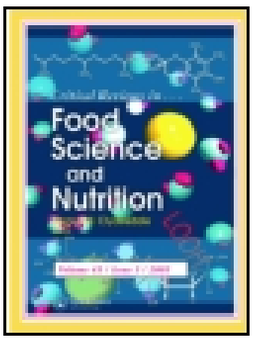

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Interactions between cell wall polysaccharides and polyphenols

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Abstract

In plant-based food systems such as fruits, vegetables, and cereals, cell wall polysaccharides and polyphenols co-exist and commonly interact during processing and digestion. The non-covalent interactions between cell wall polysaccharides and polyphenols may greatly influence the physicochemical and nutritional properties of foods. The affinity of cell wall polysaccharides with polyphenols depends on both endogenous and exogenous factors. The endogenous factors include the structures, compositions, and concentrations of both polysaccharides and polyphenols, and the exogenous factors are the environmental conditions such as pH, temperature, ionic strength, and the presence of other components (e.g., protein). Diverse methods used to directly characterise the interactions include NMR spectroscopy, size-exclusion chromatography, confocal microscopy, isothermal titration calorimetry, molecular dynamics simulation, and so on. The un-bound polyphenols are quantified by liquid chromatography or spectrophotometry after dialysis or centrifugation. The adsorption of polyphenols by polysaccharides is mostly driven by hydrophobic interactions and hydrogen bonding, and can be

described by various isothermal models such as Langmuir and Freundlich equations. Quality attributes of various food and beverage products (e.g., wine) can be significantly affected by polysaccharide-polyphenol interactions. Nutritionally, the interactions play an important role in the digestive tract of humans for the metabolism of both polyphenols and polysaccharides.

Keywords

polysaccharide, cell wall, polyphenol, non-covalent interaction, physicochemical property, nutrition

ACCEPTED MANUSCRIPT

1. Introduction

The high intake of plant-based whole foods has been associated with a low occurrence of various degenerative and chronic diseases such as obesity, type 2 diabetes, coronary heart disease, breast cancer, and prostate cancer (Campbell & Campbell, 2005; Campbell & Jacobson, 2014; Wang & Zhu, 2016). Two major functional nutrients responsible for the disease prevention are polyphenols and cell wall non-starch polysaccharides as dietary fibres (Velderrain-Rodríguez et al., 2014; Anderson et al., 2009; Campbell & Campbell, 2005). Polyphenols as natural antioxidants have been proved to have diverse health benefits such as anti-inflammation, anti-oxidation, signal transduction modulation, anti-proliferation, and anti-microbial activity (Velderrain-Rodríguez et al., 2014; Selma et al., 2009). Polyphenols also play important roles in the sensory quality, such as the formation of astringency, bitter taste, colour, turbidity, of various food products (e.g., wine and cider) (Le Bourvellec & Renard, 2012). Polyphenols as secondary plant metabolites occur in a range of medicinal and dietary plants, and the major categories include phenolic acids, flavonoids, lignans, coumarins, stilbenes, and quinones (Cai, Sun, Xing, Luo, & Corke, 2006). Various types of polyphenols with different structures have different physicochemical properties and nutritional effects (Cai et al., 2006; Velderrain-Rodríguez et al., 2014). Chemical structures of some polyphenols discussed in this review are presented in Fig. 1.

Cell wall polysaccharides are non-starch polysaccharides, making up most of the dietary fibres in vegetables, fruits, and whole grain cereals/pseudocereals (Harris & Smith, 2006). The major types of cell wall polysaccharides include cellulose (Fig. 1d), pectins, (1→3), (1→4)-β-glucans, and arabinoxylans (Harris & Smith, 2006). These polysaccharides play major roles in food

texture (e.g., as gelling agents) as well as in human nutrition as dietary fibres (Harris & Smith, 2006; Anderson et al., 2009). Increasing intake of cell wall polysaccharides as dietary fiber has many health benefits. These include lowering serum cholesterol and blood pressure levels, improving glycemia and insulin sensitivity in both diabetic and normal individuals, enhancing immunological function, and improving gastrointestinal disorders of duodenal ulcer, constipation, diverticulitis, gastroesophageal reflux disease, and haemorrhoids (Anderson et al., 2009). Therefore, a high intake of both cell wall polysaccharides and polyphenols is expected to positively impact human health with possible synergistic effects.

