Interactions of Phenolic Acids and β-Glucan: Studies of Adsorption Isotherms and Thermodynamics

P. Matić,[a,*] Š. Ukić,[b] and L. Jakobek[a]

[a]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Department of Applied Chemistry and Ecology, Franje Kuhača 18, HR 31000 Osijek, Croatia

[b]University of Zagreb, Faculty of Chemical Engineering and Technology, Department of Analytical Chemistry, Marulićev trg 19, HR 10000 Zagreb, Croatia

doi: https://doi.org/10.15255/CABEQ.2020.1902

Original scientific paper
Received: December 1, 2020
Accepted: June 30, 2021

Interactions between polyphenols and dietary fiber might play an important role in polyphenol bioactivities. These interactions can be studied through adsorption processes. The aim of this study was to investigate the adsorption of phenolic acids (*p*-coumaric acid, caffeic acid, chlorogenic acid) onto dietary fiber – β-glucan. Adsorption was carried out at different temperatures (25, 37, and 45 °C) and pH values (1.5, 5.5, and 10). Non-linear isotherm adsorption models (Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, Hill) were applied to analyze the data. Experimentally determined adsorption capacities showed some fluctuations with temperature and pH. Adsorption isotherms modelled the experimentally determined adsorption capacities well. According to isotherm parameters, it can be suggested that the adsorption was a physical process with non-covalent bonding at all temperatures and pH. Thermodynamic parameters showed that the adsorption was spontaneous (except for chlorogenic acid) and exothermic. Adsorption isotherms and thermodynamics gave useful information about phenolic acid – β-glucan interactions.

Keywords:
adsorption mechanism, phenolic acids, dietary fiber, adsorption models

Introduction

Polyphenols are secondary plant metabolites that have shown numerous positive bioactivities[1–4]. More recent studies have also shown that they can interact with macromolecules like proteins, carbohydrates, and lipids in the digestive tract, which can affect their bioactivities[5–7]. Namely, interactions with other compounds in the digestive tract can affect polyphenol accessibility for absorption (bioaccessibility), the actual amount that is being absorbed (bioavailability), and therefore their bioactivities[5]. For example, dietary fibers can interact with polyphenols, "carry" them to the lower parts of the digestive tract[8] and by doing so, possibly influence their bioactivities. That is why interactions between dietary fibers and polyphenols are in the focus of present studies.

To obtain more information about polyphenol and dietary fiber interactions, the adsorption process between polyphenols and dietary fibers has to be studied. Adsorption is a process in which molecules from the solution (adsorbate) adsorb onto the surface of the adsorbent[9]. The explanation of the adsorption process includes various aspects of the transportation of adsorbate, like transport of the adsorbate from the solution to the adsorbent surface, adsorbate diffusion across adsorbent liquid film, intraparticle diffusion, and adsorption and desorption from the surface of the adsorbent[10]. After reaching the adsorption equilibrium, data about the adsorption process can be obtained (the amount of adsorbate adsorbed onto the adsorbent and the concentration that remains unadsorbed in the solution at constant temperature), which can be modelled with equations called adsorption isotherms[9,11,12]. Some frequently used adsorption isotherm models are Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill models. These models can provide various information about the adsorption process, such as the physical or chemical nature of the adsorption, or the maximal predicted, theoretical adsorption capacity[9,11].

One of the natural dietary fibers which can be found in cereals, mushrooms, seaweed, and yeast is β-glucan[13]. Earlier studies have shown that β-glucan can interact with polyphenols[14–18]. Interactions be-

*Corresponding author: E-mail: petra.matic@ptfos.hr

tween various polyphenols and β-glucan[17], tea polyphenols and β-glucan[14,18], quercetin and β-glucan[16], vanillin-inspired phenolic derivatives and β-glucan[15] have been investigated. Only some of these studies have analyzed the experimental data with the use of adsorption isotherms[14,18]. However, interactions between polyphenols and β-glucan are yet to be completely understood. They can be affected by environmental conditions, like pH and temperature or by the chemical structure of polyphenols or β-glucan. In order to understand how polyphenols bond to β-glucan, obtain the adsorption capacity, and to understand how environmental conditions affect interactions, further studies are necessary. Moreover, by studying polyphenol – β-glucan interactions, additional insight into dietary fiber – polyphenol interactions in general can be obtained.

The objective of the present research was to study interactions between β-glucan and polyphenols that belong to the phenolic acid subclass, through the adsorption process. To understand the influence of the environment on the adsorption process, the adsorptions were conducted at three different temperatures and at three pH values. Results were analyzed with non-linear isotherm models (Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill) to help understand the adsorption process. Prediction of the adsorption process that is more precise can be obtained by applying non-linear models, rather than linear models, to the experimental results[19].

The thermodynamic parameters of adsorption were calculated (standard reaction Gibbs energy, standard reaction enthalpy, and standard reaction entropy).

Materials and methods

Reagents and solutions

The methanol (HPLC grade) was purchased from J. T. Baker (Netherlands). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) (*p*-coumaric acid – C9008 ≥ 98 %, caffeic acid – C0625, ≥ 98 %, chlorogenic acid – C3878 ≥ 95 %, β-D-glucan from barley – G6513 ≥ 95 %). Sodium carbonate anhydrous and potassium chloride were purchased from Gram-Mol (Zagreb, Croatia). Folin – Ciocalteu reagent, sodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, and sodium bicarbonate were purchased from Kemika (Zagreb, Croatia). Sodium hydroxide pellets and hydrochloric acid (37 %) were purchased from Avantor (Arnhem, Netherlands). Stock solutions of phenolic acid standards were prepared in methanol in the concentration of 1000 mg L^{-1} (*p*-coumaric acid, caffeic acid, chlorogenic acid). The β-glucan stock solution was prepared in the concentration of 190 mg L^{-1} in distilled water, heated for 15 min at 80 °C, and stored in a refrigerator at 4 °C. The solution of pH 1.5 was prepared using hydrochloric acid-potassium chloride (0.1 M). The buffer of pH 5.5 was a phosphate buffer, and of pH 10.0 a carbonate buffer (0.1 M).

