RESEARCH ARTICLE

Interaction of dietary polyphenols with bovine milk proteins: Molecular structure–affinity relationship and influencing bioactivity aspects

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Scope: Dietary flavonoids and stilbenes are important polyphenols in foods, such as, e.g. fruits, vegetables, nuts, and tea as they are of great interest for their bioactivities, which are related to the anti-oxidative property.

Methods and results: The relationship between the structural properties of dietary polyphenols and their affinities for milk proteins (MP) was investigated. Methylation and methoxylation of flavonoids decreased (or hardly affected) the affinities for MP. Hydroxylation on the rings A and B of flavones and flavonols enhanced the interaction slightly. The hydroxylation on the ring A of flavanones significantly improved the affinities. Glycosylation of flavonoids weakened the affinities by 1–2 orders of magnitude. The hydrogenation of the C2═C3 double bond of flavonoids decreased the binding affinities by 7.24–75.86-fold. Galloylation of catechins significantly improved the binding affinities by about 100–1000-fold. Glycosylation of resveratrol decreased its affinity for MP. Esterification of gallic acid increased its binding affinity. MP significantly weakened the DPPH radical scavenging activity of polyphenols. The decreasing DPPH scavenging percentages of polyphenols increased with increasing affinities of MP–polyphenol complexes.

Conclusion: The binding affinities with MP were strongly influenced by the structural differences of dietary polyphenols. The MP–polyphenol interaction weakened with the DPPH free radical scavenging potential of polyphenols.

Keywords:
DPPH radical scavengers / Milk proteins / Polyphenols / Protein–polyphenol interaction / Structure–affinity relationship

1 Introduction

Dietary flavonoids and stilbenes are important polyphenols in foods, such as, e.g. fruits, vegetables, nuts, and tea [1–5], as they are of great interest for their bioactivities, which are basically related to the anti-oxidative property [5–8]. The structural differences between the various classes occur in the chemistry of the ring C, as well as the number and distribution of hydroxyl groups and their substitutions on the rings A and B. These differences significantly affect their absorption, metabolism, and bio-activities in vivo. For instance, methylation of the free hydroxyl groups in the flavones dramatically increased their intestinal absorption and metabolic stability by preventing the formation of

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Abbreviations: EC, (−)-epicatechin; **EGCG,** (−)-epigallocatechin gallate; **GCG,** gallocatechin gallate; **MP,** milk protein

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glucuronic acid and sulfate conjugates [9, 10]. Walle concluded that the methylation appears to be a simple and effective way to increase the metabolic resistance and transport of flavonoids [11]. Courts and Williamson found that the C-glycoside aspalathin was methylated and glucuronidated in vivo in an intact form in human [12]. The flavonol moiety, i.e. the 2,3-double bond in conjugation with a 4-oxo group and a 3-hydroxyl group, as well as the 5,7-dihydroxylation at the A-ring has been found to be important structural features for significant anti-oxidant activity [13]. In addition to –OH moieties in the structural arrangements of flavonoids, the resonance of electrons between the rings A and B was reported to be essential for the anti-oxidant and biological activities of the compounds [14].

Milk proteins (MP) are natural vehicles, which evolved to deliver essential micronutrients (e.g. calcium and phosphate) and immune system components (e.g. immunoglobulins and lactoferrin), from mother to the newborn [15]. Briefly, cow MPs consist of caseins (2.4–2.8%), β-lactoglobulin (0.2–0.4%), α-lactalbumin (0.1–0.15), bovine serum albumin (BSA, 0.01–0.04%), immunoglobulins (0.06–0.1%), etc. [16]. Proteins from milk are natural vehicles for bioactive small molecules [15]. Many of their structural and physicochemical properties, such as excellent surface and self-assembly properties, superb gelation properties; pH-responsive gel swelling behavior, useful for programmable release; various shielding capabilities and essential for protecting sensitive payload, facilitate their functionality in binding of ions and small molecules [15, 17, 18]. Some of them (e.g. BSA, β-lactoglobulin, and γ-globulin) can bind with many kinds of endogenous and exogenous agents such as dietary polyphenols [19–24]. Few reports, however, have focused on the structure–affinity relationship of dietary polyphenols on the affinities for MP. The present work deals with the relationship between the molecular structures of dietary polyphenols and their affinities for MP. Fifty-five polyphenols (Table 1) were studied. The influence of MP–polyphenol interaction on the DPPH free radical scavenging potential of polyphenols was also investigated in detail.

