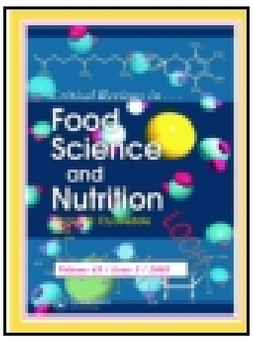

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

The inhibitory effects of flavonoids on α-amylase and α-glucosidase

Jianzhong Zhu, Chun Chen, Bin Zhang & Qiang Huang

To cite this article: Jianzhong Zhu, Chun Chen, Bin Zhang & Qiang Huang (2019): The inhibitory effects of flavonoids on α-amylase and α-glucosidase, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2018.1548428

To link to this article: https://doi.org/10.1080/10408398.2018.1548428

Published online: 13 Jan 2019.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2018.1548428

Taylor & Francis
Taylor & Francis Group

REVIEW

The inhibitory effects of flavonoids on α-amylase and α-glucosidase

Jianzhong Zhu[a,b], Chun Chen[a,c], Bin Zhang[a,b], and Qiang Huang[a,b,c]

[a]School of Food Science and Engineering, South China University of Technology, Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou, PR China; [b]Sino-Singapore International Joint Research Institute, Guangzhou, China; [c]Overseas Expertise Introduction Center for Discipline Innovation of Food Nutrition and Human Health (111 Center), Guangzhou, China

ABSTRACT

The objective of this review is to summarize knowledge on the inhibitory effects (IEs) of flavonoids on α-amylase (αA) and α-glucosidase (αG) relevant to the search of substitutes of acarbose (Aca). Flavonoids reported to be more effective at inhibiting αG than Aca have been summarized. The concept of "relative coefficient to Aca (RC_{Aca})" has been proposed to integrate data from various reports. Correlations between hydrogen bond donors (H-donors), hydrogen bond acceptors (H-acceptors), partition coefficient values (XLog P3), and RC_{Aca} are discussed. Two kinds of binding modes between flavonoids and enzymes have been observed: (i) flavonoids directly bind to amino acid residues (AARs) in the active sites of enzymes and exclude the binding of substrate; (ii) flavonoids interact with AARs near the active site and close the channel to the active center. Some groups are correlated with stronger IEs: (i) substitutions of caffeoyl, galloyl, and prenyl groups in flavonoids enhance IEs; (ii) steric hindrance attenuates IEs, and linear molecules tend to be stronger inhibitors of porcine pancreatic αA (PPA). Whilst many achievements have been made, our understanding of the combined effects of different flavonoids, and flavonoids and Aca, remain ambiguous, and the effects of food matrices and stomach digestion on IEs of flavonoids are poorly understood. This review provides a comprehensive understanding on the use of flavonoids as αA and αG inhibitors for controlling diabetes.

KEYWORDS

Diabetes; flavonoids; α-amylase inhibitors; α-glucosidase inhibitors

Introduction

Diabetes mellitus is a metabolic disease caused by diminished secretion of insulin by pancreatic *β*-cells, or poor responses of organs to insulin. Long-term complications include retinopathy, neuropathy and microangiopathy (Sales et al. 2012). This disease also causes blackouts when there is a shortage of glucose in the brain, and amputation if patients suffer from severe infection. According to the latest (8th) edition of the Diabetes Atlas released by the International Diabetes Federation in 2017, the number of people presently affected by diabetes worldwide is more than 425 million, and it may rise to 629 million by 2045. Controlling diabetes has become a challenge for scientists in the field of food science, nutrition and medical science.

Chemicals such as sulfonylureas, meglitinides, thiazolidinediones, and metformin have been developed to reduce blood sugar. They are, however, all associated with different kinds of disadvantages (Griffith 2012), including abnormally low blood sugar, bouts of extreme hunger, congestive heart failure, bone fractures, and adverse gastrointestinal effects. From the perspective of physiology, glucose levels in the bloodstream can also be controlled by limiting glucose entering blood vessels. This mainly involves inhibiting the activities of the key starch digestive enzymes α-amylase (αA) and α-glucosidase (αG). Synthetic acarbose (Aca) is clinically used to inhibit the activities of αA and αG present in the brush border of the small intestine. However, the use of Aca is accompanied by adverse effects such as diarrhea, flatulence, abdominal distention, and pneumatosis cystoides intestinalis (Sheliya et al. 2015). Therefore, interest has focused on natural products' substitutes for Aca.

Flavonoids have been widely studied for their broad bioactive benefits including anti-oxidation, cardioprotection, anti-bacteria, and anti-inflammation activities (Wang, Li, and Bi 2017). Efforts to search for better inhibitors of starch digestive enzymes have involved numerous plant extracts including medical herbs, shrubs, trees, fruits, and vegetables. Some flavonoids are more effective at inhibiting *yeast* αG (YαG) or PPA than Aca. In this review, flavonoids have been divided into seven groups, and their inhibitory effects (IEs) on αA and αG are discussed. Recently, Xiao et al. (2013) described the relationship between the structures of dietary phenolic compounds and the IEs of polyphenols on αA. Other reviews about αG inhibitors from medicinal plants (Yin et al. 2014) and seed oil (Teng and Chen 2017) have also been reported, but these do not focus on the IEs of flavonoids. This review includes (i) an overview of the IEs of flavonoids as αA and αG inhibitors, (ii) the mechanisms of inhibition, (iii) chemical structures that can

CONTACT Qiang Huang fechoh@scut.edu.cn Overseas Expertise Introduction Center for Discipline Innovation of Food Nutrition and Human Health (111 Center), Guangzhou, China.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

enhance the IEs of flavonoids, and (iv) difficulties in researching the IEs of flavonoids as αA and αG inhibitors.

IEs of different flavonoids

Flavones

The prominent flavones in foods are luteolin (Lut) and apigenin (Xiao et al. 2013). Eleven flavones, namely Lut, Lut-7-O-glucoside, baicalin, pectolinarin, linarin, bilobetin, rhoifolin, amentoflavone, lonicerin, ginkgetin, and isoginkgetin, were studied by Kim, Kwon, and Son (2000). Lut-7-O-glucoside and amentoflavone were found to strongly inhibit YαG, and the IE for Lut against YαG ($IC_{50} = 0.5–1$ mg/mL) was stronger than that of Aca ($IC_{50} = 5$ mg/mL). Lut is also a potent inhibitor of PPA (Type VI-B) (Tadera et al. 2006). The results of in vitro study on the IEs of 19 flavonoids were consistent with those of in silico study (Piparo et al. 2008), but a more recent in silico study suggested that Lut was not a potent inhibitor of αG (PDB ID: 2ZE0) or αA (PDB ID: 1HNY) (Rasouli et al. 2017). Structural differences between enzymes could be a reason for this apparent discrepancy. Although glycosylated, Lut-7-O-glucoside ($IC_{50} = 18.3\ \mu M$) and Lut-7-O-glucuronide ($IC_{50} = 14.7\ \mu M$) gave comparable IEs against YαG to Aca ($IC_{50} = 16.1\ \mu M$) (Asghari et al. 2015).

Similar to Lut, apigenin ($IC_{50} = 10.5 \times 10^{-6}$ mol L^{-1}) is more effective than Aca ($IC_{50} = 3.04 \times 10^{-4}$ mol L^{-1}) at inhibiting *Saccharomyces cerevisiae* αG (*Sc*αG) (Zeng et al. 2016). However, even though maltase and sucrase are the two components of αG, Lut and apigenin only weakly inhibited the activity these enzyme activities in a recent study, and IEs of Lut and apigenin against PPA (specific type unknown) were reportedly less than 1/75 and 1/28 that of Aca, respectively, (Zhang et al. 2017). With the same flavonoid, the inhibitory activity against the same type of enzyme should be comparable when both are compared to Aca. The IEs for apigenin differentiated greatly as reported by Zeng et al. (2016) for *Sc*αG, and Zhang et al. (2017) for maltase and sucrase. From these observations, we propose that the IEs of flavonoids are highly correlated with the structure of the enzyme. Although enzymes may have the same classification, the AARs in the active center of the same type of enzyme from different origins may differ, which can affect the affinity of flavonoids binding to the active center, resulting in differences in IEs.

Flavonols

Flavonols are the most widespread flavonoids in plants. The IEs of quercetin (Que), isoquercetin and rutin have received the most attention. Like Lut and apigenin, the inhibition performance of Que varies depending on the enzyme origin. Que is a much stronger YαG inhibitor (Ye et al. 2010) and rat small intestinal αG (RiαG) inhibitor (Kim et al. 2011) than a PPA inhibitor, consistent with a recent report that calculated an IC_{50} for Que toward αG (specific origin unstated) and pancreatic αA (specific origin unstated) of 32 and 770 μg/mL, respectively (Meng et al. 2016). However, even though maltase is a component of αG, similar inhibitory strengths and IC_{50} values were observed for rat small intestinal maltase (51.1 ± 4.7%) and PPA (52.1 ± 7.3%) enzymes with the same dose of Que (Wang, Du, and Song 2010). This difference between maltase and αG has been described in the previous section. For different types of enzymes, flavonols can exhibit different inhibitory sequences. For example, the inhibitory sequence of *Sc*αG is Que > isoquercetin ≈ rutin, whereas the inhibitory sequence for *Bacillus subtilis* αA is isoquercetin ≈ Que > rutin (Li, Song, et al. 2009; Li, Gao, et al. 2009).

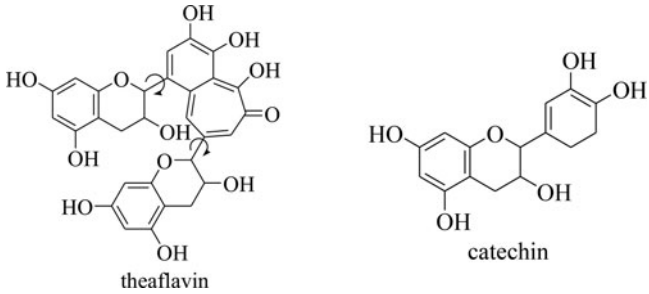

Figure 1. Chemical structures of theaflavin and catechin.

Flavanones

Among flavanones, naringin and its derivatives have received the most attention. Naringin (20 μg/L) displayed 96.6% inhibition against YαG, while 400 μg/L naringin only resulted in 9.8% inhibition against PPA (Ye et al. 2010). Nevertheless, controversial results indicated that naringin weakly inhibits RiαG (Shen, Xu, and Lu 2012). This difference is consistent with the discussion in the flavones section; IEs of flavonoids vary with the origins of enzymes. Naringenin, the aglycone of naringin, exhibited 73% inhibition (200 μM naringenin) of YαG, with an IC_{50} of 75 μM (Tadera et al. 2006). Interestingly, the binding affinities between naringin, neohesperidin, hesperidin and *B. subtilis* αA were highest at pH 6, and 0.2 M NaCl or 0.1 M $CaCl_2$ reinforced these affinities (Liu et al. 2017). Similar findings on the effect of pH and inorganic ions on the binding affinities between flavonoids and αA/αG were barely reported. This observation should arouse our attention since the small intestine in humans has a medium pH and contains numerous inorganic ions. Further research could help us to ensure that flavonoids exert maximal IEs in the digestive tract.