During food and beverage processing, the plant cells de-compartmentalize and dis-integrate under mechanical shearing forces. Various nutrients are released from their respective cell compartments and come into contact with each other (Zhu, 2015). The interactions among food components may be key factors determining the physicochemical and nutritional properties of food products (Le Bourvellec & Renard, 2012; Bordenave et al., 2014; Zhu, 2015; Jakobek, 2015). For example, the influence of grape cell wall and condensed tannin interactions on tannin extractability into wine is significant (Hanlin et al., 2010). Diet rich in digestible carbohydrates reduced the absorption of procyanidin dimers and trimers *in vivo* (rat models) (Serra et al., 2010). In contrast, in a human study, meals rich in carbohydrates (bread and sugar) facilitated the adsorption of cocoa flavanols (catechin and epicatechin) (Schramm et al., 2003). In one case, cell wall polysaccharides and polyphenols of plant foods interact with each other during processing (Hanlin et al., 2010; Le Bourvellec et al., 2014; Troszyńska et al., 2010). An early study showed that polyphenols interacted with column supporting carbohydrates of gel-permeation chromatography (e.g., Sephadex G-25 and Sephadex LH-20) (McManus et al., 1985). The non-

covalent interactions between cell wall polysaccharides and polyphenols may significantly impact the processing, storage, textural and eating quality of food and beverage (Le Bourvellec et al., 2014, 2007; Troszyńska et al., 2010). The association of cell wall polysaccharides and polyphenols in human digestive track may greatly impact the metabolism of both components (Saura-Calixto, 2011; Schramm et al., 2003; Serra et al., 2010). Polyphenols can be associated with dietary fiber in plant foods, which has a different bioavailability than the free ones (Mercado-Mercado et al., 2015). The body of knowledge on physicochemical and nutritional aspects of cell wall polysaccharides and polyphenols is individually tremendous. Understanding the nature and consequence of their noncovalent interactions should greatly contribute to the maintenance and improvement of the quality of various plant-based food systems. The cell wall polysaccharide-polyphenol interactions may also be utilised for functional food development (e.g., for controlled releasing applications).

This review summarises the quantification methods and various factors affecting the non-covalent interactions between cell wall polysaccharides and polyphenols from different plant food systems. The nature of the interactions is discussed. The impacts of the interactions on the physicochemical properties of food systems and the metabolism of nutrients in human digestive track are also reviewed. Potential research topics to better understand and utilise the interactions in food systems are suggested.

2. Analytical methods

Various direct and indirect analytical methods have been used to quantitatively and qualitatively measure the cell wall polysaccharide-polyphenol non-covalent interactions (Table 1). Each method tends to reflect different physicochemical aspects of the interactions.

2.1. Direct methods

2.1.1. Confocal laser scanning microscopy

Confocal laser scanning microscopy has been used to visualise the binding of anthocyanins with cell walls (Fig. 2a) (Padayachee et al., 2013; 2012a). Anthocyanins of purple carrot juice concentrate appeared to stack on the surface of bacterial cellulose fibrils (Padayachee et al., 2012a). The binding appeared to be non-uniformly distributed and highly localised on the surface of cellulose and cell walls (Padayachee et al., 2012a; Padayachee et al., 2013). During *in vitro* gastric and small intestinal digestion, the integrity of the cell wall-anthocyanin/phenolic acid complexes was disrupted, due to the partial removal of the polyphenols (Padayachee et al., 2013). Therefore, these polyphenols, together with the cell walls, would come into the large intestine for further metabolism.

