effects on humans. This study aimed to purify isoflavones from the crude soybean extract by the static adsorption/desorption process on macroporous resins. A screening test of four commercial resins: D101, AB-8, Amberlite® XAD4, and Diaion HP20 according to their adsorption/desorption characteristic for isoflavones was investigated. All four resins showed high adsorption and desorption characteristics in which D101 resin was chosen as the most suitable resin for purifying isoflavones. Compositional analysis showed that daidzin and genistin were the main isoflavones in the crude soybean extract. The adsorption isotherms data of total isoflavones, daidzin, and genistin fitted well with the Langmuir model with $R^2 > 0.98$. The dynamic adsorption conditions for the purification process of isoflavones on the D101 resin-packed column were selected at the bed volume (BV) of 200 mL, feed volume of 3.75 BV, and flow rate of 1.5 BV/h. The dynamic desorption was carried out with the elution solution of 70% (v/v) ethanol, elution volume of 2.5 BV, and flow rate of 1 BV/h. The total isoflavone content in the purified extract was 8.70-fold higher than its initial content in the crude soybean extract with a recovery yield of nearly 80%. The study results reveal a strong possibility for large-scale production of isoflavones for further application in functional food products or pharmaceutical products.

INTRODUCTION

Isoflavones, well-known as phytoestrogens, have been closely related to the structure of estrogen, a hormone released in a woman's body. Isoflavone compounds are naturally found in the members of the bean family (Fabaceae (Leguminosae)) [Bennetau & Pelissero, 2013; Bustamante-Rangel *et al.*, 2018; Rostagno *et al.*, 2010]. Isoflavones are a subclass of flavonoids exhibiting high antioxidant, anticancer, or anti-inflammatory activities [Lee *et al.*, 2005; Shim *et al.*, 2008]. Soybean isoflavones have been reported to induce a strong antioxidant activity both in the *in vitro* and *in vivo* studies [Li *et al.*, 2018]. Therefore, the use of these naturally-derived isoflavones as functional food supplements has been continuously gaining attraction due to the high demand of health-conscious customers [Almeida *et al.*, 2015; Uifălean *et al.*, 2015].

However, a very small amount of isoflavones in the crude soybean extract might limit its applicability or feasibility

in functional food products [Li *et al.*, 2018]. Meanwhile, crude soybean extract mostly comprises polysaccharides and other impurity parts [Liu *et al.*, 2005]. Therefore, it should be subjected to the purification process to concentrate the isoflavones to be further applied on an industrial scale. Various fractionation methods have been applied to purify these bioactive compounds, such as fractional distillation, fractional crystallization, or preparative HPLC, which are not suitable on the industrial scale as they are cost-ineffective, time-consuming and applicable only in the a small-scale production [Yang *et al.*, 2016]. Meanwhile, using column chromatography with the stationary phase of macroporous resin beads to purify isoflavones has been found to offer many advantages, such as high selectivity in adsorption, cost-effectiveness, or less consumption of solvents [Kammerer *et al.*, 2019; Li & Chase, 2009; Soto *et al.*, 2011].

Many macroporous resins, such as D101, AB-8, Amberlite® XAD4, Diaion HP20, and H103, which are safe for food

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TABLE 1. Physical and chemical properties of the macroporous resins.

Trade name	Polarity	Matrix	Surface area (m ² /g)	Mean pore size (Å)	Particle size (mm)
AB-8	Weak polar	Styrene-Divinylbenzene	480–520	130–140	0.3–1.25
D101	Non-polar	Styrene-Divinylbenzene	500–550	90–100	0.3–1.25
Diaion HP-20	Weak polar	Styrene-Divinylbenzene	500	260	0.25–0.85
Amberlite® XAD4	Non-polar	Styrene-Divinylbenzene	750	100	0.25–0.85

application [Gao *et al.*, 2018], have been studied to purify isoflavone compounds from crude extracts of soybean hypocotyls [Choi & Kim, 2007], defatted soy flakes [Wu & Lai, 2007], kudzu root (*Puerariae lobatae Radix*) [Guo *et al.*, 2015], or okara (soy pulp) [Li *et al.*, 2012; Sevillano *et al.*, 2014]. AB-8 resin, which exhibited high adsorption and desorption abilities, was reported to be a proper resin for the purification process of genistein, an isoflavone in soybean [Li *et al.*, 2012]. SP-825 and SP-207 resins were successfully used to purify catechol, 4-ethylguaiacol, 4-ethylphenol, and daidzein of soy sauce with high adsorption and desorption potentials [Kim *et al.*, 2014]. In turn, D101 resin showed high adsorption/desorption capability for purifying steroidal saponins from the *Rhizoma paridis* [Wu & Lai, 2007].

