

# Effects of Molecular Structure of Polyphenols on Their Noncovalent Interactions with Oat $\beta$ -glucan

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**ABSTRACT:** Few data were available on the interactions between polyphenols and polysaccharides. The effects of the chemical structure of polyphenols on their interactions with oat  $\beta$ -glucan were analyzed. Ultrafiltration was applied to determine the adsorption capacities of polyphenols into oat  $\beta$ -glucan. Hydroxylation favored the adsorption of flavonoids with three or fewer hydroxyl groups but deteriorated those with four or more hydroxyl groups. Among flavonoid isomers, the adsorption capacities increased in the order flavonol > flavone > flavanone > isoflavone. Glycosylation exerted complicated influences on the adsorption capacities of flavonoids into oat  $\beta$ -glucan. In most cases, methylation and methoxylation of phenolic acids lowered their adsorption capacities into oat  $\beta$ -glucan. Esterification of gallic acid weakened its adsorption capacity into oat  $\beta$ -glucan, whereas *o*-coumaric acid presented higher adsorption capacity into oat  $\beta$ -glucan than *p*- and *m*-coumaric acids. Galloylation improved the adsorption capacities of catechins into oat  $\beta$ -glucan.

**KEYWORDS:** oat  $\beta$ -glucan, polyphenols, structure–affinity relationship, adsorption, hydrophilicity

## ■ INTRODUCTION

Dietary polyphenols, widely distributed in fruits, vegetables, cereals, tea, and wine, are famous for their diverse bioactivities including antioxidation, anticancer, anti-inflammatory, antimutagenesis, anticarcinogenesis, and antiviral activities.<sup>1–5</sup> However, the varying structures of polyphenols dramatically affect their behavior in vivo, such as absorption, metabolism, and excretion. Methylated flavones are superior to their unmethylated forms in intestinal absorption and metabolic stability. Xiao and co-workers recently reported that glycosylation of flavonoids decreased their binding abilities to milk protein, whereas hydroxylation on ring A of flavanones improved the interaction and methylation, whereas methoxylation of flavonoids decreased (or hardly affected) their affinities to milk protein.<sup>6</sup> Similar findings were obtained from research on the interaction of polyphenols with bovine hemoglobin.<sup>7</sup> By interacting with other constituents of the food matrix, polyphenols changed their absorption in vivo.

The complexation of polyphenols with proteins has been extensively investigated. Interactions of polyphenols with protein and digestive enzymes are known to reduce protein digestibility and can alter the bioavailability of polyphenols.<sup>8–10</sup> On the other hand, information on interaction between polyphenols and polysaccharides was scarcely reported. Recently, the complexation of phenolic compounds with polysaccharides has attracted increasing attention due to its crucial role in regulating the free concentration of phenolic compounds in foods and the human gastrointestinal tract. This complexation was valuable in improving food flavor, inhibiting oxidation and browning, controlled-releasing of phenolics, and recovering phenolics from plant extracts.<sup>11–13</sup>

Most research on interactions between polyphenols and polysaccharides tested solid state polysaccharides such as suspension, gel, or membrane forms.<sup>14–16</sup> A few papers were available for interactions between polyphenols and soluble

polysaccharides, which play a crucial role in taste, palatability, and nutritional value of various liquid foods, such as wine, tea beverage, and clarified juice.<sup>17</sup> Oat  $\beta$ -glucan is a nonstarch polysaccharide composed of  $\beta$ -(1 $\rightarrow$ 4)-linked glucose units separated every two to three units by  $\beta$ -(1 $\rightarrow$ 3)-linked glucose. This water-soluble dietary fiber has received much attention due to its potential functions, such as increasing immunity, anticancer activity, and lowering blood cholesterol, lipids, and blood glucose.<sup>18,19</sup> In our recent works, we reported that oat  $\beta$ -glucan plays a major role in regulating the free concentration of tea polyphenols in aqueous solution.<sup>20,21</sup> Moreover, monomers of tea polyphenols were selectively adsorbed into oat  $\beta$ -glucan through noncovalent interactions.<sup>22</sup>

