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Adsorption of Apple Polyphenols onto β-Glucan

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Abstract

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The adsorption of polyphenols from apples, a good source of polyphenols in the human diet, onto β-glucan, a soluble dietary fibre were studied. Polyphenols were extracted from the flesh and peel of two apple varieties (wild apple and Slavonska srčika) and adsorbed onto β-glucan for 16 hours. The adsorption capacities (mg/g) and equilibrium polyphenol concentrations (mg/l) were modelled with Freundlich and Langmuir isotherms. Polyphenols from the flesh and peel showed different behaviours – flesh polyphenols exhibited greater affinity and peel polyphenols greater theoretical adsorption capacity. The analysis of individual polyphenols with high-performance liquid chromatography revealed that the composition of the flesh and peel differed (flesh was rich in phenolic acids, peel in flavonols) which could explain the contrasting adsorption behaviour. This study shows that polyphenols from apples can be adsorbed onto β-glucan, that the flesh and peel exhibit distinct adsorption behaviours and that the polyphenol composition can affect the adsorption mechanism.

Keywords: adsorption isotherms; Freundlich; interactions; Langmuir

Polyphenols have been studied intensively because they have shown many potential benefits for human health. One important aspect of their bioactivity is the interaction with other food constituents such as carbohydrates, lipids, and proteins (Le Bourvellec & Renard 2012; Jakobek 2015), which can have potentially important consequences. One such consequence is that polyphenols might be 'protected' and pass to the lower parts of the digestive tract without being metabolised. Once there, they might exert positive effects in their intact forms (Gorelik *et al*. 2008; Kanner *et al*. 2012). Dietary fibres are especially interesting since they can arrive in the colon in a non-metabolised form. This makes them potential 'carriers' of polyphenols (Jakobek 2015). Interactions between polyphenols and dietary fibre can be studied through adsorption processes *in vitro* (Renard *et al*. 2001; Wu *et al*. 2011; Gao *et al*. 2012; Wang *et al*. 2013). Adsorption is a process in which molecules from a solution adsorb onto the surface of an adsorbent and can be described through adsorption isotherms (Soto *et al*. 2011).

β-Glucan, a water-soluble dietary fibre, can serve as a model in adsorption processes. It can be found in different cereals where it may come into contact with fruit polyphenols. Namely, breakfast cereals or any other cereal products can be consumed in combination with fruits or fruit products. In these situations, β-glucan and polyphenols can interact. Furthermore, β-glucan is produced in the form of a dietary supplement and as such can be a part of a regular diet. Apples are a good source of polyphenols present in the everyday diet (Wojdyło *et al*. 2008), and can be consumed with breakfast cereals

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or other foods containing dietary fibres. As far as we are aware, adsorption onto β-glucan was not previously studied for apple polyphenols. Isotherms of adsorption onto β-glucan have been studied for tea polyphenols (Wu *et al*. 2011; Gao *et al*. 2012) and some other adsorption properties of polyphenolic compounds onto β-glucan have been investigated (Wang *et al*. 2013).

The aim of this work was to study the adsorption of polyphenols from the flesh and peel of apples onto β-glucan as a dietary fibre. Freundlich and Langmuir non-linear isotherm models were created and used for the interpretation of adsorption. Additionally, the polyphenol content of apple samples was determined using high-performance liquid chromatography with photo-diode array detection (HPLC-PDA) with the aim of characterising individual polyphenols and evaluating their influence on the process of adsorption.

MATERIAL AND METHODS

Chemicals. Gallic acid monohydrate, (+)-catechin hydrate, (–)-epicatechin, chlorogenic acid, *p*-coumaric acid, quercetin dihydrate, quercetin-3-β-D-glucoside and β-D-glucan from barley were purchased from Sigma-Aldrich (USA). Procyanidin B1, procyanidin B2, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, phloretin-2'-*O*-glucoside, and phloretin were from Extrasynthese (France). Orto-phosphoric acid (85% HPLC-grade) was from Fluka (Switzerland), HPLC-grade methanol was acquired from J.T. Baker (the Netherlands), and hydrochloric acid, sodium carbonate and Folin-Ciocalteau reagent were from Kemika (Croatia).