In Vietnam, soybean is a commonly used food material due to its health benefits. Therefore, functional products enriched with soybean isoflavones can gain the attention of health-conscious consumers. In this study, four macroporous resins (D101, AB-8, Amberlite® XAD4, and Diaion HP20) were screened for the purification process of isoflavones from the soybean aqueous ethanol crude extract. Besides, static adsorption and desorption characteristics of not only total isoflavones but also the predominant isoflavones from the extract were investigated. The purification of isoflavones from soybean crude extracts by an appropriate resin column was carried out and key isoflavone constituents were also identified. The result is expected to show the high feasibility of using macroporous resins in the purification process of isoflavones on a large scale so that it facilitates the development of new functional food products with the purified extract.

MATERIALS AND METHODS

Materials

Soy (*Glycine max* L. Merr) beans were collected at local farms in Dai Loc district – Quang Nam province, Vietnam. Damaged beans were removed to avoid affecting the quality of the resulting soybean flour. Soybeans were dried at 60°C until reaching the moisture content of 9.04±0.04 g/100 g. The dried beans were homogeneously ground into a powder form and stored in closed boxes at the temperature of 20±2°C to be prepared for subsequent experiments.

Six isoflavone standards (genistin, glycitin, daidzin, genistein, glycitein, daidzein) with the purity of 99% were supplied from the United States Pharmacopeia (North Bethesda, MD, USA). Ethanol and acetonitrile (HPLC grade) were supplied from Merck KGaA (Darmstadt, Germany).

D101 and Amberlite® XAD4, two non-polar resins, were from Anhui Sanxing Resin Technology Co., Ltd (Anhui, China), and Thermo Fisher Scientific Inc. (Waltham, MA, USA), respectively. Meanwhile, the two weak polar resins of AB-8 and Diaion HP20 were supplied from Anhui Sanxing Resin Technology Co., Ltd, and Mitsubishi Chemical Corporation (Tokyo, Japan), respectively. The characteristics of the studied macroporous resins are shown in Table 1.

Macroporous resins pretreatment

The resins were pretreated to remove impurity particles trapped inside the pores. First, they were soaked and allowed to swell for 24 h in 96% (v/v) ethanol solution followed by a washing step with distilled water to completely remove ethanol. The resins were then treated with HCl solution (5%, v/v) and NaOH solution (5%, w/v). Finally, distilled water was used again to wash these resins until neutralization of washing water [Guo *et al.*, 2015].

Crude soybean extract preparation

The crude soybean extract was obtained following our previous study [Tran *et al.*, 2019]. Briefly, soybean flour with an accurate weight was added to an 80% (v/v) ethanol solution. The sample was incubated in a water bath (HH-2, Wincom Company Ltd., Shanghai, China) at the temperature of 50°C under stirring (JJ-1, Hinotek Group, Ningbo, China). The mixture was then transferred to a rotary evaporator (model DHWEV01001V, Daihan Scientific Co., Ltd, Kangwon-do, South Korea) at 48–52°C to completely remove ethanol. The obtained residue was freeze-dried using an Alpha 1–2 Ldplus freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at -40°C for 16 h [Guo *et al.*, 2015]. A stock solution of the extract was prepared by diluting the freeze-dried crude soybean extract in distilled water. The initial total isoflavone content of the stock solution was tentatively determined as 432.34 µg/mL. The term “total isoflavones” is defined as the mixture of six isoflavones including the glycoside isoflavones (genistin, glycitin, daidzin) and the corresponding aglycone forms (genistein, glycitein, and daidzein).

HPLC analysis of isoflavones

Reversed phase HPLC was used to quantify the content of daidzin, genistin, and total isoflavones. Briefly, an HPLC system (1200 Agilent, Agilent Technologies, Inc., Santa Clara, CA, USA) was connected with a quaternary gradient pump (including degasser), an autosampler, a diode-array detector, and ChemStation software for controlling chromatographic

parameters and quantitative analysis. A reversed phase column (Lichrospher® 100 RP-18, 4.6×250 mm, 5 μm, Merck) was placed in the column compartment with temperature maintained at 40°C to separate the isoflavones. The mobile phase at a flow rate of 1.5 mL/min comprised eluent A (0.05% (v/v) phosphoric acid) and eluent B (acetonitrile) which were filtered through a 0.45 μm membrane. The gradient elution used for the mobile phase was in the following order: starting with 100% of A for 5 min, changing from 10% to 30% of B (linear) for over 60 min, washing with 90% of B for 5 min, equilibrating with 100% of A for 10 min. All the compounds were detected at 260 nm. Quantification of isoflavones was conducted by constructing an external calibration curve. Each isoflavone peak was compared with isoflavone standards according to its spectrum and retention time [Tran *et al.*, 2019].

