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Lijun Sun, Lijun Sun, Fredrick J. Warren, Fredrick J. Warren ...+1 more authors

Institutions: Northwest A&F University, University of Queensland, Norwich Research Park

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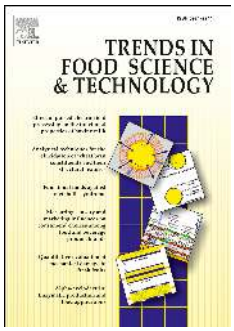

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Natural products for glycaemic control: Polyphenols as inhibitors of alpha-amylase

Lijun Sun [a, c], Fredrick J. Warren [a, b], Michael J. Gidley [*, a]

[a] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia

[b] Quadram Institute Bioscience, Norwich Research Park, Norwich NR4 7UA, UK

[c] College of Food Science and Engineering, Northwest A & F University, Yangling, Shaanxi 712100, P.R. China

[*]Corresponding author.
E-mail: m.gidley@uq.edu.au

Abstract

Background: α-Amylase plays an important role in starch digestion, the main source of exogenous glucose in the human diet. Retarding glucose absorption through delaying digestion of starchy foods by inhibiting α-amylase in the digestive tract has potential as a management and/or therapeutic approach to type II diabetes. Polyphenols have been reported to have inhibitory activity against the enzyme.

Scope and approach: This review provides an overview of structure-activity relationships of dietary polyphenols inhibiting α-amylase and the underlying mechanisms. The methods applied to characterize binding interactions between polyphenols and α-amylase, as well as the relationships between the constants obtained from these methods are discussed. As polyphenols can interact with both polysaccharides and α-amylase, the potential effects of polysaccharides on the binding of polyphenols with α-amylase are also summarised.

Key findings and conclusions: The inhibition of α-amylase by polyphenols results from binding interactions between the enzyme and polyphenols. The galloyl moiety in polyphenols plays an important role. IC_{50}, inhibition kinetics, fluorescence quenching, differential scanning calorimetry, isothermal titration calorimetry and molecular docking can be comprehensively combined to analyze the binding interactions, as the constants obtained from these methods can be correlated. Soluble polysaccharides may reduce the binding and inhibitory action of polyphenols against α-amylase. Most work reported in this review is from *in vitro* studies, so if and how the binding interactions affect starch digestion *in vivo* need to be further studied.

Keywords: Enzyme inhibition; kinetic analysis; fluorescence quenching; isothermal titration calorimetry; molecular binding; starch

1 Introduction

In recent years, some food and plant extracts have been reported to effectively inhibit α-amylase, the mammalian digestive enzyme which catalyses the first step of starch breakdown in the digestive tract. The main components exerting the inhibitory activity in such extracts are typically polyphenols (Sun et al., 2018). The inhibition of α-amylase by a polyphenol is highly related with its molecular structure, as the inhibition results from binding interactions between polyphenols and the enzyme (Jakobek, 2015; Lo Piparo et al., 2008; Sun, Warren, et al., 2016). The interaction forces between polyphenols and α-amylase are expected to include hydrogen bonding (between hydroxyl groups and the enzyme catalytic active site) and hydrophobic force (between aromatic rings of polyphenols and tryptophan residues of α-amylase) (Lo Piparo, et al., 2008). Electron delocalization between C=C (or C=O) and aromatic rings in polyphenols has been reported to enhance hydrophobic (π-π) interactions with α-amylase (Lo Piparo, et al., 2008; Xiao et al., 2013). The relationship between polyphenol structure and inhibitory activity has been investigated and reviewed for some polyphenol types, *e.g.* flavonoids (Desseaux et al., 2018; Lo Piparo, et al., 2008; Xiao, et al., 2013), but there are other polyphenol molecular structures that exhibit inhibitory activity and need to be further reviewed. Together with detailed inhibition

and biophysical studies, this can lead to a better definition of structure-activity mechanisms.

Classical methods to characterize the binding interactions between dietary polyphenols and α-amylase include Lineweaver-Burk kinetic analysis, fluorescence quenching (FQ) and molecular docking, from which Michaelis constant (K_m), fluorescence quenching constant (K_{FQ}) and binding energy (E_b) can be obtained, respectively (Fei et al., 2014; Miao et al., 2013). Recently, inhibition kinetics, isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and circular dichroism (CD) have also been applied to study the binding of polyphenols with α-amylase. From these measurements, inhibition constants (including competitive inhibition constant, K_{ic} and uncompetitive inhibition constant, K_{iu}), binding constant (K_{itc}), thermal stability and secondary structure contents can be obtained, respectively (Cai et al., 2015; Liu et al., 2017; Sun, Gidley, & Warren, 2017). The correlation between the constants obtained from these different methodologies as well as how these approaches are reasonably combined to characterize the binding interactions between polyphenols and α-amylase need to be reviewed and elucidated.

Food, as a complex system, contains starch, proteins, lipids, polysaccharides, polyphenols, *etc.* Particularly, aqueous plant extracts often contain both soluble polyphenols and polysaccharides, and the two compounds have been suggested to interact with each other (Chamorro et al., 2012). Other components are also consumed together with polyphenol-containing plant extracts when eating foods. Therefore, the effects of other food components, such as polysaccharides and proteins on inhibition

of α-amylase by dietary polyphenols should be analyzed to comprehensively evaluate the inhibitory activity of functional foods with phenolic compounds as the key constituents.

Here we summarise molecular structure-inhibitory activity studies of polyphenols and the corresponding mechanisms. Then, correlations between constants obtained from multiple techniques indicating interactions of polyphenols with α-amylase are elucidated to define a set of methods to analyze binding interactions. Potential influences of other food components (*e.g.* polysaccharides) on the binding interactions between polyphenols and α-amylase are also suggested, along with some suggested methods to study them.

2 Relationships between phenolic structure and inhibitory activity against α-amylase

Although the structure-inhibition relationships for some dietary phenolic compounds, *e.g.* flavonoids against α-amylase have been reviewed previously (Lo Piparo, et al., 2008; Xiao, et al., 2013), there have been recent studies on potential mechanisms underlying structure-activity relationships that deserve further review.

2.1 α-Amylase

α-Amylase is an enzyme that hydrolyses α-1,4-glucan polysaccharides, such as starch and glycogen, in an endo action producing primarily maltose and maltooligosaccharides prior to production of glucose. The frequency and magnitude of excursions in blood glucose trigger insulin response, which is highly related with

type II diabetes. α-Amylase is the form of amylase found in mammals. It also exists in plant seeds containing starch as a food reserve (Mundy & Rogers, 1986), and is commonly secreted by bacteria (Mohapatra, Banerjee, & Bapuji, 1998). In the three-dimensional structure determined by X-ray crystallography (Fig.1A), there are four secondary structure motifs in the enzyme, including α-helix, β-sheet, β-turn and random coil (Cai, et al., 2015). At the active site of α-amylase (Fig.1B), there are calcium and chloride ions, which are essential for maintenance of the tertiary structure and catalytic activity of the enzyme (Buisson et al., 1987). Porcine pancreatic α-amylase (PPA) is often used to study catalytic digestion of starch *in vitro*. There are 496 amino acid residues in the single polypeptide chain of the enzyme (Qian, Haser, & Payan, 1993). Through modeling based on the X-ray crystallographic structure, Asp^{300}, Asp^{197} and Glu^{233} are considered as essential catalytic residues at the active site of PPA (Fig.1B) (MacGregor, Janeček, & Svensson, 2001). In addition, some aromatic residues, like Trp^{58}, Trp^{59} and Tyr^{62} are stacking features at the entrance of the active site (Qian, Haser, & Payan, 1993). Notably, amino acid residues at the active site of α-amylase are proposed to be in positions where the main interactions between (polyphenol) inhibitors and the enzyme are thought to occur (Miao et al., 2015).

2.2 Flavonoids

Hydroxyl (-OH) groups are essential for the inhibitory activity of flavonoid compounds against α-amylase, as the inhibition is likely to depend on the formation of hydrogen bonds between -OH groups of phenolics and side chains of amino acids

(such as Asp^{197} and Glu^{233}) at the active site of α-amylase (Kawamura-Konishi et al., 2012; Lo Piparo, et al., 2008). Flavonoids without substitution of -OCH_3 at -OH in their molecular structures (Fig.2) are more likely to be effective inhibitors of α-amylase than those with such substitution patterns (Al-Dabbas et al., 2006; Lo Piparo, et al., 2008). To support the role of hydroxyl groups, quercetagetin and scutellarein were demonstrated as potential α-amylase inhibitors, with IC_{50} values of 10.2 and 9.64 μM, respectively (Lo Piparo, et al., 2008). The molecular structures of both flavonoids have hydroxyl groups at each of positions 5, 6, 7 in the A ring as well as 4' in the B ring (Fig.2). By contrast, the IC_{50} values of some other flavonoids (kaempferol, apigenin, naringenin, daidzein and catechin) with less hydroxyl groups in their molecular structures (Fig.2) were much higher (0.5 mM-6.0 mM) than quercetagetin and scutellarein (Barrett et al., 2013; Tadera et al., 2006; Wang, Du, & Song, 2010). Therefore, these confirm the role of flavonoid hydroxyl groups in inhibiting enzyme activity.