Few studies, however, have focused on the structure–affinity relationship of polyphenols and their affinities to polysaccharides. The present study concerned the relationship between the molecular structure of polyphenols and their adsorption capacities into oat  $\beta$ -glucan. To investigate the effects of hydroxylation, methylation, and glycosylation of polyphenols on their adsorption capacities into oat  $\beta$ -glucan, 23 flavonoids and 13 phenolic acids were used in this study.

## ■ MATERIALS AND METHODS

**Oat  $\beta$ -Glucan.** A commercial soluble oat  $\beta$ -glucan, extracted from hull-less oat, was bought from Zhengzhou Lion Biological Technology Co. Ltd. (Zhengzhou, China) with a  $\beta$ -glucan content of 77%. Oat  $\beta$ -glucan was pretreated according to our previous method.<sup>21</sup> The final content of oat  $\beta$ -glucan was determined as 95.7%. The weight-average molecular weight was  $9.1 \times 10^5$ .

**Polyphenols.** The following monomers of polyphenols, with purity >98.0%, were used: Apigenin, galangin, luteolin, kaempferol,

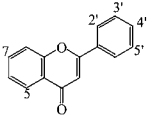
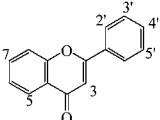
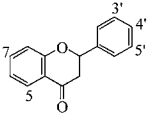
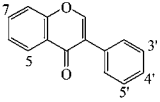
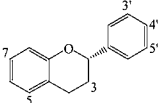
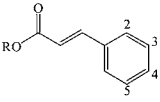
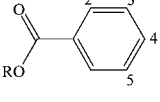
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Table 1. Chemical Structures and Adsorption Capacities of Various Polyphenols into Oat  $\beta$ -Glucan<sup>a</sup>

