# Adsorption of procyanidins B1 and B2 onto β-Glucan: adsorption isotherms and thermodynamics

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## Abstract

The interactions between phenolic compounds and dietary fibers have generally received considerable attention because these interactions have a variety of applications, for example, in the production of functional foods, in the pharmaceutical industry, or in the production of films used as packaging materials for food. The aim of this study was to investigate the interactions between two flavan-3-ols: procyanidin B1 and procyanidin B2, and dietary fiber β-glucan. The adsorption process was studied at different temperatures (25, 37 and 45 °C) and different pH values (1.5, 5.5 and 10.0). The adsorption capacity of β-glucan for procyanidins ($q_e$) ranged from 44-489 mg g$^{-1}$, depending on temperature and pH, and was generally higher for procyanidin B2. At the temperatures tested, the lowest $q_e$ values for both procyanidins were obtained at 37 °C, while at the pH values used, the lowest $q_e$ values were obtained at pH 5.5. The experimental data were fitted by Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill isotherms. Analysis of the fit of the applied isotherms led to the conclusion that the adsorption process studied was physical in nature in the range of applied temperatures and pH values. The process was spontaneous and exothermic for both procyanidins.

**Keywords** Dietary fiber · Polyphenols · Adsorption · Procyanidins · Bioaccessibility

## 1 Introduction

Polyphenols are a large family of naturally occurring phenols that are abundant in plants. Their major groups are phenolic acids and flavonoids (of which flavanols and anthocyanins are the most abundant in foods). Polyphenols are the most abundant antioxidants in the human diet and play an important role in protecting body tissues from oxidative stress and associated diseases such as cancer, coronary heart disease,

and inflammation. Therefore, it is important to know the factors that control their bioavailability [21].

Once released from the food matrix in the digestive tract, polyphenols can interact with other food components such as dietary fibers [6]. Dietary fibers are resistant to digestion and absorption in the human small intestine and are partially or completely fermented in the large intestine [1]. Therefore, they can serve as carriers that transport adsorbed polyphenols to the lower parts of the digestive tract, where they can be released and potentially exert beneficial effects. This is the main reason why the interactions between polyphenols and various dietary fibers such as β-glucan [26], resin [4], and inulin and pectin [23] have been intensively studied. Delivery system dietary fiber-polyphenols can find applications in production of functional foods, or in the pharmaceutical industry. In pharmaceutical application colon-target polyphenol delivery system with dietary fiber can be used for treatment of symptoms of disease in colon and also to maintain the gut microbial homeostasis. Binding of polyphenols and dietary fiber can be used as pharmaceutical materials in different forms like capsules, tablets and other, in order to increase the colonic health benefits and also to show potentially synergistic prebiotic effects. In this way, the

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concentration of polyphenols in the colon can be increased, and polyphenols can show their potentially positive effects [22]. The formulation of dietary fiber and polyphenols can affect different factors like pH value. Therefore, it is important to study the interactions on different pH values and at different temperature.

One of the common approaches to study the interactions between polyphenol and a dietary fiber is to study the adsorption of the polyphenol onto the dietary fiber [18]. Adsorption of a polyphenol molecule to dietary fiber can be divided into several steps (Fig. 1): I) transport of the molecule from the bulk solution to the Nernst diffusion layer surrounding the fiber surface, II) diffusion of the molecule through the Nernst diffusion layer to the fiber surface, III) intra-fiber diffusion of the molecule within the fiber, and IV) adsorption of the molecule on the fiber surface [14].

The adsorption capacity $q_e$, i.e. the maximum amount of polyphenol molecules adsorbed on the fiber, can be determined at equilibrium. This value depends on the initial amount of the polyphenol used ($c_{initial}$). Various adsorption isotherms can be used to describe the system behavior, i.e., to fit the experimental data $q_e$ *vs.* $c_e$, which provide information about the nature of adsorption process (physical or chemical), the preferred number of adsorbed layers, or the maximum theoretical adsorption capacity [2, 19]. In addition, various factors such as the pH, temperature, and chemical structure of the substances can affect the adsorption process [13]. Thermodynamic parameters such as the standard Gibbs energy change $\Delta G°$, the standard enthalpy change $\Delta H°$, and the standard entropy change $\Delta S°$ can be determined for the adsorption process and indicate whether the process is spontaneous or nonspontaneous, exothermic or endothermic, or whether it exhibits increased or decreased randomness [19].

β-glucan is one of the common dietary fibers in certain cereals, mushrooms, seaweed, and yeast. It shows potential health benefits [15, 30] and is therefore an important dietary supplement [5]. Therefore, β-glucan frequently comes into contact with polyphenols during the digestive process. Previous studies have shown that phenolic compounds can bind to
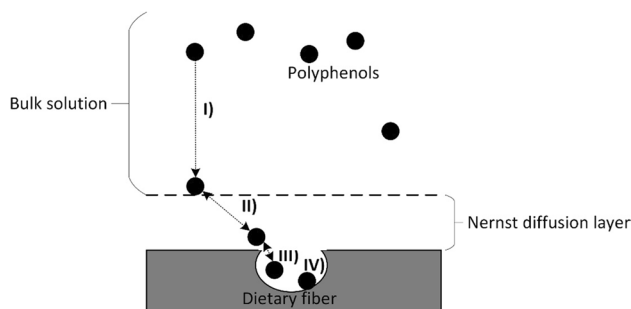
β-glucan [3, 25, 26, 28]. The interactions between β-glucan and various phenolic compounds such as quercetin [25], tea polyphenols [28], some flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and phenolic acids [26] have been studied. However, to the best of our knowledge, the adsorption of procyanidins to β-glucan has not been studied.