Adsorption between phenolic acids and β-glucan

Adsorption process was performed between phenolic acids (*p*-coumaric acid, caffeic acid and chlorogenic acid) (Fig. 1) and barley β-D-glucan in single solution. Adsorptions between phenolic acids and β-glucan were carried out according to procedure from earlier study with some modifications[17]. The model solution in a plastic tube contained β-glucan as the adsorbent (5 mg L^{-1}), a phenolic acid as the adsorbate (25 mg L^{-1}), and buffer solution. The total volume of the model solution was 500 μL. Adsorption was performed for 16 h. Concentrations of phenolic acids and β-glucan were 25 and 5 mg L^{-1}, respectively. Adsorptions were conducted at three different temperatures (25 °C, 37 °C and 45 °C, pH adjusted at 5.5). Temperature of 37 °C was chosen since it is a human body temperature. Adsorption at two additional temperatures 25, and 45 °C, enabled us to calculate thermodynamic parameters. To cover higher pH range, adsorption was conducted at pH 1.5, 5.5 and 10, with temperature adjusted to 37 °C. Phenolic acid concentrations in plastic tubes were initial concentrations ($c_{initial}$, mg L^{-1}). Solutions were vortexed (Grant Bio, Cambridgeshire, England) and put in an incubator (Incubator IN 30, Memmert, Schwabach, Germany). After reaching equilibrium (obtained experimentally, 16 h), model solutions were centrifuged (Eppendorf Minispin centrifuge, Eppendorf, Hamburg, Germany) through polyethersulfon membrane (Vivaspin 500,

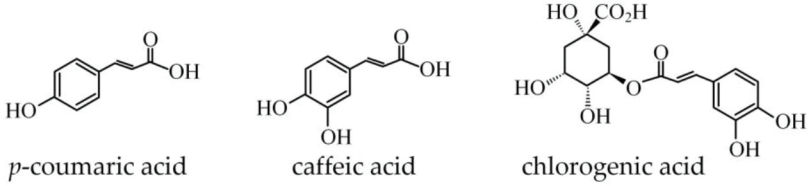

Fig. 1 – *Chemical structure of phenolic acids*

100–500 μL Sartorius, Goettingen, Germany). In the solution that passed on the other side of membrane, phenolic acids were determined using the spectrophotometric Folin-Ciocalteu method (c, mg L^{-1}). The same adsorption with the same conditions was conducted without β-glucan, which represented a blank experiment. Phenolic acids that passed through the membrane in the blank experiment, were determined using spectrophotometric Folin-Ciocalteu method validated in our laboratory for each compound in single solution[20] (c_0, mg L^{-1}). The concentration of adsorbed phenolic acids ($c_{adsorbed}$, mg L^{-1}) was calculated as:

$$c_{adsorbed} = c_0 - c \text{ (mg L}^{-1}\text{)} \quad (1)$$

The concentration of unadsorbed phenolic acid at equilibrium (c_e, mg L^{-1}) was calculated as:

$$c_e = c_{initial} - c_{adsorbed} \text{ (mg L}^{-1}\text{)} \quad (2)$$

The q_e (amount of adsorbed phenolic acids in milligrams per gram of β-glucan) was calculated according to eq. (3):

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

where $c_{adsorbed}$ is the concentration of adsorbed phenolic acids (mg L^{-1}), V_m is the total volume of a model solution (L), γ_a is the β-glucan concentration (g L^{-1}), and V_a is the volume of model solution (L). The same adsorption experiment was repeated with various initial phenolic acid concentrations (25, 50, 75, 100, and 150 mg L^{-1}) and β-glucan (5 mg L^{-1}).

Spectrophotometric Folin-Ciocalteu method

The phenolic acids in the adsorption experiment were determined using the Folin – Ciocalteu method[21] according to our earlier developed and validated procedure[20]. Aliquots of 20 μL of solution after adsorption, 1580 μL of distilled water, 100 μL of Folin – Ciocalteu reagent, and 300 μL of Na_2CO_3 (200 g L^{-1}) were added into a glass tube. The resulting solution was mixed in the vortex (Grant Bio, Cambridgeshire, England), incubated at 40 °C for 30 min (incubator Memmert IN 30, Schwabach, Germany), and then analyzed spectrophotometrically at 765 nm (UV-Vis spectrophotometer UV 2005, Selecta, Barcelona, Spain). A blank solution containing 20 μL of distilled water instead of solution after adsorption was used during the analysis. Calibration curves were constructed for each individual phenolic acid by using the same method, in the concentration range from 1 to 500 mg L^{-1}. The concentrations of phenolic acids after adsorption were determined (mg L^{-1}) using the constructed calibration curves.

Adsorption isotherms

The amount of phenolic acids in milligrams adsorbed per g of β-glucan (q_e) and the phenolic acid concentrations at equilibrium (c_e) were modelled using non-linear Langmuir (eq. 4), Freundlich (eq. 5), Dubinin-Radushkevich (eq. 6–9), Temkin (eq. 10), and Hill (eq. 11) adsorption isotherms[9,11,22–24].

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

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

Dubinin-Radushkevich
$$q_e = q_s \exp\left(-\beta\varepsilon^2\right) \quad (6)$$

$$\varepsilon = RT\ln\left(\frac{c_S}{c_e}\right) \quad (7)$$

$$E = \frac{1}{\sqrt{2\beta}} \quad (8)$$

Dubinin-Radushkevich becomes

$$q_e = q_s \exp\left(-\beta R^2 T^2 \left(\ln\frac{c_s}{c_e}\right)^2\right) \quad (9)$$

Temkin
$$q_e = \frac{RT}{b_T} \ln\left(A c_e\right) \quad (10)$$

Hill
$$q_e = \frac{q_m c_e^{n_H}}{K_D + c_e^{n_H}} \quad (11)$$

The q_e is the amount of phenolic acids adsorbed per gram of β-glucan at equilibrium state (mg g^{-1}), c_e is the phenolic acid concentration in the solution at equilibrium (mg L^{-1}), K_L is the Langmuir equilibrium constant of adsorption (L mg^{-1}) or apparent affinity constant, q_m is the theoretical maximum adsorption capacity of β-glucan (mg g^{-1}), K_F is the Freundlich constant indicative of the relative adsorption capacity of β-glucan ((mg g^{-1}) (L $mg^{-1})^{1/n}$)), $1/n$ is the intensity of adsorption, q_s is the theoretical isotherm saturation capacity (mg g^{-1}), β is a constant related to the adsorption capacity (mol^2 J^{-2}), ε is the Polanyi potential (J mol^{-1}), R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (K), E is the adsorption mean free energy (J mol^{-1}), c_s is the theoretical saturation concentration or solubility (mg L^{-1}), A is the equilibrium binding constant related to the maximum binding energy (L g^{-1}), apparent Temkin adsorption potential, b_T is the heat of adsorption (J mol^{-1}), n_H is the Hill cooperativity coefficient , and K_D is the Hill constant (mg L^{-1}).

Langmuir, Freundlich and Temkin equations are two-parameter equations and nonlinear modeling was used to determine parameters K_L and q_m from Langmuir, K_F and $1/n$ from Freundlich, and b_T and A from Temkin equation. Dubinin-Radushkevich isotherm is a three-parameter model in which q_s,

β and c_s can be determined with nonlinear modeling. The c_s (mg L^{-1}) represents a saturation concentration, a concentration of non-adsorbed phenolic acids in a theoretically saturated solution. Our experiment was not constructed to measure in such large theoretical concentrations, so the c_s values were predetermined according to our data for c_e. Due to predetermined c_s, Dubinin Radushkevich isotherm was used to determine two parameters, q_s and β. The β was finally used to calculate mean free energy of adsorption E (eq. 8). Finally, two parameters were reported, q_e and E. The Hill equation was finally used to determine parameters q_m, n_H and K_D.