Samples and sample preparation. Old apple varieties – Slavonska srčika (*Malus domestica*) and a wild variety (crab-apple) were harvested at maturity (orchard of M. Veić, Požega, Croatia). Approximately 1 kg of sampled apples were peeled. The peel was pooled and homogenised using a blender. The core and the seeds were removed from the flesh, and the flesh was then cut into smaller pieces, pooled and homogenised with a stick blender. Extracts were prepared immediately.

Polyphenol extraction. For the adsorption study, three samples of flesh or peel were weighed (0.2 g) and extracted with 1.5 ml of extraction solvent (0.1% HCl in methanol for peel samples; 80% methanol in water for flesh samples) in accordance with our previously described protocol for polyphenol extraction from apples (Jakobek *et al*. 2015). We have observed that acidified methanol was better for peel polyphenols, while 80% methanol was a good choice for flesh polyphenols (Jakobek *et al*. 2015). The samples were vortexed (Grant Bio, UK), placed in an ultrasonic bath (Bandelin Sonorex RK 100; Bandelin electronic, Germany) for 15 min and then centrifuged (Minispin; Eppendorf, Germany). Three extracts were combined and used for the adsorption study.

For the polyphenol characterisation with HPLC-PDA, polyphenols were extracted from the peel (0.1% HCl in methanol) and from the flesh (80% methanol in water). Samples were weighed (0.2 g of the flesh or peel), mixed with 1.5 ml of extraction solvents, vortexed, placed in an ultrasonic bath for 15 min and then centrifuged. The extract was removed and the residue was extracted once more in 0.5 ml of extraction solvent. These extracts were combined and filtered (0.45-μm PTFE syringe filter). Two parallel extracts were prepared for each peel or flesh sample and each was analysed once with the HPLC-PDA method.

Spectrophotometric method for total polyphenol determination. Total polyphenols were determined by the Folin-Ciocalteu method (Waterhouse 2016). Distilled water (1580 μl) was mixed with extract (20 μl), Folin-Ciocalteu reagent (100 μl), and a sodium carbonate solution (200 g/l, 300 μl). After incubation (40°C, 30 min), the absorbance was read at 765 nm on a UV-Vis spectrophotometer (JP Selecta, Spain). The results were expressed in mg/l of extract as gallic acid equivalents.

High-performance liquid chromatography with photodiode array detection. Individual polyphenols were determined on a Varian HPLC system (Varian Inc., USA); ProStar 230 solvent delivery module, ProStar 330 PDA detector, OmniSphere C18 column (250 × 4.6 mm, 5 μm), guard column (ChromSep 1 cm × 3 mm). Mobile phases were 0.1% phosphoric acid in water (A) and 100% methanol (B). The gradient was 5% B (0 min), 25% B (0–5 min), 34% B (5–14 min), 37% B (14–25 min), 40% B (25–30 min), 49% B (30–34 min), 50% B (34–35 min), 51% B (35–58 min), 55% B (58–60 min), 80% B (60–62 min), maintained at 80% B (62–65 min), down to 5% B (65–67 min) and maintained at 5% B (67–72 min). The flow rate was 0.8 ml/min; injection volume 20 μl; spectra 190–600 nm. The limits of detection and quantification are presented in the tables. Polyphenols were identified by comparison of the

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retention times and spectral data with the those of standards. Furthermore, *p*-coumaroylquinic acid, quercetin-xyloside, and phloretin-2'-xyloglucoside were tentatively identified (Tsao *et al*. 2003) and quantified using *p*-coumaric acid, quercetin and phloretin calibration curves, respectively. The results were expressed in mg/kg of the fresh weight (FW).

Adsorption experiment. The β-glucan was dissolved (190 mg/l) in distilled water. Total polyphenols in extracts (initial polyphenol concentration) were determined using the Folin-Ciocalteu method. For the adsorption study, four different volumes of polyphenol extract (10, 200, 500, and 700 µl), β-glucan (53 µl) as an adsorbent, and a phosphate buffer (0.13 mol/l, pH 5.5) were combined in plastic cuvettes (total volume was 2 ml). Solutions were mixed in a laboratory shaker (IKA KS 130; IKA Werke, Germany; 16 h, room temperature) and filtered through 0.1-µm cellulose nitrate membranes (Whatman, GE Healthcare, Germany). Unadsorbed polyphenols (polyphenol concentration at equilibrium – c_e) were determined with the Folin-Ciocalteu method. The adsorption capacity (mg of adsorbed polyphenols per g of β-glucan) was calculated (q_e):

$$q_e = \frac{(c_0 - c_e)\, V_{rs}}{c_{\beta\text{-glucan}} \times V_{\beta\text{-glucan}}} \quad (1)$$

where: c_0 – initial polyphenol concentration in the reaction solution (mg/l); c_e – equilibrium polyphenol concentration in the reaction solution (mg/l); V_{rs} – volume of reaction solution (1); $c_{\beta\text{-glucan}}$ – concentration of β-glucan (g/l); $V_{\beta\text{-glucan}}$ – volume of β-glucan in the reaction solution (1)