Procyanidins are polyphenols that belong to the group of flavan-3-ols and are very abundant in plant foods. They are present in fruits such as apples at concentrations of 34-41 mg per 100 g fresh weight [17]. They are also present in grape seeds (6.4 mg g$^{-1}$ fresh weight) [24] and cocoa (1.44-7.85 and 2.53-6.52 mg g$^{-1}$ dry weight for procyanidins B1 and C1, respectively) [16]. The aim of this work was to study the interactions of two procyanidins, procyanidin B1 and procyanidin B2, and β-glucan. Since we found few works that analyzed the interactions between phenolic compounds and β-glucan using adsorption isotherms [3, 13, 28], we decided to perform adsorption experiments and fit the obtained data with the adsorption isotherms of Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill. By analyzing the fit of the isotherm models used, we attempted to gain insight into the characteristics of this specific adsorption. In addition, the influence of pH and temperature of the solution on the adsorption process was also studied and the thermodynamic parameters of the process were determined.

## 2 Materials and methods

### 2.1 Reagents and solutions

The reagents used for the experiments were methanol (HPLC grade; J. T. Baker, Gliwice, Poland), procyanidin B1, procyanidin B2 (both ≥ 90%; Extrasynthese, Genay, France), sodium carbonate anhydrous (Gram-Mol, Zagreb, Croatia), Folin–Ciocalteu reagent, sodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, sodium bicarbonate (all four Kemika, Zagreb, Croatia), sodium hydroxide pellets, 37% hydrochloric acid (both Avantor, Arnhem, The Netherlands), and β-D-glucan from barley (≥ 95, Sigma-Aldrich, St. Louis, MO, USA).

Stock solutions of procyanidin B1 and procyanidin B2 were prepared at a concentration of 1000 mg L$^{-1}$ by dissolving the appropriate amount of the standards in distilled water. The solution of β-D-glucan was prepared at a concentration of 190 mg L$^{-1}$, also in distilled water; the solution was heated at 80°C for 15 minutes and then stored at 4°C. The solution with a pH of 1.5 was prepared by diluting 37% hydrochloric acid in distilled water. Phosphate buffer and carbonate-bicarbonate buffer were used to obtain solutions with pH of 5.5 and 10.0, respectively.



**Fig. 1** Adsorption process of a polyphenol to dietary fiber

## 2.2 Experimental design

Adsorptions of procyanidin B1 and procyanidin B2 on β-D-glucan were performed in two sets of experiments. The first set included adsorption experiments performed at three different temperatures: 25, 37 and 45°C, keeping pH of the solution at 5.5, while in the second set of experiments three different pH values were used: 1.5, 5.5, and 10.0, at a constant temperature of 37°C.

The total volume of solutions for the adsorption experiments was 500 μL. Each solution was prepared by pipetting an appropriate volume of β-D-glucan to reach the concentration of 5 mg L$^{-1}$ and an appropriate volume of procyanidin to reach the concentration of 25 mg L$^{-1}$ ($c_{initial}$). The rest of the volume was a buffer. The solutions were shaken with a vortex (Grant Bio, Cambridgeshire, England) and placed in an incubator (Incubator IN 30, Memmert, Schwabach, Germany) for 16 hours at one of the predefined temperatures. Subsequently, the solutions were filtered through a polyethersulfone membrane (Vivaspin 500, 100-500 μL Sartorius, Goettingen, Germany) in the Eppendorf Minispin centrifuge (Eppendorf, Hamburg, Germany). The concentration of non-adsorbed procyanidin ($c$, mg L$^{-1}$) was determined by the Folin-Ciocalteu spectrophotometric method. A control experiment without addition of β-D-glucan (the blank) was also performed. The concentration of adsorbed procyanidin ($c_{adsorbed}$, mg L$^{-1}$) was calculated as the difference between the concentrations of non-adsorbed procyanidin in the real ($c$, mg L$^{-1}$) and control ($c_o$, mg L$^{-1}$) experiments (Eq. (1)).

$$c_{adsorbed} = c_o - c \qquad (1)$$

The concentration of non-adsorbed procyanidin at equilibrium ($c_e$, mg L$^{-1}$) was calculated according to Eq. (2).

$$c_e = c_{initial} - c_{adsorbed} \qquad (2)$$

The amount of adsorbed procyanidins in mg per g of β-D-glucan ($q_e$) was calculated according to Eq. (3).

$$q_e = \frac{c_{adsorbed} \cdot V_m}{\gamma_a \cdot V_a} \qquad (3)$$

$V_m$ represents the total volume of solution (500·10$^{-6}$ L), $\gamma_a$ is the mass concentration of β-glucan (g L$^{-1}$), and $V_a$ is the volume of β-D-glucan (L) added into the solution.

To obtain data for adsorption isotherms, adsorption experiments were performed at five initial concentrations of procyanidins: 25, 50, 75, 100, and 150 mg L$^{-1}$. All experiments were performed in two parallel trials, with each concentration measured three times, resulting in a total number of measurements $n_{TOTAL} = 6$.

## 2.3 Folin-Ciocalteu spectrophotometric method

The concentrations of procyanidin B1 and procyanidin B2 in the adsorption experiments were determined using the Folin-Ciocalteu spectrophotometric method according to our previously developed and validated procedure [12]. An aliquot of 20 μL of the solution containing the analyte, 1580 μL distilled water, 100 μL Folin-Ciocalteu reagent, and 300 μL Na$_2$CO$_3$ solution (200 g L$^{-1}$) were added to a glass tube. The resulting solution was homogenized in a vortex, incubated at 40 °C for 30 min, analyzed spectrophotometrically at 765 nm (UV-Vis spectrophotometer Shimadzu, UV-1280, Kyoto, Japan), and compared with the blank solution. Calibration curves were prepared for each procyanidin in the concentration range of 1 to 500 mg L$^{-1}$.