Thermodynamic parameters

The standard reaction Gibbs energy (J mol^{-1}) was calculated according to eq. 12:

$$\Delta G^0 = -RT\ln K_L \tag{12}$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}), T is absolute temperature, and K_L is the Langmuir equilibrium constant of adsorption for neutral adsorbates or adsorbates with very weak charges[25] expressed in L mol^{-1}. The Langmuir constant K_L was recalculated to a dimensionless parameter[26]. Standard reaction enthalpy and standard reaction entropy were calculated from linear Van't Hoff equations[23,24] (eq. 13), which represents $\ln K_L$ *vs* $1/T$:

$$\ln K_L = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \tag{13}$$

Standard reaction enthalpy ΔH^0 was calculated from the slope ($-\Delta H^0/R$), and standard reaction entropy ΔS^0 from the intercept ($\Delta S^0/R$).

Statistical analysis

All adsorption experiments were conducted in two parallels, each concentration was measured three times (n = 6). All calculations were performed in MS Excel (Redmond, USA) software. Tool Solver in MS Excel was selected for non-linear regression analysis of adsorption isotherms. The sum of squared errors (SSE) of nonlinear least square regression was calculated according to eq. 14, where $c_{e,i}$ and $q_{e,i}$ are the means of the measured c_e and q_e values, $f(c_e, a, b)$ is the nonlinear model function with generic parameters a and b, and n_i is number of data points for the i^{th} initial concentration. Standard error of nonlinear regression was calculated according to eq. 15. (where N is the total number of observations, and k = 1, 2 or 3 is the number of parameters.

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

$$S = \sqrt{\frac{\mathrm{SSE}}{N-k}} \tag{15}$$

For statistical comparisons, a one-way analysis of variance with post hoc Tukey test was performed in Minitab (Pennsylvania, USA).

Results and discussion

Adsorption of phenolic acids onto β-glucan (influence of temperature and pH)

Table 1 shows the amount of phenolic acid adsorbed per mass of β-glucan (q_e, mg g^{-1}) determined in the experiment with initial phenolic acid concentration of 25 mg L^{-1}, at three different temperatures and three different pH values. The q_e values were in the range of 48 – 438 mg g^{-1}. It was difficult to compare these results with literature, since β-glucan can vary depending on the source (oats or barley), molecular weight[27,28], or even environmental factors during growth of barley[25], which can lead to great difference in adsorption. In addition, the ratio of polyphenol/β-glucan can affect q_e[14]. Thus, earlier studies have reported various values for q_e in adsorption onto oats β-glucan. Namely, standards of

Table 1 – *Amount of phenolic acids adsorbed per g of β-glucan (q_e, mg g^{-1}) at different temperatures (25, 37, 45 °C (pH 5.5)) and pH values (pH 1.5, 5.5, 10 (37 °C))*

Polyphenol	q_e* (mg g^{-1})		
	25 °C	37 °C	45 °C
p-coumaric acid	313±13[a]	313±0[a]	111±0[b]
caffeic acid	48±3[b]	193±10[b]	93±4[b]
chlorogenic acid	438±59[a]	185±12[b]	345±22[a]
	pH 1.5	pH 5.5	pH 10
p-coumaric acid	195±1[b]	313±0[a]	296±7[a]
caffeic acid	374±25[a]	193±10[b]	340±0[a]
chlorogenic acid	253±7[b]	185±12[b]	193±12[b]

	Tukey test**					
	Temperature			pH		
	25 °C	37 °C	45 °C	1.5	5.5	10
p-coumaric acid	a	a	b	b	a	a
caffeic acid	c	a	b	a	b	a
chlorogenic acid	a	b	a, b	a	b	a, b

q_e values obtained from adsorption experiment with the initial polyphenol concentrations 25 mg L^{-1}

*different letters in a column correspond to differences between polyphenols at the same temperature or pH (obtained with post-hoc Tukey test)

**different letters in a row correspond to differences between temperature or pH for each polyphenolic compound separately (obtained with post-hoc Tukey test)

polyphenols found in tea adsorbed onto oat β-glucan in the amount of 156 to 405 mg g^{-1} [14]. Tea polyphenols adsorbed onto β-glucan at different pH values up to 116 mg g^{-1} [18]. Some polyphenols adsorbed onto plant cell walls up to 600 mg g^{-1} [29,30], 1200 mg g^{-1} [7], 1000 mg g^{-1} [31]. The adsorption in these studies is similar to the results for adsorption of phenolic acids onto β-glucan in this study.

Furthermore, the q_e for each phenolic acid showed some significant fluctuations with temperature (Table 1). This was similar to earlier studies that had shown that temperature influences the adsorption of tea polyphenols[18] or (-)-epigallocatechin-3-gallate[14] onto oat β-glucan or the adsorption of procyanidins onto apple cell wall material[32]. Furthermore, some fluctuations in q_e with pH from 1.5 to 10 could also be seen for each compound (Table 1). This was similar to earlier studies that had shown that pH can influence the adsorption of (-)-epigallocatechin-3-gallate onto β-glucan in the range of pH 2 – 7[14] or tea polyphenols onto β-glucan in the range from pH 3 to 7[18]. Phenolic acids like caffeic and chlorogenic acid also showed pH dependence in the adsorption process onto food-grade resin[33].

To obtain a better insight into the adsorption process, the adsorption capacity was measured for more different initial concentrations of phenolic acids at three different temperatures (25, 37, and 45 °C, adjusted pH at 5.5), and three different pH values (pH 1.5, 5.5 and 10, adjusted temperature 37 °C). Those results are shown in c_e vs q_e diagrams (Fig. 2 and 3). At different temperatures, all three phenolic acids showed fluctuations with temperature (Fig. 2). All phenolic acids showed slightly higher adsorption capacity at 25 °C. With increasing temperature, the adsorption capacity decreased[32]. At different pH values, *p*-coumaric acid and chlorogenic acid showed somewhat higher adsorption capacity at pH 10 (Fig. 3). In addition, it was found in literature that adsorption capacity increased with increasing pH[14].

With the increasing concentration of phenolic acids, the adsorption capacity increased, but slight decrease in adsorption capacity was observed for concentration of 150 mg L^{-1} (Fig. 2 and 3). This could be connected with desorption that occurs in adsorption process[34]. At different temperatures, this could be seen for *p*-coumaric acid at 25 °C, caffeic acid at 25 and 37 °C, and chlorogenic acid at all temperatures (Fig. 2). At different pH values, this could be seen at pH 10 for *p*-coumaric acid, and at pH 1.5 and 10 for caffeic acid (Fig. 3).

The adsorption of *p*-coumaric acid, caffeic acid, and chlorogenic acid onto β-glucan showed some fluctuations with temperature. Adsorption capacity fluctuations with temperature can be connected to bond formation. It is known that hydrophobic interactions are an endothermic process[32,35] and accordingly, these interactions favor the increase in temperature. If the increase in temperature causes an increase in adsorption, it may indicate that mostly hydrophobic interactions are involved in the adsorption. H bond creation, on the other hand, is an exothermic process. Therefore, if the rising temperature causes decreasing adsorption, this may indicate the creation of H bonds[32,35]. Usually, both types of bonds exist in the interaction. The creation of different bonds could have affected the fluctuations of adsorption capacity with the temperature in our study. The creation of non-covalent bonds and interactions (like H bonds, Van der Waals forces and hydrophobic interactions) between phenolic acids and β-glucan in the temperature range from 25 to 45 °C, are in accordance with the predicted physical adsorption by adsorption isotherm models.