2.1.2. Saturation transfer difference (STD) NMR spectroscopy

Saturation transfer difference (STD) NMR (nuclear magnetic resonance) spectroscopy has been used to probe the interactions between anthocyanins (cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside) and pectins (Fernandes et al., 2014). ^{1}H NMR spectrum of the anthocyanins at pH 4 was compared to those of the solutions with the pectin addition (Fig. 2b). When ligand becomes

in close contact with its receptor, signals of the ligand in the STD NMR spectrum receive magnetization transfer and stay in the difference spectrum. When the delphinidin-3-*O*-glucoside-to-pectin ratio was at 200:1, the saturation on pectin was transferred to the anthocyanin proton after irradiation (Fernandes et al., 2014). Only at a much higher ratio of cyanidin-3-*O*-glucoside-to-pectin (600:1), a small resonance peak was noted. This indicates weaker interactions between cyanidin-3-*O*-glucoside and pectin than delphinidin-3-*O*-glucoside and pectin (Fernandes et al., 2014). This may be due to that cyanidin-3-*O*-glucoside has 3 hydroxyl groups while delphinidin-3-*O*-glucoside has 2 hydroxyl groups. By quantifying the relationships between the increasing peak intensity of some anthocyanin proton signal in STD NMR spectrum and the increasing concentration of anthocyanins, the dissociation constants (K_d) were calculated (Fernandes et al., 2014). This provides a quantitative basis for the anthocyanin-pectin interactions. Acidic pH (flavylium cation) gave a stronger affinity with a much smaller dissociation constant ($K_d \approx 2$ μM).

2.1.3. Molecular dynamics simulation

Computer-based molecular dynamics simulation (MDS) was used to study the non-covalent interactions between anthocyanins and pectins (Fernandes et al., 2014). Three MDS of 45 ns were employed. MDS supports the experimental results of STD NMR studies as described above and provides additional information on the interactions. Solvent-accessible surface area of pectin was obtained and the results indicated the extension of anthocyanins during binding. Representative geometries of the anthocyanin-pectin bindings are outlined (Fig. 2c).

2.1.4. UV−vis spectrophotometry

UV−vis spectrophotometry can be used to monitor the aggregation formation due to the complexation between polyphenols and soluble cell wall polysaccharides (e.g., pectins) (Watrelot et al., 2013 and 2014). Increasing absorbance at 650 nm indicated the formation of aggregates in solution with varying concentrations of polyphenols and cell wall polysaccharides as well as experimental conditions. Results of UV−vis spectrophotometry-based methods in general agreed with the results of isothermal titration calorimetry in studying the interactions between pectins and procyanidins with exceptions (Watrelot et al., 2013 and 2014). The exceptions are either because the enthalpy change of interactions was beyond the detection limit of the calorimetry or there was a lack of aggregation formation.

2.1.5. Dynamic light scattering (DLS) technique

DLS has been used to monitor the colloidal behaviours of wine tannins in the presence of wine polysaccharides (Riou et al., 2002). Wine tannins alone tend to aggregate in a model solution during storage, and the particle size evolved from a few hundred up to 1000 nm in 10 h. The presence of different pectins had no effect on the initial aggregate formation, but reduced the rate of aggregation to various extents, depending on the pectin type (Riou et al., 2002).

2.1.6. Isothermal titration calorimetry (ITC)

ITC has been used to probe the thermodynamics of the noncovalent interactions between polyphenols and macromolecules (e.g., proteins and polysaccharides) (Bourvellec & Renard, 2012; Whitesides & Krishnamurthy, 2005). Interactions between apple/citrus cell wall polysaccharides and apple procyanidins have been studied by ITC (Le Bourvellec et al., 2012; Watrelot et al., 2013 and 2014) (Fig. 2d). Positive and negative heat flows represent endothermic