2 Materials and methods

2.1 Apparatus and reagents

The fluorescence spectra were recorded on a *JASCO FP-6500* fluorometer (Tokyo, Japan). The pH measurements were carried out on a *Cole-Parmer PHS-3C* Exact Digital pH meter (IL, USA). Biochanin A, genistein, apigenin, puerarin, catechin, (−)-epicatechin (EC), and luteolin (99.0%) were purchased from Aladin (Shanghai, China). Flavone, chrysin, and baicalein (99.5%) were obtained commercially from Wako Pure Chemical Industries (Osaka, Japan). Flavanone, 7-hydroxyflavanone, 6-hydroxyflavanone, 6-methoxyflavanone, 6-hydroxyflavone, and 6-methoxyflavone were purchased from TCI Chemical Industries (Tokyo, Japan). Others polyphenol standards were obtained commercially from Shanghai Tauto Biotech (Shanghai, China). The working solutions of the polyphenols (1.0×10^{-3} mol/L) were prepared by dissolving each polyphenol in methanol. The pure bovine milk was obtained form Guangming (Shanghai, China). It contains (per 100 mL) 3.0 g protein, 3.2 g fat, and 4.7 g carbornhydrate. The working solution of MP (1:100) was prepared by directly diluting above bovine milk with double-distilled water and stored in refrigerator prior to use. All other reagents and solvents were of analytical grade and all aqueous solutions were prepared using newly double-distilled water.

2.2 Fluorescence spectra

The fluorescence spectra were recorded upon excitation at 280 nm when MPs were titrated with polyphenols. Slit widths, scan speed, and excitation voltage were kept constant within each data set. Titrations were performed manually by using trace syringes. The fluorescence emissions of these polyphenols within the range of 300–400 nm were not observed under the excitation wavelength of 280 nm. The polyphenols were stable during the fluorescence measurements, as shown by HPLC analyses (not given here). Each fluorescence intensity determination was repeated and found to be reproducible within experimental errors.

2.3 Preparation of MP–polyphenol complexes

The working solutions of the polyphenols (1.0×10^{-3} mol/L) were diluted ten times with buffer or MP solution (1:100) to obtain tested samples. These tested samples were kept at room temperature. Samples were tested periodically for DPPH radical scavenging activity analysis.

2.4 DPPH radical scavenging activity

The anti-oxidant activities of polyphenols in the absence and presence of MP were measured on the basis of the scavenging activity of DPPH free radical. One milliliter sample was added to 1 ml of DPPH solution (0.2 mM in ethanol) as the free radical source. The decrease in the solution absorbance was measured at 517 nm after 30 min. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity } (\%) = (1 - A_0/A_1) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of samples.

Each experiment was repeated thrice and found to be reproducible within experimental errors.

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Table 1. Chemical structures of the various polyphenols and their affinities for MP