Flavanols

Catechins and theaflavins are two major categories of flavanols, and both exhibit favorable IEs. Again, inhibitory performance of catechins varied with different origins of enzyme. Catechins (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate) inhibit *Sc*αG (Type I) more strongly than human salivary αA, as disclosed by IC_{50} values (Griffith 2012). By contrast, lower IC_{50} values for catechin and epicatechin against PPA than *Sc*αG were reported (Tan, Chang, and Zhang 2017).

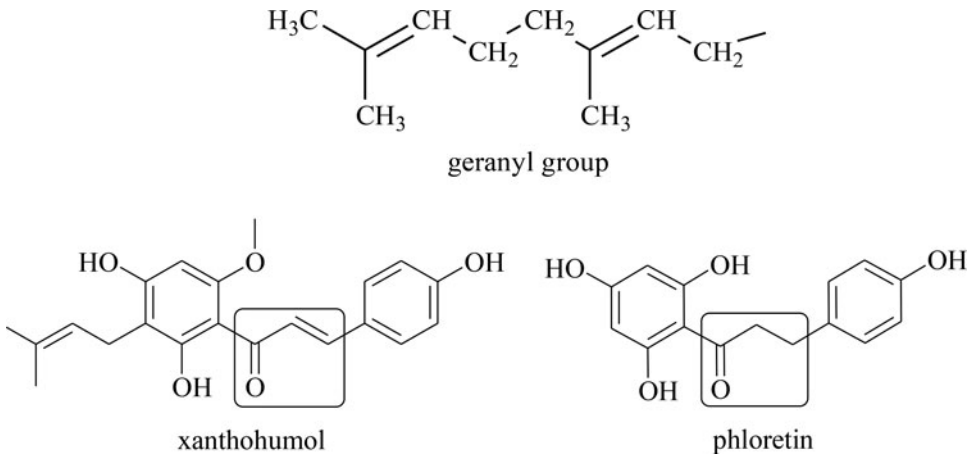

Figure 2. Chemical structures of the geranyl group, xanthohumol, and phloretin.

Therefore, the inhibitory sequence of catechin and epicatechin is likely PPA >*Sc*αG> human salivary αA. It was deduced from IC_{50} values that theaflavins (theaflavin digallate, theaflavin monogallate, and theaflavin) are generally more effective than catechins (epicatechin gallate, epigallocatechin gallate, and catechin hydrate) at inhibiting human salivary αA (Koh et al. 2010). Similar findings were also reported for PPA, which implies lower IC_{50} values for theaflavin, theaflavin-3′-gallate, and theaflavin-3,3′-digallate than catechin (Sun et al. 2016). Compared with catechin, theaflavin has a larger conjugation system (six- and seven-membered rings; Fig. 1), which may greatly stabilize its binding to AARs. The two rotatable bonds may avoid the adverse effects caused by steric hindrance in the larger theaflavin. Molecular docking analysis could provide insight on the binding differences between theaflavin and catechin to αA.

Interestingly, the IC_{50} against *Sc*αG for a polycondensate of catechin with glyoxylic acid (PCG) decreased from 239.27 μg/mL to 2.59 μg/mL compared with catechin (Geng et al. 2016). This phenomenon is uncommon because PCG has a larger molecular weight and enhanced hydrophilicity. Meanwhile, Rasouli et al. (2017) indicated that higher molecular weight is not an advantageous factor for exerting strong IEs. Additionally, increasing the hydrophilicity would not increase the affinity for the hydrophobic active center of *Sc*αG. The IC_{50} value of PCG decreased by ~90-fold compared with catechin. PCG might interact with AARs outside of the main "pocket" and thus effectively hinder substrate access due to its large size. It would be interesting to conduct further studies to understand the mechanism of action.

Isoflavones

Four isoflavones from traditional Chinese medicines are used for treating diabetes mellitus, namely daidzein (from *Panax lobata* and *Astragalus membranaceus*), calycosin (from *A. membranaceus* and *Phaseolus calcaratus*), genistein (from *A. membranaceus*, *P. calcaratus*, and *P. lobata*) and puerarin (from *Puerariae lobata*), all displayed better IEs against YαG than PPA, and calycosin was the most potent (Ye et al. 2010). Genistein was reported to be a stronger inhibitor of YαG and RiαG than daidzein (Tadera et al. 2006). Compared with daidzein, genistein has an extra hydroxyl group at the C5 position, which can potentially form an extra hydrogen bond (H-bond) with AARs that stabilize the binding of genistein to the enzyme. Although the IEs of the corresponding flavone for daidzein, calycosin, and puerarin (7,4′-dihydroxyflavone, farnisin, and bayin, respectively) were not reported, genistein has a lower IC_{50} value against YαG (7 μM) than apigenin (>200 μM), the corresponding flavone of genistein (Tadera et al. 2006). This finding is consistent with the fact that linkage of the B ring at the C3 position can enhance inhibition against YαG and PPA (Tundis et al. 2016).

Anthocyanins

Anthocyanins, included in the list of natural compounds known as potential antioxidants, are the largest group of water-soluble pigments in the plant kingdom. Cyanidin and its glycosides are natural dietary anthocyanidins and promising candidates for the prevention and treatment of diabetes mellitus (Xiao et al. 2013). Cyanidin is a more potent inhibitor of αG than αA, with an inhibition rate for YαG of 99% (200 μM) and an IC_{50} of 4 μM, compared with 37% (0.5 mM) and >0.5 mM for PPA, respectively (Tadera et al. 2006). A recent report showed that three glycosides of cyanidin (cyanidin-3-glucoside, cyanidin-3,5-glucoside, and cyanidin-3-rutinoside) also significantly inhibit PPA (Sui, Zhang, and Zhou 2016). The additional introduction of a glucose moiety to cyanidin-3-glucoside significantly decreased the IEs, consistent with a previous report (Xiao et al. 2013). Kadouh et al. (2016) observed no inhibition of procyanidins B1 and B2 against RiαG, unlike other reports. Once again, it was suggested by the author that different enzyme origins (YαG and RiαG) might account for this difference, in accordance with our explanation described above.

Chalcones

Chalcones are ring C-opened isomers of dihydroflavones responsible for color in plants, and are regarded as key precursors of flavonoid biosynthesis. Butein, okanin, and okanin-4′-O-β-D-glucopyranoside isolated from *Andromachia*

Table 1. Flavonoids reported to be more effective against αG* than Aca.

Compounds	Enzyme studied	IC_{50} values		RC_{Aca}	References
		Flavonoids	Aca		
Lut	YαG (Type I)	3.75	356	0.0105	(Collado-Gonzalez et al. 2017)[a]
chrysoeriol		1.89 IC_{20}[$]		–	
hirtacoumaroflavonoside	αG (US[#])	0.022	0.092	0.239	(Sheliya et al. 2015)[b]
hirtaflavonoside B		0.071		0.772	
vitexin	*Sc*αG	244.0	1007	0.242	(Chen et al. 2013)[b]
isovitexin		266.2		0.264	
isorhamnetin-3-O-D-rutinoside		275.4		0.273	
phloretin	*Sc*αG	31.26	38.3	0.816	(Han et al. 2017)[a]
Lut-7-O-glucuronide	YαG (Type I)	14.7 ± 2.1	16.1 ± 0.8	0.913	(Asghari et al. 2015)[b]
myricetin	αG (US[#])	3	17.8	0.169	(Meng et al. 2016)[a]
Kae	*Sc*αG	11.6	20.9	0.555	(Peng et al. 2016)[c]
xanthohumol	YαG	8.8	26.5	0.332	(Liu et al. 2014)[b]
morin	*Sc*αG	4.48 ± 0.04	304	0.0147	(Zeng et al. 2016)[c]
baohuoside I	*Sc*αG	28.9 ± 1.5	236	0.122	(Phan et al. 2013)[c]
naringenin	*Sc*αG	96.8	310.2	0.312	(Choi, Lee, and Kim 2015)[b]
catechin	*Sc*αG (Type I)	31	91.0 ± 10.8	0.341	(Griffith 2012)[a]
epicatechin gallate		18.1 ± 5.20		0.199	
epigallocatechin gallate		0.25 ± 0.01		0.00275	
gallocatechin gallate		1.35 ± 0.12		0.0148	
afzelin	*Sc*αG (Type I)	3.56	377	0.00944	(Torres-Naranjo et al. 2016)[b]
quercitrin		7.77		0.0206	
acacetin-7-O-glucopyranoside	*Sc*αG	451.8 ± 36.4	1907 ± 156	0.237	(Luyen et al. 2013)[b]
acacetin-7-O-rhamopyranoside		362.5 ± 35.1		0.190	
Kae-3-O-rutinoside	YαG	0.38 ± 0.03	0.99 ± 0.02	0.384	(Tan et al. 2013)[d]
rutin		0.10 ± 0.01		0.101	
apigenin[b]	YαG	21.85	228.16	0.0958	(Li et al. 2009)
orientin[b]		23.30		0.102	
isoorientin[b]		19.68		0.0863	
Que[c]		17	91	0.187	
3′ -prenylchalconaringenin	YαG	22.42	51.3	0.437	(Sun et al. 2017)[b]
3′-geranylchalconaringenin		1.08		0.0211	
8-prenylnaringenin		45.92		0.895	
8-geranylnaringenin		3.77		0.0735	
chalconaringenin		20.02		0.390	
narirutin	*Sc*αG	14.30 ± 3.5	54.99 ± 1.3	0.260	(Tundis et al. 2016)[b]
naringin		10.33 ± 1.1		0.188	
didymin		4.20 ± 0.6		0.0764	
hesperidin		15.89 ± 1.8		0.289	
neoeriocitrin		25.31 ± 1.2		0.460	

*All values are IC_{50} values unless otherwise stated.
[$]$IC_{20}$ = the concentration of a test substance achieving 20% of maximal inhibition in a specific reaction.
[#]Specific origin is unstated in the report.
[a]IC_{50} values are expressed as μg/mL.
[b]IC_{50} values are expressed as μM.
[c]IC_{50} values are expressed as μmol/L.
[d]IC_{50} values are expressed as mg/mL.

igniaria were evaluated for their IEs against αA and αG, butein and okanin were found to have stronger inhibition against *Sc*αG than PPA, and both presented much lower IC_{50} values than Aca against *Sc*αG, whereas okanin-4′-O-β-D-glucopyranoside displayed weaker inhibition of *Sc*αG than PPA (Saltos et al. 2015). IC_{50} values against YαG for another three chalcones (3′-prenylchalconaringenin, 3′-geranylchalconaringenin, and chalconaringenin) were also found to be much lower than those against PPA (Sun et al. 2017). Compared with chalconaringenin, IC_{50} values for 3′-geranylchalconaringenin against YαG were decreased greatly (from 20.02 to 1.08 μM), and similar findings were observed for PPA, but not for β-glucosidase (Sun et al. 2017). Similar findings were also observed for flavanones (naringenin and 8-geranylnaringenin) in the same report. It seems that the introduction of a geranyl group enhances the IEs of flavonoids. Judging from its molecular structure (Fig. 2), it lacks the potential to engage in π–π conjugation or H-bonds with AARs. The introduction of a geranyl group increases the hydrophobicity of the flavonoid, and thus elevates its affinity for the "pocket."