Freundlich (2) and Langmuir models (3) were constructed:

$$q_e = K_F c_e^{1/n} \quad (2)$$

$$q_e = \frac{q_m c_e}{1/K_L + c_e} \quad (3)$$

where: c_e – polyphenol concentration in the solution at equilibrium (mg/l); q_e – amount of polyphenol adsorbed per g of β-glucan at equilibrium (mg/g); K_F – Freundlich constant indicative of relative adsorption capacity of β-glucan (mg/g) × (mg/l)$^{-1/n}$; $1/n$ – intensity of adsorption; K_L – Langmuir equilibration constant of adsorption (l/mg) or apparent affinity constant; q_m – apparent maximum adsorption capacity of β-glucan (mg/g) (Soto *et al*. 2011)

The data (q_e vs. c_e) were fitted with nonlinear models in such a way that the sum of square differences is minimal, and adsorption parameters were determined (K_F and $1/n$ from the Freundlich isotherm, K_L and q_m from the Langmuir isotherm).

Statistical analyses. For the adsorption experiments, total polyphenols were measured at four concentration levels two times each. Nonlinear regression was performed (Minitab, USA) on q_e and c_e means by minimising the sum of square errors. The root-mean-square error (RMSE) of nonlinear least squares regression was calculated:

$$\text{RMSE} = \sqrt{1/n \sum_{i=1}^{n} (q_{e,i} - f(c_{e,i}, a, b))^2} \quad (4)$$

where: $c_{e,i} - c_e$ mean values for the i^{th} concentration level; $q_{e,i} - q_e$ mean values for the i^{th} concentration level; $f(c_{e,i}, a, b)$ – nonlinear model function with generic parameters a and b; $n = 4$ is number of concentration levels

Two extracts from each flesh and peel were prepared for individual polyphenol characterisation, each was analysed once using HPLC-PDA ($n = 2$). Means and coefficients of variation were calculated.

RESULTS AND DISCUSSION

Adsorption. The adsorption of apple polyphenols onto β-glucan was described with Freundlich and Langmuir isotherms, in an approach which can be compared to that of earlier studies (Wu *et al*. 2011; Gao *et al*. 2012). Polyphenols from the flesh of the two types of apple showed similar behaviours as evidenced by their similar curve shapes (Figures 1A and C). The curve shapes for peel polyphenols differed between the two typed of apple (Figures 1B and D), suggesting different behaviour of peel polyphenols. Moreover, polyphenols from the wild apple peel adsorbed to a greater extent (larger q_e) than polyphenols from Slavonska srčika peel.

Adsorption parameters. Table 1 displays the RMSE of each model and the parameters of the Langmuir and Freundlich isotherms. Both isotherms could be equally applied for the description of the flesh polyphenol adsorption (errors were similar). Both isotherms could also be used for the peel polyphenol adsorption, but the Langmuir model was somewhat better for the wild apple peel (smaller RMSE), and the Freundlich for the Slavonska srčika peel (smaller RMSE).

According to the K_F value (Table 1), the relative adsorption capacity of β-glucan was similar for flesh polyphenols, and different for peel polyphenols from the two apple types (somewhat higher for the Slavonska srčika peel and lower for the wild apple