Subclass	Name	Substitutions			$q_e$ (mol/mol)
		OH	OCH <sub>3</sub>	Others	
	Apigenin	5, 7, 4'			334.22 ± 16.87 <sup>b</sup>
	Luteolin	5, 7, 3', 4'			395.24 ± 14.49 <sup>a</sup>
	Diosmetin	5, 7, 3'	4'		181.78 ± 10.52 <sup>c</sup>
	Rutin	5, 7, 3', 4'		3- $\alpha$ -L-Rham -1, 6-D-Glc	166.03 ± 7.45 <sup>c</sup>
	Galangin	3, 5, 7			426.05 ± 14.21 <sup>c</sup>
	Kaempferol	3, 5, 7, 4'			525.99 ± 25.03 <sup>b</sup>
	Quercetin	3, 5, 7, 3', 4'			203.81 ± 6.86 <sup>d</sup>
	Morin	3, 5, 7, 2', 4'			64.61 ± 7.14 <sup>f</sup>
	Myricetin	3, 5, 7, 3', 4', 5'			170.94 ± 8.84 <sup>e</sup>
	Isorhamnetin	3, 5, 7, 4'	3'		829.66 ± 33.45 <sup>a</sup>
	Myricetrin	5, 7, 3', 4', 5'		3- <i>o</i> -L-rha mnoside	210.48 ± 10.74 <sup>d</sup>
	Eriodictyol	5, 7, 3', 4'			198.29 ± 17.17 <sup>a</sup>
	Naringenin	5, 7, 4'			152.22 ± 12.84 <sup>b</sup>
	Naringin	5, 4'		7-Neohespe ridose	121.19 ± 5.72 <sup>c</sup>
	Genistein	5, 7, 4'			127.05 ± 10.22 <sup>b</sup>
	Genistin	5, 4'		7-Glucoside	126.34 ± 9.26 <sup>b</sup>
	Daidzein	7, 4'			112.43 ± 4.62 <sup>c</sup>
	Daidzin	4'		7-Glucoside	155.13 ± 8.24 <sup>a</sup>
	Puerarin	7, 4'		8-C-glucose	79.68 ± 8.11 <sup>d</sup>
	EC	3, 5, 7, 4', 5'			95.52 ± 4.98 <sup>d</sup>
	ECG	5, 7, 4', 5'		3-Gallate	160.14 ± 5.88 <sup>c</sup>
	EGC	3, 5, 7, 3', 4', 5'			266.11 ± 14.14 <sup>b</sup>
	EGCG	5, 7, 3', 4', 5'		3-Gallate	197.04 ± 3.59 <sup>a</sup>
	<i>o</i> -Coumaric acid	2			178.89 ± 11.52 <sup>a</sup>
	<i>m</i> -Coumaric Acid	3			57.82 ± 14.47 <sup>e</sup>
	<i>p</i> -Coumaric Acid	4			53.27 ± 8.32 <sup>e</sup>
	Caffeic acid	3, 4			119.11 ± 7.17 <sup>c</sup>
	Ferulic acid	4	3		157.79 ± 2.16 <sup>b</sup>
	Sinapic acid	4	3, 5		103.13 ± 2.23 <sup>d</sup>
	Gallic acid	3, 4, 5		R = H	275.96 ± 14.28 <sup>a</sup>
	Methyl gallate	3, 4, 5		R = methyl	201.72 ± 19.47 <sup>b</sup>
	Ethyl gallate	3, 4, 5		R = ethyl	130.23 ± 0.83 <sup>c</sup>
	Protocatechuic acid	3, 4		R = H	292.80 ± 21.14 <sup>a</sup>
	Vanillic acid	4	3	R = H	115.38 ± 12.18 <sup>d</sup>
	Veratric acid		3, 4	R = H	87.57 ± 2.50 <sup>e</sup>
	Syringic acid	4	3, 5	R = H	202.46 ± 11.34 <sup>b</sup>

<sup>a</sup>Means with different subscripts in the last column are significantly different within the same subclass ( $p < 0.05$ ).

quercetin, isorhamnetin, rutin, morin, myricetin, myricetrin, diosmetin, naringenin, naringin, eriodictyol, genistein, genistin, daidzein, daidzin,

puerarin, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), vanillic

acid, ferulic acid, caffeic acid, and *p*-coumaric acid were commercially purchased from Chengdu Biopurify Phytochemicals Co. Ltd. (Chengdu, China). Sinapic acid, *m*-coumaric acid, and *o*-coumaric acid were obtained from Sigma-Aldrich Co. Ltd. Gallic acid was purchased from Tianjin Yifang S&T Co. Ltd. (Tianjin, China). Methyl gallate, ethyl gallate, veratric acid, and syringic acid were obtained from Shanghai Boyun Biotech Co. Ltd. (Shanghai, China). Protocatechuic acid was obtained from Shanghai Source Leaf Biological Technology Co. Ltd. (Shanghai, China). Methanol of HPLC grade was obtained from Siyou Chemical Reagent Co. Ltd. (Tianjin, China). All other reagents and solvents used were of analytical grade, and all aqueous solutions were prepared using fresh double-distilled water. Stock solution of each polyphenol (0.3 mg/mL) was prepared by dissolving each polyphenol in methanol.