Interestingly, Michael-type addition between the α,β-unsaturated keto group of xanthohumol (a prenylated chalcone; Fig. 2) and AARs is believed to be the reason for the reversible inhibition (Liu et al. 2014). However, other explanations for reversible inhibition are possible. Phloretin (dihydrochalcone; Fig. 2), which lacks a α,β-unsaturated keto group, displayed mixed-type inhibition (a type of reversible inhibition) against YαG (Han et al. 2017).

Summary and discussion

Flavonoids display significant differences in IEs against different types of enzymes, and even for the same type of enzyme from different origins. YαG, *Sc*αG, RiαG, and PPA are the most studied enzymes, and very few studies have used enzymes of human origin, although human saliva αA has occasionally been used for inhibitory studies. Human

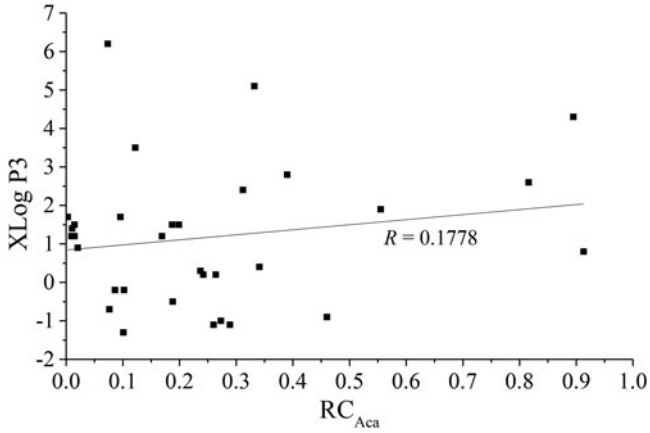

Figure 3. Relationship between XLog P3 and RC_{Aca} against αG. RC_{Aca} values are from Table 1, and the corresponding XLog P3 data for flavonoids are from the PubChem Public Chemical Database.

salivary and pancreatic αA share a high degree of amino acid sequence similarity, with 97% sequence identity overall and 92% in the catalytic domain (Piparo et al. 2008). Based on the differences in IEs against enzymes from different origins, the actual IEs of flavonoids against human pancreatic αA and αG remain poorly understood. It would be therefore be better to study enzymes of human origin to confirm the actual inhibitory performance of flavonoids. Phan et al. (2013) indicated that a better clinical outcome could be derived from specific αG inhibitors with strong IEs against αG and weak IEs against αA. Therefore, flavonoids displaying better IEs against αG than αA (such as daidzein from traditional Chinese medicine, described above in the isoflavones section) might be of considerable interest. Clearly, differences in enzyme origin should not be neglected. To ultimately apply laboratory findings to clinical practice, it would be more pragmatic to test flavonoids with different enzyme inhibitory trends such as cyanidin in the anthocyanins section against diabetic patients. This should be safe since most food-derived polyphenols are generally recognized as safe (Rasouli et al. 2017), and we can have knowledge of their actual IEs on human digestive enzymes.

Large numbers of flavonoids have been separated and identified, and their biological activities such as antioxidation and IEs against αA and αG are well studied. The IEs of many flavonoids are less effective than those of Aca, but some are more potent than Aca. It has been suggested that some side effects of Aca originate from stronger inhibition on αA, which results in abnormal fermentation of undigested starch in the colon (Phan et al. 2013). For this reason, flavonoids that exhibit better IEs against αA than Aca have not been summarized here. Flavonoids that are more effective at inhibiting αG than Aca are listed in Table 1.

Relationships between the molecular properties and IEs of flavonoids

Correlations between IEs and hydrophobicity

To investigate the importance of hydrophobic interactions between the binding of flavonoids to αG, we attempted correlation analysis between the IEs of flavonoids and XLog P3, a measure of the lipophilicity of compounds. However, one obstacle for comparing data from different studies is that IC_{50} values for inhibitors are highly dependent on assay conditions such as enzyme concentration and origin, substrate type and concentration, reaction duration, temperature, and pH, as discussed by Koh et al. (2010). Thus, different assay conditions result in differences in IC_{50} values for the same flavonoids, making data integration between different reports a difficult task. Additionally, using reported IC_{50} values directly for correlation analysis is inappropriate. Fortunately, Aca is frequently used as a positive control under various same assay conditions. Herein, we proposed the concept of "relative coefficient to Aca," abbreviated as RC_{Aca}, calculated as the ratio of the IC_{50} value relative to that of Aca under the same assay conditions. Therefore, a smaller RC_{Aca} value for an inhibitor represents stronger IEs, and $RC_{Aca} < 1, = 1$, and > 1 denotes stronger, equivalent, and weaker IEs than Aca, respectively. RC_{Aca} makes possible the comparison of general inhibitory strength of flavonoids from different reports, and RC_{Aca} values of flavonoids are listed in Table 1.

Using RC_{Aca} values (Table 1) and XLog P3 data for flavonoids gathered from the PubChem Public Chemical Database (https://pubchem.ncbi.nlm.nih.gov/search), correlation analysis between RC_{Aca} and XLog P3 was performed (Fig. 3). The regression equation was XLog P3 = 0.8417 + 1.3123 RC_{Aca} ($R = 0.1778$), and as RC_{Aca} increases, XLog P3 also increases. This indicates that hydrophobic interactions are not the main driving force. A similar finding was also reported by Xiao et al. (2013), whose regression results showed that the percentage inhibition of human αA decreases as XLog P3 increases, indicating that binding interactions between flavonoids and αA are not controlled by hydrophobic forces to a great extent.

Roles of H-bond donors/acceptors of flavonoids in determining IEs

Xiao et al. (2013) showed that the number of H-donors/acceptors of flavonoids is positive correlated with percentage inhibition of human αA. Herein, we investigated the correlation between the number of H-bond donors/acceptors and IEs against αG using RC_{Aca} values (Table 1). The number of H-bond donors/acceptors was obtained from the PubChem Public Chemical Database (https://pubchem.ncbi.nlm.nih.gov/search). As shown in Fig. 4a, the number of H-bond donors/acceptors of flavonoids was not strongly correlated with their RC_{Aca} values; RC_{Aca} decreased as the number of H-bond donors/acceptors increased. This suggests that the IEs of flavonoids increased as the number of H-bond donors/acceptors increased, as previously suggested (Xiao et al. 2013). However, the results were not exactly as we expected. R values were −0.1049 for H-acceptors and −0.1526 for H-donors, respectively, indicating a weak negative correlation between the number of H-donors/acceptors and IEs, whereas we expected a strong negative correlation. It is well known that the introduction of groups such as saccharides to the main ring of flavonoids generally increases steric hindrance and inhibits access to enzyme active sites, which is generally associated with weaker IEs. By contrast, the

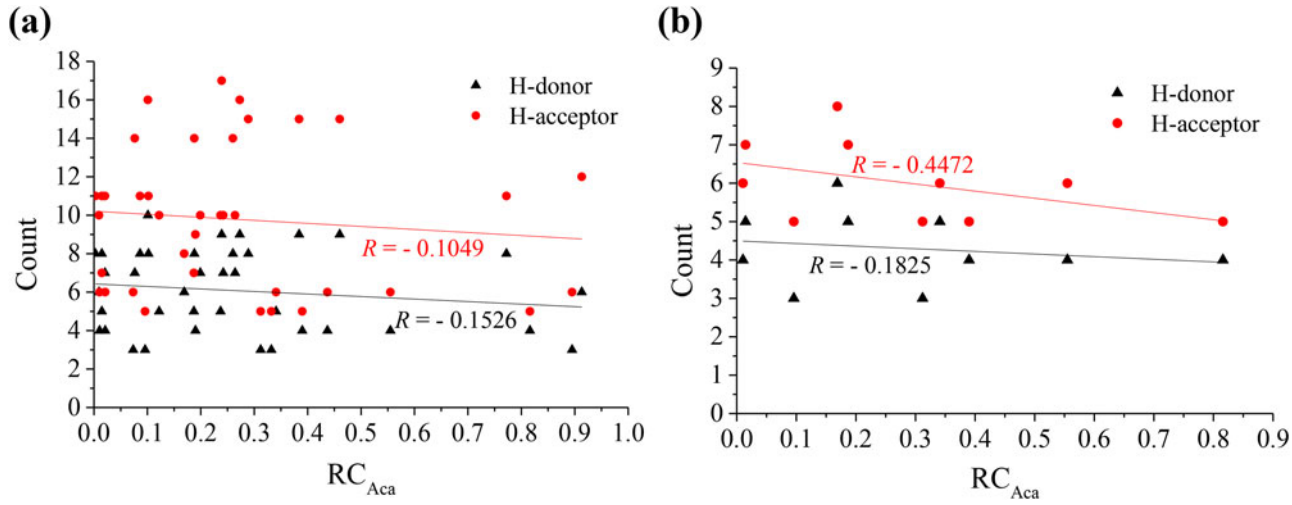

Figure 4. (a) Relationship between the number of H-donors/acceptors of flavonoids and RC_{Aca} against αG. (b) Relationship between the number of H-donors/acceptors of flavonoid aglycones and RC_{Aca} against αG. RC_{Aca} values are from Table 1, and the corresponding number of H-donors/acceptors of flavonoids is from the PubChem Public Chemical Database.