The 2,3-double bond in ring C (Fig.2) also affects the inhibitory activity of flavonoids to some extent. This double bond is conjugated with the 4-carbonyl group (Fig.2), which enhances electron delocalization between ring C and ring A; therefore, it has been proposed that the benzopyrone system (rings A and C) can form a highly stable conjugated π-π system with the indole ring of Trp^{59} at the active site of α-amylase, promoting its binding to α-amylase and reducing the catalytic activity (Lo Piparo, et al., 2008). Hydrogenation of the 2,3-double bond of flavonoids not only weakens the conjugation but also transforms the near-planar molecular structure

(flavonol and flavone, Fig.2) to a more flexible and non-planar stereochemical structure (flavanone and flavanols, Fig.2) (Todorova et al., 2013), reducing the binding ability with α-amylase due to steric hindrance. As a consequence, flavanone and flavanol compounds show weaker inhibition of α-amylase than some flavonols and flavones (Kim, Kwon, & Son, 2000; Lo Piparo, et al., 2008; Wang, Du, & Song, 2010)

There are also glycosylated forms of flavonoids in plant extracts (da Silva et al., 2013). Quercetin (Fig.2) is a flavonol (without glycosylation) that has been shown to possess competitive inhibitory activity against α-amylase (Li et al., 2009). There are some monoglycoside forms of quercetin, such as quercitrin (rhamnoside), hyperin (galactoside), guaijaverin (arabinopyranoside) and avicularin (arabinofuranoside), with rutin (rhamnosylglucoside) as a disaccharide form. The inhibiting activity of these glycosylated quercetin molecules against α-amylase was determined to be quercetin > guaijaverin > avicularin > hyperin > rutin (Li, et al., 2009; Ye et al., 2010). Komaki et al. (2003) also measured the IC_{50} value of luteolin (Fig.2) (0.01 mg/mL) against α-amylase, and found it much lower than that of luteolin-7-*O*-*β*-glucoside (0.5 mg/mL) and luteolin-4'-*O*- *β*-glucoside (0.3 mg/mL). Besides, the inhibiting effect of kaempferol (Fig.2) on α-amylase was much stronger than its glycoside form (kaempferol-3-*O*-*β*-D-diglucoside) (Ye, et al., 2010). Therefore, glycosylation on flavonoids is commonly observed to decrease the inhibitory activity. The potential reasons for the inhibition change are as follows: (i) Spatial structures of flavonoid-glycosides are transformed to bulky non-planar from near-planar, limiting

the ability to enter the hydrophobic (active) site of α-amylase due to steric hindrance effects, and (ii) as an -OH group is substituted by a glycoside, the group affinity for protein is correspondingly affected; therefore, the glycosylation of flavonoids decreases their binding affinity to α-amylase.

2.3 Phenolic acids

Phenolic acids, an important polyphenol class, are aromatic phenols of secondary plant metabolites with a carboxylic acid functional group. They are widely distributed throughout the plant kingdom (Shahidi, Janitha, & Wanasundara, 1992). The natural plant phenolic acids mainly contain two molecular groups: hydroxycinnamic and hydroxybenzoic acids (Fig.3). For individual molecular groups, although the essential structure remains the same, the substituents (hydroxyl and methoxyl groups) on the aromatic ring contribute to distinct molecular properties, such as polarity, stability and binding.

It has been reported that hydroxybenzoic acids (Fig.3), like vanillic acid and salicylic acid hardly show any inhibitory activity against α-amylase (McDougall et al., 2005; Sharma, Sharma, & Rai, 1986), while hydroxycinnamic acids (Fig.3) show inhibition (Narita & Inouye, 2011). For hydroxycinnamic acids, the C=C double bonds in the molecular structure are conjugated with the carbonyl group and are responsible for electron transfer between the acrylic acid and benzene ring moieties. As a consequence, hydroxycinnamic acids could form a highly conjugated system

198 which stabilizes the compounds when binding to the active site of α-amylase. Caffeic
199 acid (Fig.3) has been reported to have a relatively strong inhibitory activity (IC_{50}=0.4
200 mM). However, both dehydroxylation and methylation of caffeic acid lowered its
201 inhibiting activity against α-amylase, although these compounds are still structures
202 with a delocalized π-system established through carbonyl, C=C double bonds and
203 benzene (Narita & Inouye, 2011). The IC_{50} of quinic acid was determined as 26.5 mM,
204 much higher than chlorogenic acids and caffeic acid. Although there are 4 hydroxyl
205 groups in the quinic acid structure, it has no strong conjugated system (Fig.3).
206 Therefore, both conjugated structural features and multiple hydroxyl groups are
207 essential for hydroxycinnamic acids to show inhibition of α-amylase.

208 Chlorogenic acids are a set of esters between quinic acid and one or more cinnamic
209 acid derivatives like caffeic acid, ferulic acid, and *p*-coumaric acid (Fig.3). They are
210 widely distributed in e.g. green coffee beans, and include three main classes:
211 caffeoylquinic acids, dicaffeoylquinic acids and feruloylquinic acids (Clifford et al.,
212 2006). Narita et al. (2011) investigated the α-amylase inhibitory activity of 16 kinds
213 of chlorogenic acids and cinnamate derivatives from green coffee beans. It was found
214 that methylation of the 3-OH group on caffeoylquinic acids (converting 3-, 4-, and
215 5-caffeoylquinic acids to 3-, 4-, and 5-feruloylquinic acids, respectively, Fig.3)
216 decreased their inhibition effects against porcine pancreatic α-amylase isozyme I,
217 possibly as a result of weakened hydrogen bonds between feruloylquinic acids (3-, 4-,
218 and 5-feruloylquinic acids) and amino acid residues of the enzyme. This shows a
219 similar trend as methylation of flavonoids. Among the chlorogenic acids tested,

dicaffeoylquinic acids (Fig.3) showed strongest inhibitory activity, with IC_{50} values for 3,4-, 4,5-, and 3,5-dicaffeoylquinic acids of 0.02, 0.02, and 0.03 mM, respectively (Narita & Inouye, 2009). The potential reasons for their comparatively effective inhibition are (i) the dicaffeoyl groups provide more hydroxyl groups which are essential for inhibition due to the formation of hydrogen bonds between hydroxyl groups and the active catalyzing site, and/or (ii) there is an extra carbonyl, C=C double bond, and benzene ring in dicaffeoylquinic acids than caffeoylquinic acids. As a consequence, dicaffeoyl molecules are more electron-rich with *p*-π (between double bonds and benzene) and π-π (carbonyl and double bonds) conjugated systems, leading to potentially stronger π-interactions with the indole ring of Trp^{59} (Lo Piparo, et al., 2008).

Tannic acid (TA) is a common family of secondary metabolites in higher plants. The chemical structure for standard TA is decagalloyl glucose (Fig.3), but in fact it is a mixture of polygalloyl glucoses or polygalloyl quinic acid esters with 2-12 galloyl moieties per molecule depending on plant source. There are some studies reporting that TA has inhibitory activity against α-amylase *in vitro* (Kandra et al., 2004). The IC_{50} of TA was determined as 0.301 mg/mL, and it was found to demonstrate competitive inhibition (Sun, et al., 2016). Besides, the optimum pH value for pancreatic α-amylase is around 7.0 (Coronado et al., 2000), but the *PKa* of the phenolic carboxylic acid proton is between 4 and 5, making its aqueous solution acid (pH<7.0); therefore, the catalytic activity of α-amylase *in vitro* may be also partly inhibited due to the unfavorable acidity (Nielsen, Borchert, & Vriend, 2001). On the

242 other hand, the loss or decrease of the ability to inhibit α-amylase *in vivo* may occur
243 for TA. This may be because: (i) TA may be oxidized by oxygen and oxygen-derived
244 radicals in the stomach and/or (ii) TA can interact or bind with proteins present in
245 foods and stomach digesta before reaching the small intestine where starch is
246 hydrolyzed by pancreatic α-amylase. In addition, orally-taken TA may cause
247 astringent and unpleasant bitter taste due to interactions with salivary proteins. To
248 avoid these unacceptable properties, microencapsulation systems for TA have been
249 developed to control its release and to improve inhibition in the gastrointestinal tract
250 (Xing et al., 2004).