## 2.4 Adsorption isotherms

The experimentally determined $q_e$ and $c_e$ data-pairs were fitted by non-linear forms of the adsorption isotherms of Langmuir, Freundlich, Temkin, Hill, and Dubinin-Radushkevich (Eqs. (4)-(5), respectively). The first four models are two-parameter models, where $K_L$ (L mg$^{-1}$) is the Langmuir equilibrium constant of adsorption, $K_F$, ((mg g$^{-1}$)·(L mg$^{-1}$)$^{1/n}$) is the Freundlich constant related to the relative adsorption capacity of β-glucan, $K_D$ (mg L$^{-1}$) is the Hill constant, $1/n$ is the intensity of adsorption, $q_m$ (mg g$^{-1}$) is the maximum monolayer adsorption capacity, $A$ (L g$^{-1}$) is the equilibrium binding constant related to the maximum binding energy, $b_T$ (J mol$^{-1}$) is the heat of adsorption, and $n_H$ is the Hill coefficient of cooperativity. The Dubinin-Radushkevich model (Eq. (8)) is a three-parameter model that includes the Dubinin-Radushkevich constant related to the adsorption capacity ($\beta$, mol$^2$ J$^{-2}$), the theoretical saturation capacity ($q_s$, mg g$^{-1}$), and the concentration of non-adsorbed procyanidins in the theoretically saturated solution ($c_s$, mg L$^{-1}$). We specified the parameter $c_s$ according to our data for $c_e$, because $c_s$ can assume very high values for which our experiment was not designed. $R$ and $T$ represent the gas constant (8.314 J K$^{-1}$ mol$^{-1}$) and absolute temperature (K), respectively.

$$q_e = \frac{q_m K_L c_e}{1 + K_L c_e} \qquad (4)$$

$$q_e = K_F c_e^{1/n} \qquad (5)$$

$$q_e = \frac{RT}{b_T} \ln(A c_e) \qquad (6)$$

$$q_e = \frac{q_m c_e^{n_H}}{K_D + c_e^{n_H}} \qquad (7)$$

**Table 1** The amount of procyanidins adsorbed per g of β-glucan ($q_e$, mg g$^{-1}$) at different temperatures (25, 37, and 45 °C) and pH values (1.5, 5.5, and 10.0)

| Procyanidin | $q_e$ (mg g$^{-1}$) | | |
|---|---|---|---|
| | **25 °C** | **37 °C** | **45 °C** |
| B1 | $121\pm3$[aA] | $44\pm1$[bC] | $77\pm3$[bB] |
| B2 | $135\pm29$[aB] | $101\pm18$[aB] | $438\pm14$[aA] |
| | **pH 1.5** | **pH 5.5** | **pH 10.0** |
| B1 | $195\pm5$[bB] | $44\pm1$[bC] | $468\pm1$[bA] |
| B2 | $404\pm7$[aB] | $101\pm18$[aC] | $489\pm4$[aA] |

$q_e$ values obtained from adsorption experiment with the initial concentration of procyanidins 25 mg L$^{-1}$

Adsorption studies at different temperatures were conducted at constant pH value of 5.5, while adsorption studies at different pH values were conducted at constant temperature of 37 °C

Different lower-case letters correspond to differences between procyanidins obtained with post-hoc Tukey test at $P < 0.05$ (differences in a column)

Different upper-case letters correspond to differences between temperature or pH obtained with post-hoc Tukey test at $P < 0.05$ (differences in a row)

$$q_e = q_s \exp\left(-\beta R^2 T^2 \left(\ln\frac{c_s}{c_e}\right)^2\right) \tag{8}$$

Once the Dubinin-Radushkevich isotherm was used to determine $\beta$, the mean free energy of adsorption, $E$ (J mol$^{-1}$), was calculated according to (Eq. (9)).

$$E = \frac{1}{\sqrt{2\beta}} \tag{9}$$

The percentages of deviation [20] between the theoretical values of maximum adsorption capacity ($q_m$, mg g$^{-1}$) and those that are experimentally determined when the initial concentration of procyanidins was 150 mg/L ($q_e$, mg g$^{-1}$) were calculated using Eq. (10). The theoretical adsorption capacities from Langmuir, Dubinin-Radushkevich and Hill isotherms were obtained by non-linear forms of the adsorption isotherms. The theoretical adsorption capacities from Freudlich and Temkin isotherms were calculated for the initial procyanidin concentration of 150 mg L$^{-1}$ using the Freundlich and Temkin equations.

$$\% \text{ deviation} = \left(\frac{q_e - q_{\text{from model}}}{q_{\text{from model}}}\right) \cdot 100 \tag{10}$$

For the same data, root-mean-square error (*RMSE*) was calculated according to equation (11).

$$RMSE = \sqrt{\frac{\sum_i^n \left(q_{e,i} - q_{\text{from model},i}\right)^2}{n}} \tag{11}$$

## 2.5 Thermodynamics of the adsorption

Thermodynamic parameters such as the standard Gibbs energy $\Delta G°$, the standard enthalpy $\Delta H°$, and the standard entropy $\Delta S°$ of the adsorption were calculated. The $\Delta G°$ was calculated according to Eq. (12):

$$\Delta G° = -RT\ln K_L \tag{12}$$

where the Langmuir constant $K_L$ is expressed in L mol$^{-1}$. The $\Delta H°$ and $\Delta S°$ were calculated from the Van't Hoff equation [11] (Eq. (13)).