Furthermore, the influence of pH can be connected to chemical structure of phenolic acids, their functional groups, and the ratio of their deprotonated/protonated forms. Phenolic acids possess COOH groups as well as different number of OH groups. At lower pH, COOH and OH groups are in protonated (nonionic) forms. Protonated OH groups are important for the creation of H bonds at pH 1.5. As pH increases, the ratio of deprotonated/protonated functional groups (A^-/A) increases as well. This means that the attraction forces between dissociated forms of phenolic acids and β-glucan at higher pH can be stronger, which can explain the higher adsorption capacity at higher pH in some cases (for *p*-coumaric acid and chlorogenic acid). The presence of phenolic acids in different protonated or deprotonated forms could have affected the adsorption at different pH[14,17,36].

Adsorption isotherm models at different temperatures and pH values

The experimental results (c_e vs q_e) were analyzed with five different nonlinear adsorption isotherm equations: Langmuir, Freundlich, Dubinin-Radushkevich, Temkin and Hill. The goal was to fit experimental data well with those equations, to determine their parameters with least possible error, and to possibly describe the adsorption process with those parameters. Namely, from all of these five models, parameters can be obtained: K_L and q_m from Langmuir, K_F and $1/n$ from Freundlich, q_s and E from Dubinin-Radushkevich, b_T and A from Temkin, and q_m, n_H and K_D from Hill. These parameters can predict useful, theoretical information about adsorption, such as the apparent maximum predicted adsorption capacity/saturation of β-glucan (q_m and q_s), the apparent mean free energy (E) or whether there is a cooperativity in the adsorption between phenolic acids and β-glucan (n_H)[23].

Table 2 reports parameters of isotherm models at three temperatures (25, 37, 45 °C, pH 5.5). The parameters q_m from both Langmuir and Hill and q_s from Dubinin-Radushkevich can be used to gain insight into the model predicted apparent maximum adsorption capacities/saturation capacities of β-glucan[37,38]. The values of these parameters were from 518 to 778 mg g^{-1} at all temperatures, which is in the range of experimentally determined q_e values. The parameter $1/n$ presents the intensity of the adsorption. For all phenolic acids, the $1/n$ was lower than 1, which suggests that the adsorption was favored for all phenolic acids at all three temperatures. The parameter E represents apparent mean

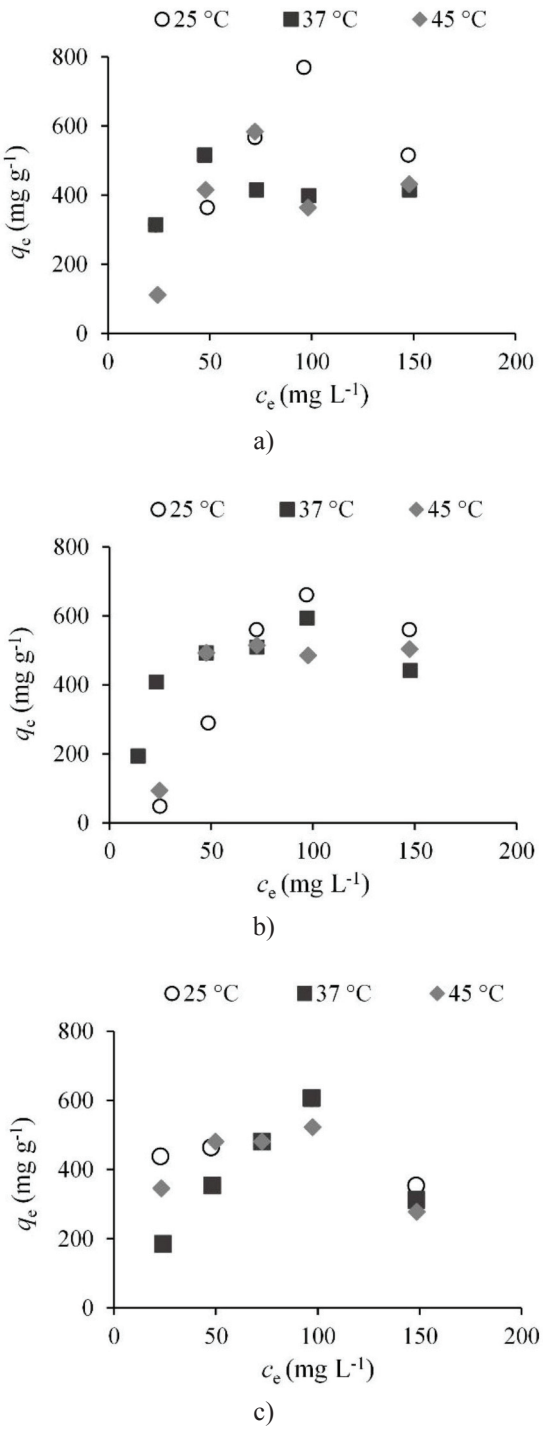

Fig. 2 – *Data for adsorption isotherms (q_e vs c_e diagrams) at 25, 37, and 45 °C (pH 5.5). Data shown are means ± standard deviation of means. a) p-coumaric acid; b) caffeic acid; c) chlorogenic acid.*

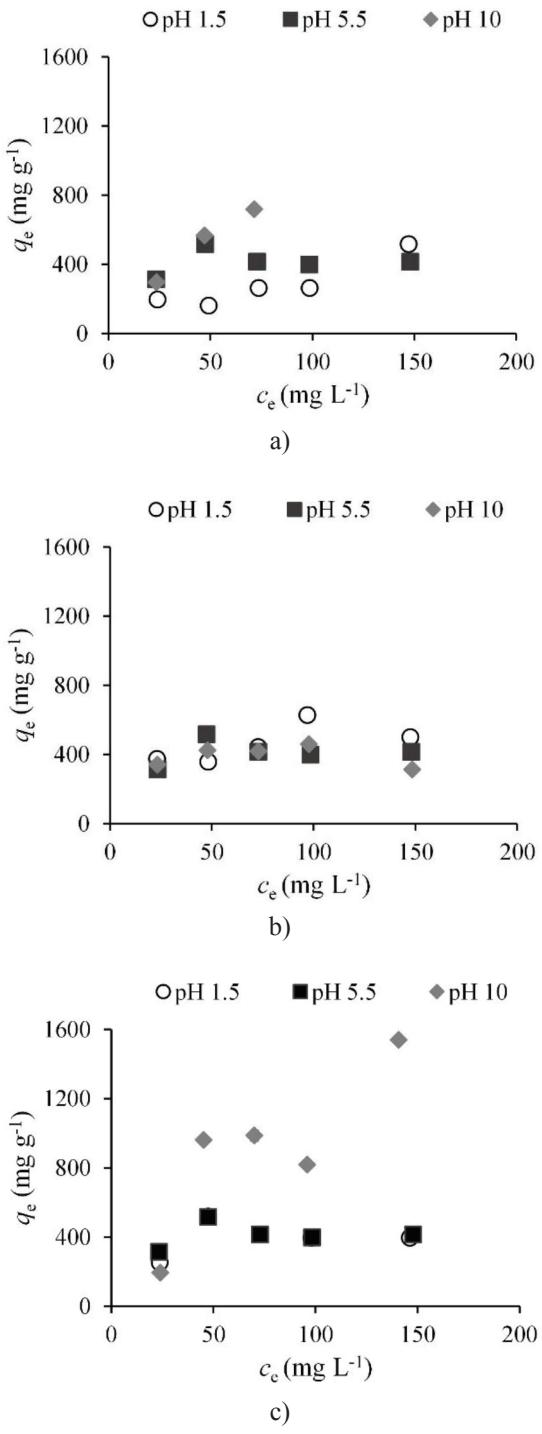

Fig. 3 – *Data for adsorption isotherms (q_e vs c_e diagrams) at pH 1.5, 5.5 and 10 (37 °C). Data shown are means ± standard deviation of means. a) p-coumaric acid; b) caffeic acid; c) chlorogenic acid.*

