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ARTICLE

Optimization of Adsorption of Tea Polyphenols into Oat β-Glucan Using Response Surface Methodology

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Information on interactions between oat β-glucan and tea polyphenols (TP) is not available in the published literatures. Equilibrium dialysis was applied to determine the adsorption of TP into β-glucan, and response surface methodology (RSM) was employed to optimize the absorbing variables (pH, temperature, and phosphate buffered saline (PBS) buffer concentration). The equilibrium data at constant temperature were fitted with the Langmuir, Freundlich, and Redlich-Peterson models. The results showed that the Freundlich model was the best method to describe the experimental data. Parabolic curves were obtained for pH and temperature. In terms of adsorption capacity, factors including temperature, pH/temperature, and buffer concentration/temperature had the greatest influence on the response. The highest adsorption capacity of TP into β-glucan was 134.55 μg mg^{-1} at the following optimized conditions: pH 5.56, PBS buffer concentration 0.13 M, and temperature 40 °C. No significant differences ($p > 0.05$) between the experimental and predicted values confirmed the adequacy of the response surface equations.

KEYWORDS: Oat β-glucan; tea polyphenols; equilibrium dialysis; adsorption isotherm; adsorption capacity; response surface methodology

INTRODUCTION

The complexation of polyphenols with proteins has been extensively investigated (*1*, *2*). However, little data are available in the literatures about the complexation of polyphenols with polysaccharides. Complexation of polyphenols with polysaccharides plays an important role in regulating the free concentration of polyphenols in foods and the human gastrointestinal (GI) tract, which results in the improvement of food flavor, inhibition of oxidation and browning, controlled-release of polyphenols, and recovery of polyphenols from plant crude extracts (*3*−*10*). Amrani-Joutei and co-workers (*11*) found that proanthocyanidins were bound to the cell wall polysaccharides. Riou et al (*12*) investigated the influence of wine polysaccharides on tannin aggregation. Furthermore, the existence of noncovalent interactions between proanthocyanidins and apple cell wall material in aqueous solution has been reported (*13*−*16*). Later work by Le Bourvellec et al. (*17*) investigated apple polyphenols (procyanidins)−cell wall interactions, and their impact on polysaccharide extractability and showed that procyanidins mainly bound to pectins as compared to other cell wall compounds. In addition, several studies demonstrated that some polysaccharides have the ability to disrupt polyphenols/proteins interactions (*18*, *19*).

In recent years, studies have demonstrated that the complexation of polyphenols with polysaccharides was affected efficiently by the properties of polysaccharides and polyphenols, especially by their structural features such as composition, state, conformational flexibility, molecular weight of polysaccharides, and degree of polymerization and percentage of galloylation (*8*, *20*, *21*). On the other hand, some environmental parameters such as pH, buffer concentration, and temperature also have a crucial influence on their complexation (*13*). Tea polyphenols (TP), the main biological active substance of tea, which consists of (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), and (−)-catechin (C), have been clearly demonstrated to provide remarkable health benefits, including anticancer, antibacterial activity, antioxidant activity, reducing blood fattiness, and decreasing blood sugar (*22*−*28*). Cereal β-glucan is a linear polysaccharide composed of consecutively (1→4)-linked β-D-glucopyranosyl residues in blocks separated by single (1→3)-linkages. It is generally classified as a soluble dietary fiber, and it possesses a number of functionalities, such as increasing immune, anticancer activity, and lowering of blood cholesterol, lipids, and blood glucose (*29*−*36*). In oriental countries, tea and β-glucan-rich cereals, such as barley and oat, are extensively consumed together in a specific diet. However, few studies have examined molecular interactions and complexes between TP and β-glucan.

In this paper, the adsorption behavior of TP into oat β-glucan in aqueous solution and optimization of interactions between TP and oat β-glucan using response surface methodology (RSM) were investigated. It will be helpful for understanding interactions between polysaccharides and other molecules in aqueous solution.

MATERIALS AND METHODS

Tea Polyphenols. TP samples were obtained from Changsha Active Ingredients Inc. (Changsha, China), with tea polyphenols content up to 98.38%. The HPLC analysis indicated that it contained (−)-catechin

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(C, 0.07%), (−)-epigallocatechin (EGC, 0.89%), (−)-epicatechin (EC, 0.34%), (−)-epigallocatechin gallate (EGCG, 74.51%), (−)-gallocatechin gallate (GCG, 4.93%), and (−)-epicatechin gallate (ECG, 17.64%).

Oat β-Glucan. A commercial soluble β-glucan, extracted from hull-less oats, was bought from Zhengzhou Lion Biological Technology Co. Ltd. (Zhengzhou, China) with a β-glucan content of 77%. The molecular weight of oat β-glucan was determined by gel-permeation chromatography (GPC) on a Sephadex G-100 column (1.6 × 100 cm) using distilled water as eluent at a flow rate of 24 mL h^{-1} (*37*). The value for the weight-average molecular weight was 9.1×10^5.