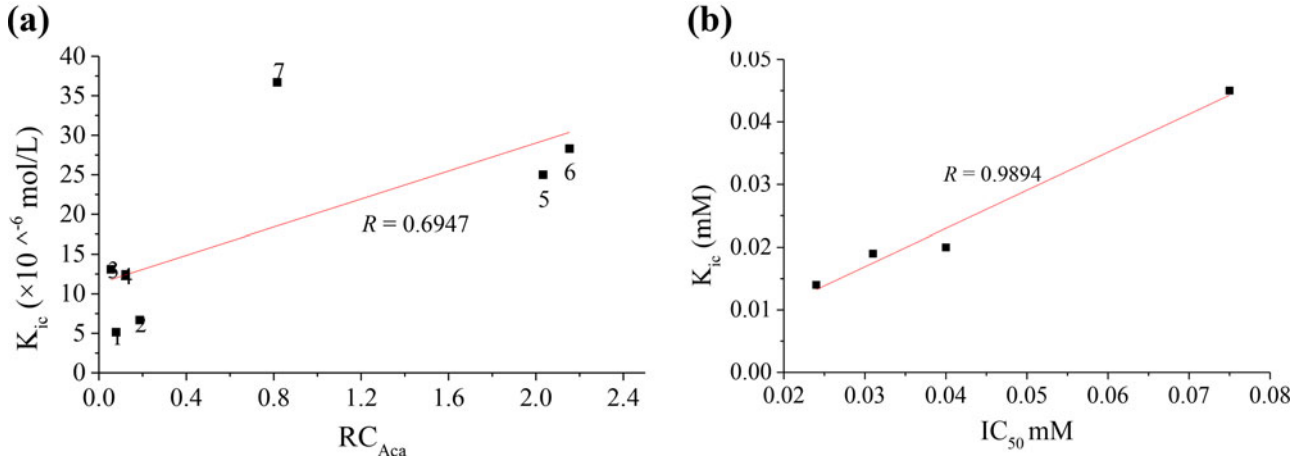

Figure 5. (a) Correlation between K_{ic} values and RC_{Aca} values (αG) of flavonoids. Data point 2 (Que), 5 (isoquercetin) and 6 (rutin) are from Li et al. (2009). Data point 1 (Kae) and 4 (baohuoside I) are from Phan et al. (2013). Data point 3 (Kae) and 7 (phloretin) are from Peng et al. (2016) and Han et al. (2017), respectively. (b) Data are from Sui, Zhang, and Zhou (2016).

introduction of groups such as glucuronide generally increases the number of H-donors/acceptors. For example, the number of H-donors and H-acceptors for Lut is 4 and 6, respectively, compared with 7 and 12 for Lut-7-O-glucuronide. The number of H-donors/acceptors is increased ~2-fold following the introduction of a glucuronic acid group. However, the IEs of Lut-7-O-glucuronide are weaker than those of Lut (RC_{Aca} is increased from 0.0105 to 0.913; Table 1). This could account for the low R values observed. However, an increase in the number of H-donors/acceptors following the introduction of extra groups to flavonoids does not necessarily translate to lower IEs. For example, the RC_{Aca} values of epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate are all lower than that of catechin (Table 1).

To verify the above statement, flavonoid derivatives with large groups on the main ring (Table 1) were excluded, and only flavonoid aglycones were used to study the correlation. As shown in Fig. 4b, the absolute value of R for H-acceptors (0.4472) is increased greatly compared to that in Fig. 4a (0.1049). By contrast, the absolute value of R for H-donors in Fig. 4b (0.1825) is not greatly different from that in Fig. 4a (0.1526). Xu and Chen (2011) investigated the molecular properties-affinity relationships of the interactions between 55 dietary polyphenols and bovine milk proteins using fluorescence spectra, and the results revealed an R of 0.4170 between XLogP3 of polyphenols and the logarithm of the binding constant, and an R of 0.2675 between the topological polar surface area of polyphenols and the logarithm of the binding constant. This result might indicate that H-acceptors of flavonoids have a more important role in determining IEs against αG than H-donors. However, only limited available data were used in the present analysis, and more data are needed to facilitate a more definitive conclusion.

In summary, expanding on the summary by Xiao et al. (2013), a larger number of H-donors/acceptors of flavonoids does not necessarily result in stronger IEs; steric hindrance should also be well considered. A favorable strong correlation between the number of H-donors/acceptors and IEs may be achievable for flavonoid aglycones.

Inhibitory mechanism study

Inhibition kinetics

Inhibition kinetics have been widely studied when investigating mechanisms. Double reciprocal equations for competitive, noncompetitive, uncompetitive, and mixed-type inhibition can be expressed as follows

$$\frac{1}{V_0} = \frac{\left(1+\frac{[I]}{K_i}\right)\times K_m}{V_{max}}\times\frac{1}{S_0}+\frac{1}{V_{max}},\ K_i = \frac{[E]\times[I]}{[EI]} \quad (1)$$

$$\frac{1}{V_0} = \frac{\left(1+\frac{[I]}{K_i}\right)\times K_m}{V_{max}}\times\frac{1}{S_0}+\frac{\left(1+\frac{[I]}{K_i}\right)}{V_{max}},$$

$$K_i = \frac{[E]\times[I]}{[EI]} = \frac{[ES]\times[I]}{[ESI]} \quad (2)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\times\frac{1}{S_0}+\frac{\left(1+\frac{[I]}{K_i}\right)}{V_{max}},\ K_i = \frac{[ES]\times[I]}{[ESI]} \quad (3)$$

$$\frac{1}{V_0} = \frac{\left(1+\frac{[I]}{K_{ic}}\right)\times K_m}{V_{max}}\times\frac{1}{S_0}+\frac{\left(1+\frac{[I]}{K_{iu}}\right)}{V_{max}},$$

$$K_{ic} = \frac{[E]\times[I]}{[EI]},\ K_{iu} = \frac{[ES]\times[I]}{[ESI]}. \quad (4)$$

where V_0 is the initial velocity, K_m is the Michaelis-Menten constant, V_{max} is the maximum initial velocity, S_0 is the initial concentration of substrate, and $[I]$ is the concentration of the inhibitor.

However, some researchers reported that Lineweaver-Burk plots cannot distinguish between uncompetitive, noncompetitive and mixed-type inhibition (Sun et al. 2016), whereas actually they are the most widely used method for studying inhibition type as used in many reports (Li, Zhou, et al. 2009; Phan et al. 2013; Sabiu, O'Neill, and Ashafa 2016; Han et al. 2017). Clarification may help future researchers better understand the mechanisms. Inhibition can generally be divided into reversible or irreversible inhibition, and reversible inhibition can be further classified into competitive, noncompetitive, uncompetitive, and mixed-type inhibition. To study the specific inhibition type, we first need to know whether inhibition is reversible or not. There are two main methods to determine the reversibility of enzyme inhibition; dialysis (Liu et al. 2014) and kinetics (Sun et al. 2017). In most cases, inhibition of αA and αG by flavonoids is reversible (Yan et al. 2014; Peng et al. 2016; Zeng et al. 2016; Han et al. 2017). Competitive, noncompetitive, uncompetitive and mixed-type inhibition can be further distinguished by Lineweaver-Burk plots; intersection at the y-axis (Yang, He, and Lu 2014), x-axis (Zeng et al. 2016), second quadrant (Han et al. 2017) or third quadrant (Geng et al. 2016) corresponds to competitive, noncompetitive, mixed inhibition (competitive and noncompetitive, $K_{ic}<K_{iu}$) and mixed inhibition (noncompetitive and uncompetitive, $K_{ic} > K_{iu}$), respectively. For uncompetitive inhibition, Lineweaver-Burk plots are parallel in the coordinate, as reported previously (Sabiu, O'Neill, and Ashafa 2016).

To assess the relationship of K_{ic}, K_{iu} and the IEs of flavonoids, the correlation between K_{ic} and K_{iu} values collected from various reports and corresponding RC_{Aca} values were investigated. Unfortunately, to date, only a small number of flavonoid monomers have been studied in terms of detailed kinetics, and some reports include IC_{50} values for flavonoid monomers but not K_{ic} or K_{iu} values, whereas some reports include K_{ic} or K_{iu} values but not IC_{50} values. For these reasons, only limited K_{ic} data against αG are available for analysis, and thus only K_{ic} vs. RC_{Aca} plots were created herein. As shown in Fig. 5a, a positive correlation ($R = 0.6947$, $p = 0.083$) between K_{ic} and RC_{Aca} was observed, which indicates that IEs of flavonoids increased as K_{ic} decreased. This result is consistent with the report by Sun et al. (2016), who found a strong correlation ($R = 0.9951$, $p = 0.005$) between K_{ic} and IC_{50} for PPA with pure phenolic compounds in tea extracts. Using data from another report, a strong positive correlation was also observed between K_{ic} and IC_{50} for PPA (type VI-B; Fig. 5b). Differences in data may result in deviations during regression analysis (we noticed different K_{ic} values for the same flavonoid). A K_{ic} of Kae of 5.14 μM/L for *Sc*αG was reported by Phan et al. (2013), while Peng et al. (2016) reported a K_{ic} of Kae for *Sc*αG of 13.1 μM/L. This indicates that more data are needed for a statistically significant conclusion to be drawn.

Molecular docking

Molecular docking software such as AutoDock (Scripps Research Institute, La Jolla, CA) and Sybyl 2.0 (Tripos Inc., St. Louis, USA) has been used to study the binding sites between flavonoids and enzymes. Such studies can provide significant insight into ligand-protein binding strength as well as ligand binding mode and mechanism. Recently, molecular docking has been used as a virtual screening tool to study the IEs of plant-derived phenolic compounds against αA and αG (Rasouli et al. 2017). Computational drug design may be a promising method to preliminarily screen digestive enzyme inhibitors.