Table 2 – *Apparent, theoretical, predicted parameters of Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill adsorption isotherms at 25, 37, and 45 °C (pH 5.5)*

Phenolic acid	Langmuir			Freundlich			Dubinin-Radushkevich			Temkin			Hill			
	q_m (mg g^{-1})	K_L (L mg^{-1})	S	$1/n$	K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	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
25 °C																
p-coumaric acid	770	0.033	15	0.80	12	29	775	5245	35	40	90	24	778	0.89	25	22
caffeic acid	665	0.053	17	0.65	25	14	667	6000	15	45	120	19	699	0.75	25	21
chlorogenic acid	605	0.033	13	0.84	12	24	608	5898	16	49	135	13	609	0.92	20	9
37 °C																
p-coumaric acid	520	0.048	6	0.81	12	16	518	5778	10	61	178	8	522	0.75	10	5
caffeic acid	590	0.053	8	0.80	10	22	589	4821	21	50	140	9	590	0.81	5	10
chlorogenic acid	610	0.029	13	0.78	10	20	611	5981	15	47	122	15	613	0.93	35	18
45 °C																
p-coumaric acid	585	0.029	10	0.76	13	13	589	6966	12	68	158	11	590	0.92	20	13
caffeic acid	518	0.040	16	0.75	15	18	520	4984	24	60	150	20	522	0.68	10	19
chlorogenic acid	525	0.010	13	0.88	8	29	527	5839	22	70	250	24	530	0.75	15	15

S represents standard errors reported as values ·10^{-4}

Table 3 – *Apparent, theoretical, predicted parameters of Langmuir, Freundlich, Dubinin-Radushkevich, Temkin, and Hill adsorption isotherms at pH values 1.5, 5.5, 10 (37 °C)*

Phenolic acid	Langmuir			Freundlich			Dubinin-Radushkevich			Temkin			Hill			
	q_m (mg g^{-1})	K_L (L mg^{-1})	S	$1/n$	K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	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
1.5																
p-coumaric acid	520	0.020	9	0.78	10	6	522	5556	12	69	163	11	521	0.79	20	14
caffeic acid	626	0.67	10	0.87	10	21	628	6466	12	42	130	11	629	0.75	8	19
chlorogenic acid	520	0.10	8	0.74	15	14	521	6511	8	80	295	7	523	0.85	10	9
5.5																
p-coumaric acid	520	0.048	6	0.81	12	16	518	5778	10	61	178	8	522	0.75	10	5
caffeic acid	590	0.053	8	0.80	10	22	589	4821	21	50	140	9	590	0.81	5	10
chlorogenic acid	610	0.029	13	0.78	10	20	611	5981	15	47	122	15	613	0.93	35	18
10																
p-coumaric acid	720	0.045	8	0.88	17	3	725	6718	11	35	65	9	722	0.91	15	10
caffeic acid	462	0.033	14	0.80	10	23	460	4767	24	82	482	14	461	0.89	15	14
chlorogenic acid	1540	0.022	34	0.82	25	5	1542	6236	4	20	30	4	1545	0.86	30	4

S represents standard errors reported as values ·10^{-4}

Table 4 – *Thermodynamic parameters for the adsorption of phenolic acids and β-glucan*

Polyphenol	ΔG^0 (J mol^{-1})			R^2	ΔH^0 (J mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
	25 °C	37 °C	45 °C			
p-coumaric acid	–21311	–23135	–22399	0.0136*	–2385	64
caffeic acid	–22716	–23630	–23495	0.6278	–10063	43
chlorogenic acid	–23218	–23819	–21617	0.7202	–43465	–67

*in these cases, the data were inconsistent with a linear fit

free energy of adsorption. All free energies of adsorption were lower than 8000 J mol^{-1}, which indicates a physical bond creation between phenolic acids and β-glucan, like H bonds or Van der Waals forces[23] at all three temperatures. The creation of these non-covalent bonds is in agreement with earlier studies [14,18,32]. The parameter n_H is lower than 1 for all studied phenolic acids at all temperatures, which suggests a negative cooperation in the adsorption. This means that when a molecule of phenolic acid is adsorbed onto the surface of β-glucan, the affinity for other molecules decreases. Temkins parameters b_T and A are shown in relation to q_e, in Fig. 3. The parameter b_T, Temkin's apparent heat of adsorption, and parameter A, apparent maximum bonding energy, both decreased as q_e increased, suggesting that compounds with higher maximum adsorption capacities had lower apparent adsorption heat (Fig. 3).

Table 3 reports parameters of isotherm models at three pH values (pH 1.5, 5.5, 10, at 37 °C). Parameters q_m from Langmuir and Hill, and q_s from Dubinin-Radushkevich isotherm were in the range from 460 to 1545 mg g^{-1}, which is in the range of experimentally determined q_e values or somewhat higher. For all phenolic acids, the $1/n$ was lower than 1, which suggested that the adsorption was favored for all phenolic acids at all pH. The mean free energy of adsorption E was lower than 8000 J mol^{-1} at all pH, which still indicated physical bonds between phenolic acids and β-glucan. The apparent heat of the adsorption b_T and bonding energy A, decreased as q_e increased, suggesting that compounds with higher maximum adsorption capacities had lower apparent adsorption heat (Fig. 4). The n_H was lower than 1 at all pH values, which suggested a negative cooperation in adsorption. As already mentioned, this means that when a molecule of polyphenol is adsorbed onto the surface of β-glucan, the affinity for other molecules decreases.

Furthermore, predicted parameters q_m from Langmuir and Hill models, q_s from Dubinin-Radushkevich model were correlated to experimentally determined q_e to see how well the predicted values described the experimental values (Fig. 5). Predicted values q_m and q_s correlated well with the experimentally determined q_e at different temperatures and pH, with high R^2 (0.9562 to 0.9999). This suggested well conducted modelling process.