Static adsorption and desorption characteristics of isoflavones on four macroporous resins

Static adsorption and desorption tests

Four adsorption resins, including Diaion HP20, Amberlite® XAD4, D101, and AB-8, were selected to evaluate the purification capacity of isoflavones from the crude ethanolic soybean extract. The static adsorption was conducted in 250 mL conical flasks with stoppers which contained 10 g of resins and an exact volume of crude extract solutions. The flasks were shaken for 1 h at 25°C with the speed of 120 rpm, then allowed to stand for 1 h. This step was repeated in 3 cycles to attain the adsorption equilibrium. The resins were washed with distilled water followed by the addition of 50 mL of a 70% (v/v) ethanol solution to desorb isoflavones [Tang *et al.*, 2018; Yang *et al.*, 2016]. The extracts from the desorption process were concentrated under a vacuum rotary evaporator then diluted to 50 mL by acetonitrile and double distilled water mixture at a 1:1 ratio (v/v). The equilibrium concentrations of daidzin, genistin, and total isoflavones in adsorption solutions (C_e , mg/mL) and desorption solution (C_d , mg/mL) were determined by HPLC. The adsorption capacity (q_e , mg/g dry resin) adsorption ratio (A, %), desorption capacity (q_d , mg/g dry resin), desorption ratio (D, %) and recovery ratio (H, %) of the resins were calculated as follows:

$$q_e = \frac{(C_0 - C_e) \times V_0}{m} \quad (1)$$

$$A = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (2)$$

$$q_d = \frac{C_d \times V_d}{m} \quad (3)$$

$$D = \frac{C_d \times V_d}{C_0 \times V_0 - C_e \times V_e} \times 100 \quad (4)$$

$$H = \frac{C_d \times V_d}{C_0 \times V_0} \times 100 \quad (5)$$

where: C_0 is the initial concentration of daidzin, genistin and total isoflavone in sample solutions (mg/mL); V_0 is the volume

of initial sample solution (mL); m is the weight of dry resin used (g); V_d is the volume of desorption solution (mL); and V_e is the volume of elution solution (mL).

Equilibrium adsorption isotherms

To plot the adsorption isotherms of daidzin, genistin, and total isoflavone on the D101 resin, 50 mL of crude extract solution were added at varying concentrations to 2.5 g of the resin at 25°C. The content of daidzin, genistin, and total isoflavones in the sample was measured after reaching their equilibrium concentration. Langmuir and Freundlich models were used to describe the isoflavones adsorption behaviors on D101 resin [Duran *et al.*, 2011; Guo *et al.*, 2015; Liu *et al.*, 2010; Shazeli *et al.*, 2020].

The Langmuir adsorption equation is as follows:

$$\frac{q_e}{q_{\max}} = \frac{K_L \times C_e}{1 + K_L \times C_e} \quad (6)$$

where: C_e (mg/mL) and q_e (mg/g dry resin) are equilibrium concentrations of analytes in the adsorption solutions and adsorption capacity, respectively; K_L (L/mg) is the Langmuir constant; q_{\max} (mg/g dry resin) is the theoretical maximum adsorption capacity. Another important characteristic of Langmuir isotherms is a dimensionless constant (separation factor, R_L). It was calculated as follows:

$$R_L = \frac{1}{1 + K_L C_0} \quad (7)$$

where: C_0 (mg/mL) is the initial concentration of sorbate.

The Freundlich equation can be expressed as:

$$q_e = K_F \times C_e^{\frac{1}{n}} \quad (8)$$

where: K_F (mg/g) is the Freundlich constant, and $1/n$ is an empirical constant having the value of $0.1 < 1/n < 1$ or $1 < n < 10$.

Static desorption ratio at different ethanol concentrations

After the adsorption process, the D101 resin was washed by distilled water, then soaked in 50 mL of an ethanol solution at different concentrations, 30, 40, 50, 60, 70, 80, and 90% (v/v), for 2 h to reach the equilibrium desorption. The mixtures were then filtered and subjected to the composition analysis using the HPLC. The desorption ratio (D, %) was calculated according to the previous papers [Ma *et al.*, 2015; Tungmunithum *et al.*, 2020] from the content of eluted daidzin, genistin, and total isoflavones.

Dynamic adsorption and desorption of isoflavones on the D101 resin-packed column

Dynamic adsorption test

Dynamic adsorption tests were evaluated by using a glass column (inner diameter of 3 cm, length of 50 cm) wet-packed with 100 g of D101 resins and with the bed volume (BV) of 200 mL. The stock extract solution ($C_0 = 432.34$ mg/mL) was gradually loaded on the resin column at a flow rate

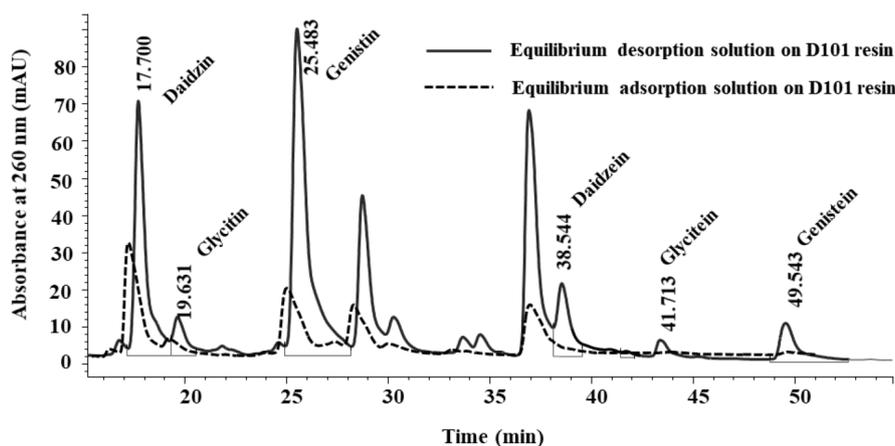


FIGURE 1. HPLC chromatograms of both equilibrium adsorption and equilibrium desorption solutions on the D101 macroporous resin.