Molecular docking has identified two types of flavonoid binding to αA and αG (Yang, He, and Lu 2014; Milella et al. 2016; Peng et al. 2016; Zeng et al. 2016; Zhen et al. 2017); flavonoids can bind with AARs in the "pocket," which is usually considered as competitive inhibition. According to the "lock and key" theory, the substrate has to bind in the active center of an enzyme first before the catalytic AARs break the glycosidic bond. If the inhibitor binds with the contact residues of the enzyme, the substrate will not be hydrolyzed. The main factors affecting binding include H-bonds, hydrophobic interactions and π–π conjugation. Generally, stronger IEs are associated with more hydrophobic inhibitors, more H-bonds, and more extensive π–π conjugation formed between the inhibitor and AARs. Lots of flavonoids have been shown to form H-bonds with the

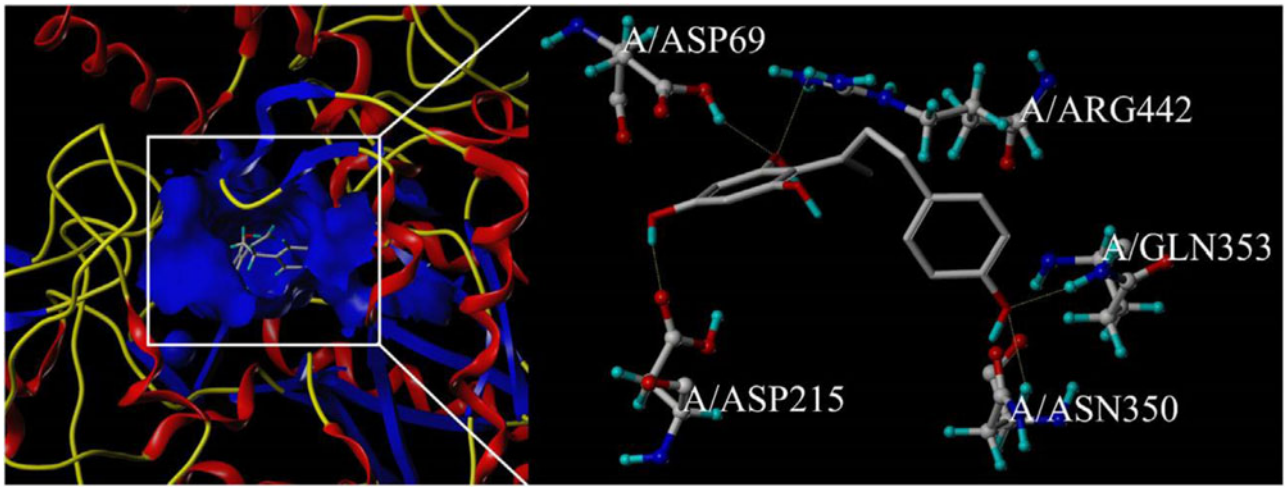

Figure 6. Expected binding of phloretin to αG. Phloretin binds in the hydrophobic "pocket" (left), and H-bonds (dashed green lines) are formed between phloretin and amino acid residues (right) (Han et al.2017).

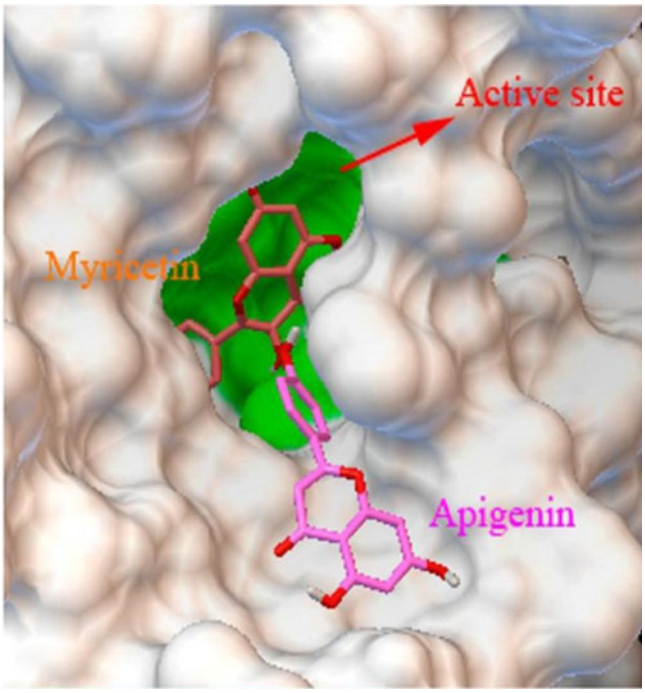

Figure 7. Predicted binding of apigenin and myricetin to αG. The crystal structure of αG (PDB ID: 3A4A) was downloaded from the Protein Data Bank (http://www.rcsb.org/pdb) (Zeng et al.2016).

AARs in the active center of αA and αG. Yang, He, and Lu (2014) found that isoorientin interacts with Asp197 and Glu233 via H-bonds, and Asp 197, Glu233 and Asp300 through hydrophobic interactions. Asp197, Asp300, and Glu233 are putative active site residues in αA that cleave starch chains (Nahoum et al. 2000). Regarding αG, due to the lack of a 3D structure of YαG (Peng et al. 2016), a structural template of αG was used to carry out docking analysis. Five H-bonds were predicted between the hydroxyl groups of the phloretin molecule and Asp69, Asp215, Arg442, Gln353, and Asn350 of the enzyme based on the high sequence homology of αG (PDB ID: 3A4A; gi number: 411229; Fig. 6) (Han et al. 2017). Hydroxyl and benzyl groups of 3′-geranylchalconaringenin were also able to interact with key AARs based on homology of YαG (isomaltase, PDB entry 3A4A), through H-bonds and π–π conjugation (Sun et al. 2017).

The other type of flavonoid binding is with residues outside the active center that are important for sustaining the conformation of the enzyme. Binding of flavonoids with these structural residues may close the channel to the active center, and this is usually associated with noncompetitive inhibition. Yan et al. (2014) found that interactions between Lut (a noncompetitive inhibitor) and AARs (Phe303, Ser311, Pro312, and His351) near the active site of αG might contribute to inducing cleft closure, hereby preventing entry of the substrate. Recently, Zeng et al. (2016) also reported that apigenin, a noncompetitive inhibitor of *Sc*αG, binds to residues of αG (PDB ID: 3A4A) near the active site, and the channel to the active center might be closed due to these interactions (Fig. 7). Some molecules with a large molecular weight may act in this way, such as PCG, discussed above in the flavanols section.

Structure–activity relationships

Some special structures such as the C2 = C3 double bond, C3–OH, C = O at the C4 position, linkage of the B ring at the 3 position, and the number of hydroxyl groups on ring B, have been found to impact the IEs of flavonoids. Although structure–activity relationships (methylation, methoxylation, hydrogenation, hydroxylation, glycosylation, and galloylation) between dietary polyphenols and αA have been reviewed previously (Xiao et al. 2013), some new findings are included here.

Effects of different groups on flavonoid molecules

The caffeoyl group (Fig. 8) is important for inhibiting αG. Que-3-O-(6-O-caffeoyl)-*β*-galactoside isolated from flowers of *Spiraea cantoniensis* demonstrated stronger maltase inhibition than Que and its glucoside. The introduction of a caffeoyl group on the galactose moiety markedly enhanced the IEs against αG (Yoshida et al. 2008). Other evidence has been found using non-flavonoids substances; quinic acid showed less than 20% inhibition (1 mM) against maltase,

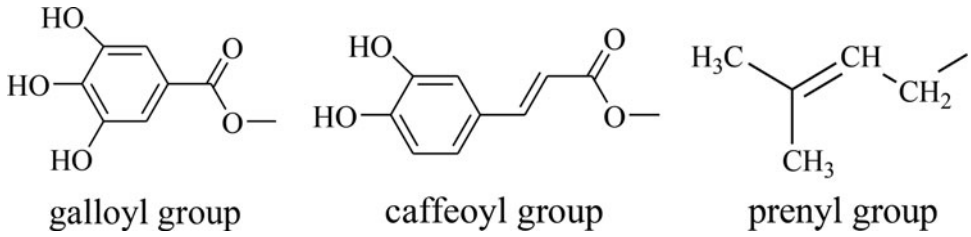

Figure 8. Chemical structures of the galloyl, caffeoyl, prenyl groups.

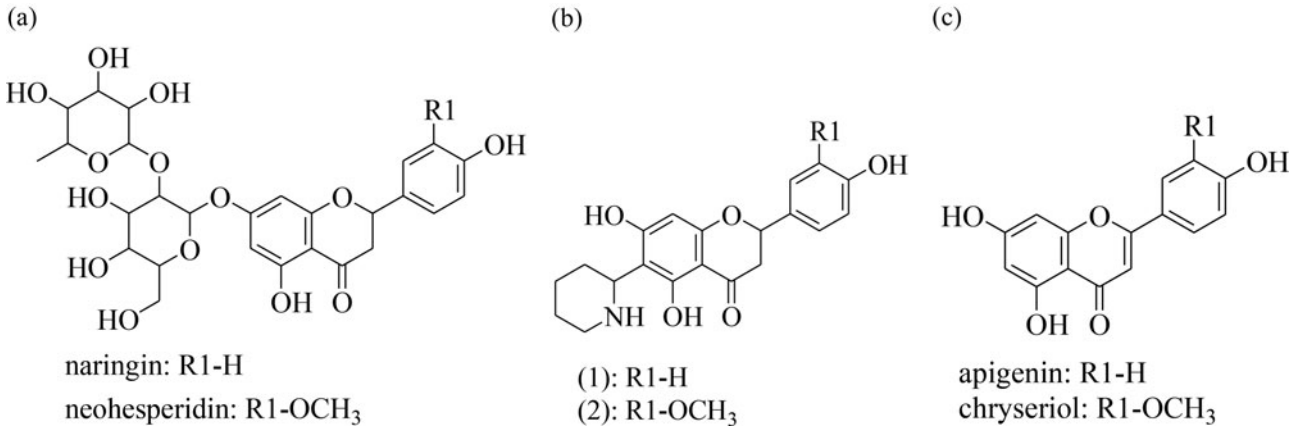

Figure 9. Structures of some flavonoids with C3′–OCH_3. (a) Naringin and neohesperidin. (b) Cyclic imine Δ1 piperidine-modified naringenin (1) and hesperetin (2). (c) Apigenin and chryseriol.

whereas 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid inhibited 65%, 64%, and 62% of maltase activity at the same concentration (1 mM) (Gao et al. 2008). There is a large conjugation system in caffeic acid, in which C = O and C = C double bonds are conjugated with the benzene ring. Thus, the introduction of a caffeoyl group may stabilize the binding of flavonoids and flavones to enzymes via π–π conjugation, as reported for the active site of human salivary αA (Piparo et al. 2008). In addition, there are an extra two hydroxyl groups on the benzene ring when this group is present, providing additional opportunities for forming H-bonds with AARs.

Galloyl moieties (Fig. 8) strengthen the IEs of flavonoids against both αA and αG. IC_{50} values of epicatechin and epigallocatechin against human saliva αA (Type IX-A) were >1 mg/mL, compared with ~27 μg/mL and ~24 μg/mL, respectively, for epicatechin gallate and epigallocatechin gallate (Griffith 2012). Similarly, IC_{50} values of epicatechin and epigallocatechin against *Sc*αG (Type I) were >290 μg/mL and >1 mg/mL, respectively, compared with 18.1 ± 5.20 μg/mL and 0.25 ± 0.01 μg/mL, respectively, for epicatechin gallate and epigallocatechin gallate (Griffith 2012). Similar observations were also reported for theaflavins; the IEs of theaflavin against human saliva αA and Riαg increased with an increasing number of galloyl groups (Koh et al. 2010). The effects of galloylated catechins on the IEs of αA and αG were further confirmed by the work of Sun et al. (2016) and Liu et al. (2017). The importance of the galloyl group was also noted in an earlier report in which a known maltase inhibitor (1,2,3,4,6-penta-O-galloyl-b-D-glucopyranose) displayed enhanced inhibitory potency with a larger number of galloyl groups (Gao et al. 2008). Like caffeic acid, gallic acid has three –OH groups and one –COOH group on the benzene ring, which could expand the conjugation system and form more H-bonds with AARs, and thus increase the affinities of flavonoids to enzymes. Gallic acid has also been reported to interact with critical residues (Asp197, Asp300, Glu233, and His299) of αA (Chi et al. 2017). This could explain why the introduction of galloyl moieties can enhance IEs.