Thermodynamics

Thermodynamic parameter ΔG^0 is shown in Table 4, together with ΔS^0 and ΔH^0 obtained from the Van't Hoff plots. It should be mentioned that the calculation of these thermodynamic parameters involves the use of constants from Langmuir isotherm (K_L (L mol^{-1})), which were determined with modelling, and depend on precisely conducted modelling process. This could affect the determination of thermodynamic parameters. Since K_L values showed differences with temperature (Table 2), they were used in this study for the evaluation of thermodynamic parameters. For all phenolic acids, the standard reaction Gibbs energy ΔG^0 was negative at all temperatures, which was similar to literature data[23,39] and suggested a spontaneous process. Van't Hoff plots were then used to evaluate ΔS^0 and ΔH^0. More precisely, standard reaction enthalpy ΔH^0 was calculated from the slope, and standard reaction entropy ΔS^0 from the intercept of the Van't Hoff plot. Lines in Van't Hoff plots had R^2 from low values (0.0136) to higher values (0.7202) (Table 4), which suggested that the lines were not linear in all cases (*p*-coumaric acid), and that they should be interpreted with caution. This could be connected to the complexity of adsorption process[38]. Nevertheless, ΔS^0 and ΔH^0 were obtained from slopes and intercepts of Van't Hoff plots. The results showed the positive value of standard reaction entropy ΔS^0, which supported a spontaneous process in the interaction of phenolic acids with β-glucan (except for chlorogenic acid). ΔH^0 was found to be negative, indicative of an exothermic reaction[35].

Conclusions

By studying adsorption, this study showed that phenolic acids interacted with β-glucan in a complex process affected by the concentration of phenolic acids, chemical structure of phenolic acids or pH value and temperature of environment. The

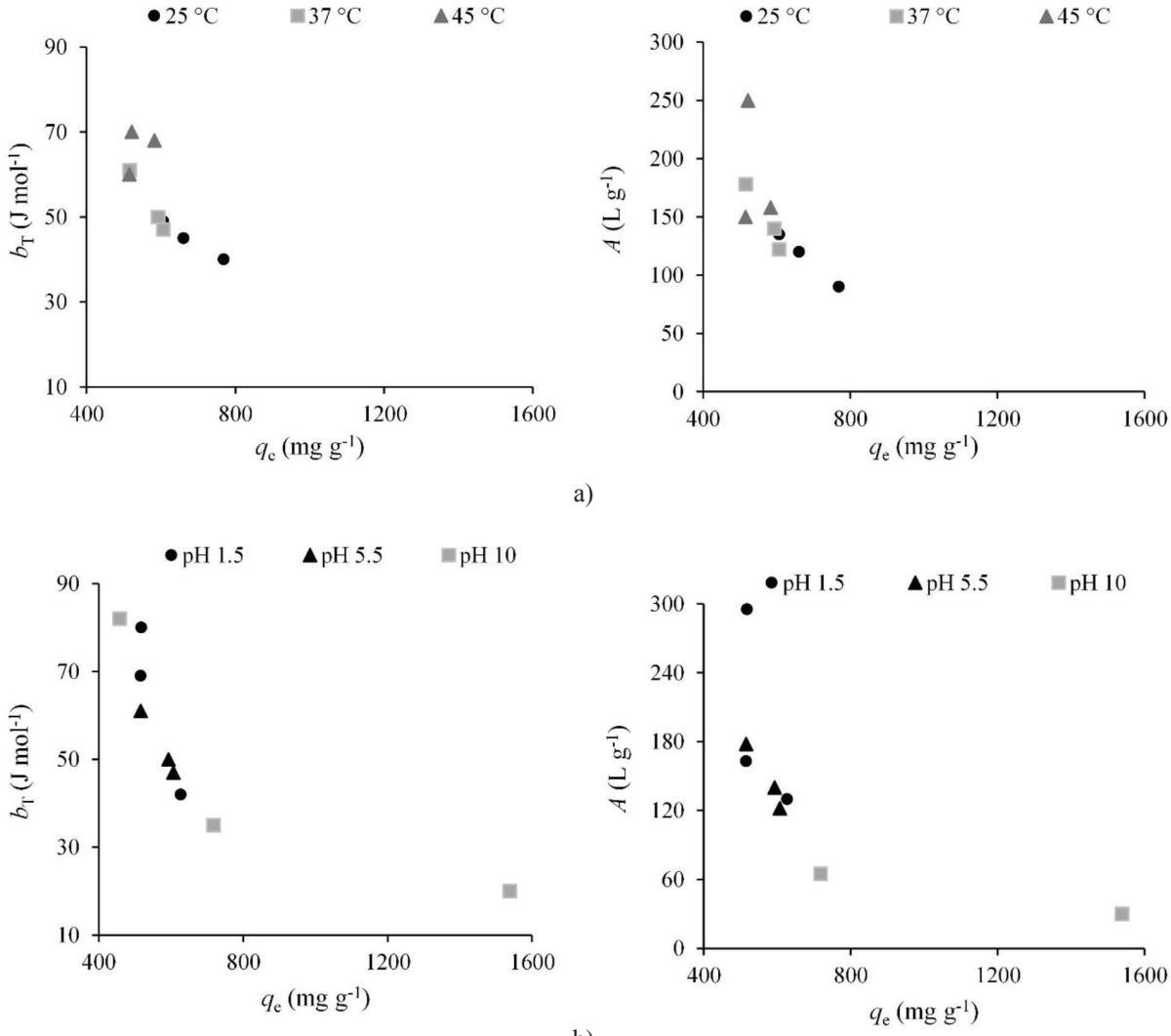

Fig. 4 – *Relationship between Temkin adsorption parameters (b_T and A) and the adsorption capacity q_e a) at different temperatures; b) at different pH values (adsorption capacities obtained in the experiment with the initial concentration of 150 mg L^{-1} in the reaction solution)*

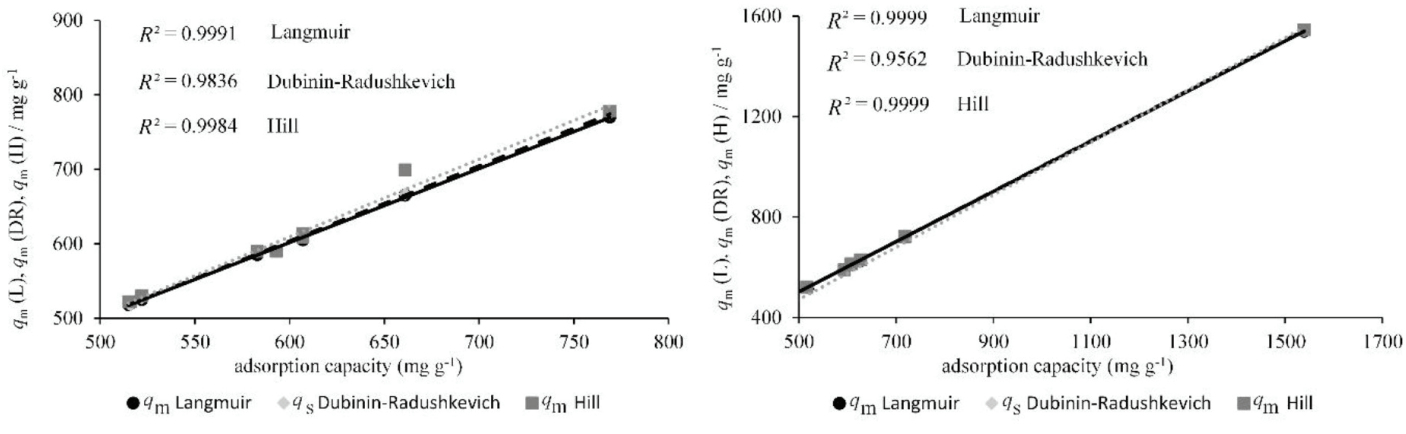

Fig. 5 – *Correlation between adsorption parameters (q_m from Langmuir, q_s from Dubinin-Radushkevich, q_m from Hill) and adsorption capacities (q_e) obtained experimentally a) at different temperatures; b) at different pH values (adsorption capacities obtained in the experiment with the initial concentration of 150 mg L^{-1} in the reaction solution)*