of 1.5 BV/h. The effluents were taken at intervals of 50 mL to determine the concentrations of daidzin, genistin, and total isoflavones (C_d). These results were used to plot the adsorption breakthrough curves of daidzin, genistin, and total isoflavones. The adsorption capacity (q_e , mg/g) and the adsorption ratio (A , %) on the D101 resin-packed column were also calculated according to the previous papers [Li *et al.*, 2012; Liu *et al.*, 2010].

Dynamic desorption test

After attaining the adsorption equilibrium, the impurities in the resin column were removed by washing with double distilled water at a flow rate of 3 BV/h. The isoflavones were desorbed by 70% (v/v) ethanol solution at a flow rate of 1 BV/h. Eluted solution was collected at intervals of 50 mL until no isoflavones were left in the sample. The eluted sample was subjected to composition analysis to determine the concentrations of daidzin, genistin, and total isoflavones (C_d). These results were used to build up the desorption curves of isoflavones on the D101 resin packed column followed by the determination of desorption capacity (q_d , mg/g), desorption ratio (D , %), and recovery ratio (H , %) using the procedure described by Li *et al.* [2012] and Liu *et al.* [2010].

Statistical analysis

The results were analyzed by using a Minitab 18 software (Minitab, LLC, State College, PA, USA). Experimental values were expressed as mean \pm standard deviation of three replicates. Analysis of variance (ANOVA) and Fishers least significant difference (LSD) test were utilized to compare the mean values with the significance level at $p < 0.05$.

RESULTS AND DISCUSSION

Static adsorption and desorption characteristics of four macroporous resins

A suitable macroporous resin for the purification process of isoflavones was evaluated based on the adsorption and desorption performance. Adsorption capacity depicts the amount of isoflavones absorbed on resins, whereas the desorption capacity describes the amount of isoflavones

desorbed from resins in the desorption solution, respectively [Gao *et al.*, 2018]. In this study, the HPLC results showed that all six isoflavones, including glycitin, daidzin, genistin, daidzein, glycitein, and genistein, were present in both equilibrium adsorption and desorption solutions on the four evaluated resins. That was, all macroporous resins exhibited the capacity in the adsorption and desorption of six isoflavones, indicating their applicability in the purification of isoflavones from the crude soybean extract. A typical chromatogram for the recognition of these isoflavones on the D101 resin is depicted in Figure 1. The peaks of isoflavones in the chromatography were consistent with a previous study of Tran *et al.* [2019]. As shown in Figure 1, the predominant compounds in the adsorption/desorption solution were daidzin and genistin, thus the total isoflavones and two of these dominant compounds were selected as the responses for the adsorption/desorption performances on the four resins.

The adsorption and desorption characteristics of total isoflavones, daidzin, and genistin on the four resins were distinct, as presented in Figure 2. The adsorption capacities of the total isoflavones, daidzin, and genistin were 4.89–5.32 mg/g, 1.31–1.45 mg/g, and 2.19–2.35 mg/g, respectively. The adsorption ratios of the resins were 76–82% for total isoflavones, 64–71% for daidzin, and 79–85% for genistin. The adsorption of phenolics on macroporous resins is a result of physical interaction via van der Waals forces, hydrogen bonds, or the π - π conjugation between phenolic compounds and benzene rings of macroporous resins [Gao *et al.*, 2018; Yang *et al.*, 2016]. It was found that the adsorption and desorption capacities were dependent on many noticeable variables such as surface area, pore diameters, and polarity of resins [Gao *et al.*, 2018; Sun *et al.*, 2015; Tang *et al.*, 2018], or molecular weight of absorbed substances [Dong *et al.*, 2015]. The chemical constituents of macroporous resins were also considered a key factor influencing the adsorption and desorption characteristics [Fu *et al.*, 2006]. In our study, the adsorption performances of D101 and Amberlite® XAD4 resins were considerably better than the others, and there was no significant difference in adsorption capacity between these two resins ($p \geq 0.05$) (Figure 2a). This phenomenon could be due to the different