251 **2.4 Galloyl moiety**

252 The galloyl group is a common substituent of polyphenols, especially tea
253 polyphenols. Catechin (C), epicatechin (EC), epigallocatechin (EGC),
254 epigallocatechin gallate (EGCG), epicatechin gallate (ECG), theaflavin (TF),
255 theaflavin-3'-gallate (TF1), theaflavin-3, 3'-digallate (TF2) (Fig.4) are the prominent
256 polyphenols in aqueous green, oolong or black tea extracts (Sun, et al., 2016). The
257 inhibitory activities of tea polyphenols against α-amylase have been studied, and the
258 results show that polyphenols with a galloyl moiety in their molecular structures have
259 greater enzyme inhibition than those without a galloyl moiety, indicated by IC_{50}
260 values (Miao, et al., 2015; Miao, et al., 2013; Sun, et al., 2016). The binding of tea
261 polyphenols with α-amylase has been studied through inhibition kinetics
262 (Lineweaver-Burk, Dixon and Cornish-Bowden equations), fluorescence quenching,
263 isothermal titration calorimetry and molecular docking methods, confirming that the

binding of galloylated polyphenols with α-amylase is higher than for non-galloylated polyphenols (Miao, et al., 2013; Sun, Gidley, & Warren, 2017; Sun, et al., 2016). These results indicate that the higher inhibitory activities of galloylated polyphenols are attributed to the greater binding of the polyphenols with the enzyme.

It should be noted that for the theaflavin family in black tea, the number of galloyl groups at the 3-position on the C ring or the corresponding 3'-position on the C' ring in the TF, TF1 and TF2 molecular structure is 0, 1, and 2, respectively (Fig.4). The inhibitory potency is enhanced as the number of galloyl moieties increased. Additionally, ECG and EGCG, the respective 3-gallate forms of EC and EGC (Fig.4), have inhibitory activities against the enzyme around 5 times greater than EC and EGC, respectively (Sun, Gidley, & Warren, 2017). Each galloyl group provides three hydroxyl groups that can potentially interact with the catalytic amino acid side-chains of α-amylase (Asp^{197}, Glu^{233} and Asp^{300}) through formation of hydrogen bonds (Fei, et al., 2014), and the benzene ring may develop hydrophobic π-π (aromatic-aromatic) interactions at the active site of the enzyme (Miao, et al., 2013). In addition, in the galloyl group, the C=O double bond is conjugated to the benzene ring and is responsible for electron delocalization, which has been proposed to lead to enhanced π-π interactions with the indole ring of Trp^{59} of α-amylase (Lo Piparo, et al., 2008). Therefore, 3 or 3'-galloyl groups on the C or C' ring are likely to be responsible for enhancing the inhibitory activities of catechins and theaflavins against α-amylase.

3 Methods used to characterize interactions between polyphenols and α-amylase

3.1 Inhibition of α-amylase

In vitro assays to measure enzyme inhibition are relatively convenient to conduct. Generally, a real or synthetic substrate at a physiological concentration is used with α-amylase, and the assay is carried out at a suitable pH value. The inhibition is assayed based on the determination of initial amylase-catalyzed reaction velocity against a starch substrate in the absence and presence of polyphenols that can be monitored through iodo-starch reaction, reducing sugars method, *p*-nitrophenyl-α-$_D$-maltoside method or fluorescently-labelled starch method (Fei, et al., 2014; Karim, Holmes, & Orfila, 2017; Kawamura-Konishi, et al., 2012; Warren et al., 2011). Then, the inhibitory activity of a polyphenol is typically described by its half inhibitory concentration value, IC_{50}. It should be noted that the IC_{50} value, for a competitive inhibitor, as many polyphenols are, can only be considered a value for comparative purposes within a given study, or with reference to a known inhibitor such as acarbose. This is because the IC_{50} value is dependent on the competition between inhibitor and substrate for enzyme active site, and is therefore dependent not only on the inhibitor concentration, but also on the substrate concentration. The consequence of this is that IC_{50} values for competitive inhibitors are highly dependent on the assay conditions used, and specifically substrate concentration, making direct comparison between studies difficult. Therefore, more detailed kinetics analyses are required to reproducibly characterize the inhibitory activity of competitive enzyme inhibitors such as polyphenols.

3.2 Inhibition kinetics

Usually, the Lineweaver-Burk plot, a double-reciprocal plot of the Michaelis-Menten equation is applied in inhibition analysis to identify the inhibition type, maximum initial reaction velocity (V_{max}) and Michaelis constant (K_m) (Lineweaver & Burk, 1934). In practice, there are commonly experimental and statistical errors when applying the Lineweaver-Burk equation, which may affect the full fitting of the plot (Fei, et al., 2014; Yang, He, & Lu, 2014).

Although the Lineweaver-Burk equation can give the V_{max} and K_m, there is a relatively large error in this plot when an inappropriate range of substrate concentrations is used, particularly for very low substrate concentrations relative to K_m. Also, the Lineweaver-Burk plot cannot distinguish between uncompetitive, non-competitive and mixed-type inhibition (Cornish-Bowden & Eisenthal, 1974). Therefore, additional inhibition analysis methods may be used to assist in analyzing the kinetics of inhibition. The use of a Dixon plot, in which the reciprocal of initial reaction velocity ($1/v$) is plotted against inhibitor concentration (i) at various substrate concentrations (a), along with a Cornish-Bowden plot, plotting a/v against i at several values of a, are useful in the case that the interaction between inhibitor and enzyme is more complex than competitive or uncompetitive inhibition mechanisms (Cornish-Bowden & Eisenthal, 1974). In addition, the inhibition type, competitive (K_{ic}) and uncompetitive inhibition constant (K_{iu}) can be obtained by use of these plots. As defined, K_{ic} suggests the dissociation of an inhibitor-enzyme complex; therefore $1/K_{ic}$ demonstrates the association of an inhibitor with enzyme (competitive inhibition). Similarly, $1/K_{iu}$ describes the binding of an inhibitor-enzyme-substrate

ternary complex (uncompetitive inhibition). It should be noted that a lower K_{ic} value means a shift of equilibrium position in favour of enzyme-inhibitor complex, or in other words, it means the inhibitor binds more tightly with the enzyme (Cornish-Bowden & Eisenthal, 1974).

Inhibition kinetics, including Lineweaver-Burk, Dixon and Cornish-Bowden plots have been applied to analyse the inhibition mechanisms of polyphenols against α-amylase. It was found that EGCG, TF2 and TA are competitive inhibitors of α-amylase, while ECG, TF1 and TF are mixed-type inhibitors that have both competitive and uncompetitive inhibition characteristics (Sun, et al., 2016). Additionally, the galloyl moiety was found to play an important role in binding (association) of catechins and theaflavins with the enzyme (Sun, Gidley, & Warren, 2017). Correlations between IC_{50} and inhibition constants (K_{ic} and K_{iu}) for tea polyphenols were established in the above study (Fig.5A). It was found that there is a positive linear relationship between IC_{50} and K_{ic} and between IC_{50} and K_{iu} for the tea polyphenols (Sun, et al., 2016), indicating that the inhibition of polyphenols against α-amylase results from binding (association) of polyphenols with the enzyme, and that IC_{50} and inhibition kinetics (K_{ic} and K_{iu}) can be combined to characterize the interactions between dietary polyphenols and α-amylase (Fig.6).

3.3 Fluorescence quenching (FQ)

Fluorescence quenching (FQ) has been used to clarify if and how phenolic compounds interact with proteins at a molecular level. As there are fluorophores in α-amylase, like tryptophan and tyrosine, the enzyme can emit fluorescence at certain

352 excitation wavelengths, and the fluorescence intensity is directly related with the
353 amount of enzyme in solution (Soares, Mateus, & de Freitas, 2007). Polyphenols can
354 interact with any surface-accessible tyrosine or tryptophan or its vicinity, including
355 the tryptophan at the active site of α-amylase (Trp^{59}), decreasing the fluorescence
356 properties of the fluorophores; therefore, the fluorescence of α-amylase can be
357 quenched and should at least in part be modified by prior binding of a polyphenol to
358 the same site(s). From the Stern-Volmer equation ($\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{FQ}[Q]$)
359 or its modified form ($\frac{F_0}{F} = e^{(K_{FQ}[Q])}$), the fluorescence quenching constant (K_{FQ}) can
360 be calculated (Soares, Mateus, & de Freitas, 2007). As K_{FQ} can reflect the interaction
361 (binding) of polyphenols with α-amylase, higher K_{FQ} values correspond to higher
362 binding properties of polyphenols. FQ can be classified into dynamic and static
363 patterns, in which the former results from collisional encounters between fluorophore
364 and quencher, and the latter is caused by formation of a ground state complex between
365 the two compounds (Soares, Mateus, & de Freitas, 2007). Usually, a linear
366 Stern-Volmer plot indicates that there is a single class of fluorophore in the protein
367 interacting with the quencher in the same way and that only one quenching
368 mechanism (dynamic or static) takes place. However, positive deviations from the
369 equation are frequently observed when the quenching extent is large. In this case, the
370 plot of F_0/F against [Q] is an upward curve, concave towards the y axis. Commonly,
371 the upward curvature indicates that there are several mechanisms responsible for the
372 quenching effects on fluorophores in the protein, or it suggests the existence of a
373 'sphere of action', *i.e.*, apparent static quenching. The bimolecular quenching constant,

374 k_q, which reflects the efficiency of quenching or the availability of quenchers to
375 fluorophores, can be used to determine if the quenching results from complex
376 formation between proteins and quenchers. The k_q is close to 1×10^{10} $M^{-1}s^{-1}$ for the
377 typical dynamic mechanism (collision-controlled quenching) (Soares, Mateus, & de
378 Freitas, 2007). Therefore, in recent studies, EGCG was shown to quench the
379 fluorescence of α-amylase by a dynamic mechanism because its Stern-Volmer plot
380 was linear and its k_q was determined to be lower than 1×10^{10} $M^{-1}s^{-1}$ (Fei, et al., 2014;
381 Miao, et al., 2015), while sorghum procyanidins were shown to apparently statically
382 quench the α-amylase fluorescence with an upward Stern-Volmer plot and a k_q value
383 that was much higher than 1×10^{10} $M^{-1}s^{-1}$ (Cai, et al., 2015).