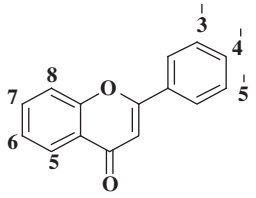
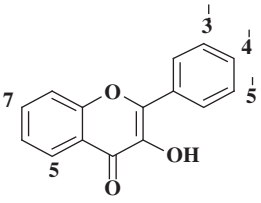
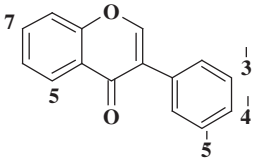
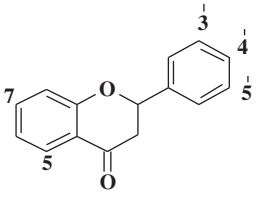
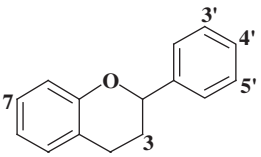
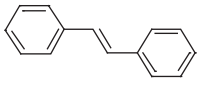
Subclass	Name	Substitutions			Affinity	
		OH	OCH_3	Others	lg K_a	*n*
Flavones	Flavone				4.81	1.05
	7-Hydroxyflavone	7			5.09	1.05
	6-Hydroxyflavone	6			6.02	1.16
	6-Methoxyflavone		6		5.63	1.10
	Chrysin	5,7			5.19	1.04
	Baicalein	5,6,7			6.14	1.17
	Baicalin	5,6		7-β-D-glucuronide	4.76	0.99
	Apigenin	5,7, 4′			5.82	1.14
	Luteolin	5,7,3′, 4′			5.82	1.13
	Hispidulin	5,7, 4′	6		5.29	1.07
	Wogonin	5,7	8		5.13	1.07
	Tangeretin		5,6,7,8,4′		5.76	1.12
	Nobiletin		5,6,7,8,4′,5′		5.69	1.11
Flavonols	Galangin	3,5,7			5.40	1.08
	Kaempferide	3,5,7	4′		5.36	1.05
	Kaempferol	3,5,7, 4′			5.57	1.06
	Kaempferitrin	5,4′		3,7-Dirhamnoside	4.99	1.03
	Quercetin	3,5,7,3′, 4′			5.72	1.12
	Quercitrin	5,7,3′, 4′		3-*o*-β-D-glucoside	5.65	1.09
	Myricetin	3,5,7,3′, 4′, 5′			6.04	1.17
	Fisetin	3,7,3′, 4′			5.40	1.07
	Rutin	5,7,3′, 4′		3-α-L-Rham-1,6-D-Glc	3.98	0.87
Isoflavones	Formononetin	7	4′		4.12	0.91
	Genistein	5,7,4′			4.76	1.01
	Daidzein	7,4′			5.29	1.04
	Daidzin	4′		7-Glucoside	5.21	1.09
	Genistin	5,4′		7-Glucoside	4.65	1.04
	Biochanin A	5,7	4′		4.79	0.96
	Tectorigenin	5,7,4′	6		5.47	1.11
	Puerarin	7,4′		8-C-glucose	4.21	0.90
	Sophoricoside	5,7		4′-*O*-glucoside	3.39	0.79
Flavanone	Naringenin	5,7, 4′			3.94	0.86
	Naringin	5,4′		7-Neohesperidose	3.76	0.83
	Narirutin	5,4′		7-α-L-Rham-1,6-D-Glc	3.64	0.82
	Hesperitin	5,7, 3′	4′		5.14	1.05
	Hesperitin-7-*O*-rutinose	5,3′	4′	7-α-L-Rham-1,6-D-Glc	4.80	0.99
	Dihydromyricetin	3,5,7,3′, 4′, 5′			5.07	1.04
	Flavanone				3.58	0.84
	7-Hydroxyflavanone	7			5.59	1.12
	6-Hydroxyflavanone	6			4.87	1.08
	6-Methoxyflavanone		6		4.77	1.06
	Silibinin				5.28	1.07
	Alpinetin	7	5		5.43	1.17
Flavanonol	GCG (2,3-trans)	5,7,3′,4′,5′		3-Gallate	4.98	1.02
	EGCG (2,3-cis)	5,7,3′,4′,5′		3-Gallate	4.54	0.97
	ECG (2,3-cis)	5,7,4′,5′		3-Gallate	4.96	1.01
	EC (2,3-cis)	3,5,7,4′,5′			3.01	0.79
	EGC (2,3-cis)	3,5,7,3′,4′,5′			2.05	0.65
	C (2,3-trans)	3,5,7,4′,5′			2.29	0.66
Stilbene	Resveratrol	3,5,4′			4.94	1.02
	Polydatin	5,4′		3-glucoside	4.54	0.95

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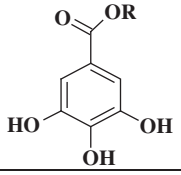
Table 1. Continued.