and exothermic changes, respectively. Addition of procyanidins into pectin solution gave the exothermic peaks, and increasing procyanidin concentration decreased the peak height. This is due to the gradual saturation of the binding sites of the polysaccharides. Thermodynamic parameters, including stoichiometry (n), association constant (K_a), and enthalpy change (ΔH) of binding, can be obtained from the titration curve (Le Bourvellec et al., 2012). For example, n of the interactions between procyanidins (DP 9) and apple pectin was 0.0236 and suggested that 1 mol equivalent of flavan-3-ol monomer can bind to 42 mol equivalents of galacturonic acid. K_a was $1.4 \times 10^4\ M^{-1}$ ($> 1 \times 10^4\ M^{-1}$), suggesting strong interactions (Le Bourvellec et al., 2012). The relationships between Gibbs free energy (ΔG), enthalpy change (ΔH), and entropy (ΔS) of binding can be defined by the Van't Hoff equation as:

$\Delta G = -RT\ln K_a = \Delta H - T\Delta S$, where R is the ideal gas constant and T is the absolute temperature.

By comparing the ΔH (enthalpy contribution) and $-T\Delta S$ (entropy contribution), the nature of the interactions (entropy-driven or enthalpy-driven) may be derived. In a ligand-design approach, entropy-driven interactions are of hydrophobic interactions and enthalpy-driven ones are of hydrogen bonds (Whitesides & Krishnamurthy, 2005). Most of the interactions between pectins and procyanidins were mainly hydrophobic, while some of the interactions were moderated through hydrogen bonding (Hanlin et al., 2010; Le Bourvellec et al., 2012; Watrelot et al., 2013 and 2014). The presence of aromatic groups in grape procyanidins favours the hydrophobic interactions (Watrelot et al., 2013). During the pectin-procyanidin complex formation, water molecules got expelled from the interface due to hydrophobic contacts (Le Bourvellec et al., 2012).

2.2. Indirect methods

The unbound polyphenols after their interactions with cell wall polysaccharides are usually recovered through dialysis assay or centrifugation before quantification by HPLC (high performance liquid chromatography) and/or spectrophotometry (Padayachee et al., 2012a, 2012b, and 2013; Phan et al., 2015 and 2016; Lin et al., 2016; Le Bourvellec et al., 2004, 2005a; 2005b; Renard et al., 2001; Le Bourvellec et al., 2007; Le Bourvellec et al., 2012; Bindon et al., 2010a and 2010b; Bautista-Ortín et al., 2014 and 2016;). Fig. 2e presents the molecular size distribution of polyphenols (tannins and anthocyanins) before and after the addition of cell wall (grape) polysaccharides in solution as analysed by HPLC. The changes in the molecular size reflect their binding behaviours with cell walls. The unbound polyphenols may also be quantitatively reflected by using *in vitro* antioxidant assays to measure the changes in the antioxidant activity (Sun-Waterhouse et al., 2008a and 2008b). The extractability of cell wall polysaccharides (e.g., pectins) also indirectly reflects their interactions with the polyphenols as the bound polysaccharides became less extractable (Le Bourvellec et al., 2009).

3. Factors affecting non-covalent interactions between polyphenols and cell wall polysaccharides

3.1. Polyphenol structure

Various studies reported the impact of polyphenol structure and composition on the affinity between polyphenols and cell wall polysaccharides (Tang et al., 2003; Padayachee et al., 2012a; 2012b; 2013; Phan et al., 2015 and 2016; Fernandes et al., 2014; Renard et al., 2001; Le Bourvellec et al., 2004, 2005a, 2005b, 2007, 2009; Bindon & Kennedy, 2011; Gonçalves et al.,

2012; Bautista-Ortín et al., 2014; Bautista-Ortín et al., 2016; Simonsen et al., 2009; Gao et al., 2012a; Wang et al., 2013; Quirós-Sauceda et al., 2014). Structurally-diverse polyphenols (anthocyanins, tannins, flavonoids, and phenolic acids) from various sources (fruits, vegetables, and tea) have been employed in different systems (Table 2).