384 As both K_{FQ} and $1/K_{ic}$ indicate binding interactions between polyphenols and
385 α-amylase, it is interesting and necessary to establish the relationship between the two
386 indices to evaluate the feasibility of and relationship between the two measurement
387 methods. By doing this, it was found that there was a positive linear correlation (K_{FQ}
388 $= 13.97\cdot 1/K_{ic} + 1517.1$, $R^2 = 0.9590$, Fig.5B) between K_{FQ} and $1/K_{ic}$ for 6 pure
389 polyphenols (Sun, Gidley, & Warren, 2017). This indicates that lower K_{ic} corresponds
390 with higher K_{FQ} for individual polyphenols, and that FQ and inhibition kinetics may
391 be combined to characterize the binding interactions between dietary polyphenols and
392 α-amylase (Fig.6).

393 It is worth noting that in some fluorescence spectra of enzyme in the presence of
394 polyphenols, a red-shift of maximum emission wavelength (λ_{em}) is observed (Fei, et
395 al., 2014; Sun, Chen, et al., 2016; Sun, Gidley, & Warren, 2017). This indicates that

partial structural unfolding may occur for α-amylase upon binding with these polyphenols (Soares, Mateus, & de Freitas, 2007). The interaction between polyphenols and α-amylase is assumed to involve Typ and Tyr residues via their aromatic heterocyclic and hydrophobic groups which are exposed, causing microenviromental changes in the local spatial structure and backbone conformation of the protein (Fei, et al., 2014). In addition, polyphenol-amylase interactions have been found to make the structure of the enzyme more flexible (Cai, et al., 2015). Interestingly, the potential structural unfolding of α-amylase upon binding with polyphenols indicated by the red-shift of λ_{em} may also be detected by techniques that report on the thermal stability and spatial structure of a protein, such as differential scanning calorimetry (DSC) and circular dichroism (CD). This will be discussed in the following sections.

3.4 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) can be applied to monitor phase and conformational transitions through measurement of specific heat capacity as a function of temperature for a sample (Tang, Covington, & Hancock, 2003). In DSC analysis, the parameters indicating thermostability of a protein include denaturation temperature, T_d and denaturation enthalpy, ΔH *i.e.* the energy required to denature the protein. DSC offers an objective and quantitative way of evaluating the effects of dietary polyphenols on thermal stability of proteins (Barrett, et al., 2013; Prigent et al., 2003; Raghavendra, Kumar, & Prakash, 2007). It has been reported that phenolic acids, such as chlorogenic acid and caffeic acid could decrease the thermostability of

418 lipase indicated by a decreased T_d (Raghavendra, Kumar, & Prakash, 2007). Some
419 earlier studies also indicate that phenolic compounds decreased the thermal stability
420 of some proteins by binding with them (Muralidhara & Prakash, 1995; Prigent, et al.,
421 2003; Rawel et al., 2002). A decrease in thermal stability of an enzyme is usually
422 associated with protein conformational changes. Thus, the partial unfolding of
423 α-amylase by dietary polyphenols may be suggested by the T_d and ΔH values from
424 DSC thermograms. The thermal denaturation process for a protein usually takes place
425 in two steps. One is reversible, arising from the protein unfolding process. In this step,
426 there is a partial loss of activity for the protein due to the disruption of intramolecular
427 non-covalent interactions (Lumry & Eyring, 1954; Violet & Meunier, 1989). The
428 second step is irreversible, leading to the denaturation of the initially-unfolded
429 molecule (Cueto et al., 2003). Therefore, the reversible unfolding process of
430 α-amylase under external force is expected to promote the denaturation process of the
431 enzyme during a DSC experiment. As reported, four tea polyphenols, including TA,
432 ECG, EGCG and TF2 could potentially cause α-amylase structure to unfold, as
433 suggested by the red-shift of λ_{em}, while EC, EGC, TF1 and TF did not cause the
434 red-shift of λ_{em} of the enzyme. TA, ECG, EGCG and TF2 were each found to decrease
435 the thermal stability of α-amylase as indicated by the decreased T_d and ΔH, while EC,
436 EGC, TF1 and TF did not affect the enzyme thermal stability (Sun, Gidley, & Warren,
437 2017). It is therefore proposed that it is the partial unfolding of α-amylase by TA,
438 ECG, EGCG and TF2 (suggested by the red-shift of λ_{em} in the presence of the four
439 polyphenols) that promotes the complete unfolding (denaturation) of the enzyme in

the DSC experiment. Therefore, the combination of DSC and FQ to characterize the unfolding effects of dietary polyphenols on α-amylase structure is feasible (Fig.6).

3.5 Circular Dichroism (CD)

CD spectroscopy has been widely used to investigate the secondary structures of proteins, from which the contents of α-helix, β-sheet, β-turn and random coil in a protein can be estimated. CD has also been applied to study the effect of dietary polyphenols on the secondary structures of α-amylase, with the effect found to be dependent on polyphenol type. For example, sorghum procyanidins were found to retain α-helix but reduce β-sheet content of α-amylase, suggesting that the polyphenols made the enzyme structure looser (Cai, et al., 2015). Red rice polyphenols were able to reduce α-helix but increase β-sheet content of α-amylase (Liu, et al., 2017), while tea polyphenols could increase both α-helix and β-sheet contents of the enzyme (Liu, Ou, & Huang, 2016). Changes in α-helix and β-sheet contents have been reported to be related with the folding and/or unfolding extent of a protein (Cai, et al., 2015; Liu, et al., 2017), which may be compared with the folding/unfolding extent obtained from FQ and DSC. Therefore, in future studies the conformational changes of α-amylase after binding with dietary polyphenols may be characterized by a combination of FQ, DSC and CD methods (Fig.6).

3.5 Isothermal titration calorimetry (ITC)

Thermodynamic surveys of complexation may be performed using isothermal titration (micro)calorimetry, a technique that permits the determination of binding enthalpy and binding constant of the reaction between a macromolecule and a ligand

462 (Jelesarov & Bosshard, 1999). This technique has been successfully applied to
463 characterize the binding interactions between polyphenols and proteins (Frazier,
464 Papadopoulou, & Green, 2006; Karonen et al., 2015; Wu et al., 2013). Through ITC
465 analysis, the binding constant (K_{itc}) can be obtained, with higher K_{itc} indicating higher
466 binding affinity of a polyphenol to a protein. Usually, the binding of polyphenol with
467 protein is an exothermic process, as hydrogen bonding and hydrophobic interactions
468 occur (Poncet-Legrand et al., 2007). During the ITC experiment, a plot of heat flow
469 (μcal/min) against time (min) can be obtained. To calculate the binding energy
470 released by the binding interactions, ΔH_{itc}, a plot of observed enthalpy change per
471 mole of injectant (ΔH, J/mol) against molar ratio of polyphenol to enzyme is obtained
472 by peak-to-peak integration of a plot of heat flow against time. Here, higher ΔH_{itc}
473 corresponds to higher binding interactions (Karonen, et al., 2015). One feature that
474 should be noted in ITC experiments is that heat flow may be observed for the titration
475 of some polyphenols (especially polymers) into blank (buffer) solution. This is caused
476 by the heat of dilution, which affects the detected heat resulting from the binding of
477 polyphenol with enzyme. Therefore, the dilution heat should be subtracted from the
478 total observed heat obtained from the titration of polyphenol solution into enzyme
479 solution. Two binding models are usually used to fit ITC data, including a one-site
480 binding model in which specific binding is expected and a two-site binding model in
481 which both specific and non-specific binding occur (Karonen, et al., 2015). As the
482 reversible inhibition of an enzyme by polyphenol mainly involves the binding of
483 polyphenol with the active site of the enzyme (Kromann-Hansen et al., 2016), the

484 interactions between polyphenol and enzyme more likely involve specific binding.
485 Therefore, to simplify the analysis, a one-site binding model is often used to analyze
486 the interactions between them (Le Bourvellec & Renard, 2012; Wu, et al., 2013).