Subclass	Name	Substitutions			Affinity	
		OH	OCH_3	Others	lg K_a	*n*
Gallic acid 	Gallic acid			R = H	5.06	1.18
	Methyl gallate			R = methyl	5.71	1.20
	Ethyl gallate			R = ethyl	5.58	1.18
	Propyl gallate			R = propyl	5.50	1.15

3 Results and discussion

3.1 The binding constants (K_a) and the number of binding sites (*n*)

As representative examples, the fluorescence spectra of MP after addition of daidzein and daidzin are shown in Supporting Information Fig. 1 (the fluorescence spectra of MP quenched by other polyphenols are not given here). The maximum λ_{em} of MP was hardly shifted in the presence of daidzein and daidzin. However, in these and all other cases, the fluorescence intensities of MP decreased remarkably with increasing concentration of polyphenols. The quenching degrees of polyphenols depend on their structures. For daidzein and daidzin, daidzein (A) showed stronger quenching effect on MP fluorescence than that of daidzin (B). Daidzein resulted in quenching about 48% MP fluorescence; however, daidzin only quenched about 35% MP fluorescence.

The binding constants were calculated according to the double-logarithm equation [20–25]:

$$\log_{10}(F_0 - F)/F = \log_{10}K_a + n\log_{10}[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensities of MP in the absence and in the presence of polyphenols, K_a is the binding constant, n is the number of binding sites, and [Q] is the concentration of polyphenols. Table 1 summarizes the calculated results according to Eq (1). The values of $\log_{10}K_a$ are proportional to the number of binding sites (n) (data not shown), which indicates that the Eq. (1) used here is suitable to study the interaction between polyphenols and MP [26, 27]. The magnitudes of apparent binding constants for MP were almost all in the range of 10^4–10^5 L/mol, which were similar to the recent reports for bovine γ-globulin [24]. However, these data were much smaller than the affinities of polyphenols for serum albumins from our previous reports (10^4–10^8 L/mol) [20–23].

3.2 Flavonoids

3.2.1 Methylation and methoxylation

As shown in Fig. 3, the methylation and methoxylation of flavonoids weakened or little affected their binding affinities for MP. In general, the methylation of hydroxyl group in flavonoids decreased their binding affinities for MP by 1.10–14.79 times. The affinity of dadzein for MP was found to be 14.79-times higher than that of its methylated form (formononetin) (Fig. 1). On the other side, 4′-methoxylation of galangin hardly affected the affinity for MP. This result supports that the methylation of hydroxyl group in flavonoids reduced the binding affinities for MP.

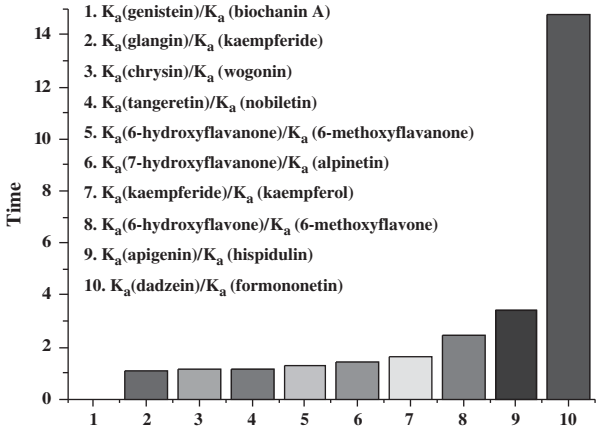


Figure 1. The methoxylation and methylation of flavonoids weakens the affinity of the polyphenols for MP.

3.2.2 Hydroxylation

Table 2 shows the effects of hydroxylation of flavonoids on the affinities for MP. As seen from the data, the hydroxylation on the rings A and B of flavones and flavonols slightly enhanced the binding affinities for MP. The hydroxylation on the ring A of flavanones significantly improved the affinities for MP. However, the hydroxylation on the ring C of flavones hardly influenced the binding affinities for MP and the hydroxylation on ring A of isoflavones reduced or little affected the affinities for MP.