$$\ln K_L = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R} \tag{13}$$

## 2.6 Statistical analysis

The MS Excel tool Solver (Redmond, Washington, USA) was used to preform non-linear regression analysis. The sum of squared errors (SSE) was calculated according to the Eq. (14), where $m$ is the number of initial concentrations used in the adsorption experiments, $c_{e,i}$ and $q_{e,i}$ are the means of the measured $c_e$ and $q_e$ values for the $i^{th}$ initial concentration,

**Fig. 2** Chemical structures of: **a**) procyanidin B1, and **b**) procyanidin B2. The part of the molecular structure that distinguishes procyanidin B1 from procyanidin B2 is circled

a)　　　　　　　　　b)
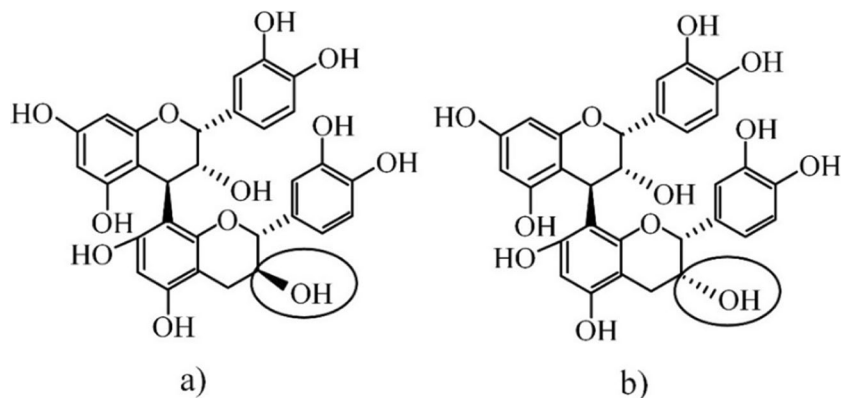
$f(c_e, a, b)$ is the non-linear model function with generic parameters $a$ and $b$, and $n_i$ is number of data points for the $i^{th}$ initial concentration. The standard error of nonlinear regression, $S$, was calculated according to Eq. (15), where $N$ is the total number of initial concentration cases and $k = 1, 2$ or $3$ is the number of parameters to be determined.

$$\text{SSE} = \sum_{i=1}^{m} n_i \left[ q_{e,i} - f(c_{e,i}, a, b) \right]^2 \quad (14)$$

$$S = \sqrt{\frac{SSE}{N - k}} \quad (15)$$

All these calculations were done in MS Excel (Redmond, Washington, USA).

One-way analysis of variance with post hoc Tukey test was performed in Minitab Statistical Software (Minitab LLC, State College, Pennsylvania, USA).

## 3 Results and discussion

### 3.1 The influence of temperature and pH value on the adsorption process

To see the influence of temperature and pH on the amount of procyanidins adsorbed onto β-glucan ($q_e$, mg g$^{-1}$), the $q_e$ was first studied with the initial concentration of procyanidins of 25 mg L$^{-1}$. The obtained $q_e$ values are in range 44-489 mg g$^{-1}$ (Table 1), which are results similar to those reported by Gao et al. [3] for the adsorption of tea polyphenols onto β-glucan (156 to 405 mg g$^{-1}$).

Adsorption studies at different temperatures shown that each procyanidin adsorbed differently at the studied temperatures (differences in a row). Statistically, the adsorption capacity of procyanidin B1 was significantly different at all three temperatures. Procyanidin B2 adsorbed similarly at 25 and 37 °C, and showed higher $q_e$ at 45 °C. These fluctuations of $q_e$ values with temperature can be connected to bond formation [7]. Hydrophobic interactions have endothermic nature and accordingly favor an increase in the temperature. H-bond creation, on the other hand, is an exothermic process and if raising the temperature causes a decreasing adsorption, this may indicate the creation of H-bonds. It is not completely clear whether the bonds between procyanidins and β-glucan are H-bonds or hydrophobic interactions. H-bonds can be created between the OH groups of procyanidins and β-glucan, while the aromatic ring on procyanidin molecules might be responsible for the hydrophobic interactions. The creation of non-covalent interactions (like H-bonds and hydrophobic interactions) are in accordance with observations from other studies [6], [8]. Moreover, the amounts of

**Table 2** Predicted parameters of Langmuir, Freundlich, Dubinin Radushkevich, Temkin, and Hill adsorption isotherms at 25, 37, and 45 °C and constant pH value of 5.5