Various phenolic acids interacted with cell wall polysaccharides to different extents (Padayachee et al., 2012b; Wang et al., 2013). Among the phenolic acids of purple carrot juice concentrate (ferulic acid, chlorogenic acid, and caffeic acid), caffeic acid had the maximum adsorption by pectin-cellulose composite and ferulic acid had the minimum adsorption (Padayachee et al., 2012b). Among 13 phenolic acids (including both hydroxybenzoic and hydroxycinnamic acids), methylation and methoxylation were negatively correlated with their binding to soluble oat β-glucans (Wang et al., 2013). Among the 3 different types of coumaric acids (*o*-, *m*-, *p*-coumaric acids), *o*-coumaric acid was more bound to soluble oat β-glucans than the *m*- and *p*-coumaric acids which had similar binding (Wang et al., 2013). Various types of flavonoids (flavones, flavonols, flavanones, and isoflavones) interacted with soluble oat β-glucans to different degrees (Wang et al., 2013). The binding capacities for the flavonoid isomers increased by the order of flavonol > flavone > flavanone > isoflavone. Three or less number of OH groups on flavonoids facilitated the affinity while four and more OH groups decreased it (Wang et al., 2013). Glycosylation increased or decreased the binding of flavonoids onto the oat β-glucans, depending on the type of flavonoids (Wang et al., 2013). Both the non-acylated and acylated anthocyanins of purple carrot juice concentrate showed a similar binding pattern to cellulose and cellulose--pectin composite (Padayachee et al., 2012a). Anthocyanins of grape wine bound to the wine polymeric material (Gonçalves et al., 2012). Anthocyanins with coumaroyl and acetyl

moieties showed a stronger binding with wine polymeric material as compared with the non-acylated anthocyanins (Gonçalves et al., 2012). This suggested that the interactions between wine anthocyanins and polymeric material were most hydrophobic (Gonçalves et al., 2012). The difference in the results of the two anthocyanin studies (Padayachee et al., 2012a; Gonçalves et al., 2012) suggested the important role of the cell wall polysaccharides in the interactions. Two anthocyanins (cyanidin-3-*O*-glucoside and delphinidin- 3-*O*-glucoside) interacted with citrus pectins (Fernandes et al., 2014). Delphinidin-3-*O*-glucoside (with 3 OH groups) had a much higher affinity with pectin than cyanidin-3-*O*-glucoside (with 2 OH groups) (Fernandes et al., 2014), suggesting the role of hydroxyl groups involving in the interactions (possibly through hydrogen bonds). Procyanidins from various sources (grape, apple, and pear) showed affinity with cell wall polysaccharides to various degrees (Table 2). The binding capacity of apple cell walls with procyanidins greatly depended on the structure and composition of this polyphenol (Le Bourvellec et al., 2005a; 2005b). The molecular size, degree of galloylation, and portion of (+)-catechin of procyanidins were positively correlated with the binding capacity of cell walls (Renard et al., 2001; Le Bourvellec et al., 2005a; Bindon & Kennedy, 2011; Bautista-Ortín et al., 2014; Watrelot et al., 2013). Compared with the degree of galloylation, the molecular size appeared to be more important in determining the interactions (Bautista-Ortín et al., 2014). Procyanidins have multiple sites with simultaneous binding to various regions of the pectins (Baxter et al. 1997). Therefore, larger procyanidins have more binding sites for the interactions. Oxidation of procyanidins increased their binding to the cell walls (Bautista-Ortín et al., 2014; Le Bourvellec et al, 2009). Tea polyphenols, including (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, (−)-gallocatechin gallate, and (−)-catechin,