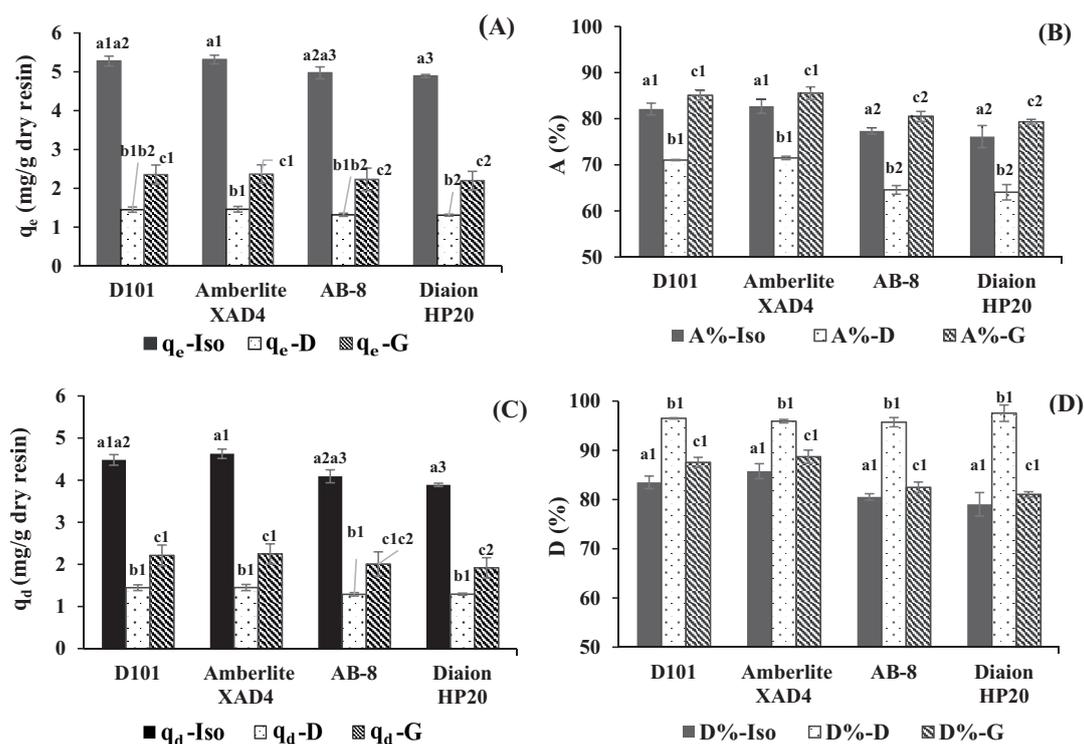


FIGURE 2. Adsorption capacity, q_e (A), adsorption ratio, A (B), desorption capacity, q_d (C), and desorption ratio D (D) of total isoflavones (-Iso), daidzin (-D), genistin (-G) on the four resins.

Error bars show standard deviations. Different numbers for the same letter above bars indicate significant differences between resins ($p < 0.05$).

polarity of these resins. The D101 and Amberlite® XAD4 resins with non-polar property could better absorb non-polar isoflavones compared to AB-8 and HP-20, which are weak polar resins. This complied with the principle of “like dissolve like” indicating which non-polar compounds are easier absorbed by non-polar resins [Yan & Tang, 2003]. The desorption capacities of daidzin, genistin, and total isoflavones on D101 resin were 1.45 mg/g, 2.21 mg/g, and 4.48 mg/g, respectively, and there were insignificant difference ($p \geq 0.05$) between D101 and Amberlite® XAD4 resins (Figure 2c). The significantly ($p < 0.05$) lower desorption capacity was determined on AB-8 and Diaion HP20 resins for total isoflavones. The desorption ratios of total isoflavone for four resins were from 79 to 86%. Interestingly, inversely to the adsorption ratios, the desorption ratios of daidzin (95–98%) were higher than those of genistin (81–88%).

Generally, both two non-polar resins (Amberlite® XAD4, D101) and two weak polar resins (Diaion HP20, AB-8) showed good adsorption and desorption characteristics of isoflavones from crude soybean extract and only slightly better parameters were found for the non-polar ones. Our results support previous report showing that Amberlite® XAD4 resin was efficient in purifying the major isoflavones from the defatted soy flakes [Wu & Lai, 2007]. However, compared to high-priced Amberlite® XAD4 resin, the D101 resin used in this study, which is cheaper, fairly possessed the same adsorption and desorption properties. Therefore, D101 resin could be a proper material for the purification process of isoflavones from crude soybean extract; therefore, subsequent experiments were conducted on the D101 resin.