analysis of adsorption of selected phenolic acids onto β-glucan indicated that temperature and pH affected the adsorption process causing some fluctuations in adsorption capacities. Furthermore, the adsorption capacity was higher for *p*-coumaric acid and chlorogenic acid at pH 10. Experimental results in the adsorption process can be modelled well with adsorption isotherms, which can give useful information about the adsorption process. According to adsorption isotherm parameters, the adsorption process could be physical and noncooperative at all temperatures and pH values. It can be suggested that bonds created between all phenolic acids and β-glucan were physical bonds like H bonds and Van der Waals forces. According to thermodynamic parameters, the adsorption process was spontaneous (except for chlorogenic acid) and exothermic for all phenolic acids. All this might have an important effect on the polyphenol bioactivities in the human organism. Further studies are needed to completely understand interactions.

ACKNOWLEDGEMENTS

Special thanks to Professor Andrew R. Barron for his help and advice in statistical analysis of the data and adsorption isotherm modelling.

FUNDING

This work has been fully supported by Croatian Science Foundation under the project number HRZZ-IP-2016-06-6777.

References

1. *Camouse*, *M. M.*, *Hanneman*, *K. K.*, *Conrad*, *E. P.*, *Baron*, *E. D.*, Protective effects of tea polyphenols and caffeine, Expert Rev. Anticancer Ther. **5** (2005) 1061. doi: https://doi.org/10.1016/j.lfs.2007.06.011
2. *Kampa*, *M.*, *Nifli*, *A. P.*, *Notas*, *G.*, *Castanas*, *E.*, Polyphenols and cancer growth, Rev. Physiol. Biochem. Pharmacol. **159** (2007) 79. doi: https://doi.org/10.1007/112_2006_0702
3. *Pandey*, *K. B.*, *Rizvi*, *S. I.*, Plant polyphenols as dietary antioxidants in human health and diseases, Oxid. Med. Cell. Longev. **2** (2009) 270. doi: https://doi.org/10.4161/oxim.2.5.9498
4. *Scalbert*, *A.*, *Manach*, *C.*, *Morand*, *C.*, *Remesy*, *C.*, Dietary polyphenols and the prevention of diseases, Crit. Rev. Food Sci. Nutr. **45** (2007) 287. doi: https://doi.org/10.1080/1040869059096
5. *Jakobek*, *L.*, Interactions of polyphenols with carbohydrates, lipids and proteins, Food Chem. **175** (2015) 556. doi: https://doi.org/10.1016/j.foodchem.2014.12.013
6. *Jakobek*, *L.*, *Matić*, *P.*, Non-covalent dietary fiber – polyphenol interactions and their influence on polyphenol bioaccessibility, Trends Food Sci. Technol. **83** (2019) 235. doi: https://doi.org/10.1016/j.tifs.2018.11.024
7. *Le Bourvellec*, *C.*, *Renard*, *C. M. G. C.*, Interactions between polyphenols and macromolecules: Quantifications methods and mechanism, Crit. Rev. Food Sci. Nutr. **52** (2012) 213. doi: https://doi.org/10.1080/10408398.2010.499808
8. *Quirós-Sauceda*, *A. E.*, *Palafox-Carlos*, *H.*, *Sáyago-Ayerdi*, *S. G.*, *Ayala-Zavala*, *J. F.*, *Bello-Perez*, *L. A.*, *Álvarez-Parrilla*, *E.*, *de la Rosa*, *L. A.*, *González-Córdova*, *A. F.*, *González-Aguilar*, *G. A.*, Dietary fiber and phenolic compounds as functional ingredients: Interaction and possible effect after ingestion, Food Funct. **5** (2014) 1063. doi: https://doi.org/10.1039/c4fo00073k
9. *Soto*, *M. L.*, *Moure*, *A.*, *Domínguez*, *H.*, *Parajó*, *J. C.*, Recovery, concentration and purification of phenolic compounds by adsorption: A review, J. Food Eng. **105** (2011) 1. doi: https://doi.org/10.1016/j.jfoodeng.2011.02.010
10. *Plazinski*, *W.*, *Rudzinski*, *W.*, *Plazinska*, *A.*, Theoretical models of sorption kinetics including a surface reaction mechanism: A review, Adv. Colloid Interface Sci. **152** (2009) 2. doi: https://doi.org/10.1016/j.cis.2009.07.009
11. *Foo*, *K. Y.*, *Hameed*, *B. H.*, Insight into the modeling of adsorption isotherm systems, Chem. Eng. J. **156** (2010) 2. doi: https://doi.org/10.1016/j.cej.2009.09.013
12. *Limousin*, *G.*, *Gaudet*, *J. P.*, *Charlet*, *L.*, *Szenknect*, *S.*, *Barthès*, *V.*, *Krimissa*, *M.*, Sorption isotherms: A review on physical bases, modeling and measurement, J. Appl. Geochem. **22** (2007) 249. doi: https://doi.org/10.1016/j.apgeochem.2006.09.010
13. *Laroche*, *C.*, *Michaud*, *P.*, New developments and prospective applications for β (1,3) glucans, Recent Pat. Biotechnol. **1** (2007) 59. doi: https://doi.org/10.2174/187220807779813938
14. *Gao*, *R.*, *Liu*, *H.*, *Peng*, *Z.*, *Wu*, *Z.*, *Wang*, *Y.*, *Zhao*, *G.*, Adsorption of (-)-epigallocatechin-3-gallate (EGCG) onto oat β-glucan, Food Chem. **132** (2012) 1936. doi: https://doi.org/10.1016/j.foodchem.2011.12.029
15. *Simonsen*, *H. T.*, *Nielsen*, *M. S.*, *Christensen*, *N. J.*, *Christensen*, *U.*, *La Cour*, *T. V.*, *Motawia*, *M. S.*, *Jespersen*, *B. P. M.*, *Engelsen*, *S. B.*, *Møller*, *B. L.*, Molecular interactions between barley and oat β-glucans and phenolic derivatives, J. Agric. Food Chem. **57** (2009) 2056. doi: https://doi.org/10.1021/jf802057v
16. *Veverka*, *M.*, *Dubaj*, *T.*, *Gallovič*, *J.*, *Jorík*, *V.*, *Veverková*, *E.*, *Mičušík*, *M.*, *Šimon*, *P.*, Beta-glucan complexes with selected nutraceuticals: Synthesis, characterization, and stability, J. Funct. Foods **8** (2014) 309. doi: https://doi.org/10.1016/j.jff.2014.03.032
17. *Wang*, *Y.*, *Liu*, *J.*, *Chen*, *F.*, *Zhao*, *G.*, Effects of molecular structure of polyphenols on their noncovalent interactions with oat β-glucan, J. Agric. Food Chem. **61** (2013) 4533. doi: https://doi.org/10.1021/jf400471u
18. *Wu*, *Z.*, *Li*, *H.*, *Ming*, *J.*, *Zhao*, *G.*, Optimization of adsorption of tea polyphenols onto oat β-glucan using response surface methodology, J. Agric. Food Chem. **59** (2011) 378. doi: https://doi.org/10.1021/jf103003q
19. *Sreńscek-Nazzal, J., Narkiewicz, U., Morawski, A. W.*, Comparison of optimized isotherm models and error functions for carbon dioxide adsorption on activated carbon, J. Chem. Eng. Data **60** (2015) 3148. doi: https://doi.org/10.1021/acs.jced.5b00294
20. *Matić*, *P.*, *Sabljić*, *M.*, *Jakobek*, *L.*, Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content, J. AOAC Int. **100** (2012) 1795. doi: https://doi.org/10.5740/jaoacint.17-0066