**Interaction of Polyphenols with Oat  $\beta$ -Glucan.** Solution of polyphenol (0.3 mg/mL) was diluted 10 times with 0.13 M, pH 5.56, phosphate buffer to obtain a working solution (30  $\mu$ g/mL). Twenty milliliters of working solution of polyphenol and a solution of 0.4 mg of pretreated  $\beta$ -glucan in 2 mL of 0.13 M, pH 5.56, PBS buffer were mixed in a capped 50 mL conical flask and magnetically stirred at 40 °C for 90 min until completely blended. Then the flask was transferred to a 40 °C water bath and incubated for 16 h to complete the adsorption of polyphenol into oat  $\beta$ -glucan. After reaction, 1 mL solutions of flavonoid (except flavanone) with oat  $\beta$ -glucan were dissolved in 2 mL of methanol and immediately ultrafiltered at 2000g for 15 min, whereas solution containing flavanone or phenolic acid with better solubility in PBS buffer was treated without addition of methanol. Ultrafiltration was carried out on an Amicon Ultra-15 centrifugal filter (10 kDa), Millipore (USA), using a Beckman Coulter Avanti J-30 centrifuge. After ultrafiltration, unbound polyphenol successfully passed through the ultrafiltration membrane, whereas  $\beta$ -glucan–polyphenol complex ( $M_w > 10$  kDa) was retained. Subsequently, 10  $\mu$ L of the ultrafiltrate was directly injected onto the HPLC system. A blank was simultaneously treated following the same procedure except the solution of oat  $\beta$ -glucan was substituted with 2 mL of 0.13 M, pH 5.56, PBS buffer.

Adsorption capacity ( $q_e$ , see values in Table 1), in terms of moles of polyphenol absorbed by 1 mol of oat  $\beta$ -glucan, can be represented by the following equation and employed as an evaluation indicator.

$$\text{adsorption capacity } (q_e, \text{ mol/mol}) = (C_0 - C_e) \times V \times A/M \times M_w/M_{w_e} \quad (1)$$

$C_e$  and  $C_0$  are the concentrations of free polyphenol in solution with or without oat  $\beta$ -glucan ( $\mu$ g/mL), respectively.  $V$  is the total volume of solution (mL) in the conical flask, and  $A$  is a dilution factor (1 for flavanones and phenolic acids and 3 for the others).  $M$  is the mass of oat  $\beta$ -glucan (mg).  $M_w$  and  $M_{w_e}$  are the molecular mass of oat  $\beta$ -glucan and polyphenol, respectively.

**RP-HPLC Analysis.** An LC-20A HPLC instrument (Shimadzu, Japan) was equipped with a photodiode array (PDA) detector. Analysis of free polyphenols was performed on a reverse phase Thermo BDS  $C_{18}$  column (250  $\times$  4.6 mm i.d.) with 5  $\mu$ m particle diameter. The optimal mobile phase was a mixture of methanol–aqueous 2% phosphoric acid with different volume ratios, for example, 70:30 for morin and genistein; 65:35 for diosmetin; 60:40 for apigenin, galangin, luteolin, kaempferol, quercetin, isorhamnetin, genistin, naringenin, and eriodictyol; 55:45 for myricetin, rutin, and daidzein; 50:50 for myricetin, daidzin, ethyl gallate, and naringin; 45:55 for *m*-coumaric acid and *o*-coumaric acid; 35:65 for puerarin, vanillic acid, ferulic acid, caffeic acid, *p*-coumaric acid, veratric acid, syringic acid, protocatechuic acid, methyl gallate, and sinapic acid; and 30:70 for gallic acid. The flow rate was 0.8 mL/min. The analysis was monitored at optimal absorption wavelength of each polyphenol according to the HPLC system. The method previously used in our group was applied and modified to individually analyze EC, ECG, EGC, and EGCG, employing an isocratic elution method in which the mobile phase was methanol–aqueous 2% glacial acetic acid (30:70, v/v).<sup>22</sup>

**Statistical Analysis.** All results were reported as the mean  $\pm$  standard deviation (SD) of three independent repetitions ( $n = 3$ ).

Duncan's multiple-range tests were performed to determine significance using a SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA) ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

**Effects of Hydroxylation of Flavonoids on Their Adsorption Capacities into Oat  $\beta$ -Glucan.** The effects of hydroxylation of flavonoids on their adsorption capacities into oat  $\beta$ -glucan are shown in Table 2. As seen from the effect data, hydroxylation on rings A, B, and C significantly affected the adsorption capacities of flavonoids into oat  $\beta$ -glucan.