3.2.2.1 Hydroxylation on ring A of flavones

Hydroxylation on ring A of flavones obviously improved the affinities for MP with increasing hydroxyl group number. It

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Table 2. Effects of hydroxylation of flavonoids on the affinities for MP

Class	Ring	Position	Example	Effect (times)
Flavone	A	7 H→OH	Flavone→7-OHflavone	↑ 1.91
		5 H→OH	7-OHflavone→chrysin	↑ 1.26
		6 H→OH	Chrysin→baicalein	↑ 8.91
			Flavone→6-Ohflavone	↑ 16.22
	B	4′H→OH	Chrysin→apigenin	↑ 4.27
		3′H→OH	Apigenin→luteolin	No effect
	C	3 H→OH	Chrysin→galangin	↑ 1.62
			Apigenin→kaempferol	↓ 1.78
			Luteolin→quercetin	↓ 1.26
Flavonol	A	5 H→OH	Fisetin→quercetin	↑ 2.09
	B	3′H→OH	Kaempferol→quercetin	↑ 1.41
		4′H→OH	Galangin→kaempferol	↑ 1.48
		5′H→OH	Quercetin→myricetin	↑ 2.09
Isoflavone	A	5 H→OH	Daidzein→genistein	↓ 3.39
			Daidzin→genistin	↓ 3.63
			Formononetin→biochanin A	Little effect
Flavanone	A	6 H→OH	Flavanone→6-OHflavanone	↑ 19.95
		7 H→OH	Flavanone→7-OHflavanone	↑ 104.71

appears that the optimal hydroxyl groups introduced to the ring A of flavones is 5,6,7-position, as the highest binding was observed with baicalein (containing three hydroxyl groups).

3.2.2.2 Hydroxylation on ring B of flavones

Hydroxylation on ring B of flavones increased or little affected the apparent binding constants (K_a) between flavones and MP. For example, the affinity of apigenin (5,7,3′) for MP was found to be 4.27-times higher than that of chrysin (5,7). On the other hand, the affinity of apigenin (5,7,3′) for MP was the same as that of luteolin (5,7,3′,4′).

3.2.2.3 Hydroxylation on ring C of flavones

The affinities of kaempferol (3,5,7,3′), galangin (3,5,7), and quercetin (3,5,7,3′,4′) for MP are almost the same as those of apigenin (5,7,3′), chrysin (5,7), and luteolin (5,7,3′,4′), respectively. It illustrated that the hydroxylation on the ring C of flavones hardly influenced the binding affinities for MP.

3.2.2.4 Hydroxylation of rings A and B of flavonols

It was found that the hydroxylation on rings A and B of flavonols slightly improves the binding affinity for MP. The hydroxylation on ring B of flavonols obviously improved the affinities for MP with increasing hydroxyl group number. Flavonols are the most prominent flavonoids in plants. The most prominent flavonols such as quercetin and kaempferol in foods carry 5,7-dihydroxyl groups on ring A. Here, it was found that the hydroxylation on position 5 of flavonol slightly enhances the binding affinity for MP by 2.09 times.

3.2.2.5 Hydroxylation on ring A of isoflavones

The affinity of genistein (5,7,4′) for MP was about 3.39-times lower than that of daidzein (7,4′). The affinity of genistin (5,7,4′) for MP was about 3.63-times lower than that of daidzein (7,4′). However, the affinity of biochanin A (5,7) for MP was similar to that of formononetin (7). These data revealed that the hydroxylation on ring A of isoflavones reduced or little affected the affinities for MP.

3.2.2.6 Hydroxylation on ring A of flavanones

Hydroxylation on ring A of flavanones significantly increased the affinities for MP. 6-OHflavanone and 7-OHflavanone showed 19.95- and 104.71-fold higher affinities than that of flavanone.