| Procyanidin | $t$ (°C) | Langmuir $q_m$ (mg g$^{-1}$) | $K_L$ (L mg$^{-1}$) | $S$ | Freundlich $1/n$ | $K_F$ (mg g$^{-1}$)·(L mg$^{-1}$)$^{1/n}$ | $S$ | Dubinin–Radushkevich $q_s$ (mg g$^{-1}$) | $E$ (J mol$^{-1}$) | $S$ | Temkin $b_T$ (J mol$^{-1}$) | $A$ (L g$^{-1}$) | $S$ | Hill $q_m$ (mg g$^{-1}$) | $n_H$ | $K_D$ (mg L$^{-1}$) | $S$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 25 | 350 | 0.15 | 25 | 0.65 | 14 | 12 | 342 | 6000 | 19 | 65 | 70 | 25 | 331 | 0.62 | 8 | 29 |
| B2 |    | 583 | 0.18 | 36 | 0.82 | 10 | 24 | 562 | 5898 | 37 | 40 | 43 | 56 | 609 | 0.92 | 20 | 49 |
| B1 | 37 | 615 | 0.10 | 56 | 0.80 | 11 | 39 | 598 | 4821 | 52 | 35 | 35 | 87 | 590 | 0.81 | 5 | 10 |
| B2 |    | 629 | 0.10 | 96 | 0.78 | 13 | 72 | 638 | 5981 | 95 | 35 | 38 | 13 | 631 | 0.93 | 35 | 97 |
| B1 | 45 | 680 | 0.08 | 66 | 0.75 | 16 | 65 | 670 | 4984 | 60 | 33 | 30 | 78 | 660 | 0.68 | 10 | 79 |
| B2 |    | 720 | 0.09 | 26 | 0.88 | 9.5 | 7 | 682 | 5839 | 26 | 24 | 5 | 25 | 673 | 0.75 | 5 | 26 |

adsorbent adsorbed were the lowest at 37°C for both procyanidins, which is the temperature of the human body. This might have an importance for the adsorption of procyanidins on the β-glucan in the digestive tract.

Adsorption studies at different pH values gave highest $q_e$ values at pH 10.0, for both procyanidins tested (Table 1). Flavan-3-ols are weak acids with p$K_a$ values in the range 9-10 (Le Bourvellec et al., 2004). Considering specifically procyanidins B1 and B2, we found reported p$K_a$ values of 8.69 (ContaminantDB, Version 1.0, Wishart Research Group, Edmonton, Canada; available online at: https://contaminantdb.ca/) for both substances. At pH values lower than p$K_a$, procyanidins B1 and B2 exist mostly in a non-dissociated form, while at pH values above p$K_a$, dissociated form dominates. Therefore, at pH 10.0, procyanidins B1 and B2 are dominantly dissociated which might be a related with the highest adsorption capacities obtained for both procyanidins, indicating that dissociated form has stronger interactions with β-glucan than the non-dissociated form. The influence of the degree of flavan-3-ol dissociation (depending on pH) on the adsorption process was also found by other authors [9], [29].

Furthermore, procyanidins B1 and B2 were adsorbed in different amounts onto β-glucan (Table 1). At each temperature procyanidin B2 had higher $q_e$ than procyanidin B1, and this difference was statistically significant at 37 and 45 °C (differences in columns). Likewise, procyanidin B2

showed significantly higher $q_e$ than procyanidin B1 at all tested pH values (differences in columns). Procyanidins differ by their constitutive units, their interflavanic linkages, and their degree of polymerization [27]. Interactions of procyanidins and dietary fibers can be influenced by structure, molar mass, conformation, or interflavanic bonds [10]. With increased number of hydroxyl groups, aromatic rings, molar mass and the higher the degree of polymerization of procyanidins, the affinity for adsorption of procyanidins onto pectin increase [10]. Due to the higher number of hydroxyl groups and aryl rings, a higher number of hydrogen bonds and hydrophobic interaction occurs. Procyanidin B1 and procyanidin B2 have the same number of hydroxyl groups and molar mass. It is possible that this difference in adsorption capacity is due to the slight divergence in the chemical structure of the two procyanidins tested, as procyanidin B1 and procyanidin B2 differ in the spatial arrangement of one OH group (Fig. 2) [26, 29].

### 3.2 Application of adsorption isotherms

In the next stage of this study, the adsorption experiments were conducted with different initial procyanidin concentrations from 25 to 150 mg L$^{-1}$. The experiments were divided in two sets: the first set included adsorption experiments at different temperatures (25, 37, and 45 °C) while keeping the pH constant (5.5) and the second set had experiments



**Fig. 3** The relationships between the apparent Temkin adsorption parameters ($b_T$ and $A$) and the experimentally determined adsorption capacities, $q_e$, for both procyanidins. The adsorption capacities were obtained in the experiment with the initial concentration of procyanidins of 150 mg/L. The gray and white circles represent the data for procyanidin B1 and procyanidin B2, respectively. Cases **a**) and **b**) refer to the first set of experiments in which different temperatures were applied while the pH of the solution was maintained at 5.5. Cases **c**) and **d**) refer to the second set of experiments in which different pH values were applied while the temperature was kept constant at 37 °C
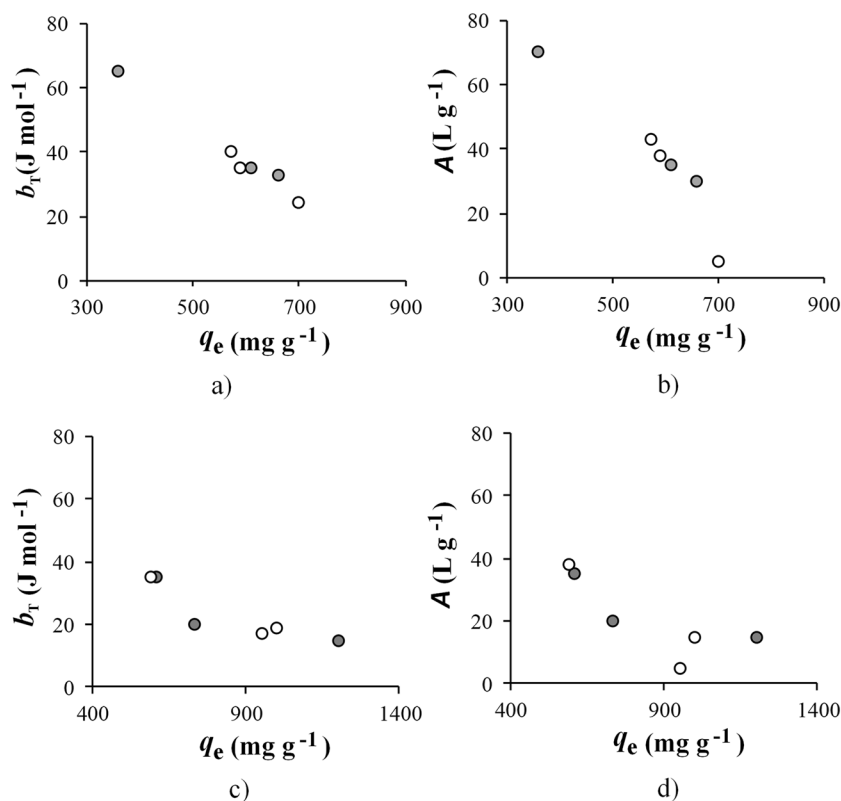
at different pH values (1.5, 5.5, and 10.0) but at constant temperature (37 °C). Nonlinear forms of Langmuir, Freundlich, Temkin, Hill, and Dubinin-Radushkevich adsorption isotherms (Eqs. (4)-(5)) were used to fit the experimental data ($c_e$ vs. $q_e$).