UV Absorption Spectroscopy of TP. TP stock solution (0.5 mg mL^{-1}) was prepared by dissolving 10 mg of TP in 20 mL of doubly distilled water. A series of TP solutions (0.004−0.040 mg mL^{-1}) were obtained by diluting TP stock solution with doubly distilled water. The ultraviolet (UV) absorption spectroscopy of the above-mentioned TP solutions was recorded using an UV-2450 spectrophotometer (Shimadzu, Japan) in the wavelength range from 200 and 400 nm.

Pretreatment of β-Glucan. Oat β-glucan (10 mg) was heated at 80 °C with magnetic stirring in 15 mL of distilled water for 2 h until complete solubilization. The solution of oat β-glucan was cooled to room temperature and successively dialyzed against tap water for 24 h and distilled water for 24 h in dialysis bag (Union Carbide) with a size exclusion of 1.4 kDa for globular molecules. Then, it was adjusted to 20 mL with distilled water to achieve 0.5 mg mL^{-1} β-glucan solution. The resulting solution of oat β-glucan was stored at +4 °C prior to performance of the dialysis experiment.

Equilibrium Dialysis Assays. Six milliliters pretreated 0.5 mg mL^{-1} oat β-glucan and 2 mL of 0.5 mg mL^{-1} TP were totally mixed and applied into a dialysis bag. Then it was dialyzed against 30 mL of phosphate buffered saline (PBS) buffer for 16 h in a capped 50 mL plastic tube to reach equilibrium that was judged with the constant value of absorbance outside the dialysis bag at 273 nm and to confirm that the free TP concentrations were equal on both sides of the dialysis bag. The influence of temperature (20−60 °C), equilibrated pH (3−7) and concentration (0.05−0.5 M) of PBS buffer on adsorption capacity were investigated. To determine the working curve of UV absorbance−TP concentration, the UV absorption spectroscopy method was used (*38*). A well fitted regression equation of the UV absorbance (Y, the maximum absorbance of TP solution at 273 nm) to the concentration (X), $Y = 0.00391 + 23.561X$, was obtained with R square (R^2) of 0.99997 (**Figure 1**). Then, the absorbance of the TP solution at 273 nm was measured and the equilibrium concentration of the TP solution, C_e (μg mL^{-1}), was calculated based on the working curve. The adsorption capacity (Q_e), in terms of μg of TP absorbed by 1 mg of β-glucan, can be represented by the following equation (*39*−*41*).

$$\text{Adsorption capacity } (Q_e, \mu g\ mg^{-1}) = \frac{M_{tp}\text{-}C_e V}{M_{beta}} \quad (1)$$

where M_{tp} is the mass of TP (μg), C_e is the concentration of TP outside the dialysis bag (μg mL^{-1}), V is the total volume of solution in plastic tube (mL), and M_{beta} is the mass of oat β-glucan (mg).

Adsorption Isotherm Models. Langmuir Isotherm Model. Langmuir isotherm is usually used with an ideal assumption of monolayer adsorption on a homogeneous surface (*42*, *43*). It is derived from simple mass kinetics and based on two assumptions that the forces of interaction between sorbed molecules are negligible and once a molecule occupies a site no further sorption takes place at that site (*44*, *45*). This model is commonly represented by the following equation (*46*).

$$Q_e = \frac{Q_m b C_e}{1 + b C_e} \quad (2)$$

where Q_e is the amount of TP adsorbed per unit mass of β-glucan (μg mg^{-1}), C_e is the equilibrium concentration of TP outside the dialysis bag (μg mL^{-1}), Q_m is the maximum adsorption capacity (μg mg^{-1}), and b is the constant related to the free energy of adsorption.

Freundlich Isotherm Model. Freundlich isotherm is usually suitable for nonideal adsorption on heterogeneous surfaces. The heterogeneity is caused by the presence of different functional groups on the surface and several interactions. The Freundlich model assumes that there are many types of sites acting simultaneously, each with a different free energy of sorption and that there is a large amount of available sites (*47*, *48*). The Freundlich exponent is indicative of the diversity of free energies associated with the solute sorption by the multiple components of a heterogeneous sorbent. This model is expressed by the following equation (*49*).