**Table 2. Effects of Hydroxylation of Flavonoids on Their Adsorption Capacities into Oat  $\beta$ -Glucan**

class	ring	position	example	effect <sup>a</sup> (%)
flavones	B	3'H $\rightarrow$ OH	apigenin $\rightarrow$ luteolin	18.26 <sup>b</sup>
	C	3H $\rightarrow$ OH	apigenin $\rightarrow$ kaempferol	57.38 <sup>b</sup>
	C	3H $\rightarrow$ OH	luteolin $\rightarrow$ quercetin	-48.43 <sup>b</sup>
flavonols	B	3'H $\rightarrow$ OH	kaempferol $\rightarrow$ quercetin	-61.25 <sup>b</sup>
	B	4'H $\rightarrow$ OH	galangin $\rightarrow$ kaempferol	23.46 <sup>b</sup>
	B	5'H $\rightarrow$ OH	kaempferol $\rightarrow$ morin	-87.72 <sup>b</sup>
	B	2'H $\rightarrow$ OH	quercetin $\rightarrow$ myricetin	-16.13 <sup>b</sup>
isoflavones	A	5H $\rightarrow$ OH	daidzein $\rightarrow$ genistein	13.01 <sup>b</sup>
flavanone	B	4'H $\rightarrow$ OH	naringenin $\rightarrow$ eriodictyol	30.27 <sup>b</sup>

<sup>a</sup>Effect (%) =  $(q_{e2} - q_{e1})/q_{e1} \times 100\%$ , where  $q_{e1}$  and  $q_{e2}$  refer to the adsorption capacity of polyphenol before and after the arrow in the "example" column. <sup>b</sup>The adsorption capacities of example compounds are significantly different within the same row ( $p < 0.05$ ).

**Hydroxylation on Rings B and C of Flavones.** As illustrated in Table 2, introduction of a hydroxyl group to ring B (apigenin  $\rightarrow$  luteolin) or ring C (apigenin  $\rightarrow$  kaempferol) significantly increased the adsorption capacities of flavones into oat  $\beta$ -glucan. Although both luteolin (5-, 7-, 3', 4'-OH) and kaempferol have the same number of hydroxyl groups, kaempferol showed higher adsorption capacity into oat  $\beta$ -glucan than luteolin. This suggests that hydroxylation at the 3-position on ring C is more favorable than that on the 3'-position on ring B in terms of adsorption capacity. However, when luteolin was further hydroxylated on ring C, resulting in quercetin, its adsorption capacity greatly decreased.

**Hydroxylation on Ring B of Flavonols.** Galangin, kaempferol, morin, quercetin, and myricetin share the same structure of rings A and C. Hydroxylation on ring B of galangin, resulting in kaempferol (3-, 5-, 7-, 4'-OH), significantly increased its adsorption capacity into oat  $\beta$ -glucan by 23.46%. However, compared to kaempferol, morin (3-, 5-, 7-, 2', 4'-OH) and quercetin (3-, 5-, 7-, 3', 4'-OH) presented decreased adsorption capacities into oat  $\beta$ -glucan by 87.72 and 61.25%, respectively (Table 2). Similarly, the adsorption capacity of myricetin (3-, 5-, 7-, 3', 4', 5'-OH) was lower than that of quercetin by 16.13%. Although the same numbers of hydroxyl groups on ring B are present, the adsorption capacities of morin (64.61  $\pm$  7.14 mol/mol) and quercetin (203.81  $\pm$  6.86 mol/mol) into oat  $\beta$ -glucan differed dramatically, suggesting that the hydroxyl group of ring B of flavonols substituted at the 3'-position is more favorable than at the 2'-position in the adsorption capacities into oat  $\beta$ -glucan.