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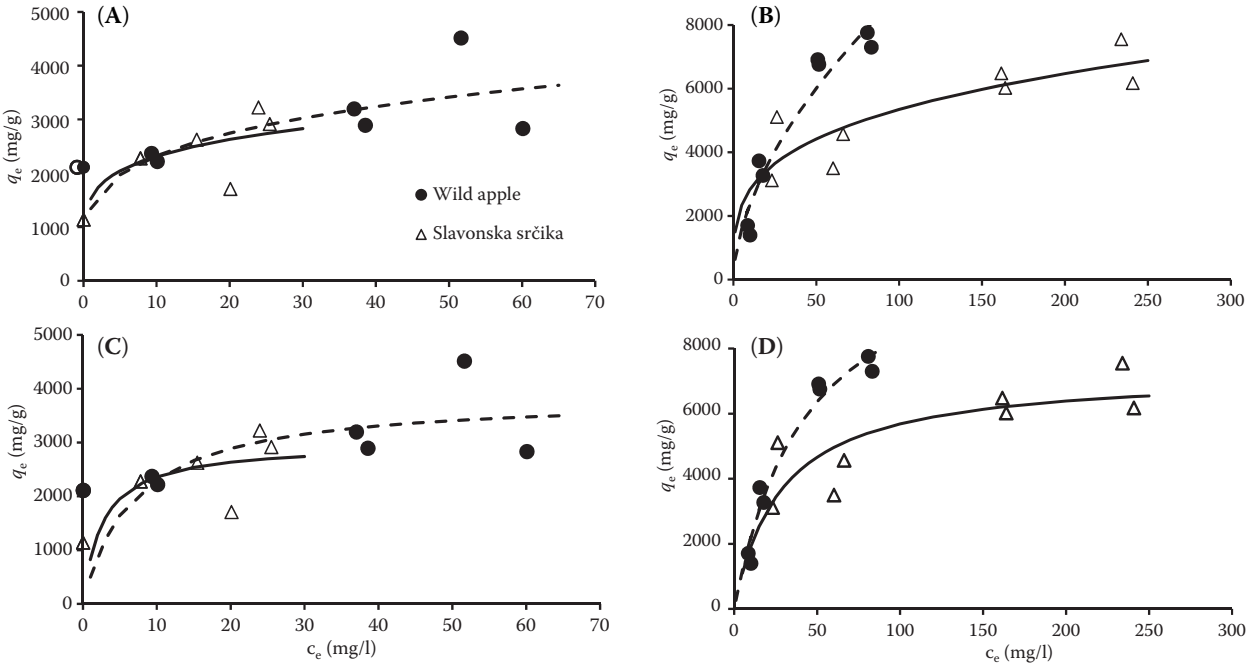

Figure 1. The adsorption isotherms representing the adsorption of apple polyphenols onto β-glucan (28°C, 16 h, nonlinear models): Freundlich isotherms of flesh polyphenols (**A**), Freundlich isotherms of peel polyphenols (**B**), Langmuir isotherm of flesh polyphenols (**C**), and Langmuir isotherm of peel polyphenols (**D**)
q_e – (polyphenols mg/g β-glucan) as a function of c_e (polyphenols mg/l)

peel polyphenols). According to the estimated q_m values of maximum adsorption, β-glucan may have the capacity to adsorb more peel polyphenols onto its surface than flesh polyphenols (q_m value 11 949 and 7254 mg/g for peel; 3927 and 3114 mg/g for flesh). The adsorption intensity was shown to be similar for peel and flesh polyphenols ($1/n$ was similar) except for wild apple peel polyphenols which showed much higher adsorption capacity. The K_L values showed that the apparent affinity of polyphenols for β-glucan was higher for the flesh polyphenols (0.29 and 0.14 l/mg) than for peel polyphenols (0.036 and 0.023 l/mg). In general, differences between polyphenols from the flesh and peel could be seen.

Polyphenol composition in apples. Figure 2 shows the polyphenols identified in apples, Table 2 their levels and Figure 3 the percentages of polyphenolic subgroups. The identification and levels of different polyphenols are in accordance with earlier studies (Tsao *et al*. 2003; Jakobek *et al*. 2013). Differences between samples were found – higher flavonol content and proportion in the peel, and a much higher phenolic acid proportion in the flesh. Furthermore, the two flesh samples differed in their polyphenol content (higher in wild apple). There was a higher total polyphenol content in the Slavonska srčika peel compared to that of wild apple, while the former also contained phenolic acids in contrast to the latter.