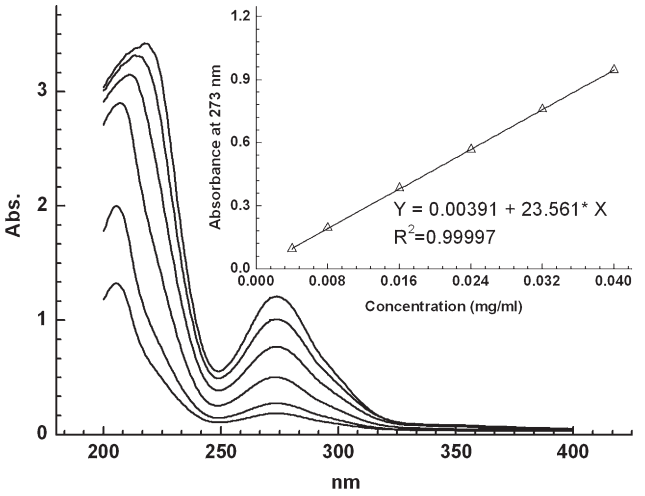

Figure 1. Ultraviolet (UV) absorption spectrometry of different concentrations of tea polyphenols (TP) solution. In order of increasing peak absorbance at 273 nm, TP concentrations are 0.004, 0.008, 0.016, 0.024, 0.032, and 0.040 mg mL^{-1}. (Inset): the working curve of UV absorbance (Y)−TP concentration (X) at 273 nm. A well fitted regression equation, $Y = 0.00391 + 23.561X$, was obtained with a correlation coefficient R^2 of 0.99997. Y represents the absorbance of TP solution. X represents the concentration of TP solution.

$$Q_e = K_F C_e^n \quad (3)$$

where K_F is the Freundlich adsorption constant, n is the Freundlich exponent indicating adsorption intensity of the system, and C_e is the equilibrium concentration of TP outside the dialysis bag (μg mL^{-1}).

Redlich-Peterson Isotherm Model. The Redlich-Peterson model may be used to represent adsorption equilibrium over a wide concentration range and can be applied either in homogeneous or heterogeneous systems due to its versatility (*43*). This model contains three parameters and incorporates the features of the Langmuir and Freundlich isotherms. It can be described as follows (*50*−*52*):

$$Q_e = \frac{K_R C_e}{1 + a C_e^{\beta}} \quad (4)$$

where K_R and a are Redlich-Peterson isotherm constants, respectively. β is the exponent which lies between 1 and 0. C_e is the equilibrium concentration of TP outside the dialysis bag (μg mL^{-1}).

Curve Fitting of Isotherm Data. For the study of sorption models, data have been plotted as adsorption capacity, Q_e (μg mg^{-1}), as a function of the equilibrium concentration, C_e (μg mL^{-1}). The analysis of the resulting isotherms to the different models (Langmuir, Freundlich, and Redlich-Peterson model) was performed by means of a nonlinear fitting procedure using an Origin Software (Systat Software Inc., Version 7.5) (*45*, *47*, *53*).

Experiment Design and Statistical Analysis. Box-Behnken design (BBD) was used for optimization of the adsorption process and to evaluate the effects and interactions of the process variables, that is, pH, temperature, and PBS buffer concentration, on the adsorption of TP into oat β-glucan. The coded and uncoded independent variables used in the RSM design were listed in **Table 1**. Fifteen experiments (**Table 2**), which included 12 factorial points and 3 center points, were randomly performed. Experiments at the center point were conducted for evaluation of the experimental error. All trials were performed in triplicate. A Design-Expert Software Version 7.0 (STAT-EASE Inc., Minneapolis, USA) was used to generate the experimental designs, statistical analysis, and regression model. A second-order polynomial equation was used to express the adsorption capacity (Q_e) as a function of the independent variables

$$Q_e = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j>1}^{3} \beta_{ij} X_i X_j \quad (5)$$

where Q_e represents the predicted response, β_0 is the constant coefficient, β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interaction coefficients, respectively. The model was built based on the variables with confidence levels of 95%.

RESULTS AND DISCUSSION

Influence of pH on the Adsorption Capacity of TP into Oat β-Glucan. As shown in **Figure 2A**, the pH was changed from 3 to 7, while other variables were set as follows: buffer concentration 0.05 M and temperature 25 °C. The adsorption capacity of TP into oat β-glucan increased with pH until 6 and began to decrease, and the maximum adsorption capacity was 116.08 ± 5.69 μg mg^{-1} at pH 6, which showed a resulting parabolic curve. The intensity of molecular interaction between TP and β-glucan under the conditions of pH values of 5 and 6, which are typical of the conditions in the upper and lower part of the small intestine, respectively, could provide evidence for β-glucan behavior in solution in the gastrointestinal (GI) tract. Several studies indicated that pH had a great influence on interactions between cell wall material and small molecules (*54*, *55*). Therefore, it is important to optimize the pH of the solution in order to obtain effective interactions between TP and β-glucan. However, Le Bourvellec and co-workers (*13*) showed that complex formation between procyanidins and cell wall material was not affected by pH in the range 2−7. Similarly, Silva and co-workers (*56*) reported that the pH had no influence on the adsorption of polyphenols on resin using an experimental design methodology.