**Hydroxylation on Ring A of Isoflavone and Ring B of Flavanone.** As shown in Table 2, hydroxylation on ring A of

daidzein (7-, 4'-OH), resulting in genistein (5-, 7-, 4'-OH), slightly enhanced the adsorption capacity. Riihimäki et al.<sup>23</sup> observed similar results when they investigated the affinities of daidzein and genistein with  $\beta$ -lactoglobulin. The adsorption capacity of eriodictyol (5-, 7-, 3', 4'-OH) into oat  $\beta$ -glucan was 1.30 times higher than that of naringenin (5-, 7-, 4'-OH). Interestingly, hydroxylation favored the adsorption of flavonoids with three or fewer hydroxyl groups but deteriorated for those with four or more hydroxyl groups.

**Comparing the Adsorption of Flavonoid Isomers into Oat  $\beta$ -Glucan.** To investigate the effects of core structure of flavonoid isomers on their adsorption capacities into oat  $\beta$ -glucan, two isomer groups (apigenin, galangin, naringenin, and genistein and luteolin, kaempferol, and eriodictyol) were concerned. Their adsorption capacity was determined as galangin > apigenin > naringenin > genistein and kaempferol > luteolin > eriodictyol. Combined with classification of these compounds, it was concluded that adsorption capacities of flavonoid isomers into oat  $\beta$ -glucan increased in the order flavonol > flvaone > flavanone > isoflavone.

**Effects of Glycosylation on Adsorption Capacities of Flavonoids into Oat  $\beta$ -Glucan.** The majority of dietary flavonoids naturally occur as  $\beta$ -glycosides. These  $\beta$ -glycosides are often found as 3- and 7-O-glycosides, whereas 8-C-glycoside also exists in a small proportion.<sup>24,25</sup> In the present study (Table 3), effects of glycosylation on the adsorption capacities

**Table 3. Effects of Glycosylation of Flavonoids on Their Adsorption Capacities into  $\beta$ -Glucan**

class	ring	position	example	effect <sup>a</sup> (%)
flavonols	B	3OH $\rightarrow$ 3-O- $\alpha$ -L-rhamnoside	myricetin $\rightarrow$ myricetrin	23.13 <sup>b</sup>
		3OH $\rightarrow$ 3- $\alpha$ -L-Rham-1,6-D-Glc	quercetin $\rightarrow$ rutin	-18.54 <sup>b</sup>
flavanone	A	7OH $\rightarrow$ 7-neohesperidose	naringenin $\rightarrow$ naringin	-20.39 <sup>b</sup>
isoflavones		7OH $\rightarrow$ 7-glucoside	genistein $\rightarrow$ genistin	-0.56
			daidzein $\rightarrow$ daidzin	37.98 <sup>b</sup>
		8H $\rightarrow$ 8-C-glucose	daidzein $\rightarrow$ puerarin	-29.12 <sup>b</sup>

<sup>a</sup>Effect (%) =  $(q_{e2} - q_{e1})/q_{e1} \times 100\%$ , where  $q_{e1}$  and  $q_{e2}$  referred to the adsorption capacity of polyphenol before and after the arrow in the "example" column. <sup>b</sup>The adsorption capacities of example compounds are significantly different within the same row ( $p < 0.05$ ).

of flavonoids into oat  $\beta$ -glucan were shown to vary. Adsorption capacities of rutin, naringin, and puerarin were significantly lower than those of their unglycosylated forms. This was in agreement with the result reported by Simonsen et al. that glucosides were significantly less adsorbed than their aglycons by oat and barley  $\beta$ -glucans.<sup>17</sup> Glycosylation hardly affected the adsorption capacity of genistein. On the other hand, 7-glucosylation of daidzein and 3-O- $\alpha$ -L-rhamnosylation of myricetin enhanced their adsorption capacities into oat  $\beta$ -glucan. Therefore, no definitive correlation between glycosylation and adsorption was observed. This may be related to the multiaspect changes in physicochemical properties of flavonoids induced by different types of glycosylation, such as

the difference in hydrophilicity of adsorbate<sup>26</sup> and steric hindrance of glycosyl groups.<sup>27,28</sup>