Prenylation also enhances IEs. It was found that hirtacoumaroflavonoside and hirtaflavonoside B (prenylated flavonoids) showed higher maximum inhibition of αG than non-prenylated flavonoids (quercetin and dimethoxyquercetin) (Sheliya et al. 2015). More recently, Ye, Fan, and Ma (2017) found that the IC_{50} (2.3 μg/mL) of 5′-prenylquercetin against αG (from *Bacillus stearothermophilus*) was much lower than that of Que (25.8 μg/mL). Similarly, prenylated eriodictyol showed much higher inhibition (IC_{50} = 31.2 μg/mL for 6-prenyleriodictyol and 57.4 μg/mL for 5′-prenyleriodictyol) against αG than eriodictyol (IC_{50} >100 μg/mL) (Ye, Fan, and Ma 2017). Similar to the discussion of the geranyl group in the chalcones section, the lack of H-donors/acceptors in the prenyl group (Fig. 8) prevents the formation of π–π conjugation interactions with AARs, and this may be related to lipophilic properties that enhance the affinity of flavonoids to the hydrophobic "pocket" (Ye, Fan, and Ma 2017).

Steric effects

The C3′ methoxy group was found to have an adverse effect on the C4′ hydroxyl group. Liu et al. (2017) studied the interactions of naringin and neohesperidin (Fig. 9a) with *B. subtilis* αA, and the binding ability was ordered naringin > neohesperidin despite sharing the same number of hydroxyl groups on the B ring. The inferior IEs of

neohesperidin were concluded to be the result of steric hindrance due to the methoxy group in the ortho-position instead of the hydroxyl group. Similarly, cyclic imine Δ1 piperidine-modified naringenin (1) and hesperetin (2) (Fig. 9b), with IC_{50} values against YαG of 249.4 and >500 μM, respectively, might also suffer from this effect (Zhen et al. 2017). Similar results were also found for apigenin and chrysoeriol (Fig. 9c), with IC_{20} values against YαG of 0.29 and 1.89 μg/mL, respectively (Collado-Gonzalez et al. 2017). The O atom of the C4′ hydroxyl group should be sp^2 hybridized for conjugation with the benzene ring, leaving two positions for the H atom (Fig. 10). An AAR serving as an H-acceptor on the left side of the O atom requires the H atom to remain in position 1 to form the H-bond, but the collocation of the C3′ methoxy group may push the H atom into position 2, resulting in the failure to form H-bonds between this H atom and the AAR due to the increased distance, which decreases the affinity of the flavonoid for the enzyme.

Linear molecules are beneficial for higher IEs against PPA. Yang, He, and Lu (2014) observed that both isoorientin and isovitexin had stronger IEs against PPA than orientin and vitexin. Orientin and vitexin are flavone C8-glycosides, while isoorientin and isovitexin are flavone C6-glycosides. Molecular docking results for these four compounds showed that the "V" shaped flavone C8-glycosides could easily occupy a small hollow near the active site of αA, resulting in less coverage of the active center and more space for the substrate. Meanwhile, flavone C6-glycosides (isoorientin and isovitexin) fits the hydrophobic "pocket" of PPA better due to its linearity. For this reason, isoorientin and isovitexin have lower IC_{50} values than orientin and vitexin (Yang, He, and Lu 2014). Similar findings for αG have not been reported. Obviously, this factor is dependent on the shape of the active center of the enzyme; if it is linear, then linear molecules would better accommodate to the active center, whereas V-shaped molecules will better occupy a V-shaped active site.

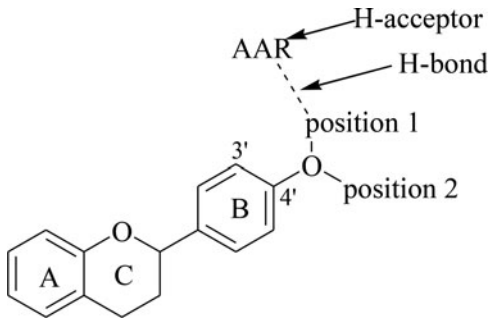

Figure 10. Schematic diagram showing the steric effect of C3′–OCH_3 on C4′–OH.

Challenges of determining the IEs of flavonoids

Effects of food matrices and in vitro digestion on the IEs of flavonoids

Flavonoids bind to proteins, but are more likely to disperse into lipid surfaces due to their high lipophilicity. Thus, their binding to other proteins or lipids in complex food systems may decrease their effective concentrations for binding to αA and αG, potentially lowering the IEs. Some evidence supporting this hypothesis is summarized in Table 2. For example, the IEs of Lut against αG decrease in the presence of oleic acid or palmitic acid (Collado-Gonzalez et al. 2017). Similarly, the IEs of grape skin extract-fortified tomato juice and bread against RiαG and PPA were lower than expected (Lavelli et al. 2016). These observations are important since proteins, fatty acids and dietary fiber, all of which are present in a typical daily diet, could work together to diminish the IEs of flavonoids. Moreover, evaluation of IEs against αA and αG is usually conducted in phosphate-buffered saline at pH 6.9. However, flavonoids must pass through the stomach to reach pancreatic αA and αG, and there is a huge pH difference between the stomach and the small intestine, which may cause structural transformation of flavonoids. The IEs of products from these transformations remain unknown. Besides, flavonoids are well mixed with food

Table 2. Effect of food matrices and stomach digestion on the IEs of different samples.

Samples	Enzyme	Impact factor	IEs (before)	IEs (after)	References
proanthocyanidins-rich sorghum bran extract	PPA	bovine serum albumin	91.9	14.6	(Hargrove et al. 2011)[a]
	aromatase		55.3	33	
grape skin extracts	RiαG	tomato puree	28	26	(Lavelli et al. 2016)[a]
		wheat flat bread	51	34	
	PPA	tomato puree	41	26	
		wheat flat bread	100	29	
Lut	YαG	palmitic acid	0.25	1.65	(Collado-Gonzalez et al. 2017)[b]
		oleic acid	0.25	0.80	
lutein		palmitic acid	2.76	7.15	
		oleic acid	2.76	85.0	
Tribulus terrestris water extract	αG	in vitro simulated digestion	11.6 ± 1.77	8.13 ± 0.90	(Ercan and El 2016)[a]
	αA		73.0 ± 1.63	66.0 ± 1.86	
Chickpea water extract	αG		18.1 ± 1.75	10.9 ± 1.32	
	αA		87.8 ± 2.20	72.6 ± 0.84	
Ribes magellanicum polyphenol enriched extracts	PPA	in vitro simulated digestion	21.7 ± 0.5	>100	(Burgos-Edwards et al. 2017)[c]
	*Sc*αG		0.4 ± 0.0	1.4 ± 0.0	
R. punctatum polyphenol enriched extracts	PPA		20.1 ± 1.7	>100	
	*Sc*αG		0.3 ± 0.0	1.9 ± 0.2	

[a]Results are expressed as inhibition rate (%).
[b]Results are expressed as IC_{20} values (μg/mL).
[c]Results are expressed as IC_{50} values (μg polyphenol enriched extracts/mL).

matrices in the stomach. Studies related to these two aspects are scarce, and the influence of stomach digestion on the IEs of flavonoids should be addressed in the future.

Combinatorial studies

To potentially reduce the clinical dose of Aca and lower its side-effects, flavonoids have been combined with Aca, and the combined effects vary with flavonoid variety and concentration. The IC_{20} value of Lut decreased from 0.25 μg/mL to 0.14 μg/mL after combining with lutein (Collado-Gonzalez et al. 2017). Zeng et al. (2016) reported a synergistic effect between myricetin and morin, and apigenin and myricetin at low concentrations, whereas the combined effects tended to be additive or antagonistic as the concentration was increased. Similar findings concerning the combined effects between (+)-catechin, apigenin, baicalein and Aca were also reported by Zhang et al. (2017). The IEs of each compound in combination may be necessary for exerting synergistic inhibition, and antagonistic inhibition between flavonoids and Aca might be ascribed to competitive binding at the active sites of αG at higher concentrations, whereas noncompetitive binding in αG would enhance synergistic inhibition (Zhang et al. 2017). Antagonistic effects may be caused by the binding of one component to a site outside the "pocket," which could decrease the affinity of the other inhibitory component to the "pocket" (Boath, Stewart, and McDougall 2012). The principles of such interactions are not comprehensive. Both aspects (synergism and antagonism) are meaningful because if flavonoids display synergistic or additive effects toward αG with Aca, it would be promising to include them in Aca tablets to lower its side effects. For example, co-administration of baicalein (80 mg/kg) and Aca (1 mg/kg), both displaying synergistic effects in vivo, exhibited a comparable blood sugar lowering effect with 8 mg/kg Aca, which reduced the effective dose of Aca by 87.5% (Zhang et al. 2017). Flavonoids exerting antagonistic effects with Aca can provide guidance for designing diets for diabetic patients to ensure the IEs Aca are not adversely affected.

The methods employed by some researchers to study synergism, additive effects, and antagonism between flavonoids (or between flavonoids and Aca) might suffer a common shortcoming because their assessment is generally based on the arithmetic sum method. This simple method can be inaccurate since if two individual compounds exert 60% and 70% inhibition, respectively, their additive effect cannot be 130% (Chou 2010). Rather, we recommend studying the combined effects of flavonoids and Aca using the combination index approach introduced by Chou and Talalay in 1984, which has been cited over 4681 times [Google Scholar Citations—Ting-Chao Chou] in over 711 different biomedical journals (Fu et al. 2016). The combination index is regarded as the "gold standard" for evaluating combined drug effects (Tallarida 2001). Unfortunately, to date, it has rarely been employed for studying the combined IEs against digestive enzymes for flavonoids and Aca. Indeed, only two studies investigating the combined IEs between flavonoids and Aca against αG (Zhang et al. 2017) and αA (Gao et al. 2013) using the combination index have been reported.