Table 1. Level of Various Independent Variables at Coded Values of Response Surface Methodology Experimental Design

		coded levels		
symbol	independent variables	−1	0	1
X_1	pH	5	6	7
X_2	PBS buffer concentration (M)	0.05	0.1	0.15
X_3	temperature (°C)	20	30	40

Table 2. Box-Behnken Design Matrix (in Uncoded Level of Three Variables), Experimental Data and Predicted Values for Three-Level-Three-Factor Response Surface Analysis

	pH	PBS buffer concentration (M)	temperature (°C)		
number[a]	X_1	X_2	X_3	predicted desirability[b]	observed desirability[c]
1	7	0.1	40	88.81	91.72 ± 13.98
2	5	0.15	30	95.48	97.09 ± 11.58
3	7	0.15	30	102.65	106.95 ± 10.21
4	7	0.1	20	107.71	102.11 ± 5.99
5	6	0.15	40	132.44	125.23 ± 8.60
6	6	0.1	30	125.05	127.02 ± 8.21
7	6	0.1	30	125.05	127.74 ± 17.21
8	6	0.05	40	107.18	105.88 ± 3.53
9	6	0.05	20	114.08	121.29 ± 8.61
10	5	0.05	30	105.34	101.04 ± 5.66
11	6	0.1	30	125.05	120.39 ± 15.33
12	7	0.05	30	92.61	91.00 ± 13.45
13	5	0.1	40	128.77	134.37 ± 7.53
14	5	0.1	20	73.30	70.39 ± 12.90
15	6	0.15	20	88.98	90.28 ± 2.99

[a] Experiments were conducted in a random order. [b] μg of TP/mg of oat β-glucan. [c] Each value represented the mean ± SD (n = 3).

Influence of Buffer Concentration on the Adsorption Capacity of TP into Oat β-Glucan. The experiments were carried out for different PBS buffer concentrations (0.05−0.5 M) at fixed pH of 7 and temperature 25 °C. The adsorption capacity of TP into oat β-glucan decreased from 86.69 ± 11.61 to 2.11 ± 2.34 μg mg^{-1} with the buffer concentration increasing from 0.05 to 0.5 M (**Figure 2B**). To elucidate the role of hydrophobic interaction during complex formation, varying buffer concentration (or ionic strength) was used (*13*, *53*). The buffer concentration had a significant impact on the retention of TP into β-glucan. The decrease of interaction between TP and oat β-glucan with increasing ionic strength suggested that hydrophobic interactions were weak. A similar observation has been reported that the total binding number of Calcofluor per β-glucan molecule decreased with increasing buffer concentration by Wu and co-workers (*53*).

Influence of Temperature on the Adsorption Capacity of TP into Oat β-Glucan. The adsorption capacity influenced by temperature from 20 to 60 °C was shown in **Figure 2C**. The adsorption studies were carried out at pH 7 and PBS buffer concentration of 0.05 M. The results showed that the adsorption capacity of oat β-glucan increased until 30 °C and then slightly decreased, and the maximum adsorption capacity was 71.82 ± 11.58 μg mg^{-1} at 30 °C. It can be easily inferred that the adsorption of TP by oat β-glucan was not a simple endothermic process and the mechanism was not physical adsorption (*57*). In order to investigate the interaction mechanism, varying buffer concentration (or ionic strength) and temperature can be used to investigate the roles of hydrogen bonding and hydrophobic interaction (*53*). The decrease of interaction between TP and oat β-glucan with increasing ionic strength suggested that the hydrophobic interactions were

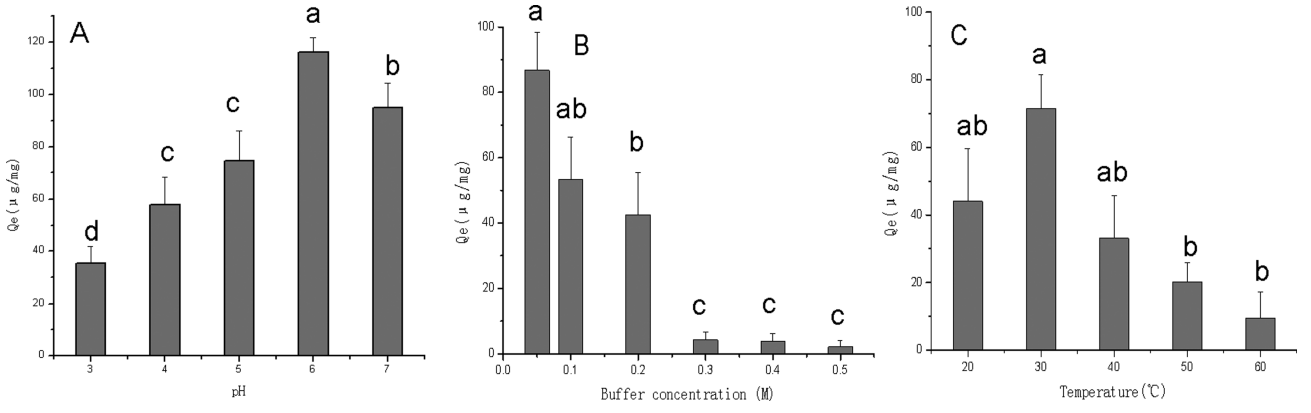

Figure 2. Effects of pH (**A**), PBS buffer concentration (**B**), and temperature (**C**) on the adsorption capacity of tea polyphenols (TP) into oat β-glucan. Comparisons of means are carried out into the technique of least-significant difference (LSD), and different lowercase letters in the same bar chart indicate significant difference (p < 0.05). Each value represents the mean ± SD (n = 3). Q_e represents the adsorption capacity of TP into oat β-glucan (μg mg^{-1}).