**Effects of Methylation and Methoxylation on Their Adsorption Capacities into Oat  $\beta$ -Glucan.** As presented in Table 4, methylation considerably affected the adsorption

**Table 4. Effects of Methylation and Methoxylation of Phenolics on Their Adsorption Capacities into Oat  $\beta$ -Glucan**

class	position	example	effect <sup>a</sup> (%)
flavonols	B-ring, 3-OH $\rightarrow$ OCH <sub>3</sub>	quercetin $\rightarrow$ isorhamnetin	307.08 <sup>b</sup>
flavones	B-ring, 4-OH $\rightarrow$ OCH <sub>3</sub>	luteolin $\rightarrow$ diosmetin	-54.01 <sup>b</sup>
hydroxycinnamic acids	3-OH $\rightarrow$ OCH <sub>3</sub>	caffeic acid $\rightarrow$ ferulic acid	32.48 <sup>b</sup>
	5-H $\rightarrow$ OCH <sub>3</sub>	ferulic acid $\rightarrow$ sinapic acid	-34.64 <sup>b</sup>
hydroxybenzoic acids	3-OH $\rightarrow$ OCH <sub>3</sub>	protocatechuic acid $\rightarrow$ vanillic acid	-60.59 <sup>b</sup>
	4-OH $\rightarrow$ OCH <sub>3</sub>	vanillic acid $\rightarrow$ veratric acid	-24.11 <sup>b</sup>
	3, 4-OH $\rightarrow$ OCH <sub>3</sub>	protocatechuic acid $\rightarrow$ veratric acid	-70.09 <sup>b</sup>
	3,5-OH $\rightarrow$ OCH <sub>3</sub>	gallic acid $\rightarrow$ syringic acid	-26.64 <sup>b</sup>

<sup>a</sup>Effect (%) =  $(q_{e2} - q_{e1})/q_{e1} \times 100\%$ , where  $q_{e1}$  and  $q_{e2}$  referred to the adsorption capacity of polyphenol before and after the arrow in the "example" column. <sup>b</sup>The adsorption capacities of example compounds are significantly different within the same row ( $p < 0.05$ ).

capacities of flavonols and flavones into oat  $\beta$ -glucan. Introduction of a methoxy group to ring B of quercetin (3-, 5-, 7-, 3', 4'-OH), resulting in isorhamnetin (3-, 5-, 7-, 4'-OH), sharply increased its adsorption capacity into oat  $\beta$ -glucan by 307.08%. However, the adsorption capacity of luteolin (5-, 7-, 3', 4'-OH) was dramatically decreased by 54.01% when methylated to result in diosmetin (5-, 7-, 3'-OH). The unexpected improvement in adsorption capacity resulting from methylation of quercetin should be ascribed to the excellent solubility of isorhamnetin.<sup>29</sup>

**Effects of Methylation and Methoxylation of Phenolic Acids on Their Adsorption Capacities into Oat  $\beta$ -Glucan.**

Methylated ( $-\text{OH} \rightarrow -\text{OCH}_3$ ) and methoxylated ( $-\text{C} \rightarrow -\text{C}-\text{OCH}_3$ ) phenolic acids are widely distributed in plants. Effects of methylation and methoxylation on the adsorption capacities of phenolic acids are shown in Table 4. With regard to hydroxycinnamic acid derivatives, ferulic acid, a methylated derivative from caffeic acid, presented a higher adsorption capacity than its unmethylated analogue. This was different from the result reported by Li and co-workers that methylation of caffeic acid at 3-OH lowered its affinity with BSA.<sup>30</sup> Unlike methylation, methoxylation of ferulic acid, resulting in sinapic acid, significantly decreased its adsorption capacity into oat  $\beta$ -glucan by 34.64%. As for hydroxybenzoic acids, methylation deteriorated their adsorption capacities into oat  $\beta$ -glucan.