487 ITC has been applied to study the interactions between dietary polyphenols and
488 α-amylase (Gyémánt et al., 2018; Sun, Gidley, & Warren, 2017), through which the
489 binding constant (K_{itc}) is obtained. It was found that higher K_{itc} corresponded to higher
490 inhibitory activity of tea polyphenols against α-amylase (Sun, Gidley, & Warren,
491 2017), which indicates that the enzyme inhibition of polyphenols results from the
492 binding between them. This conclusion can also be obtained by comparison of
493 fluorescence quenching results with inhibition results for inhibition of α-amylase by
494 polyphenols (Dai et al., 2018; Miao, et al., 2015; Zhao et al., 2018), as FQ results also
495 reflect the binding of an inhibitor with the enzyme. Additionally, it should be noted
496 that similar to K_{FQ} and $1/K_{ic}$, K_{itc} characterizes the binding interactions as well. If so,
497 there should be a correlation between K_{itc} and K_{FQ} and between K_{itc} and $1/K_{ic}$. The
498 constants of binding interactions between tea polyphenols and α-amylase support this.
499 It was found that there is a positive linear relationship between K_{itc} and K_{FQ} (Fig.5C)
500 and between K_{itc} and $1/K_{ic}$ (Fig.5D) (Sun, Gidley, & Warren, 2017), indicating that it
501 is feasible to combine the inhibition kinetics, FQ and ITC to characterize the binding
502 interactions between dietary polyphenols and α-amylase (Fig.6).

503 **3.6 Molecular docking**

504 Molecular docking is a useful tool in structural molecular biology and
505 computer-assisted drug design. The aim of ligand-protein docking is to predict the

main binding modes of a ligand with a protein of known three-dimensional structure (Vriend, 1990). Molecular docking has been applied to investigate the binding interactions between polyphenols and enzymes (Schwartz et al., 2018). Through docking studies, hydrogen bonding between the hydroxyl groups of polyphenols and amino acid residues (Asp^{197}, Glu^{233} and Asp^{300}) at the active site of α-amylase was suggested, as well as hydrophobic interactions between the aromatic groups of polyphenols (benzene rings) and the enzyme (Trp^{59}) (Lo Piparo, et al., 2008; Miao, et al., 2015). The total binding energy, E_b (kJ/mol), of a polyphenol with α-amylase can be predicted from the docking study. It was found that higher E_b corresponds to higher binding interactions between a polyphenol and α-amylase and thus higher inhibitory activity (Hua et al., 2018; Miao, et al., 2015). Notably, similar to K_{itc}, K_{FQ} and $1/K_{ic}$, E_b can also indicate the binding of polyphenols with α-amylase; therefore, there may be positive relationships between E_b and the three constants. Although this has not yet been reported, a combination of molecular docking, inhibition kinetics, FQ and ITC, as well as establishment of correlations between the binding constants obtained from these methods is a reasonable approach to characterize the binding interactions between dietary polyphenols and α-amylase in future work (Fig.6).

4 Effect of polysaccharides on binding interactions between polyphenols and α-amylase

4.1 Potential influence

Many plant extracts have been studied for their inhibitory activity against α-amylase, and the main components with inhibiting effects have been shown to be phenolic compounds. However, aqueous extracts from plants are likely to be a complex mixture containing not only polyphenols but also other components that they may bind to, such as soluble polysaccharides (Chamorro, et al., 2012). Besides, even though pure phenolic or phenolic extracts containing high contents of polyphenols are consumed, polyphenols, in practice may interact with other food components (like proteins, polysaccharides, *etc.*) in the digestive tract (Le Bourvellec & Renard, 2012). Therefore, it is necessary to investigate if and how other food components affect the binding and inhibitory activity of dietary polyphenols against α-amylase.

Previous studies have suggested that some carbohydrates, like arabinogalactan, dextran, xanthan, *etc.* are able to interrupt the binding of polyphenols with proteins (de Freitas, Carvalho, & Mateus, 2003). Besides, some soluble polysaccharides (arabic gum, pectin, β-cyclodextrin and polygalacturonic acid) have also been reported to inhibit protein-polyphenol aggregation through two possible mechanisms (Soares, Mateus, & de Freitas, 2012; Soares et al., 2009). One is that a ternary protein-polyphenol-polysaccharide complex is formed that increases the solubility of protein-polyphenol aggregates. Another is that polysaccharide is able to interact with polyphenol, competing with the binding of polyphenol to protein (Soares, Mateus, & de Freitas, 2012; Soares, et al., 2009). As inhibitors, polyphenols are able to exhibit inhibitory activity against α-amylase through binding with the protein. The presence of polysaccharides in the reaction solution may be a factor influencing these binding

549 interactions because of the potential binding interactions between soluble
550 polysaccharides and dietary polyphenols.

551 **4.2 Characterization of polysaccharide effects on binding interactions**

552 To study the influence of polysaccharides on protein/polyphenol aggregates,
553 nephelometry and dynamic light scattering have been applied, because these two
554 methods can be used to determine the formation and dissolution of insoluble
555 aggregates (Soares, Mateus, & de Freitas, 2012; Soares, et al., 2009). However, the
556 competitive or uncompetitive inhibition of an enzyme is attributed to the reversible
557 (soluble) binding of an inhibitor with the enzyme or enzyme-substrate complex, rather
558 than irreversible (insoluble) aggregation. Therefore, IC_{50} value, inhibition kinetics and
559 FQ methods that have been applied to characterize binding and inhibitory activity of
560 polyphenols against α-amylase may also be used to characterize the influence of
561 polysaccharides on the binding interactions between polyphenols and α-amylase. It
562 was found that three soluble polysaccharides, including citrus pectin, wheat
563 arabinoxylan and oat β-glucan are able to increase IC_{50} and decrease $1/K_{ic}$ and K_{FQ}
564 values of tea polyphenols interacting with α-amylase (Sun, Warren, & Gidley, 2018).
565 This indicates that the soluble polysaccharides can decrease the inhibitory activity of
566 tea polyphenols against the enzyme through decreasing the binding interactions
567 between them.

568 In addition, the binding interactions between polyphenols and soluble
569 polysaccharides have been studied using ITC (Watrelot et al., 2013, 2014), from
570 which the binding constant (K_{itc}') between the two compounds can be obtained

571 (Renard, Watrelot, & Le Bourvellec, 2017). The presence of a K_{itc} for polyphenol
572 binding with α-amylase (Sun, Gidley, & Warren, 2017) as well as a K_{itc}' for
573 polyphenol binding with polysaccharides (Renard, Watrelot, & Le Bourvellec, 2017)
574 indicates that there may be a competitive mechanism between polysaccharides and
575 α-amylase in terms of binding with polyphenols. Furthermore, by comparing the two
576 K_{itc} values, it should be possible to determine how polysaccharides affect the
577 inhibition of α-amylase by polyphenols. Therefore, in the study of effects of soluble
578 polysaccharides on the binding interactions between dietary polyphenols and
579 α-amylase, ITC may be combined with IC_{50} value, inhibition kinetics and FQ methods
580 to provide a comprehensive understanding. Besides, the above methods may also be
581 applied to study the influence of other food macromolecules, such as proteins, lipids
582 and insoluble polysaccharides on binding of polyphenols with α-amylase. This
583 analysis is also suggested to be performed to understand the consequence of whole
584 food intake containing functional polyphenols rather than only pure polyphenols. To
585 get a full understanding of food-delivered polyphenols on amylase activity, it is
586 necessary and valuable to evaluate comprehensively the inhibitory activity of food
587 components complexed against α-amylase in addition to the polyphenol itself.

588 **4.2 From *in vitro* to *in vivo***

589 Most work investigating binding interactions between polyphenols and α-amylase
590 is *in vitro*. The bioavailability of polyphenols and the inhibition of α-amylase *in vivo*
591 should be further studied. There are studies reporting that dietary polyphenols can
592 retard the increase in postprandial blood sugar in mice (Cao et al., 2018; Murray et al.,

593 2018; Xu et al., 2018). However, there are several possible mechanisms including (i)
594 the inhibition of starch digestion enzymes *in vivo*, including α-amylase, α-glucosidase,
595 *etc.* (Figueiredo-González et al., 2018; Kato et al., 2017), and/or (ii) the inhibition of
596 expression of glucose transporters (*e.g.* SGLT1 and GLUT2) in enterocytes of the
597 small intestine which are responsible for transporting glucose from the intestine
598 lumen into the blood (Muller et al., 2018; Villa‐Rodriguez et al., 2017). Therefore,
599 to study if and how binding interactions affect starch digestion *in vivo*, the binding
600 constants obtained as described above are suggested to be compared and correlated
601 with postprandial blood sugar level by use of mice models fed with starch and dietary
602 polyphenols. Furthermore, the *in vivo* effects of polysaccharides on the binding
603 interactions between polyphenols and α-amylase should also be investigated, which
604 should give a better understanding of how functional food components (polyphenols
605 and polysaccharides) affect starch digestion and postprandial blood sugar level *in*
606 *vivo*.
607

608 **5 Conclusion**

609 The inhibitory activity of a polyphenol against α-amylase is determined by its
610 molecular structure. Hydroxyl groups, galloyl substituents and conjugated systems are
611 some of the important characteristics of polyphenols for effective inhibition of the
612 enzyme. IC_{50} value, inhibition kinetics, FQ and ITC can be combined to characterize
613 the binding and inhibitory activity of dietary polyphenols against α-amylase because
614 the constants obtained from these techniques are correlated due to common origins.