Table 1. Parameters of Freundlich and Langmuir isotherms obtained with nonlinear models

Apple	Freundlich isotherm			Langmuir isotherm		
	K_F	$1/n$	RMSE	K_L	q_m	RMSE
Wild apple flesh	1230.5	0.26	1059.6	0.137	3926.8	1066.7
Slavonska srčika flesh	1325.1	0.23	626.8	0.292	3114.4	636.5
Wild apple peel	635.6	0.57	590.5	0.023	11 949.0	375.0
Slavonska srčika peel	1510.5	0.27	421.4	0.036	7254.0	639.9

K_F – indicative constant of the relative adsorption capacity of β-glucan (mg/g)(mg/l)$^{-1/n}$; $1/n$ – intensity of adsorption; K_L – Langmuir equilibration constant of adsorption (l/mg), apparent affinity constant; q_m – apparent maximum adsorption capacity of β-glucan (mg polyphenols/g β-glucan); RMSE – root mean square error

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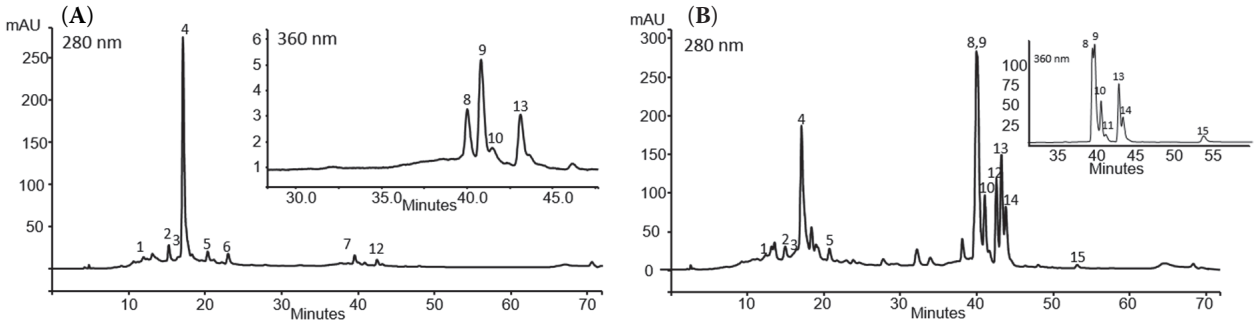

Figure 2. HPLC-PDA chromatogram of wild apple flesh (**A**) and Slavonska srčika peel (**B**)

1 – procyanidin B1; 2 – (+)-catechin; 3 – procyanidin B2; 4 – chlorogenic acid; 5 – (-)-epicatechin; 6 – *p*-coumaroylquinic acid; 7 – phloretin-2'-xyloglucoside; 8 – quercetin-3-galactoside; 9 – quercetin-3-glucoside; 10 – quercetin derivative 1; 11 – quercetin derivative 2; 12 – phloretin-2'-glucoside; 13 – quercetin-3-xyloside; 14 – quercetin-3-rhamnoside; 15 – quercetin

Apple polyphenol – β-glucan adsorption. The contrasting behaviour of polyphenols from flesh and peel in terms of adsorption could be explained by their different polyphenol compositions, manifested as higher flavonol content in peel samples, higher phenolic acid portion in flesh samples and the pres-

Table 2. The content of polyphenols in the flesh and peel of old apple varieties (mg/kg of fresh weight)

	Slavonska srčika		Wild	
	flesh	peel	flesh	peel
Flavan-3-ols				
Procyanidin B1	12.6	23.3	31.3	22.8
(+)-Catechin	9.2	248.4	277.7	86.5
Procyanidin B2	20.5	135.2	84.9	36.8
(–)-Epicatechin	Nd	253.7	196.5	114.0
Total	42.3	660.6	590.4	260.1
Phenolic acids				
Chlorogenic acid	338.3	438.4	855.0	nd
p-Coumaroylquinic acid[a]	10.6	nd	17.8	nd
total	348.9	438.4	872.8	nd
Flavonols				
Quercetin-3-galactoside	nd	728.1	49.9	152.4
Quercetin-3-glucoside	1.5	1182.1	17.5	337.3
Quercetin derivative 1	0.4	164.7	1.2	61.0
Quercetin derivative 2	nd	22.7	nd	13.0
Quercetin-3-xyloside[a]	0.3	224.5	4.9	68.3
Quercetin-3-rhamnoside	2.1	404.2	nd	52.7
Quercetin	nd	21.3	nd	13.6
Total	4.3	2747.6	73.5	698.3
Dihydrochalcones				
Phloretin-2'-xyloglucoside[a]	26.0	nd	24.4	nd
Phloretin-2'-glucoside	25.2	207.1	22.1	19.3
Total	51.2	207.1	46.5	19.3
Total	446.7	4053.7	1583.2	977.7