weak, while the notable decrease with increasing temperature indicated hydrogen bonding (*13*, *58*). On the other hand, the hydroxyl groups in TP and β-glucan are functional groups responsible for hydrogen bonding between TP and β-glucan. After the formation of hydrogen bonding, the distance between aromatic rings in TP and sugar rings in β-glucan becomes very short and permits the generation of van der Waals interactions (*53*). Therefore, hydrogen bonding and van der Waals forces were involved in the binding of TP with β-glucan. In previous studies, dextran gels were able to encapsulate polyphenols inside their pores via hydrogen bonds between the hydroxyls groups of polyphenols and the oxygen atoms of the cross-linking ether bonds (*4*, *5*), as in the apolar cavity of cyclodextrin (*59*). Similar observations have been reported that multiple hydrogen bonding, hydrophobic interaction, and $\pi-\pi$ stacking were responsible for the adsorption of TP onto polymeric adsorbents in aqueous solution by Huang and co-workers (*60*). In addition, study on interactions between TP and β-glucan will be helpful to understand their digestion and absorption in our bodies. The phenomenon of the adsorption of TP into oat β-glucans will influence the biological effects of individual compounds. In other words, complexation of TP with oat β-glucans plays an important role in regulating the free concentration of polyphenols in foods and the human gastrointestinal tract, which improves the stability of polyphenols, modifies aggregation of oat β-glucans, and therefore may influence the physiological effects of oat β-glucans. Free levels of tea polyphenols are reduced as well, which improves controlled-release of polyphenols. However, this simple in vitro study cannot provide an exact description of physiological actions of β-glucans in the gastrointestinal tract but rather affords knowledge on its molecular affinities toward small molecules.

According to single-factor analysis, adsorption isotherms determination was under the conditions of pH 6, buffer concentration 0.05 M, and temperature 30 °C. Then, we adopted pH from 5 to 7, PBS buffer concentration from 0.05 to 0.15 M, and temperature from 20 to 40 °C for RSM experiments.

Adsorption Model Analysis. Adsorption isotherms can describe qualitative information on the nature of the adsorbent−adsorbate interaction as well as the specific relation between the concentration of adsorbate and its degree of accumulation onto adsorbent surface at constant temperature (*43*). The adsorption modeling experiment was studied at a temperature of 30 °C, pH 6, PBS buffer concentration 0.05 M, 6 mL of 0.5 mg mL^{-1} oat β-glucan, and 2 mL of TP aqueous solution with known concentrations.

According to the adsorption isotherms, the adsorption process and adsorption mechanism can be deduced as well (*60*). **Figure 3** shows the fitted equilibrium data in Langmuir, Freundlich, and Redlich-Peterson models. The fitting results, that is, isotherm equations and the coefficient of determination, R^2, are shown in **Table 3**. It can be seen in **Figure 3** that the Freundlich sorption model fitted the data better than the Langmuir and Redlich-Peterson sorption models in the whole concentration range; this is also confirmed by the relatively high value of R^2 in the case of Freundlich (0.9790) compared to Langmuir (0.7734) and Redlich-Peterson (0.9170) models. This indicated that the adsorption process was involved multilayer coverage (*43*, *44*). Therefore, the experiment verified that the adsorption of TP into oat β-glucan was composed of multiple properties. A similar phenomenon can be found in the research done by Simonsen et al. (*41*). Additionally, Marsal and co-workers (*61*) reported the adsorption of polyphenols present in vegetable extracts (mimosa) in wastewater by organo-bentonites and indicated that the experimental results were fitted to the Freundlich equation.

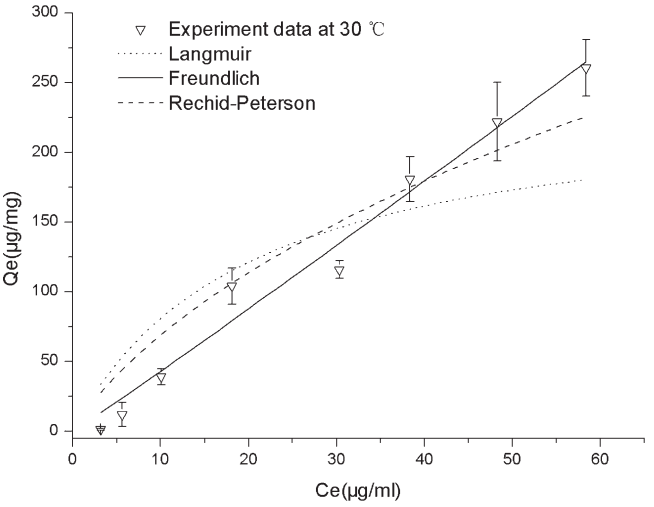

Figure 3. Isotherms obtained using the nonlinear method for the adsorption of tea polyphenols (TP) into oat β-glucan under the conditions of temperature 30 °C, pH 6, PBS buffer concentration 0.05 M, 6 mL of 0.5 mg mL^{-1} oat β-glucan and 2 mL of TP solution with known concentrations. Q_e is the adsorption capacity of TP into oat β-glucan (μg mg^{-1}), and C_e is the concentration of TP outside the dialysis bag (μg mL^{-1}).