3.2.3 Comparing the affinities of flavonoid isomers with MP

In this study, we can compare two isomer groups (apigenin, galangin, baicalein, genistein and luteolin, kaempferol, fisetin). The binding constants (K_a) were determined as: baicalein > apigenin > galangin > genistein and luteolin > kaempferol > fisetin. These data illustrated that flavones showed higher binding capacity with MP than flavonols. The current results are in good agreement with data reported previously about the interaction between flavonoid isomers with BSA [28].

3.2.4 Glycosylation

The dietary flavonoids in nature occur mostly as β-glycosides. The flavonols are found mainly as the 3- and 7-*O*-glycoside, although the 4′ position may also be glycosylated in some plants (Table 1). Most recently, we have reported the glycosylation of flavonoids lowered the affinity for BSA by 1–3 orders of magnitude depending on the conjugation

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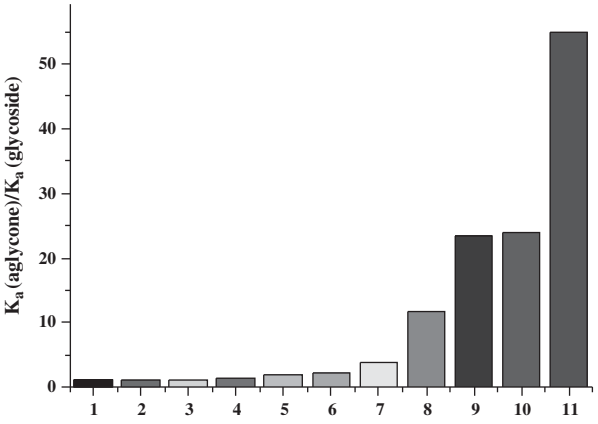

Figure 2. The glycosylation of flavonoids decreases the affinity of the polyphenols for MP. 1. K_a (quercetin)/K_a (quercitrin), 2. K_a (daidzein)/K_a(daidzin), 3. K_a (genistein)/K_a (genistin), 4. K_a (naringenin)/K_a (naringin), 5. K_a (naringenin)/K_a (narirutin), 6. K_a (hesperitin)/K_a (hesperitin-7-*O*-rutinose), 7. K_a (kaempferol)/K_a (kaempferitrin), 8. K_a (daidzein)/K_a (puerarin), 9. K_a (genistein)/K_a (sophoricoside), 10. K_a (baicalein)/K_a (baicalin), 11. K_a (quercetin)/K_a (rutin).

site and the class of sugar moiety [23]. Herein, the effect of glycosylation of dietary flavonoids on the affinities for MP was investigated. The sugar moieties are in 3, 7, or 4′-positions of flavonoids. In our present study (Fig. 2), the glycosylation of flavonoids lowered the affinity for MP 1–2 order of magnitude. The affinity of quercetin for MP was about 54.95-fold higher than that of rutin, but the affinity of quercetin for MP is only 1.02 times higher than that of quercitrin. The affinities of naringenin, naringin and narirutin for MP were determined as 3.94, 3.76, and 3.64, respectively. It revealed that the monoglycosides of flavonoids showed stronger binding affinities with MP than their polyglycoside forms. The decreasing affinity for MP after glycosylation may be caused by the non-planar structure. After the hydroxyl group is substituted by a glycoside, steric hindrance may take place, which weakens the affinity for proteins [23].

3.2.5 Hydrogenation of the C2=C3 double bond

The C2=C3 double bond in conjugation with a 4-oxo group plays a very important role for the affinity for MP. As shown in Table 2, it was found that the hydrogenation of the C2=C3 double bond of flavonoids decreased the binding affinities for MP about 7.24 to 75.86 times. The affinities of apigenin and myricetin for MP were about 75.86-times and 8.51-times higher than those of naringenin and dihydromyricetin, respectively. Previously, we have investigated the effect of hydrogenation of the C2=C3 double bond in flavonoids on the affinities for BSA [29]. Hydrogenation of the C2=C3 double bond for many flavonoids decreased the binding affinity for BSA by 2–4 orders of magnitude. Planarity of the C ring in flavonoids may be important for binding interaction with proteins, as the molecules with saturated C2–C3 bonds (flavanones and certain others) permit more twisting of the B ring with reference to the C ring. A C2=C3 double bond increases the π-conjugation of the bond linking the B and C rings, which favors near-planarity of the two rings [30]. Molecules with near-planar structure easier enter the hydrophobic pockets in proteins.