615 FQ, DSC and CD are suggested to be combined to indicate conformational changes of
616 α-amylase after binding with polyphenols. Soluble polysaccharides are indicated to
617 reduce the binding interactions between polyphenols and α-amylase. IC_{50} value,
618 inhibition kinetics, FQ and ITC may be applied to study the effects of other food
619 components on binding of polyphenols with the enzyme. In addition, the influences of
620 binding interactions between polyphenols and α-amylase on starch digestion *in vivo*
621 needs to be further studied.

622

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Figure Captions

883 **Figure 1** Three-dimensional structure, determined by crystallography, of porcine
884 pancreatic α-amylase (Qian, Haser, & Payan, 1993) (**A**), and the active site of
885 α-amylase (**B**) (MacGregor, Janecek and Svensson, 2001), in which the calcium,
886 chloride ions and essential amino acids (Glu^{233}, Asp^{197}, Asp^{300}) are indicated. The
887 yellow structure is a short chain of five sugar units connected through α-1,4-link
888 (coloured pink).

889 **Figure 2** Molecular structures of flavonoids (Lo Piparo et al., 2008).

890 **Figure 3** Molecular structures of phenolic acids (Narita & Inouye, 2011).

891 **Figure 4** Molecular structures of tea polyphenols, including catechins and theaflavins.

892 **Figure 5** Correlations between the constants characterizing binding and inhibitory
893 activity of tea polyphenols against α-amylase (Sun et al., 2016; Sun, Gidley, &
894 Warren, 2017).

895 **Figure 6** Scheme for study of binding interactions between dietary polyphenols and
896 α-amylase.

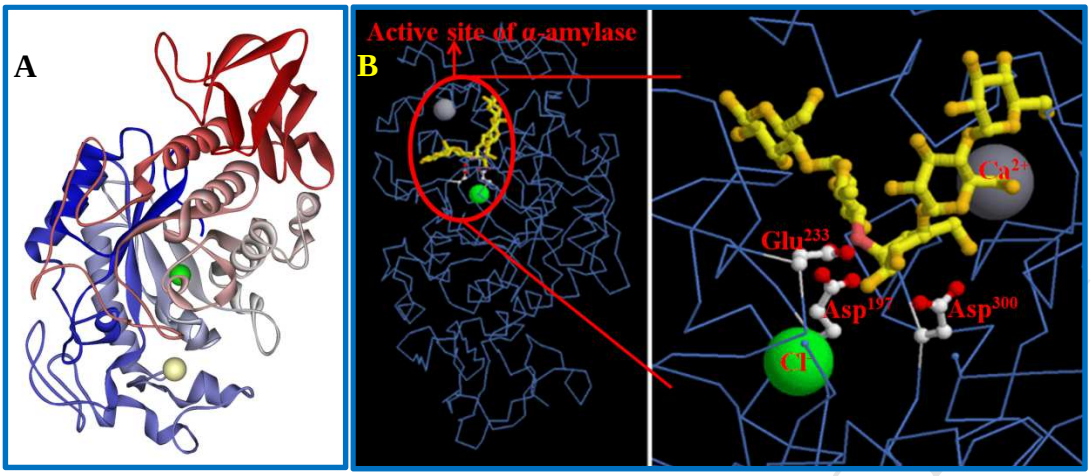
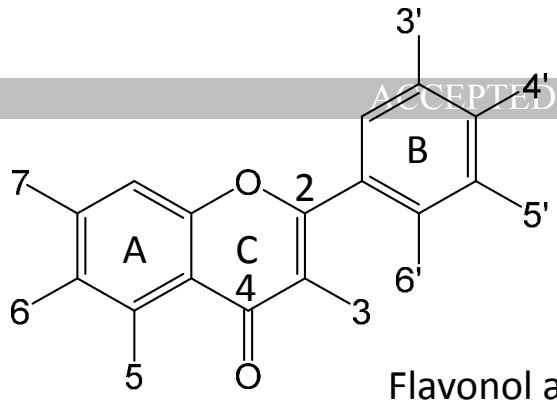
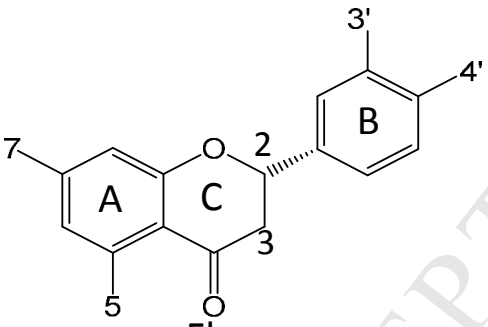


Figure 1 Three-dimensional structure, determined by crystallography, of porcine pancreatic α-amylase (Qian, Haser, & Payan, 1993) (**A**), and the active site of α-amylase (**B**) (MacGregor, Janecek and Svensson, 2001), in which the calcium, chloride ions and essential amino acids (Glu^{233}, Asp^{197}, Asp^{300}) are indicated. The yellow structure is a short chain of five sugar units connected through α-1,4-link (coloured pink).

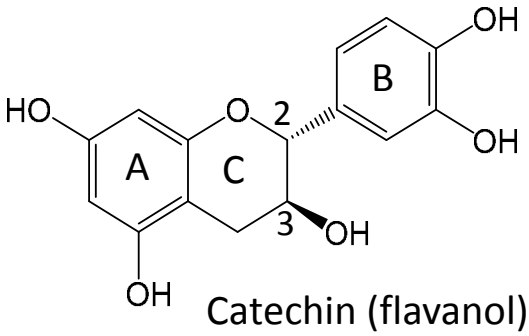


Flavonol and Flavone

3	5	6	7	3'	4'	5'	6'	
OH	H	H	OH	H	OH	OH	H	fisetin
OH	OH	H	OH	H	OH	H	H	kaempferol
OH	OH	H	OH	OH	OH	OH	H	myricetin
OH	OH	OH	OH	OH	OH	H	H	quercetagetin
OH	OH	H	OH	OH	OH	H	H	quercetin
OH	OH	H	OH	H	OH	OCH3	H	isorhamnetin
OH	OH	H	OCH3	OH	OH	H	H	rhamnetin
H	OH	H	OH	H	OH	H	H	apigenin
H	OH	H	OH	H	OCH3	H	H	acacetin
H	OH	H	OH	OH	OCH3	H	H	diosmetin
H	OH	OCH3	OH	H	OH	OH	H	eupafolin
H	OH	H	OCH3	H	OCH3	H	H	genkwanin
H	OH	H	OH	OH	OH	H	H	luteolin
H	OH	OH	OH	H	OH	H	H	scutellaretin

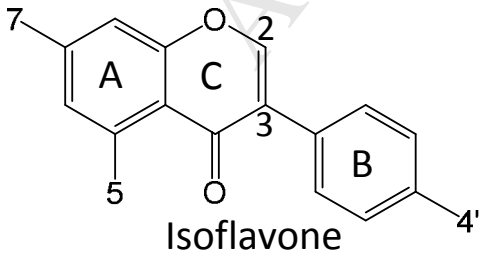


Flavanone

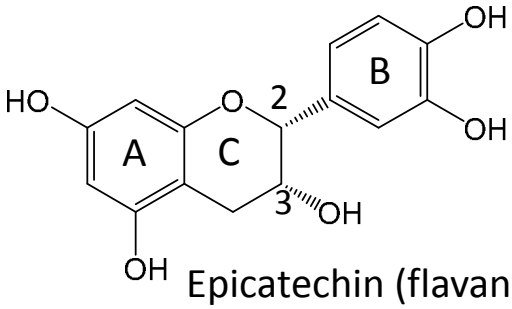


Catechin (flavanol)

5	7	3'	4'	
OH	OH	OH	OCH3	heperetin
OH	OH	H	OH	naringenin

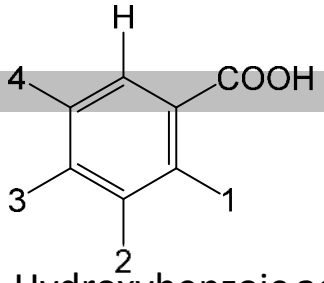


Isoflavone

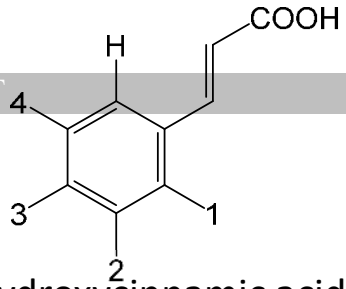


Epicatechin (flavanol)