interacted with the soluble oat β-glucans (Gao et al., 2012a; Wang et al., 2013). (−)-Epigallocatechin gallate (EGCG) had the highest binding capacity with the β-glucans (Gao et al., 2012a). The binding with oat β-glucans was negatively correlated with the degree of gallic acid esterification and positively correlated with that of catechin galloylation (Wang et al., 2013). Another study on the interactions between cellulose and 24 polyphenols (gallotannins and ellagitannins) showed that the molecular weight, hydrophobicity, and number of galloyl groups were positively correlated with the strength of binding (Tang et al., 2003). This suggests that hydrophobic interaction plays a role in the association. Gallotannins had a much lower affinity with cellulose than ellagitannins (Tang et al., 2003). Through monitoring the interactions between β-glucans and 21 vanillin-inspired phenolic derivatives, Simonsen et al. (2009) found that the glucosides of phenolics had little binding (Fig. 1a). Phenolics with an OH group in para-position to a CHO group had the strongest binding, and additional functional groups reduced the binding. The binding capacity among different types of polyphenols was explored in comparative studies under the same experimental conditions (Phan et al., 2015; Wang et al., 2013) (Supplementary Fig. 1). Ferulic acid, gallic acid, chlorogenic acid, (+/−)-catechin, and cyanidin-3-glucoside had similar binding patterns with cellulose, and the native charges had little impact on the interactions (Phan et al., 2015). Compared with hydroxycinnamic acids and epicatechin, procyanidins had strong binding with the apple cell walls (Renard et al., 2001). Addition of anthocyanins into the tannin-cell wall system facilitated the extraction of tannins from the grape seeds and skins, indicating their competitive binding with the cell walls (Bautista-Ortín et al., 2016). A systematic study on 36 polyphenols from different categories with varying

structures showed that there is no clear cut on their binding with cell wall polysaccharides (Wang et al., 2013).

It should be noted that different results from different studies may not be compared, and that the impact of polyphenol structure and composition on the polyphenol-cell wall polysaccharide interactions also depends on the type of cell wall polysaccharides as well as the experimental conditions as described below.

3.2. Cell wall polysaccharide structure

Various studies reported the impact of structure and composition of cell wall polysaccharides on the interactions with polyphenols (Padayachee et al., 2012a and 2012b; Lin et al., 2016; Le Bourvellec et al., 2005a, 2005b, 2012; Sun-Waterhouse et al., 2008a and 2008b; Simonsen et al., 2009; Ruiz-Garcia et al., 2014; Quirós-Sauceda et al., 2014; Le Bourvellec et al., 2004 and 2007; Wu et al., 2011; Simonsen et al., 2009). Increasing the proportion of pectin in cellulose-pectin composite increased the adsorption of anthocyanins of purple carrot juice concentrate (Padayachee et al., 2012a). This suggests that the pectin has a higher binding capacity with the anthocyanins than the cellulose. Grape skin cell walls were fractionated by sequential washing with CDTA solution and NaOH solutions (0.05 M, 1 M, and 4 M), and these fractions showed different affinity with grape skin procyanidins (Ruiz-Garcia et al., 2014). The amount of proanthocyanidins bound to cell walls was 54%. Removal of galacturonan-rich fractions by CDTA solution greatly reduced the binding capacity of cell walls, while the hemicellulose fractions retained a good affinity with the procyanidins. The remaining lignocellulosic residues (mostly cellulose and lignin) after the removal of hemicellulose had a greatly reduced

polyphenol affinity (Ruiz-Garcia et al., 2014). Due to the complex nature of the fractions, single type cell wall polysaccharide should be employed as model systems. In a comparative study on the interactions of pectin, xyloglucan, starch, and cellulose with apple procyanidins, the apparent affinity constants for the individual polysaccharides followed the order of pectin, xyloglucan, starch, and cellulose (Le Bourvellec et al., 2005b). Conformation of cellulose and xyloglucans favours the stacking and cooperativity with higher apparent saturation levels. Pectin had a lower level of apparent saturation due to the steric hindrance (Le Bourvellec et al., 2005b). It was suggested that pectin has the ability to form hydrophobic pockets through gelling to capture the procyanidins (Le Bourvellec et al., 2005b). In contrast, compared with cellulose-pectin composite, cellulose had a higher initial adsorption (within 1h) of various phenolic acids (ferulic acid, chlorogenic acid, and caffeic acid). Cellulose and cellulose-pectin composite had a similar degree of affinity with these phenolic acids after a few days (Padayachee et al., 2012b). The discrepancy between these two studies may be attributed to the types of polyphenol, composition and structure of the cell wall polysaccharides, as well as the experimental conditions. Two β-glucans from oat and barley, with rather different composition, structure, and rheology, showed rather similar binding behaviours with 21 vanillin-derived phenolic compounds (Fig. 1a) (Simonsen et al., 2009). The analytical method was by dialysis assay and may not be able to reflect any possible impact of polysaccharide type on their binding properties under the experimental conditions (Simonsen et al., 2009).