Table 2 summarizes determined values of parameters of the isotherm models for the first set of experiments. The apparent maximum adsorption/saturation capacities of β-glucan were in range from 331 to 720 mg g$^{-1}$ ($q_m$ and $q_s$ values). The Freundlich parameter $1/n$ was lower than 1 which indicates favoring of the adsorption at all three applied temperatures. Determined values of free energy of adsorption ($E$) were lower than 8000 J mol$^{-1}$, indicating the presence of physical interactions between procyanidins and β-glucan at all three temperatures, like the formation of H-bonds, hydrophobic interactions or Van der Waals forces. The Hill parameter $n_H$ was lower than 1 for both procyanidins at all temperatures, implying a negative cooperation in the adsorption. A negative cooperation means that when a molecule of procyanidin is adsorbed onto the surface of β-glucan, the affinity for other molecules decreases. Figure 3a and b presents Temkin parameters $b_T$ and $A$ in relation to $q_e$. Both parameters decreased as $q_e$ increased, indicating that high values of maximum adsorption capacities ($q_e$) could be the result of the lower apparent adsorption heat and binding energy.

Parameters of the isotherm models for the second set of experiments are given in Table 3. The predicted $q_m$ and $q_s$ values were in the range 590-1230 mg g$^{-1}$. The analysis showed favoring of the adsorption at all pH values ($1/n <$ 1), accompanied by the formation of physical bonds between procyanidins and β-glucan ($E < 8000$ J mol$^{-1}$). Once again, negative cooperation during the adsorption is detected ($n_H <$ 1). The parameters $b_T$ and $A$ decreased as $q_e$ increased which indicating that high values of maximum adsorption capacities could be the result of the lower apparent adsorption heat and binding energy (Fig. 3c and d).

Finally, to see the matching of predicted and experimentally determined values, the $q_m$ values from the Langmuir and Hill models, and the $q_s$ from the Dubinin-Radushkevich model were compared with $q_e$ values experimentally determined from experiment with the highest initial concentration of procyanidins (150 mg L$^{-1}$). As shown by the Figs. 4 and 5, the compared data follows the ideal scenario, i.e. the straight line $y = x$, very well. Further, root-mean-square error (RMSE) was calculated as a measure of the deviation of the experimental values from the values obtained by the isotherm models. The data for both procyanidins were included in the calculation of the RMSE value. Calculated RMSE values were not higher than 50.06 mg g$^{-1}$ indicating the suitability of the applied isotherm models. In addition, the percentage deviations between the predicted and experimentally determined adsorption capacities were calculated (Fig. 6). The deviations varied from -6.2% to

**Table 3** Predicted parameters of Langmuir, Freundlich, Dubinin Radushkevich, Temkin, and Hill adsorption isotherms at pH values 1.5, 5.5, and 10.0 and constant temperature of 37 °C