5	7	4'	
OH	OH	OH	genistein
H	OH	OH	daidzein



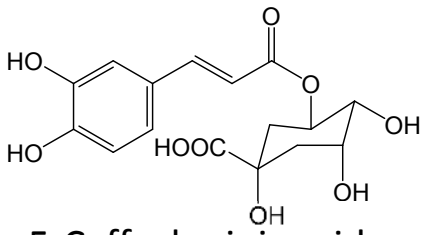
Hydroxybenzoic acid



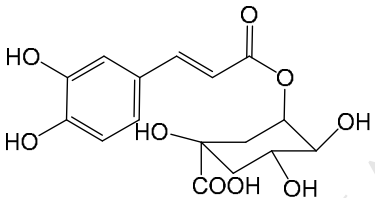
Hydroxycinnamic acid

1	2	3	4	
H	H	H	H	benzoic acid
H	H	OH	H	*p*-hydroxybenzoic acid
H	OCH_3	OH	H	vanillic acid
H	OH	OH	OH	gallic acid
H	OH	OH	H	protocatechuic acid
H	OCH_3	OH	OCH_3	syringic acid
OH	H	H	OH	gentistic acid
H	OCH_3	OCH_3	H	veratric acid
OH	H	H	H	salicylic acid

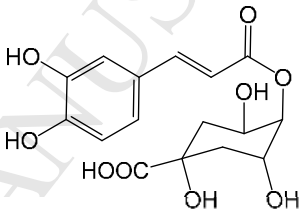
1	2	3	4	
H	H	H	H	cinnamic acid
OH	H	H	H	*o*-coumaric acid
H	OH	H	H	*m*-coumaric acid
H	H	OH	H	*p*-coumaric acid
H	OCH_3	OH	H	ferulic acid
H	OCH_3	OH	OCH_3	sinapic acid
H	OH	OH	H	caffeic acid



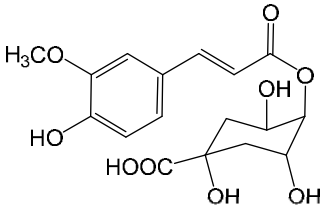
5-Caffeylquinic acid



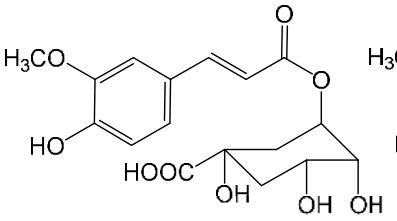
3-Caffeylquinic acid



4-Caffeylquinic acid



4-Feruloylquinic acid



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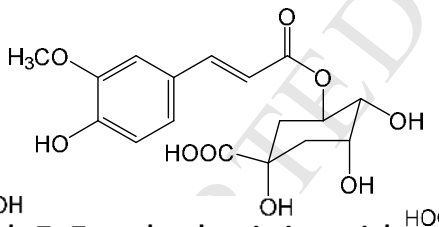
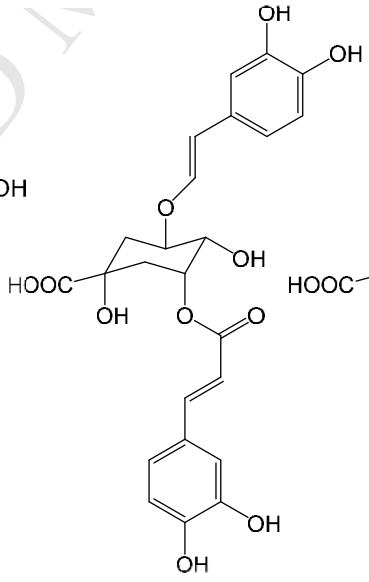
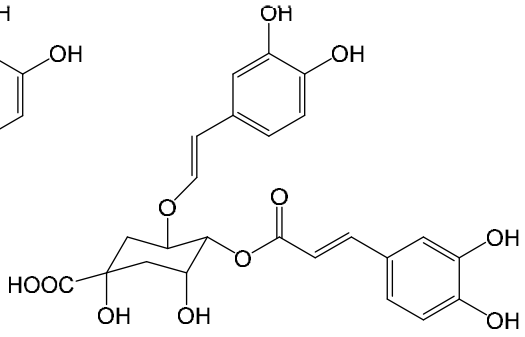