Table 3. Isotherm Parameters and Equations Obtained Using the Nonlinear Method for the Adsorption of Tea Polyphenols (TP) into Oat β-Glucan[a]

isotherm type	equation[b]	R^2
Langmuir	$Q_e = 241.8 \times (0.0504 \times C_e)/(1 + 0.0504 \times C_e)$	0.7734
Freundlich	$Q_e = 3.997 \times C_e^{1.031}$	0.9790
Redlich-Peterson	$Q_e = 11.55 \times C_e/(1 + 0.1641 \times C_e^{0.6131})$	0.9170

[a] Under the conditions of temperature 30 °C, pH 6, PBS buffer concentration 0.05 M, 6 mL of 0.5 mg mL^{-1} oat β-glucan, and 2 mL of TP solution with known concentrations. [b] Q_e is the adsorption capacity of TP into oat β-glucan (μg mg^{-1}), C_e is the concentration of TP outside the dialysis bag (μg mL^{-1}), and R^2 represents the coefficient of determination.

Predicted Model and Analysis of Variance. The design matrix of the variables in the uncoded units and the corresponding results of RSM experiments to determine the effects of the three independent variables were shown in **Table 2**. By applying multiple regression analysis methods on the experiment data, the predicted model was obtained by a second-order polynomial function, which was given as follows:

$$Q_e = 125.05 - 1.39X_1 + 0.043X_2 + 9.14X_3 + 4.98X_1X_2 - 18.59X_1X_3 + 12.59X_2X_3 - 18.53X_1^2 - 7.50X_2^2 - 6.88X_3^2 \quad (6)$$

where Q_e is the adsorption capacity (μg mg^{-1}), X_1 is the pH, X_2 is the PBS buffer concentration (M), and X_3 is the temperature (°C).

The predicted values of adsorption capacity were calculated using the regression model and compared with experimental values in **Figure 4**, and the experimental data were analyzed by analysis of variance (ANOVA), which was listed in **Table 4**. In **Table 4**, a low probability '*P*' value of 0.0128 indicated high significance of the regression model, and the lack-of-fit *F*-value of 4.66 implied that the lack-of-fit was not significant relative to the pure error. The goodness of the model can be checked by the determination coefficient R^2 and the adjusted R^2. The total determination coefficient (R^2) was 94.25%, indicting a reasonable fit of the model to the experimental data. By analyzing the *F*-values and '*P*' values from **Table 4**, it was found that X_3, X_1X_3, X_2X_3, X_1^2 had high significance to explain the individual and interaction effect of variables on the adsorption capacity of TP into oat β-glucan to

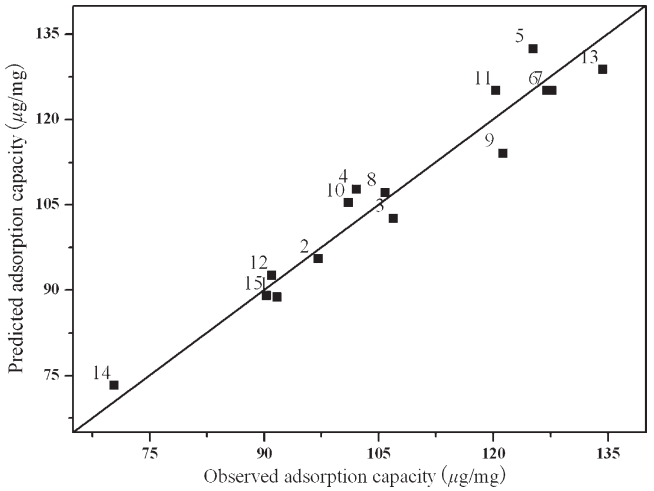

Figure 4. Comparison between observed and predicted adsorption capacity. The numbers indicate experiment numbers presented in **Table 2**.