| Procyanidin | pH | Langmuir | | | Freundlich | | | Dubinin-Radushkevich | | | Temkin | | | Hill | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $q_m$ (mg g$^{-1}$) | $K_L$ (L mg$^{-1}$) | $S$ | $K_F$ (mg g$^{-1}$)(L mg$^{-1}$)$^{1/n}$ | $1/n$ | $S$ | $q_s$ (mg g$^{-1}$) | $E$ (J mol$^{-1}$) | $S$ | $b_T$ (J mol$^{-1}$) | $A$ (L g$^{-1}$) | $S$ | $q_m$ (mg g$^{-1}$) | $n_H$ | $K_D$ (mg L$^{-1}$) | $S$ |
| B1 | 1.5 | 700 | 0.03 | 62 | 10 | 0.87 | 45 | 680 | 6466 | 77 | 28 | 20 | 79 | 740 | 0.82 | 16 | 75 |
| B2 | | 981 | 0.03 | 30 | 12 | 0.91 | 28 | 976 | 6511 | 29 | 19 | 15 | 32 | 983 | 0.85 | 10 | 30 |
| B1 | 5.5 | 615 | 0.10 | 56 | 11 | 0.80 | 39 | 598 | 4821 | 52 | 35 | 35 | 87 | 590 | 0.81 | 5 | 10 |
| B2 | | 629 | 0.10 | 96 | 13 | 0.78 | 72 | 638 | 5981 | 95 | 35 | 38 | 13 | 631 | 0.93 | 35 | 97 |
| B1 | 10.0 | 1230 | 0.05 | 29 | 24 | 0.80 | 27 | 1105 | 4767 | 38 | 16 | 15 | 19 | 1110 | 0.89 | 15 | 34 |
| B2 | | 950 | 0.02 | 61 | 18 | 0.82 | 40 | 962 | 6236 | 67 | 17 | 5 | 73 | 947 | 0.86 | 20 | 56 |
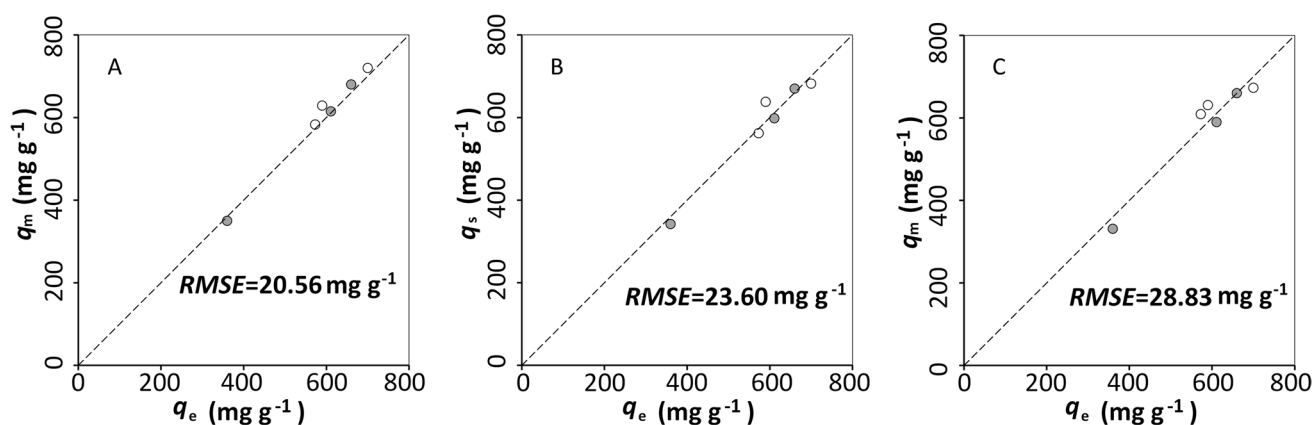
**Fig. 4** The comparison of experimentally determined adsorption capacities ($q_e$) and the apparent adsorption parameters: **A)** $q_m$ from the Langmuir isotherm, **B)** $q_s$ from the Dubinin-Radushkevich isotherm, and **C)** $q_m$ from the Hill isotherm. The data shown are for the first set of experiments (three different temperatures at constant solution pH of 5.5). The gray and white circles represent the data for procyanidin B1 and procyanidin B2, respectively. The dashed line is the expected correlation (the $y = x$ line). The data for both procyanidins were included in the calculation of the *RMSE* value
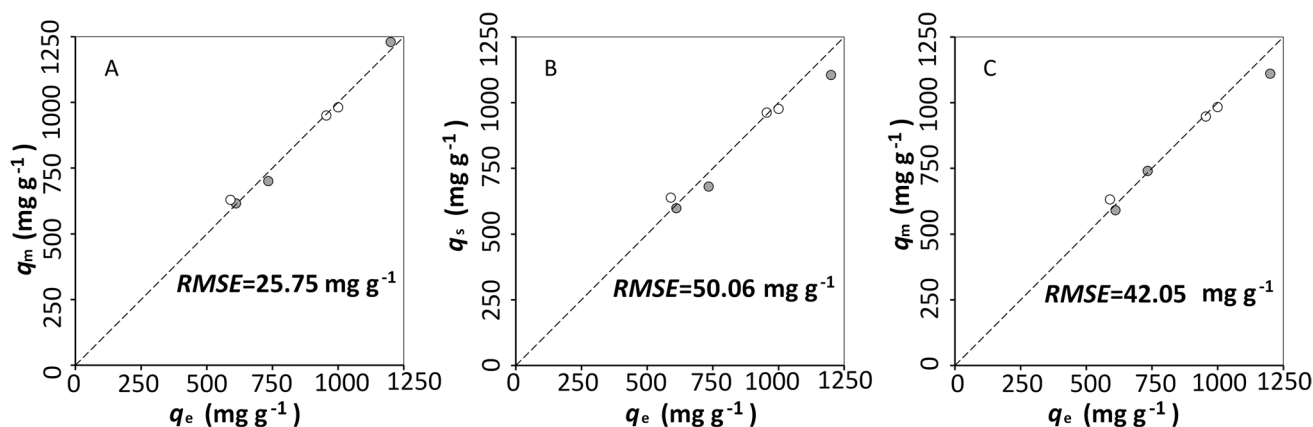


**Fig. 5** The comparison of experimentally determined adsorption capacities ($q_e$) and the apparent adsorption parameters: **A)** $q_m$ from the Langmuir isotherm, **B)** $q_s$ from the Dubinin-Radushkevich isotherm, and **C)** $q_m$ from the Hill isotherm. The data shown are for the second set of experiments (three different pH values at constant temperature of 37 °C). The gray and white circles represent the data for procyanidin B1 and procyanidin B2, respectively. The dashed line is the expected correlation (the $y = x$ line). The data for both procyanidins were included in the calculation of the *RMSE* value

4.8%, -1.4% to 11.3%, -8.2 to 3.3%, -11.5 to 1.6%, and -8.0% to 6.9%, for Langmuir, Dubinin-Radushkevich, Hill, Freundlich, and Temkin model, respectively. Obviously, all the applied adsorption models fit the experimental data well and were suitable for description of the adsorption process studied in this work.