Adsorption isotherms of isoflavones on the D101 resin

Adsorption isotherms are usually described by the Langmuir and Freundlich equations due to their simplicity and high accuracy [Jia & Lu, 2008; Yin *et al.*, 2010]. The adsorption isotherms of daidzin, genistin, and total isoflavones on the D101 resin at 25°C, described by the Langmuir and Freundlich models, are presented in Figure 3. The Langmuir model depicts the adsorption behavior of the mono-molecular layer, whereas the Freundlich model is applied to describe that of both mono-molecular layer and multi-molecular layer [Sun *et al.*, 2015]. The adsorption capacity of total isoflavones, daidzin, and genistin increased with increasing equilibrium concentration (C_e) (Figure 3). This was possible because the initial concentration served as a driving force to attenuate the resistance of mass transfer for the isoflavones movement from the crude extract to the D101 resin surface [Gao *et al.*, 2018]. The parameters of Langmuir and Freundlich isotherms at the temperature of 25°C are listed in Table 2. Both models showed a very good fit to the experimental data with the high coefficient of determinations (R^2). However, this coefficient for the Langmuir model was higher for daidzin, genistin, and total isoflavones ($R^2 > 0.98$) compared to the Freundlich model (0.862–0.951). Hence, the Langmuir model was found to better describe the adsorption capacity of isoflavones on the resin and this finding was in agreement with a study of Tang *et al.* [2018]. In other words, the adsorption behavior of soybean isoflavones on the D101 resin was based on the formation of a mono-molecular layer. The R_L values of Langmuir model, in this study, ranged from 0.019 to 0.155, indicating the favorable isotherms of isoflavones

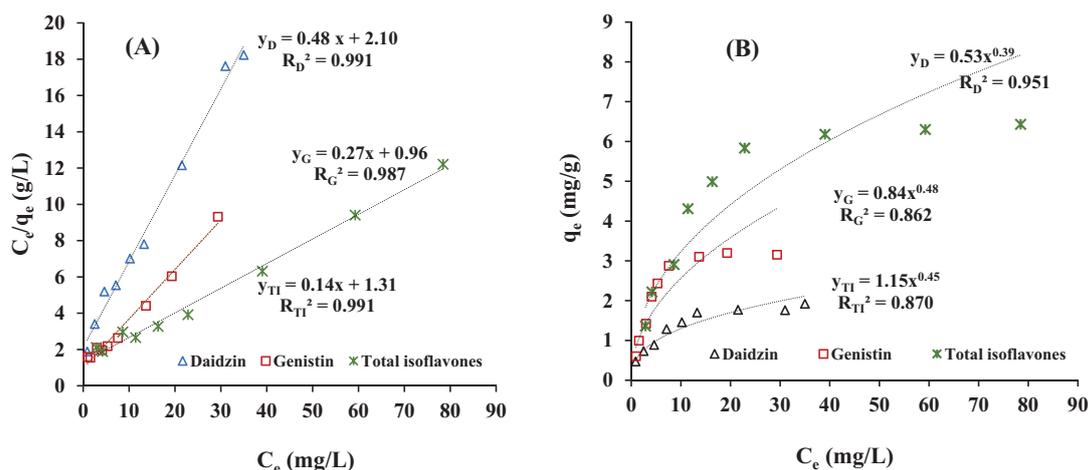


FIGURE 3. Langmuir (A) and Freundlich (B) adsorption isotherms of daidzin, genistin, and total isoflavones on the D101 resin at 25°C. C_e : equilibrium concentration of analytes in the adsorption solutions, q_e : adsorption capacity.

on the D101 resin. Previous studies also highlighted that R_L values in the range of 0–1 showed the favorable isotherms of analytes adsorbed on the macroporous resins [Duran *et al.*, 2011; Tang *et al.*, 2018]. In addition, the low values of R_L indicated the strong interaction between bioactive compounds and macroporous resins [Duran *et al.*, 2011]. Besides, the $1/n$ value in the range from 0.220 to 0.483 in the Freundlich model (Table 2) was adequate. The $1/n$ value from 0.1 to 0.5 indicates that it is easy to carry out the adsorption process but it is impossible when this value exceeds 1.0 [Jia & Lu, 2008; Liu *et al.*, 2010].

Static desorption of isoflavones on the D101 resin by different ethanol solution

The desorption process is the second step to purify the isoflavones. The proper ethanol concentration is vital to obtain better recovery of isoflavones. The effects of ethanol concentrations on the desorption ratio of total isoflavones, daidzin, and genistin on the D101 resin are presented in Figure 4. The effect of ethanol concentration on the desorption ratio was significant ($p < 0.05$). The increment in ethanol concentration was found to increase the desorption ratio, which reached the maximal value at the ethanol concentration of 70% (v/v). Further increase in the ethanol concentration did not promote better desorption ratio. This finding was in agreement with previous studies [Guo *et al.*, 2015; Jia & Lu, 2008; Shazeli *et al.*, 2020; Tungmunthum

et al., 2020] that most flavonoids were desorbed by aqueous-ethanol solution at the ethanol concentration range from 50% to 90% (v/v). In this context, the weak polarity of isoflavones facilitates their dissolution in ethanol [Tran *et al.*, 2019]. However, the increment in the ethanol concentration led to a reduction in the polarity of the desorption solution, and the polarity of ethanol at the concentration above 70% (v/v) was too low to effectively extract isoflavones from the resin. A similar problem was previously discussed when desorbing dioscin from *Dioscorea nipponica* on the D101 resin [Yin *et al.*, 2010]. Finally, the ethanol concentration of 70% (v/v) was considered a proper concentration to desorb isoflavones on the D101 resin in the studied conditions.