Table 4. Analysis of Variance (ANOVA) for the Response Surface Quadratic Polynomial Model for Adsorption Capacity of Tea Polyphenols (TP) into Oat β-Glucan

source[a]	sum of squares	df	mean squares	*F*-value	probability prob > *F*[b]
model	4298.19	9	477.58	9.11	0.0128
X_1	15.43	1	15.43	0.29	0.6108
X_2	0.014	1	0.014	2.763×10^{-4}	0.9874
X_3	668.50	1	668.50	12.75	0.0160
X_1X_2	99.01	1	99.01	1.89	0.2278
X_1X_3	1382.72	1	1382.72	26.37	0.0037
X_2X_3	634.03	1	634.03	12.09	0.0177
X_1^2	1267.27	1	1267.27	24.17	0.0044
X_2^2	207.90	1	207.90	3.96	0.1031
X_3^2	174.59	1	174.59	3.33	0.1276
residual	262.18	5	52.44		
lack of fit	229.35	3	76.45	4.66	0.1818
pure error	32.83	2	16.42		
cor total	4560.37	14			

[a] X_1 = pH, X_2 = PBS buffer concentration (M), X_3 = temperature (°C). [b] $p < 0.05$ indicates statistical significance.

predict the response. All the other terms (X_1, X_2, X_1X_2, X_2^2, X_3^2) were insignificant in the equation and were not required to explain the adsorption process. Similarly, Bretag and co-workers (*62*) found that temperature played a critical role in the adsorption of rutin onto a food-grade styrene-divinylbenzene copolymer in a model system using RSM. Kammerer et al (*63*) reported that temperature and pH values were crucial parameters determining the adsorption behavior of individual polyphenols.

Response Surface Analysis. As shown in **Figure 5**, three-dimensional (3D) response surface and two-dimensional (2D) contour plots were the graphical representations of regression function. They explained the type of interactions between two tested variables and the relationships between responses and experiment level. **Figure 5A**,**B** shows the 3D response surfaces and the elliptical contour plot, the combined effect of pH and buffer concentration on the adsorption capacity of TP into oat β-glucan at constant temperature, and it revealed that at low and high levels of the pH and buffer concentration the adsorption capacity was minimal. Shown in **Figure 5C**,**D**, when the buffer concentration was fixed at 0.10 M level, pH and temperature displayed a quadratic effect on the response adsorption capacity. When the temperature was kept at a lower level, the adsorption capacity increased at first and then decreased with the increase of pH. From **Figure 5E**,**F**, it indicated that the mutual interactions between buffer concentration and temperature were significant when the pH was fixed at 6.

Verification of Predictive Model. The suitability of the model equation for predicting optimum response value was investigated under the following conditions: pH 5.56, PBS buffer concentration 0.13 M, and temperature 40 °C. The conditions were determined to be optimum by the RSM optimization process and were also used to predict the values of the responses. In the optimal conditions, the experimental adsorption capacity of TP by oat β-glucan was 134.55 ± 15.55 μg mg^{-1}, which agreed with the predicted value of 136.15 μg mg^{-1}. No significant difference ($p > 0.05$) was reported between the experimental and the predicted values. Therefore, the results indicated the suitability of the model employed and the success of RSM in optimizing the adsorption conditions.

Further, the present study investigated the adsorption capacity of TP into oat β-glucan in vitro, which provided evidence for molecular interactions between oat β-glucan and small molecules in the GI tract and confirmed the ability of oat β-glucan to adsorb small molecules. The approach of equilibrium dialysis was chosen because it has been proven useful in interaction studies on β-glucan and small molecules, such as barley and oat β-glucan and phenolic derivatives (*41*) and barley β-glucan and bile salts (*64*). On the other hand, the equilibrium dialysis can offer a relatively fast method for the analysis of small molecules. In addition, the efficacy of various water-soluble fibers to adsorb bile salts and other small compounds was evaluated and investigated in several studies (*41*, *65*, *66*). Those studies indicated that the binding of the different small compounds by water-soluble fibers was due to several and interacting properties of the fiber matrix and not a few single parameters.

According to our findings, detailed evaluation of such polyphenolic complex systems may contribute to optimize the selective use of plant ingredients, for example, by providing polyphenols enriched extracts with promising health-promoting properties. The retention of small compounds by cereal β-glucan or water-soluble fiber is of great interest from a food composition point of view and from a health perspective. Therefore, the investigation of the adsorption of TP into oat β-glucan would add new information not only to understand the molecular interaction mechanism but also to provide supporting evidence for the function of combined usage of cereal β-glucan and TP. In addition, encapsulation of polyphenols with natural polymers such as mixed linked (1 → 3, 1 → 4)-β-D-glucan gel has been reported to enhance their stability and potentially provide controlled-release of those functional ingredients (*7*, *67*).

In conclusion, the isotherm study and response surface methodology were successfully applied for modeling analysis and optimization of adsorption capacity of TP into oat β-glucan. The adsorption capacity exhibited a typical response to the pH, PBS buffer concentration, and temperature effect. Different adsorption isotherms, including Langmuir, Freundlich, and Redlich-Peterson sorption model, were used to evaluate the actual experimental data, and it was found that the Freundlich isotherm was suitable to characterize the adsorption process of TP into oat β-glucan. Analysis of the adsorption process suggested that multilayer adsorption was responsible for the adsorption of TP into oat β-glucan. ANOVA of response surface methodology implied that temperature was the most significant factor affecting the adsorption capacity of TP into oat β-glucan. It was also predicted that the optimum adsorption conditions within the experimental range would be the pH of 5.56, PBS buffer concentration of 0.13 M, and temperature of 40 °C. Under the optimum adsorption conditions, the predicted value of adsorption capacity of TP into oat β-glucan was 136.15 μg mg^{-1}.