## 3.3 Thermodynamics study of adsorption

The determined values of thermodynamic parameters of the adsorption processes are given in Table 4. For both procyanidins studied, $\Delta G°$ had negative values at all temperatures applied, indicating that adsorption on β-glucan was a spontaneous process. $\Delta H°$ and $\Delta S°$ were obtained from the Van't Hoff equation which fitted the data with $R^2$ of 0.9989 and 0.9390 for procyanidin B1 and procyanidin B2, respectively. The obtained positive values of $\Delta S°$ supported an increased randomness in the interactions of procyanidins B1 and B2 with β-glucan. The $\Delta H°$ had negative values, indicating the adsorptions of both procyanidins are exothermic.

**Fig. 6** The percentage deviation between adsorption capacities determined with adsorption isotherms (Langmuir (L), Dubinin-Radushkevich (DR), Hill (H), Freundlich (F) and Temkin (T)) and experimentally obtained adsorption capacities ($q_e$) for both procyanidins at: **a**) different temperatures, and **b**) different pH values
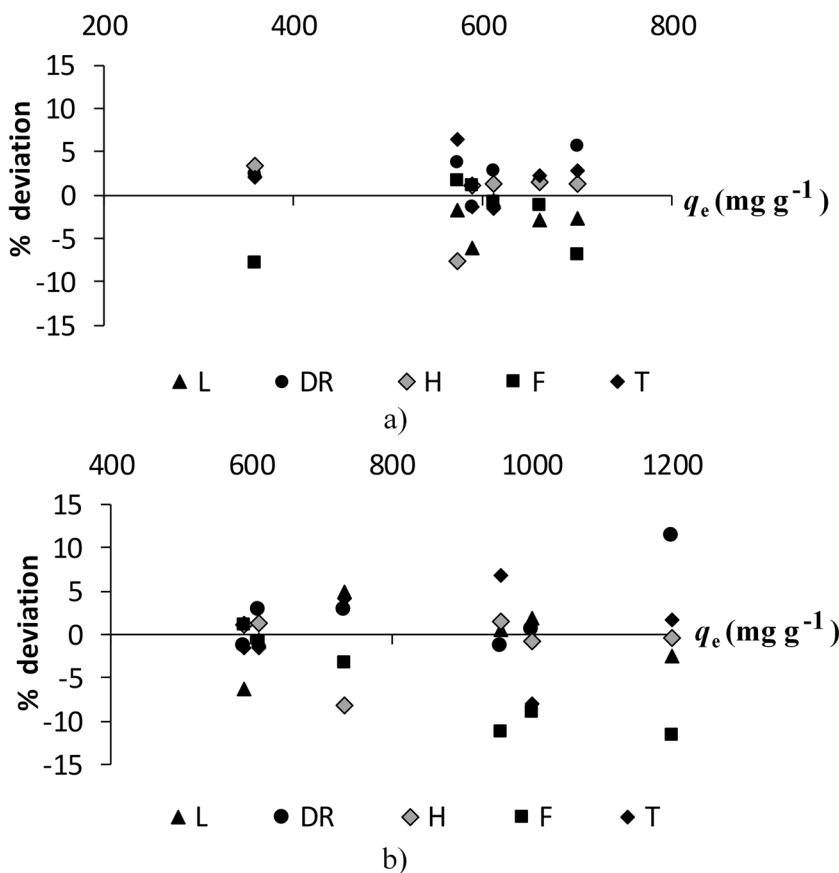


**Table 4** Thermodynamic parameters for the adsorptions of procyanidins B1 and B2 on β-glucan

| Procyanidin | $\Delta G°$(J mol$^{-1}$) | | | $\Delta H°$ (J mol$^{-1}$) | $\Delta S$ (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|
| | 25 °C | 37 °C | 45 °C | | |
| B1 | -28186 | -28275 | -28419 | -24898 | 11 |
| B2 | -28639 | -28276 | -28727 | -28293 | 1 |

## 4 Conclusions

This study showed that procyanidins B1 and B2 interact with β-glucan. The adsorption capacity is between 44-489 mg g$^{-1}$ depending on the temperature and pH value. At the temperature of the human body (37°C), the amount of procyanidins that can be adsorbed on β-glucan is somewhat lower than at 25 and 45°C. Considering the influence of pH value, the lowest $q_e$ was obtained at pH 5.5. Higher adsorption capacity was obtained for procyanidin B2. As procyanidins B1 and B2 differs only in spatial arrangement of one OH group, it seems very likely that this was the reason for higher adsorption capacity in case of procyanidin B2. The application of five adsorption isotherms gave additional information on the adsorption process. The adsorption process was favored for both procyanidins. It was assumed that non-covalent interactions like H-bonds and hydrophobic interactions, occur between the studied procyanidins and β-glucan. Adsorption of the procyanidins on β-glucan decreases the affinity of β-glucan toward other adsorbates. Relatively high maximum adsorption capacities could be the result of the lower apparent adsorption heat and binding energy. Thermodynamic parameters showed that the adsorption process was spontaneous and exothermic for both procyanidins studied.

The study of interactions between procyanidins B1 and B2, as common antioxidants in human diet, and β-glucan as common dietary fiber is important at it helps in revealing of the fate of procyanidins in digestive tract and might help in achieving procyanidins' more efficient action. Furthermore, such kind of information can be beneficial for pharmaceutical industry while creating various dietary supplements containing together β-glucan and procyanidins. Future study can be focused on investigating the application of the adsorption process to porous liquids.

## Declarations

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