3.3 Catechins

Catechins are the major polyphenols in green tea leaves. The major catechins of green tea extract are (−)-catechin (C), EC, (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), and gallocatechin gallate (GCG). Here, we determined the affinities between catechins and MP by fluorescence quenching method with double-logarithm regression curve (Table 1). The binding constants ($\log_{10}K_a$) between ECG, EGCG, and GCG for MP were 4.96, 4.54, and 4.98, respectively. However, the binding constants of EC, EGC, and C for MP were within the range of 10^2–10^3 L/mol. It illustrates that the galloylation of catechins significantly improved the binding affinities with MP about 100–1000 times. The pyrogallol-type catechins showed lower affinities than catechol-type catechins. Moreover, the affinity of the catechin with 2,3-*trans* structure (GCG) for MP exhibited higher than that of the catechin with 2,3-*cis* structure (EGCG).

3.4 Stilbenes

Stilbenes are phytoalexins that become activated when plants are stressed and are important polyphenols with the C_6-C_2-C_6 structure [31]. The typical natural stilbenes are resveratrol and its 3-glucoside, polydatin [32]. These compounds exist in foods and are widely consumed. Resveratrol is a grape-derived polyphenol, which possesses a wide range of bioactivities including anti-oxidant, anti-inflammatory, and anti-tumor effects [33, 34]. Here, we determined the affinities between resveratrol/polydatin with MP. The binding constants ($\log_{10}K_a$) between resveratrol and polydatin for MP were 4.94 and 4.54, respectively. It illustrated that the glycosylation of resveratrol slightly reduced the affinity for MP. The affinity of resveratrol for MP was about 2.51-times higher than that of polydatin.

3.5 Gallic acid

Gallic acid is a type of phenolic acid found in gallnuts (http://en.wikipedia.org/wiki/Gallnut), sumac (http://en.wikipedia.org/wiki/Sumac), witch hazel (http://en.wikipedia.org/wiki/Witch_hazel), tea leaves (http://en.wikipedia.org/wiki/Tea), oak bark (http://en.wikipedia.org/wiki/Oak), and

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other plants (http://en.wikipedia.org/wiki/Plant). Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect our cells against oxidative damage [35]. Gallic acid was found to show cytotoxicity against cancer cells [36]. The gallates of gallic acid showed more effective inhibition of hemolysis than gallic acid [37]. The esters of gallic acid are termed as 'gallates'. The typical gallates are methyl gallate, ethyl gallate, and propyl gallate (Table 1). As seen from Table 1, the esterification of gallic acid significantly improved the affinity for MP. The affinities of gallic acid and its esters with α-amylase were determined as: methyl gallate > ethyl gallate > propyl gallate > gallic acid.

3.6 MP weakened the DPPH radical scavenging activity of polyphenols

The working solutions of the polyphenols (1.0×10^{-3} mol/L) were diluted ten times with double-distilled water or MP solution (1:100) to obtain tested samples. The immediate DPPH radical scavenging activities of the tested samples were detected to illustrate the effect of MP on the DPPH scavenging potential of polyphenols. The result showed that MP rapid weakened the DPPH radical scavenging activity of polyphenols (Fig. 3). Many reports found the similar results with the current work that proteins weakened the anti-oxidant capacity of polyphenols. Polyphenols–protein interaction is expected to modulate the bio-availability of polyphenols. Smith et al. found that binding of polyphenols, such as quercetin, fisetin, myricetin, and morin, to albumin reduced their prooxidant activity [38]. Arts et al. showed the antioxidant capacity of quercetin, rutin, and (+)-catechin in blood plasma was not additive [39]. Arts et al. further checked the effect of the flavonoid–protein interaction on the anti-oxidant capacity of flavonoids with the Trolox equivalent antioxidant capacity assay [40]. It looks like that