The influence of pectin structure and composition on the binding capacity of polyphenols has been studied (Table 2) (Fig. 3a). Highly methylated pectins had strong interactions with procyanidins, indicating that the hydrophobic interaction plays an important role (Watrelot et al.,

2013). Esterification of pectin may also increase the chain flexibility, making their association with procyanidins easier. Citrus pectin and apple pectin had different binding patterns with procyanidins (Watrelot et al., 2013). The higher portion of rhamnose in the citrus pectin than apple pectin may give the former a higher flexibility of conformation to bind the procyanidins. The neutral sugar side chains limited the interactions between pectin and procyanidins, possibly due to the steric hindrance (Watrelot et al., 2013 and 2014). Monitoring the interactions of hairy regions of pectins and rhamnogalacturonans II with procyanidins showed that the binding capacity of pectins followed the sequence of rhamnogalacturonan, arabinans + galactan I + xylogalacturonans, galactan I, arabinans + galactans II, and arabinans (Watrelot et al., 2014). The linear part of the pectin (e.g., backbone) may feasibly allow the stacking/association of procyanidins. The arabinan side chains tend to be more mobile than the galactan chains, therefore, conformationally limiting their associations with procyanidins (Watrelot et al., 2014). Type II galactans have highly branched structure, thus, having limited interactions with procyanidins. Rhamnogalacturonan II bound procyanidins inefficiently, which disagreed with the results of Riou et al. (2002). This discrepancy may be due to the differences in the experimental conditions of different studies (Riou et al., 2002; Watrelot et al., 2014) as described in the next section. Two types of β-glucans from oat and barley, with rather different composition, structure, and rheology, showed rather similar binding behaviours with 21 vanillin-derived phenolic compounds (Simonsen et al., 2009). The analytical method was by dialysis assay and may not be able to reflect any possible impact of polysaccharide type on their binding properties under the experimental conditions (Simonsen et al., 2009).

Modifications of cell wall polysaccharides by chemical (cross-linking and oxidation) and physical (drying) means greatly impacted their interactions with polyphenols (Le Bourvellec et al., 2005a and 2005b; Simonsen et al., 2009). Harsh drying of apple cell walls (100 °C, 72 h) decreased the porosity (from 2.15 to 0.52 m^2/g) and greatly increased both apparent saturation level and apparent affinity per surface unit (Le Bourvellec et al., 2005a). This may be due to the disrupted physical structure of cellulose matrix, making the polyphenols easier to attach to the cellulose molecules. Cross-linking increased the apparent affinity constant of pectins and xyloglucans with procyanidins (Le Bourvellec et al., 2005a). This may be due to the increased hydrophobicity at the surface of these polysaccharides as a result of cross-linking. Enzymatic degradation of barley β-glucans greatly decreased the binding capacity with vanillin-derived phenolic compounds (Simonsen et al., 2009). This could be readily attributed to the partially disrupted tertiary and secondary structures of β-glucans.

3.3. Environmental factors

Environmental factors, including the ratios of polyphenol to polysaccharide, pH, ionic strength, temperature, and reaction time, may greatly impact the interactions between polyphenols and cell wall polysaccharides (Le Bourvellec et al., 2004; Simonsen et al., 2009; Wu et al., 2011; Phan et al., 2016; Lin et al., 2016; Padayachee et al., 2013). These factors are related to the common food processing and digestive tract conditions.