Purification of isoflavones on the D101 resin-packed column

Dynamic adsorption breakthrough curves for total isoflavones, daidzin, and genistin on the D101 resin are the function of the effluent volume and the concentration ratio (C_e/C_0) of isoflavones herein, as presented in Figure 5. Normally, the 10% ratio of solutes to the initial concentration of the feed in the effluent is defined as the breakthrough point or leak point. After reaching the leak point, the adsorption affinity reduces significantly, leading to solute leakage from the resins [Liu *et al.*, 2013; Tang *et al.*, 2018]. The breakthrough volume is defined as the volume of feed extract when

TABLE 2. The parameters of Langmuir and Freundlich adsorption isotherms of isoflavones on the D101 resin.

Compound	Langmuir model				Freundlich model		
	q_{max} (mg/g dry resin)	K_L L/mg)	R_L	R^2	$1/n$	K_F	R^2
Daidzin	2.121	0.224	0.032–0.154	0.991	0.390	0.523	0.951
Genistin	3.672	0.283	0.019–0.102	0.987	0.483	0.843	0.862
Total isoflavones	7.369	0.104	0.023–0.119	0.991	2.220	0.450	0.870

q_{max} , theoretical maximum adsorption capacity; K_L , Langmuir constant; R_L , separation factor; $1/n$, empirical constant; K_F , Freundlich constant.

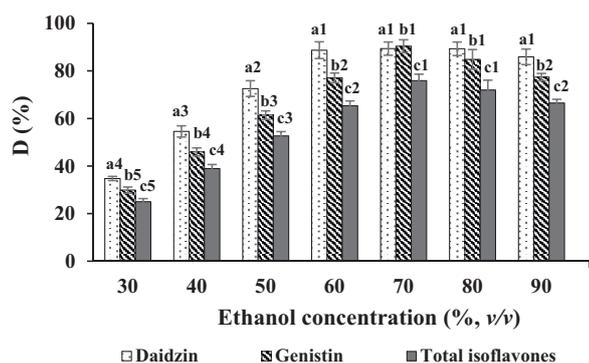


FIGURE 4. Effect of ethanol concentrations on the static desorption ratio (D) of total isoflavones, daidzin, and genistin on the D101 resin. Error bars show standard deviations. Different numbers for the same letter above bars indicate significant differences between resins ($p < 0.05$).

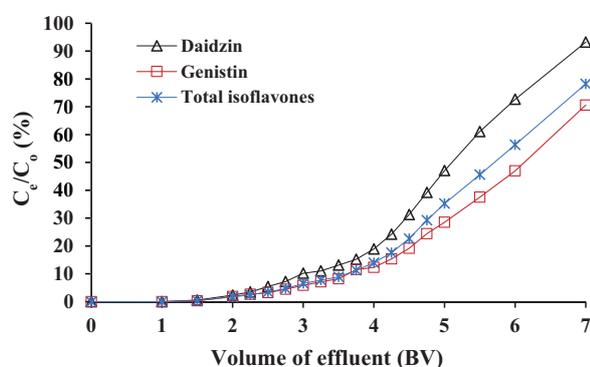


FIGURE 5. Dynamic adsorption breakthrough curves for total isoflavones, daidzin, and isoflavones on the D101 resin packed column.

C_0 : concentration of isoflavones in stock extract resin solution, C_e : concentration of isoflavones in effluent.

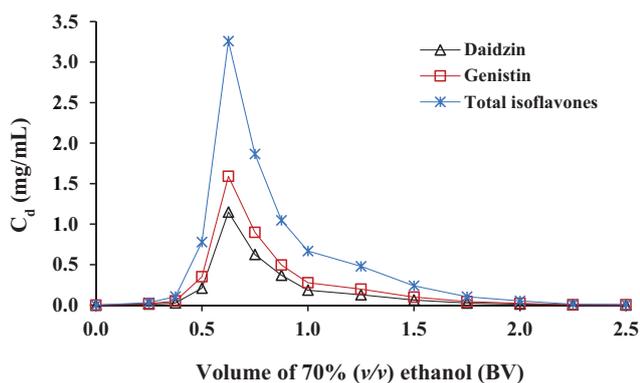


FIGURE 6. Dynamic desorption curves for total isoflavones, daidzin, and genistin on the D101 resin packed column.

C_d : concentration of isoflavones in the eluted solution.

the leak point is reached [Gao *et al.*, 2018]. Breakthrough volume helps determine when the sample volume can be pre-concentrated to limit the loss of analytes [Liu *et al.*, 2013]. As shown in Figure 5, the leak points of daidzin and genistin were approximately 3.0 BV and 3.75 BV, respectively. The breakthrough point of total isoflavones was nearly the same as that

TABLE 3. Isoflavone contents in the crude soybean solid extract and purified isoflavone solid extract.