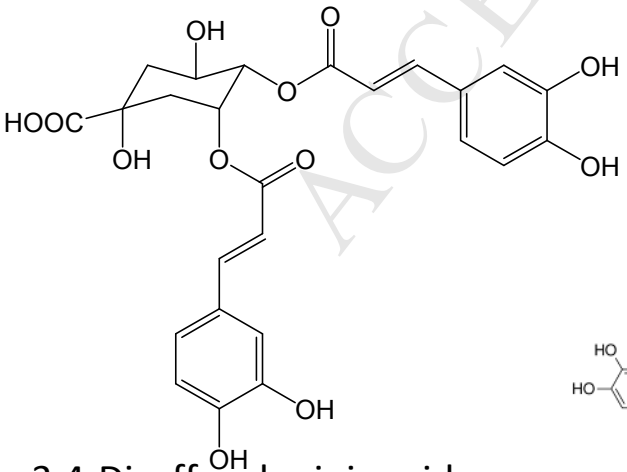
5-Feruloylquinic acid



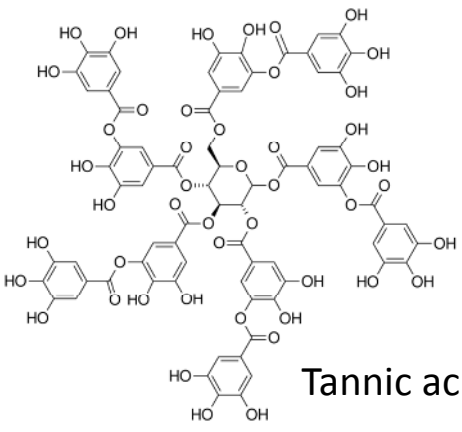
3,5-Dicaffeoylquinic acid



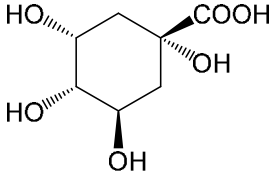
4,5-Dicaffeoylquinic acid



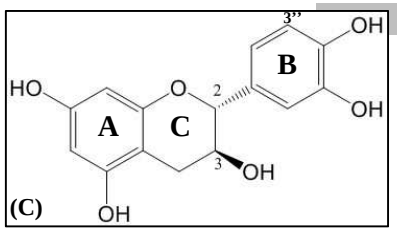
3,4-Dicaffeoylquinic acid

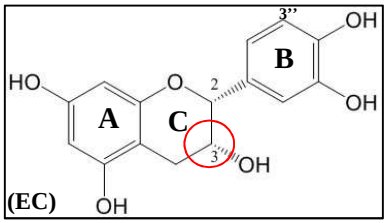


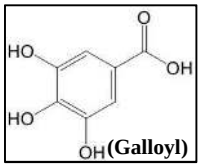
Tannic acid

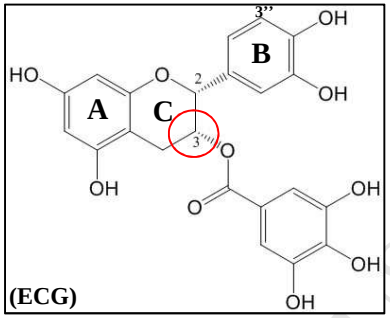


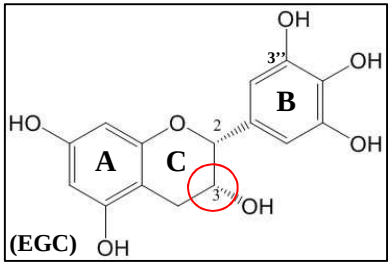
quinic acid

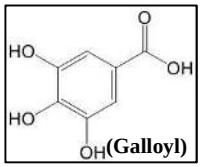


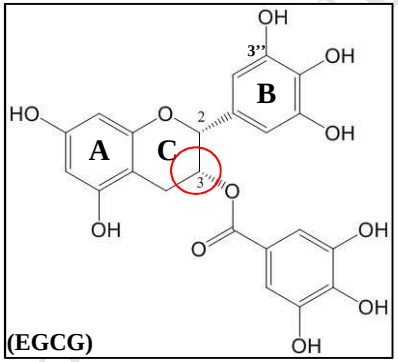


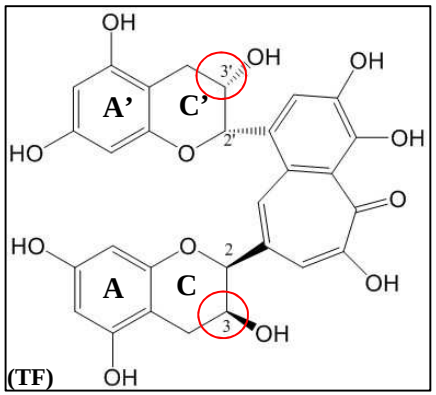


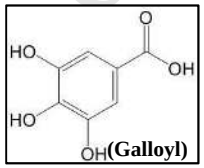


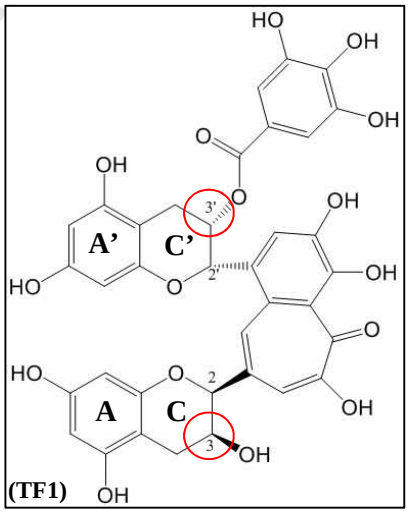


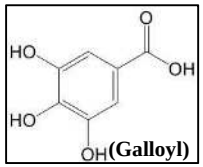


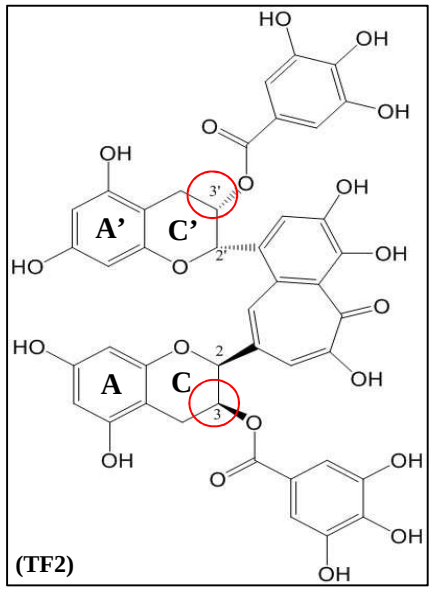












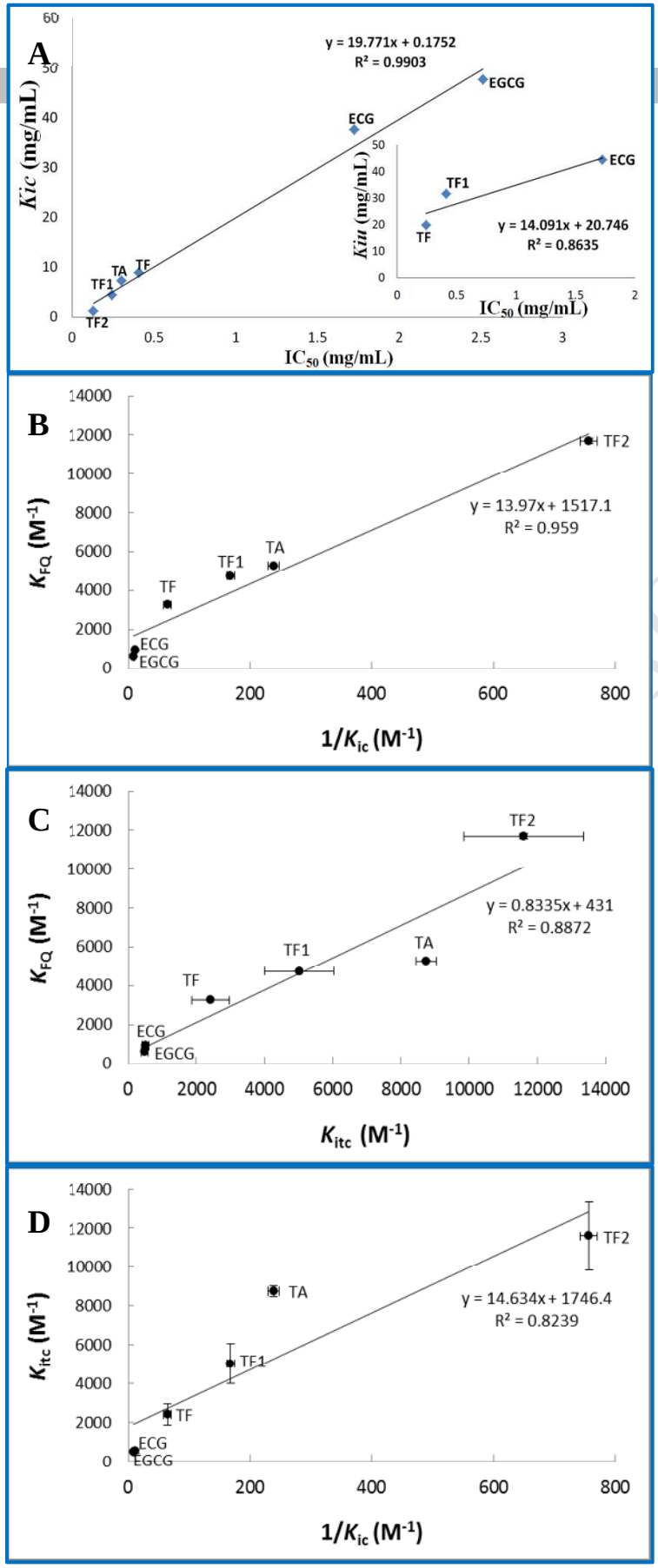


Figure 5 Correlations between the constants characterizing binding and inhibitory activity of tea polyphenols against α-amylase (Sun et al., 2016; Sun, Gidley, & Warren, 2017).

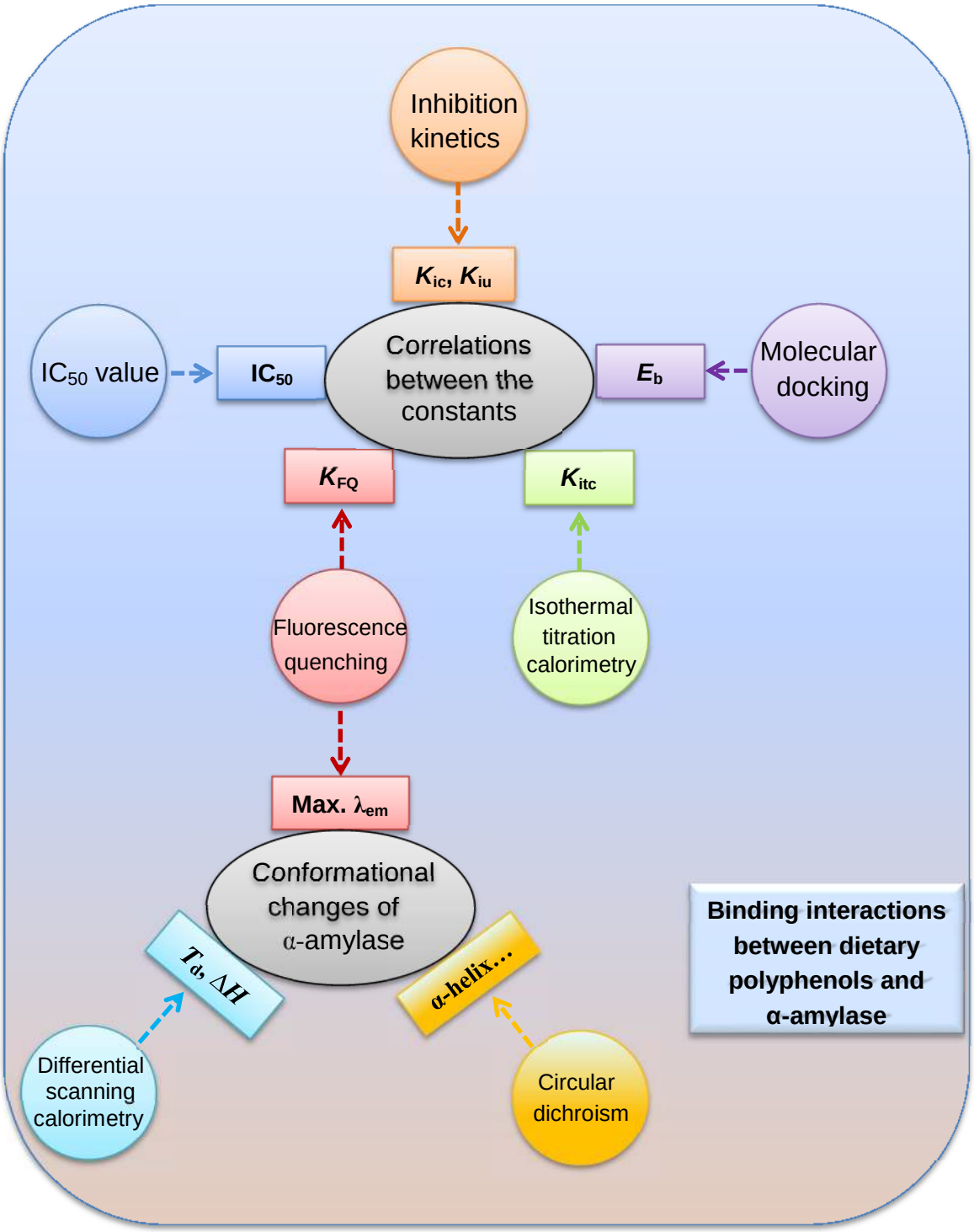


Figure 6 Scheme for study of binding interactions between dietary polyphenols and α-amylase.

Highlights

Inhibition of α-amylase by polyphenols results from molecular binding interactions.

The galloyl moiety in polyphenols plays an important role in binding.

Calorimetry, fluorescence quenching, molecular modelling and kinetic analysis combined.

Soluble polysaccharides reduce amylase binding and inhibition action of polyphenols.

Most insights from *in vitro* studies, more *in vivo* studies needed.