nd – not detected; LOD and LOQ were: (+)-catechin – 0.2 and 0.7; (–)-epicatechin – 0.3 and 1; procyanidin B1 – 0.3 and 0.9; procyanidin B2 – 1.2 and 3.9; *p*-coumaric acid – 0.1 and 0.3; chlorogenic acid – 0.14 and 0.4; phloretin – 0.15 and 0.5; phloretin-2'-glucoside – 0.13 and 0.43; quercetin – 0.03 and 0.1; quercetin-3-rhamnoside – 0.3 and 1; quercetin-3-galactoside – 0.6 and 2.0; quercetin-3-glucoside – 0.08 and 0.3; *data based on two extracts, each measured once (n = 2); variation coefficient range 1–25% for flesh and 1 – 28% for peel; [a]tentatively identified

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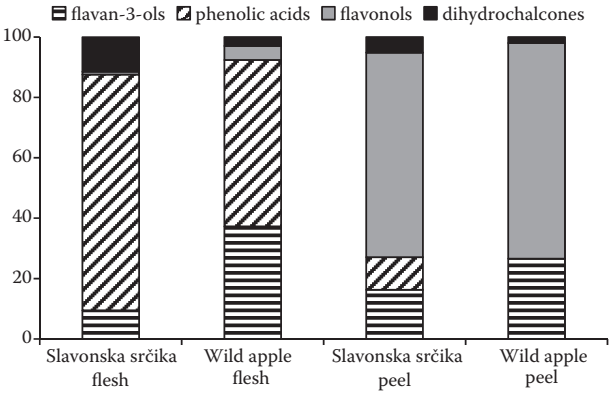

Figure 3. Percentage distribution of polyphenolic subgroups in apples

ence of phenolic acids in Slavonska srčika peel but not in wild apple. This would be in agreement with earlier studies where it was shown that individual apple polyphenols have different affinities toward resin (Kammerer *et al*. 2007), that different tea polyphenols have different affinities for β-glucan (Gao *et al*. 2012) and procyanidins with different degrees of polyperisation towards polysaccharides (Le Bourvellec *et al*. 2005).

It has been shown that flavonols adsorbed with higher adsorption capacity than phenolic acids onto β-glucan (Wang *et al*. 2013) and resins (Kammerer *et al*. 2007). In our study, peel polyphenols had higher flavonol content and showed higher maximum adsorption capacity (q_m). Thus, in accordance with an earlier study (Wang *et al*. 2013), it appears that flavonols from the peel were adsorbed in higher amounts. Phenolic acids, on the other hand, predominated in flesh which might be the reason for the lower adsorption capacity of flesh polyphenols, in accordance with earlier studies (Kammerer *et al*. 2007; Wang *et al*. 2013). Moreover, Slavonska srčika peel contained phenolic acids, which might be the reason for its lower adsorption capacity in comparison to wild apple peel.

The bonds created between polyphenols and β-glucan have been described to be non-covalent in nature, i.e., hydrogen bonds, Van der Waals forces and hydrophobic bonding (Wu *et al*. 2011; Veverka *et al*. 2014; Nguela *et al*. 2016). H-bonds and Van der Waals forces might be created between OH groups of polyphenols and β-glucan (Wu *et al*. 2011). Hydrophobic bonding is possible due to hydrophobic aromatic rings on polyphenols. The same type of bonding could be responsible for the adsorption in this study.

Interactions of apple polyphenols with β-glucan (dietary fibres) might be important for apple bioactivity. If apple polyphenols create associations with dietary fibre, there is a possibility that they can reach the colon which might influence their bioaccessibility, bioavailability and different beneficial activities in the lower parts of the digestive tract. Since apples are present in the everyday diet understanding their actual bioactivity is important.

CONCLUSIONS

We have here reported that polyphenols from the flesh and peel of apples adsorb onto the surface of β-glucan and that the adsorption could be described with Freundlich and Langmuir models. Contrasting behaviour between flesh and peel polyphenols was found – flesh polyphenols exhibited greater affinity towards β-glucan, while peel polyphenols showed greater adsorption capacity. The differences in the polyphenol composition between flesh and peel might have influenced the adsorption process. Flavonols may have been partially responsible for higher maximum adsorption capacity of peel polyphenols, and the lower adsorption of flesh polyphenols might have been due to the presence of phenolic acids in the flesh. Further studies are necessary to confirm and explain this adsorption process.

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