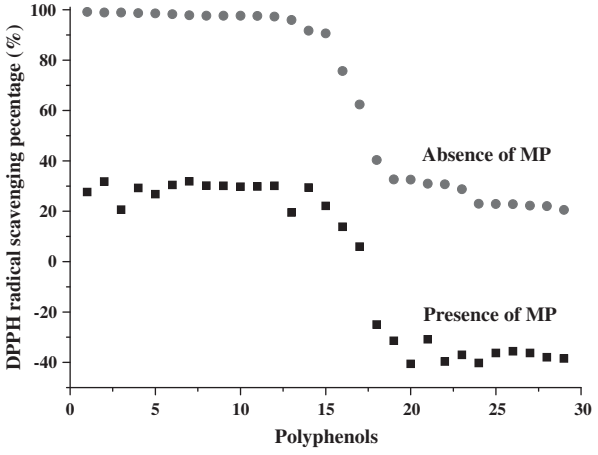

Figure 3. The DPPH radical scavenging capacity of polyphenols in the presence and absence of MP under aerobic condition.

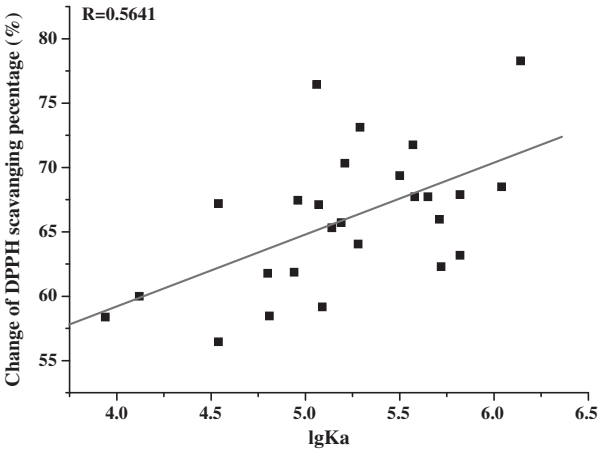

Figure 4. The relationship between the decreased DPPH scavenging percentages (%) and lg K_a values for polyphenols.

the effect of polyphenol–protein interaction on the bioavailability of flavonoids is not equivocal and the interaction of flavonoids with proteins will weaken the antioxidant capacity of the flavonoids both in products and in vivo.

The bioactivity of polyphenols is often executed in complex biological systems where various interactions took place. Polyphenol–protein interactions are expected to modulate the bioavailability of polyphenols. The relationship of the affinities of MP–polyphenol complexes and decreasing in DPPH scavenging percentages of polyphenols in the presence of MP is shown in Fig. 4. As seen from these data, the decreasing DPPH scavenging percentages of polyphenols increased with increasing affinities of MP–polyphenol complexes. According to the free drug hypothesis [41], if a molecule is highly bound to proteins, it is difficult to disassociate this complex to form free molecule, which will weak its efficacy. Here, it looks like that the antioxidant ability of the polyphenol–MP complex does not depend on the sum of the antioxidant ability of polyphenol molecules bound but simply on the number of polyphenols unbound. The significance of current research lies in the effect of polyphenol–protein interaction on the food–drug interaction.

4 Concluding remarks

Some of the structural elements that influence the affinities of polyphenols for γ-globulin are the following: (i) Methylation and methoxylation of flavonoids decreased or little affected the affinities; (ii) Hydroxylation on the rings A and B of flavones and flavonols slightly enhanced the interaction and hydroxylation on the ring A of flavanones significantly improved the affinities; (iii) Glycosylation of polyphenols weakened the affinities; (iv) Hydrogenation of the C2 = C3 double bond of flavonoids decreased the binding affinities;

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(v) Galloylation of catechins and esterification of gallic acid significantly improved the binding affinities. The MP–polyphenol interaction weakened on the DPPH free radical scavenging potential of polyphenols.

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