Concluding remarks and future directions

Flavonoids have been widely studied as αA and αG inhibitors, which is of potential relevance to their use as a treatment for diabetes. Some flavonoids are stronger inhibitors of αG than Aca, and inhibitory mechanisms and structural explanations for their stronger IEs have been summarized. Though some achievements have been made, the following aspects require further investigation

1. Numerous studies have focused on evaluating IEs in vitro, while some reports clearly indicate that food matrices such as proteins could lower the IEs. Whether flavonoids can remain fully functional in the sophisticated human digestive system remains unknown. Systematic studies of the effects of food matrices and/or stomach digestion on the IEs of flavonoids are lacking. Additionally, the IEs of flavonoids vary with enzyme origin. It would be more pragmatic to test flavonoids on humans to elucidate their actual real performance under the influence of food matrices.
2. Some in vivo tests have demonstrated that some flavonoids can effectively reduce the dose of Aca (such as baicalein, as described above). Thus, combining flavonoids with Aca might be a promising way to lower the side effects of Aca. It would be interesting to develop Aca tablets combined with flavonoids, and future studies should focus on human trials to put laboratory findings into practice.
3. Based on the known mechanisms of action and structure–activity relationships, it is possible to virtually screen compounds *in silico*. The IEs of promising compounds identified *in silico* could then be confirmed by in vitro and in vivo studies. This approach could prove effective for identifying and developing digestive enzyme inhibitors as novel medicines.
4. Due to the favorable IEs of flavonoids against digestive enzymes, functional foods could be designed for diabetic patients to help control blood sugar. However, few studies concerning antidiabetic activity have been reported, and future studies related to the application of flavonoids to specific foods are needed.

Acknowledgments

The authors thank the financial support received from the National Key Research and Development Program of China (2017YFD0400502), Guangzhou Science Technology and Innovation Commission (201803050001), the Fundamental Research Funds for the Central Universities of China (2018KZ13), the 111 Project (B17018), and Science and Technology Planning Project of Nansha, Guangzhou (2016GJ001), China. We are grateful to prof. Jay-Lin Jane, Department of Food Science and Human Nutrition, Food Sciences Building, Iowa State University, for her kind help in checking the first draft.

Abbreviations

IEs	inhibitory effects
αA	α-amylase

αG	α-glucosidase
YαG	*yeast* α-glucosidase
*Sc*αG	*Saccharomyces cerevisiae* αG
RiαG	rat small intestinal αG
PPA	porcine pancreatic α-amylase
AARs	amino acid residues
Aca	acarbose
Kae	kaempferol
Que	quercetin
Lut	luteolin
PCG	polycondensate of catechin with glyoxylic acid
H-bond	hydrogen bond
H-donor	hydrogen bond donor
H-acceptor	hydrogen bond acceptor

ORCID

Qiang Huang http://orcid.org/0000-0002-7162-1221

References

Asghari, B., P. Salehi, A. Sonboli, and S. N. Ebrahimi. 2015. Flavonoids from *Salvia chloroleuca* with α-amylsae and α-glucosidase inhibitory effect. *Iranian Journal of Pharmaceutical Research* 14 (2):609–615.

Boath, A. S., D. Stewart, and G. J. McDougall. 2012. Berry components inhibit alpha-glucosidase in vitro: synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chemistry* 135 (3):929–936. doi:10.1016/j.foodchem.2012.06.065.

Burgos-Edwards, A., F. Jimenez-Aspee, S. Thomas-Valdes, G. Schmeda-Hirschmann, and C. Theoduloz. 2017. Qualitative and quantitative changes in polyphenol composition and bioactivity of *Ribes magellanicum* and *R. punctatum* after in vitro gastrointestinal digestion. *Food Chemistry* 237 :1073–1082. doi:10.1016/j.foodchem.2017.06.060.

Chen, Y. G., P. Li, P. Li, R. Yan, X. Q. Zhang, Y. Wang, X. T. Zhang, W. C. Ye, and Q. W. Zhang. 2013. α-Glucosidase inhibitory effect and simultaneous quantification of three major flavonoid glycosides in *Microctis folium*. *Molecules* 18 (4):4221–4232. doi:10.3390/molecules18044221.

Chi, C., X. Li, Y. Zhang, L. Chen, L. Li, and Z. Wang. 2017. Digestibility and supramolecular structural changes of maize starch by non-covalent interactions with gallic acid. *Food & Function* 8 (2):720–730. doi:10.1039/c6fo01468b.

Choi, C.-I., S. R. Lee, and K. H. Kim. 2015. Antioxidant and α-glucosidase inhibitory activities of constituents from *Euonymus alatus* twigs. *Industrial Crops and Products* 76 :1055–1060. doi:10.1016/j.indcrop.2015.08.031.

Chou, T. C. 2010. Drug combination studies and their synergy quantification using the Chou–Talalay method. *Cancer Research* 70 (2):440–446. doi:10.1158/0008-5472.CAN-09-1947.

Collado-Gonzalez, J., C. Grosso, P. Valentao, P. B. Andrade, F. Ferreres, T. Durand, A. Guy, J. M. Galano, A. Torrecillas, and A. Gil-Izquierdo. 2017. Inhibition of α-glucosidase and α-amylase by Spanish extra virgin olive oils: the involvement of bioactive compounds other than oleuropein and hydroxytyrosol. *Food Chemistry* 235 :298–307. doi:10.1016/j.foodchem.2017.04.171.

Ercan, P., and S. N. El. 2016. Inhibitory effects of chickpea and *Tribulus terrestris* on lipase, alpha-amylase and alpha-glucosidase. *Food Chemistry* 205 :163–169. doi:10.1016/j.foodchem.2016.03.012.

Fu, J., N. Zhang, J. H. Chou, H.-J. Dong, S.-F. Lin, G. S. Ulrich-Merzenich, and T.-C. Chou. 2016. Drug combination in vivo using combination index method: Taxotere and T607 against colon carcinoma HCT-116 xenograft tumor in nude mice. *Synergy* 3 (3):15–30. doi:10.1016/j.synres.2016.06.001.

Gao, H., Y.-N. Huang, B. Gao, P.-Y. Xu, C. Inagaki, and J. Kawabata. 2008. α-Glucosidase inhibitory effect by the flower buds of *Tussilago farfara* L. *Food Chemistry* 106 (3):1195–1201. doi:10.1016/j.foodchem.2007.07.064.

Gao, J., P. Xu, Y. Wang, Y. Wang, and D. Hochstetter. 2013. Combined effects of green tea extracts, green tea polyphenols or epigallocatechin gallate with acarbose on inhibition against alpha-amylase and alpha-glucosidase in vitro. *Molecules* 18 (9):11614–11623. doi:10.3390/molecules180911614.

Geng, S., S. Shan, H. Ma, and B. Liu. 2016. Antioxidant activity and α-glucosidase inhibitory activities of the polycondensate of catechin with glyoxylic acid. *PloS One* 11 (3):e0150412. doi:10.1371/journal.pone.0150412.

Griffith, A. M. T. 2012. *The inhibition of α-amylase and α-glucosidase by bioflavonoids*. Corvallis, OR: Oregon State University.

Han, L., C. Fang, R. Zhu, Q. Peng, D. Li, and M. Wang. 2017. Inhibitory effect of phloretin on α-glucosidase: Kinetics, interaction mechanism and molecular docking. *International Journal of Biological Macromolecules* 95 :520–527. doi:10.1016/j.ijbiomac.2016.11.089.

Hargrove, J. L., P. Greenspan, D. K. Hartle, and C. Dowd. 2011. Inhibition of aromatase and α-Amylase by flavonoids and proanthocyanidins from *Sorghum bicolor* bran extracts. *Journal of Medicinal Food* 14 (7–8):799–807. doi:10.1089/jmf.2010.0143.

Kadouh, H. C., S. Sun, W. Zhu, and K. Zhou. 2016. α-Glucosidase inhibiting activity and bioactive compounds of six red wine grape pomace extracts. *Journal of Functional Foods* 26 :577–584. doi:10.1016/j.jff.2016.08.022.

Kim, J. S., C. S. Kwon, and K. H. Son. 2000. Inhibition of α-glucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology, and Biochemistry* 64 (11):2458–2461. doi:10.1271/bbb.64.2458.

Kim, S. H., S. H. Jo, Y. I. Kwon, and J. K. Hwang. 2011. Effects of onion (*Allium cepa* L.) extract administration on intestinal α-glucosidases activities and spikes in postprandial blood glucose levels in SD rats model. *International Journal of Molecular Sciences* 12 (6):3757–3769. doi:10.3390/ijms12063757.

Koh, L. W., L. L. Wong, Y. Y. Loo, S. Kasapis, and D. Huang. 2010. Evaluation of different teas against starch digestibility by mammalian glycosidases. *Journal of Agricultural and Food Chemistry* 58 (1):148–154. doi:10.1021/jf903011g.

Lavelli, V., P. S. Sri Harsha, P. Ferranti, A. Scarafoni, and S. Iametti. 2016. Grape skin phenolics as inhibitors of mammalian α-glucosidase and α-amylase – Effect of food matrix and processing on efficacy. *Food & Function* 7 (3):1655–1663. doi:10.1039/c6fo00073h.

Li, H., F. Song, J. Xing, R. Tsao, Z. Liu, and S. Liu. 2009. Screening and structural characterization of α-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MS^n and SORI-CID FTICR MS. *Journal of the American Society for Mass Spectrometry* 20 (8):1496–1503. doi:10.1016/j.jasms.2009.04.003.

Li, Y., F. Gao, F. Gao, F. Shan, J. Bian, and C. Zhao. 2009. Study on the interaction between 3 flavonoid compounds and α-amylase by fluorescence spectroscopy and enzymatic kinetics. *Journal of Food Science* 74 (3):C199–C203. doi:10.1111/j.1750-3841.2009.01080.x.

Li, Y. Q., F. C. Zhou, F. Gao, J. S. Bian, and F. Shan. 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. *Journal of Agricultural and Food Chemistry* 57 (24):11463–11468. doi:10.1021/jf903083h.

Liu, M., H. Yin, G. Liu, J. Dong, Z. Qian, and J. Miao. 2014. Xanthohumol, a prenylated chalcone from beer hops, acts as an α-glucosidase inhibitor in vitro. *Journal of Agricultural and Food Chemistry* 62 (24):5548–5554. doi:10.1021/jf500426z.

Liu, S., Z. Ai, F. Qu, Y. Chen, and D. Ni. 2017. Effect of steeping temperature on antioxidant and inhibitory activities of green tea extracts against α-amylase, α-glucosidase and intestinal glucose uptake. *Food Chemistry* 234 :168–173. doi:10.1016/j.foodchem.2017.04.151.

Liu, X., F. Luo, P. Li, Y. She, and W. Gao. 2017. Investigation of the interaction for three citrus flavonoids and α-amylase by surface plasmon resonance. *Food Research International* 97 :1–6. doi:10.1016/j.foodres.2017.03.023.

Luyen, N. T., L. H. Tram, T. T. H. Hanh, P. T. Binh, N. H. Dang, C. V. Minh, and N. T. Dat. 2013. Inhibitors of α-glucosidase, α-amylase and lipase from *Chrysanthemum morifolium*.

Phytochemistry Letters 6 (3):322–325. doi:10.1016/j.phytol.2013.03.015.