Compound	Crude soybean solid extract (mg/g)	Purified isoflavone solid extract (mg/g)	Purified/crude ratio of isoflavones
Daidzin	5.40±0.14 ^b	49.69±0.24 ^b	9.20±0.19
Glycitin	0.89±0.02 ^c	7.35±0.13 ^d	8.21±0.12
Genistin	8.01±0.02 ^a	68.51±0.36 ^a	8.56±0.24
Daidzein	1.18±0.10 ^c	11.16±0.43 ^c	9.47±0.37
Glycitein	0.10±0.01 ^d	0.47±0.01 ^e	7.94±0.12
Genistein	1.05±0.03 ^c	7.49±0.14 ^d	7.12±0.01
Total isoflavones	16.63±0.12	144.67±1.21	8.70±0.01

Different superscripts (a-e) indicate the significant differences between the analyzed compounds within the same column ($p < 0.05$).

TABLE 4. Dynamic adsorption and desorption characteristics of isoflavones on the D101 resin packed column.

Compound	Dynamic adsorption		Dynamic desorption		
	q_e (mg/g)	A (%)	q_d (mg/g)	D (%)	H (%)
Daidzin	0.91±0.01	94±2	0.762±0.01	84±2	79±1
Genistin	1.40±0.02	98±1	1.11±0.01	79±1	77±1
Total isoflavones	2.95±0.02	97±2	2.39±0.02	81±1	78±2

q_e , adsorption capacity; A, adsorption ratio; q_d , desorption capacity; D, desorption ratio; H, recovery ratio.

of genistin due to the fact that the genistin content in the crude soybean extract was the highest, as shown in Table 3. Daidzin leaked with a lower volume compared to the others. This was possibly attributed to the discrepancies in polarity, initial concentrations, and adsorption rates [Tang *et al.*, 2018]. In summary, all isoflavones in the feed extract were mostly absorbed by the resin before 3.75 BV, thus it was selected as the feed volume at the fixed flow rate of 1.5 BV/h as it reached the maximal treating capacity of the resin. Parameters of dynamic adsorption of isoflavones on the D101 resin-packed column are presented in Table 4. The adsorption ratios for total isoflavones, daidzin, and genistin were high (>94%), confirming the suitability of D101 resin in the purification process of isoflavones.

The dynamic desorption curves of total isoflavones, daidzin, and genistin are illustrated in Figure 6. The concentration of isoflavones increased with the elution volume, reaching a maximum at the elution volume of 0.68 BV. Then, it decreased gradually. Similar behaviors were also discussed in many previous reports [Gao *et al.*, 2018; Li *et al.*, 2012; Wu & Lai, 2007]. The desorption process was completed when reaching the elution volume of 2.5 BV (Figure 6). The hydrogen bonds and hydrophobic interactions between the functional groups of phenolic compounds and the resin

are broken during their elution with ethanol [Gao *et al.*, 2018]. The dynamic desorption parameters are presented in Table 4. As a result, the desorption ratios (ranging from 79 to 84%) were fairly lower than the adsorption ratios. The recovery ratio of the adsorption/desorption process achieved a relatively high percentage, *i.e.* around 79%. Therefore, the desorption volume of 70% (*v/v*) ethanol as eluate was selected at 2.5 BV at the fixed flow rate of 1 BV/h.

The composition analysis results of the purified isoflavones solid extract compared to the crude soybean solid extract are shown in Table 3. The total isoflavone content in the purified isoflavones solid extract was 144.67 mg/g, being more than 8.70-fold higher than that in the crude soybean solid extract. The contents of daidzin and genistin increased by 9.20 and 8.56 times, respectively. The obtained results were possibly comparable with prior reports that the phenolic compound content was 3.5–11-fold higher than their initial extracts with the recovery yield of 70–95% [Gao *et al.*, 2018; Liu *et al.*, 2010; Liu *et al.*, 2013; Shazeli *et al.*, 2020; Sun *et al.*, 2015; Tang *et al.*, 2018]. For example, the flavonoids from the extract of Chinese wolfberry were concentrated from 0.58% to 10.77% when using D101 resin [Wu *et al.*, 2015]. The podophyllotoxin content in the extract from *Sinopodophyllum hexandrum* purified using D101 resin was 7.41-fold higher than that of the crude extract with a recovery ratio of 74.6% [Wang *et al.*, 2018].

CONCLUSION

Static adsorption and desorption characteristics of isoflavones from crude soybean extract on four resins were successfully evaluated. The D101 resin was the best resin to purify the isoflavones due to its high adsorption and desorption characteristics. Daidzin and genistin were the main isoflavones in the crude soybean extract. The experimental data of adsorption isotherms of total isoflavones, daidzin, and genistin showed better fit with the Langmuir model compared to the Freundlich model. The use of the D101 resin-packed column allowed an 8.70-fold increase in the content of total isoflavones in the extract with the recovery yield of nearly 80%. The result suggested the high feasibility of D101 resin in the purification process of isoflavones from the crude soybean extract in large-scale production. This result highlighted the practical aspect of using macroporous resins in the purification process so that the purified isoflavones could be easily applied to develop functional food products instead of using crude extract.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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