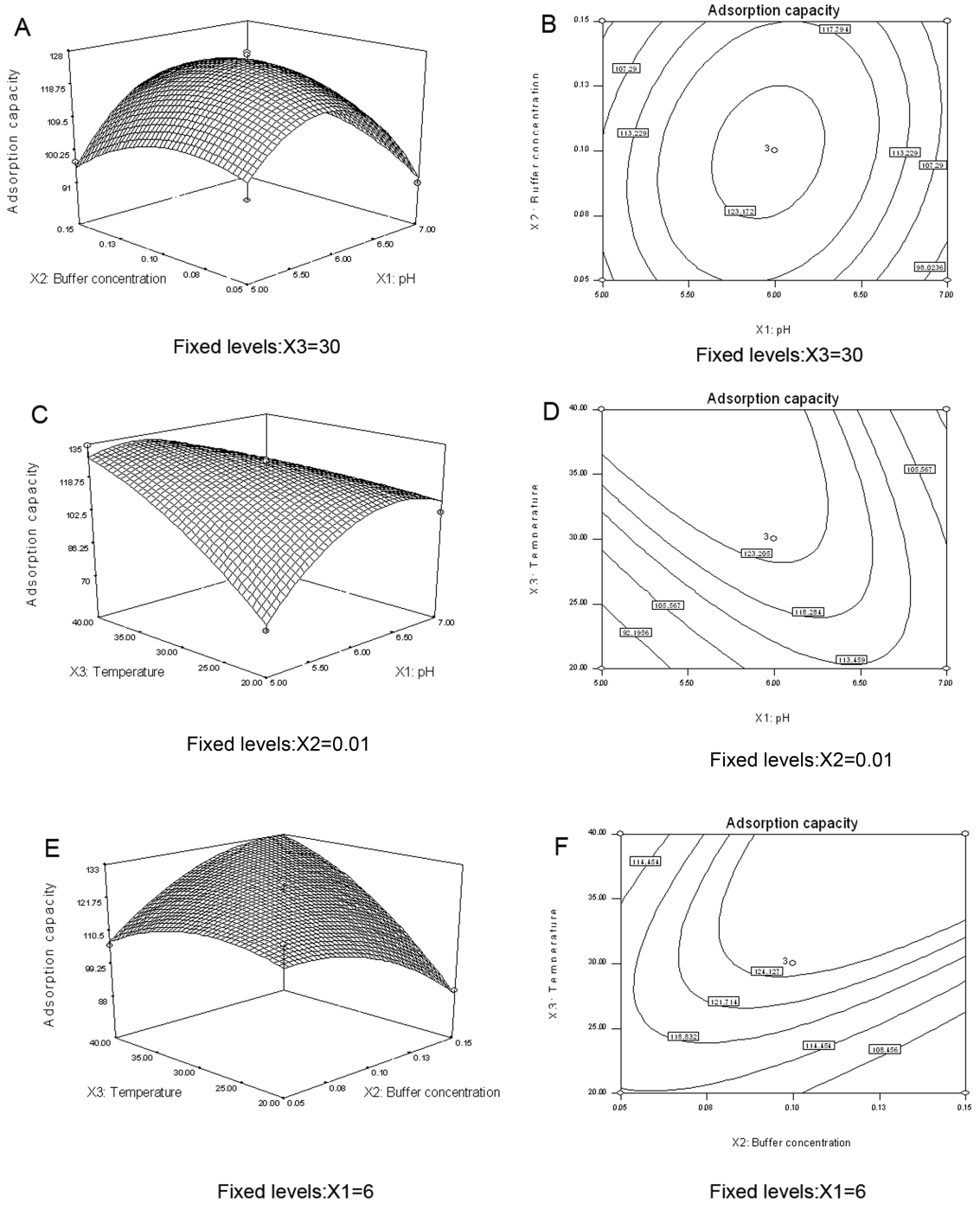

Figure 5. Response surface plots (**A**, **C**, and **E**) and contour plots (**B**, **D**, and **F**) showing the interactive effects of pH (X_1), PBS buffer concentration (X_2), and temperature (X_3) on the adsorption capacity of tea polyphenols (TP) into oat β-glucan. Experimental data and conditions are shown in **Table 2**.

It is confirmed that β-glucans from oat are able to adsorb TP. However, the influence of the adsorption of TP into oat β-glucan on the individual compound in vivo was not clear. Therefore, we will continue to investigate the influence of molecular interactions between TP and oat β-glucan on their biological effects.

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