Meng, Y., A. Su, S. Yuan, H. Zhao, S. Tan, C. Hu, H. Deng, and Y. Guo. 2016. Evaluation of total flavonoids, myricetin, and quercetin from *Hovenia dulcis* thunb. As inhibitors of α-amylase and α-glucosidase. *Plant Foods for Human Nutrition* 71 (4):444–449. doi:10.1007/s11130-016-0581-2.

Milella, L., S. Milazzo, M. De Leo, M. B. Vera Saltos, I. Faraone, T. Tuccinardi, M. Lapillo, N. De Tommasi, and A. Braca. 2016. Glucosidase and α-amylase inhibitors from *Arcytophyllum thymifolium*. *Journal of Natural Products* 79 (8):2104–2112. α doi:10.1021/acs.jnatprod.6b00484.

Nahoum, V., G. Roux, V. Anton, P. RougÉ, A. Puigserver, H. Bischoff, B. Henrissat, and F. Payan. 2000. Crystal structures of human pancreatic α-amylase in complex with carbohydrate and proteinaceous inhibitors. *Biochemical Journal* 346 (1):201. doi:10.1042/0264-6021:3460201.

Peng, X., G. Zhang, Y. Liao, and D. Gong. 2016. Inhibitory kinetics and mechanism of kaempferol on α-glucosidase. *Food Chemistry* 190 :207–215. doi:10.1016/j.foodchem.2015.05.088.

Phan, M. A. T., J. Wang, J. Tang, Y. Z. Lee, and K. Ng. 2013. Evaluation of α-glucosidase inhibition potential of some flavonoids from *Epimedium brevicornum*. *Lebensmittel-Wissenschaft & Technologie* 53 (2):492–498. doi:10.1016/j.lwt.2013.04.002.

Piparo, E. L., H. Scheib, N. Frei, G. Williamson, M. Grigorov, and C. J. Chou. 2008. Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *Journal of Medicinal Chemistry* 51 (12):3555–3561. doi:10.1021/jm800115x.

Rasouli, H., S. M. Hosseini-Ghazvini, H. Adibi, and R. Khodarahmi. 2017. Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: A virtual screening perspective for the treatment of obesity and diabetes. *Food & Function* 8 (5):1942–1954. doi:10.1039/c7fo00220c.

Sabiu, S.,. F. H. O'Neill, and A. O. T. Ashafa. 2016. Kinetics of alpha-amylase and alpha-glucosidase inhibitory potential of zea mays Linnaeus (Poaceae), stigma maydis aqueous extract: an in vitro assessment. *Journal of Ethnopharmacology* 183 :1–8. doi:10.1016/j.jep.2016.02.024.

Sales, P. M. d., P. M. D Souza, L. A. Simeoni, P. D. O. Magalhães, and D. Silveira. 2012. α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy & Pharmaceutical Sciences* 15 (1):141–183. doi: 10.18433/J35S3K.

Saltos, M. B. V., B. F. N. Puente, I. Faraone, L. Milella, N. D. Tommasi, and A. Braca. 2015. Inhibitors of α-amylase and α-glucosidase from *Andromachia igniaria* humb. & Bonpl. *Phytochemistry Letters* 14 :45–50. doi:10.1016/j.phytol.2015.08.018.

Sheliya, M. A., B. Rayhana, A. Ali, K. K. Pillai, V. Aeri, M. Sharma, and S. R. Mir. 2015. Inhibition of α-glucosidase by new prenylated flavonoids from *Euphorbia hirta* L. herb. *Journal of Ethnopharmacology* 176 :1–8. doi:10.1016/j.jep.2015.10.018.

Shen, W., Y. Xu, and Y. H. Lu. 2012. Inhibitory effects of citrus flavonoids on starch digestion and antihyperglycemic effects in HepG2 cells. *Journal of Agricultural and Food Chemistry* 60 (38):9609–9619. doi:10.1021/jf3032556.

Sui, X., Y. Zhang, and W. Zhou. 2016. In vitro and in silico studies of the inhibition activity of anthocyanins against porcine pancreatic α-amylase. *Journal of Functional Foods* 21 :50–57. doi:10.1016/j.jff.2015.11.042.

Sun, H., D. Wang, X. Song, Y. Zhang, W. Ding, X. Peng, X. Zhang, Y. Li, Y. Ma, R. Wang, and P. Yu. 2017. Natural prenylchalconaringenins and prenylnaringenins as antidiabetic agents: α-Glucosidase and α-amylase inhibition and in vivo antihyperglycemic and antihyperlipidemic effects. *Journal of Agricultural and Food Chemistry* 65 (8):1574–1581. doi:10.1021/acs.jafc.6b05445.

Sun, L., F. J. Warren, G. Netzel, and M. J. Gidley. 2016. 3 Or 3'-Galloyl substitution plays an important role in association of catechins and theaflavins with porcine pancreatic α-amylase: The kinetics of inhibition of α-amylase by tea polyphenols. *Journal of Functional Foods* 26 :144–156. doi:10.1016/j.jff.2016.07.012.

Tadera, K., Y. Minami, K. Takamatsu, and T. Matsuoka. 2006. Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology* 52 (2):149–153. doi: 10.3177/jnsv.52.149.

Tallarida, R. J. 2001. Drug synergism: Its detection and applications. *The Journal of Pharmacology and Experimental Therapeutics* 298(3):865–872.

Tan, C., Q. Wang, C. Luo, S. Chen, Q. Li, and P. Li. 2013. Yeast α-glucosidase inhibitory phenolic compounds isolated from *Gynura medica* leaf. *International Journal of Molecular Sciences* 14 (2):2551–2558. doi:10.3390/ijms14022551.

Tan, Y., S. K. C. Chang, and Y. Zhang. 2017. Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry* 214 :259–268. doi:10.1016/j.foodchem.2016.06.100.

Teng, H., and L. Chen. 2017. α-Glucosidase and α-amylase inhibitors from seed oil: A review of liposoluble substance to treat diabetes. *Critical Reviews in Food Science and Nutrition* 57 (16):3438–3448. doi:10.1080/10408398.2015.1129309.

Torres-Naranjo, M., A. Suárez, G. Gilardoni, L. Cartuche, P. Flores, and V. Morocho. 2016. Chemical constituents of *Muehlenbeckia tamnifolia* (Kunth) meisn (Polygonaceae) and its in vitro α-amilase and α-glucosidase inhibitory activities. *Molecules* 21 (11):1461. doi:10.3390/molecules21111461.

Tundis, R., M. Bonesi, V. Sicari, T. M. Pellicanò, M. C. Tenuta, M. Leporini, F. Menichini, and M. R. Loizzo. 2016. *Poncirus trifoliata* (L.) raf.: chemical composition, antioxidant properties and hypoglycaemic activity via the inhibition of α-amylase and α-glucosidase enzymes. *Journal of Functional Foods* 25 :477–485. doi:10.1016/j.jff.2016.06.034.

Wang, H., Y.-J. Du, and H.-C. Song. 2010. α-Glucosidase and α-amylase inhibitory activities of guava leaves. *Food Chemistry* 123 (1):6–13. doi:10.1016/j.foodchem.2010.03.088.

Wang, T-y, Q. Li, and K.-S Bi. 2017. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian Journal of Pharmaceutical Sciences* 13 :12–23. doi:10.1016/j.ajps.2017.08.004

Xiao, J., G. Kai, K. Yamamoto, and X. Chen. 2013. Advance in dietary polyphenols as alpha-glucosidases inhibitors: A review on structure–activity relationship aspect. *Critical Reviews in Food Science and Nutrition* 53 (8):818–836. doi:10.1080/10408398.2011.561379.

Xiao, J., X. Ni, G. Kai, and X. Chen. 2013. A review on structure–activity relationship of dietary polyphenols inhibiting α-amylase. *Critical Reviews in Food Science and Nutrition* 53 (5):497–506. doi:10.1080/10408398.2010.548108.

Xu, C., and X. Chen. 2011. Molecular property–affinity relationship of the interaction between dietary polyphenols and bovine milk proteins. *Food & Function* 2 :368–372. doi:10.1039/c1fo10074b.

Yan, J., G. Zhang, J. Pan, and Y. Wang. 2014. α-Glucosidase inhibition by luteolin: Kinetics, interaction and molecular docking. *International Journal of Biological Macromolecules* 64 :213–223. doi:10.1016/j.ijbiomac.2013.12.007.

Yang, J. P., H. He, and Y. H. Lu. 2014. Four flavonoid compounds from *Phyllostachys edulis* leaf extract retard the digestion of starch and its working mechanisms. *Journal of Agricultural and Food Chemistry* 62 (31):7760–7770. doi:10.1021/jf501931m.

Ye, R., Y. H. Fan, and C. M. Ma. 2017. Identification and enrichment of α-glucosidase-inhibiting dihydrostilbene and flavonoids from *Glycyrrhiza uralensis* leaves. *Journal of Agricultural and Food Chemistry* 65 (2):510–515. doi:10.1021/acs.jafc.6b04155.

Ye, X.-P., C.-Q. Song, P. Yuan, and R.-G. Mao. 2010. α-Glucosidase and α-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. *Chinese Journal of Natural Medicines* 8 (5):349–352. doi:10.1016/s1875-5364(10)60041-6.

Yin, Z., W. Zhang, F. Feng, Y. Zhang, and W. Kang. 2014. α-Glucosidase inhibitors isolated from medicinal plants. *Food Science and Human Wellness* 3 (3–4):136–174. doi:10.1016/j.fshw.2014.11.003.

Yoshida, K., A. Hishida, O. Iida, K. Hosokawa, and J. Kawabata. 2008. Flavonol caffeoylglycosides as α-glucosidase inhibitors from *Spiraea*

cantoniensis flower. *Journal of Agricultural and Food Chemistry* 56 (12):4367–4371. doi:10.1021/jf8007579.

Zeng, L., G. Zhang, S. Lin, and D. Gong. 2016. Inhibitory mechanism of apigenin on α-glucosidase and synergy analysis of flavonoids. *Journal of Agricultural and Food Chemistry* 64 (37):6939–6949. doi:10.1021/acs.jafc.6b02314.

Zhang, B-w, X. Li, W-l Sun, Y. Xing, Z-l Xiu, C-l Zhuang, and Y-s Dong. 2017. Dietary flavonoids and acarbose synergistically inhibit α-glucosidase and lower postprandial blood glucose. *Journal of Agricultural and Food Chemistry* 65 (38):8319–8330. doi:10.1021/acs.jafc.7b02531.

Zhen, J., Y. Dai, T. Villani, D. Giurleo, J. E. Simon, and Q. Wu. 2017. Synthesis of novel flavonoid alkaloids as α-glucosidase inhibitors. *Bioorganic & Medicinal Chemistry* 25 (20):5355–5364. doi:10.1016/j.bmc.2017.07.055.