21. *Singleton*, *V. L.*, *Orthofer*, *R.*, *Lamuela-Raventós, R. M.,* Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Meth. Enzymol. **299** (1999) 152. doi: https://doi.org/10.1016/S0076-6879(99)99017-1
22. *Dalal*, *R. C.*, Application of Dubinin-Radushkevich adsorption isotherm for phosphorus sorption by soils, Soil Sci. **128** (1979) 65.
23. *Marsal*, *A.*, *Maldonado F.*, *Cuadros*, *S.*, *Bautista*, *M. E.*, *Manich*, *A. M.*, Adsorption isotherm, thermodynamics and kinetics studies of polyphenols onto tannery shavings, Chem. Eng. J. **183** (2012) 21. doi: https://doi.org/10.1016/j.cej.2011.12.012
24. *Samatya*, *S.*, *Kabay*, *N.*, *Yüksel*, *Ü.*, *Arda*, *M.*, *Yüksel*, *M.*, Removal of nitrate from aqueous solution by nitrate selective ion exchange resins, React. Funct. Polym. **66** (2006) 1206. doi: https://doi.org/10.1016/j.reactfunctpolym.2006.03.009
25. *Liu*, *Y.*, Is the free energy change of adsorption correctly calculated, J. Chem. Eng. Data **54** (2009) 1981. doi: https://doi.org/10.1021/je800661q
26. *Lima, E. C., Hosseini-Bandegharaei, A. H., Moreno-Piraján, J. C., Anastopoulos, I.*, A critical review of the estimation of the thermodynamic parameters on adsorption equilibria. Wrong use of equilibrium constant in the Van't Hoff equation for calculation of thermodynamic parameters of adsorption, J. Mol. Liq. **273** (2019) 425. doi: https://doi.org/10.1016/j.molliq.2018.10.048
27. *Ajithkumar*, *A.*, *Andersson*, *R.*, *Åman*, *P.*, Content and molecular weight of extractable β-glucan in American and Swedish oat samples, J. Agric. Food Chem. **53** (2005) 1205. doi: https://doi.org/10.1021/jf040322c
28. *Zheng*, *X.*, *Li*, *L.*, *Wang*, *Q.*, Distribution and molecular characterization of β-glucans from hull-less barley bran, shorts and flour, Int. J. Mol. Sci. **12** (2011) 1563. doi: https://doi.org/10.3390/ijms12031563
29. *Phan*, *A. D. T.*, *Flanagan*, *B. M.*, *D'Arcy*, *B. R.*, *Gidley*, *M. J.*, Binding selectivity of dietary polyphenols to different plant cell wall components: Quantification and mechanism, Food Chem. **233** (2017) 216. doi: https://doi.org/10.1016/j.foodchem.2017.04.115
30. *Renard*, *C. M. G. C.*, *Baron*, *A.*, *Guyot*, *S.*, *Drilleau*, *J. F.*, Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences, Int. J. Biol. Macromol. **29** (2001) 115. doi: https://doi.org/10.1016/S0141-8130(01)00155-6
31. *Le Bourvellec*, *C.*, *Renard*, *C. M. G. C.*, Non-covalent interaction between procyanidins and apple cell wall material. Part II: Quantification and impact of cell wall drying, Biochim. Biophys. Acta **1725** (2005) 1. doi: https://doi.org/10.1016/j.bbagen.2004.04.001
32. *Le Bourvellec*, *C.*, *Guyot*, *S.*, *Renard*, *C. M. G. C.*, Non-covalent interaction between procyanidins and apple cell wall materials. Part I. Effect of some environmental parameters, Biochim. Biophys. Acta **1672** (2004) 192. doi: https://doi.org/10.1016/j.bbagen.2004.04.001
33. *Bretag*, *J.*, *Kammerer*, *D.*, *Jensen*, *U.*, *Carle*, *R.*, Evaluation of the adsorption behavior of flavonoids and phenolic acids onto a food-grade resin using a D-optimal design, Eur. Food Res. Technol. **228** (2009) 985. doi: https://doi.org/10.1007/s00217-009-1017-0
34. *Plazinski, W., Rudzinski, W., Plazinska, A.*, Theoretical models of sorption kinetics including a surface reaction mechanism: A review, Adv. Colloid Interface Sci. **152** (2009) 2. doi: https://doi.org/10.1016/j.cis.2009.07.009
35. *Nguela*, *M. J.*, *Poncet-Legrand*, *C.*, *Sieczkowski*, *N.*, *Vernhet*, *A.*, Interactions of grape tannins and wine polyphenols with a yeast protein extract, mannoproteins and β-glucan, Food Chem. **210** (2016) 671. doi: https://doi.org/10.1016/j.foodchem.2016.04.050
36. *Gennaro-Matos, T. C., Maurío, A. Q., Rettori, D., Alonso, A., Hermes-Lima, M.*, Antioxidant activity of caffeic acid against iron-induced free radical generation – a chemical approach, Plos One **10** (2015) 1. doi: https://doi.org/10.1371/journal.pone.0129963
37. *Phan*, *A. D. T.*, *Netzel*, *G.*, *Wang*, *D.*, *Flanagan*, *B. M.*, *D'Arcy, B. R.*, *Gidley*, *M. J.*, Binding of dietary polyphenols to cellulose: Structural and nutritional aspects, Food Chem. **171** (2015) 388. doi: https://doi.org/10.1016/j.foodchem.2014.08.118
38. *Zhang*, *D., Zhu*, *J.*, *Ye*, *F.*, *Zhao*, *G.*, Non-covalent interaction between ferulic acid and arabinan-rich pectic polysaccharide from rapeseed meal, Int. J. Biol. Macromol. **103** (2017) 307. doi: https://doi.org/10.1016/j.ijbiomac.2017.05.053
39. *Chowdhury*, *S.*, *Mishra*, *R.*, *Saha*, *P.*, *Kushwaha*, *P.*, Adsorption thermodynamics, kinetics and isosteric heat of adsorption of malachite green onto chemically modified rice husk, Desalination **265** (2011) 159. doi: https://doi.org/10.1016/j.desal.2010.07.047