**Gallic Acid.** Gallic acid and its derivatives are phenolic acids widely distributed in plants with strong antioxidative activities.<sup>31</sup> Gallic acid showed cytotoxicity against cancer cells without damaging healthy cells and was renal-protective in long-term treatment of chronic kidney disease.<sup>32,33</sup> Recent research has revealed that structure and hydrophobicity of gallic acid and its derivatives were crucial factors determining their

biological activities, such as the protective effects on cell systems.<sup>31,34</sup> As presented in Table 1, esterification notably reduced the adsorption capacity of gallic acid into oat  $\beta$ -glucan. In detail, ethyl esterification presented more serious effects than methyl esterification with respect to the adsorption. As commonly known, the bigger the acyl group attached to gallic acid, the lower the hydrophilicity of the product was. In comparison with gallic acid, esterified gallic acids presented deteriorated adsorption capacities, which could be ascribed to enhancement of hydrophobicity by esterification. This evidence implied that adsorption of polyphenols into oat  $\beta$ -glucan was governed by hydrophilic driving forces, such as hydrogen bonding.

**Coumaric Acid.** Coumaric acids, especially *p*-coumaric acid (*p*-CA), are widely distributed in plants in free or bound form.<sup>35</sup> *o*-Coumaric acid (*o*-CA) and *m*-coumaric acid (*m*-CA) are often found in bound form, such as *o*-coumaric acid glucoside in mesophyll cells of sweet clover leaves.<sup>36</sup> As shown in Table 1, *o*-CA was more feasible to be adsorbed by  $\beta$ -glucan than *m*-CA and *p*-CA. No difference in adsorption capacity was observed for *m*-CA and *p*-CA. It is imperative to point out that Stražišar and coauthors found that apparent binding constants of coumaric acids to  $\beta$ -cyclodextrin ( $\beta$ -CD) increased in the order *p*-CA > *m*-CA > *o*-CA.<sup>37</sup> This was explained by the different driving forces involved in interaction between  $\beta$ -CD and specific coumaric acid. In detail, *p*-CA was retained in the cavity of  $\beta$ -CD by hydrophobic interaction, whereas *m*-CA and *o*-CA formed hydrogen bonds with  $\beta$ -CD.

**Catechins.** Catechins are the most important components in tea polyphenols with substantial antioxidant activity. Catechins mainly refer to (–)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), and (–)-gallocatechin gallate (GCG).<sup>38</sup> In our present study, effects of the galloyl group on adsorption of catechins into oat  $\beta$ -glucan were investigated with four catechin monomers. As presented in Table 1, galloylation of EC, resulting in ECG, dramatically decreased its adsorption capacity into oat  $\beta$ -glucan. Likewise, EGC, the galloylation mother of EGCG, presented a lower value in adsorption capacity than EGCG. These facts illustrated that pyrogallol-type catechins showed lower adsorption capacities than catechol-type catechins. This confirmed previous findings that the galloyl group was of great importance in interactions of phenolics with polysaccharides.<sup>22,39,40</sup>

It has to be pointed out that our work is not without some limitations, the most prominent being (1) that to observe the effects of structural parameters of polyphenols on their adsorption into oat  $\beta$ -glucan, the concentration of tested polyphenols in the present study was set as the same level (30  $\mu$ g/mL). Indeed, the concentration of polyphenol monomers varied too much in various foodstuffs. (2) The conditions of the present experiment system were much different from those of the actual food samples and in vivo situation, especially the utilization of menthol in the adsorption process. Consequently, further investigations should be carried out in this area to address these limitations and reinforce the actual implications of this study.

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## Notes

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## ABBREVIATIONS USED

EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate

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