Phan et al. (2016) found that the pH (3−7) was the most dominant factor affecting the binding between cellulose and some polyphenols (cyanidin-3-glucoside, ferulic acid, (+/-)-catechin). The extents of influence depended on the type of polyphenol. Cyanidin-3-glucoside binding increased

with increasing pH from 3 to 5 before decreasing with pH up to 7. This is possibly due to the structural changes of the anthocyanins at various pH (Fig.1b). The pH (2.0−4.5) greatly influenced the binding of anthocyanins with pectins (Lin et al., 2016). The pH 3.6 favoured the binding while other pH values gave less affinity. The forms (quinoidal base) of anthocyanins at this pH may favour their stacking (Goto & Kondo, 1991) (Fig. 1b and Fig. 3b). At pH 7, the anthocyanins may also be degraded. Increasing pH increased the adsorption of ferulic acid (pK_a value 4.6), and had little effect on that of (+/-)-catechin (pK_a value 8.6). This suggests that the ionic interactions may not play an important role in the binding. Wu et al. (2011) showed that tea polyphenols had the maximum adsorption with oat β-glucans at pH 6 when varying the pH from 3 to 7. Altering pH (2−7) had no effect on the associations between procyanidins and apple cell walls, suggesting that the ionic or electrostatic interactions contributed little to this type of interactions (Le Bourvellec et al., 2004; Renard et al., 2001). Therefore, the pH effect appeared to be greatly dependent on the type of polyphenols. Compared with pH, the temperature (4−37°C) was the second dominant factor affecting the interactions (Phan et al., 2016). Increasing temperature slightly decreased the adsorption of cyanidin-3-glucoside and ferulic acid except for catechin. This may suggest that hydrogen bonding was involved in the interactions (Phan et al., 2016). Increasing temperature (20−60 °C) decreased the adsorption of tea polyphenols onto β-glucans, again suggesting the involvement of hydrogen bonds in the interactions (Wu et al., 2011). Increasing buffer concentration (up to 0.5 M) decreased the binding of tea polyphenols with β-glucans, suggesting that the hydrophobic interaction is small (Wu et al., 2011). NaCl (0--100 mM) (related to ionic strength) had little influence on the polyphenol binding (Phan et al., 2016). This may be due to the rather low concentrations used in

this study. Le Bourvellec et al. (2007) found that the binding of procyanidins with apple cell walls increased slightly with the increasing ionic strength (up to 1 M). Le Bourvellec et al. (2004) studied the effects of adding solvents on the interactions between procyanidins and apple cell walls. Addition of urea which disrupts the hydrogen bonds decreased the association, suggesting the involvement of hydrogen bonding. The involvement of hydrogen bonds in the polyphenol-polysaccharide interactions is schematically illustrated in Fig. 3c. Addition of ethanol or dioxane which decreases the solvent polarity disrupted the association, suggesting the importance of hydrophobic interactions. Gao et al. (2012a) also found that increasing concentration of NaCl and ethanol up to 0.5 M decreased the binding of (-)-epigallocatechin gallate with oat β-glucan in a linear manner, suggesting the hydrogen bonding is a major player in this type of interactions. Procyanidins bound to apple cell walls to saturation within a few mins (Le Bourvellec et al., 2004). Little change in the affinity between procyanidins and apple cell walls in the course of 60 min was noted (Renard et al., 2001).

Once the polyphenols are bound with the cell walls, altering the environmental conditions may release them from the complexes (Padayachee et al., 2013). The anthocyanins and phenolic acids bound to carrot cell walls were subjected to various conditions for re-releasing (Padayachee et al., 2013). Acidified methanol released 30% of phenolic acids and 20% of total anthocyanins, while only 2% of the polyphenols were unbound after *in vitro* simulated gastric and small intestinal digestions (Padayachee et al., 2013). The bound polyphenols with cell walls would pass into the colon. Overall, the effects of environmental factors and experimental conditions depend on their interactions of these factors, the types of both polyphenols and polysaccharides.

4. Isothermal adsorption models

The isothermal adsorption behaviours of cell wall polysaccharides with polyphenols in different food models have been well described by various mathematical models including Langmuir, Freundlich, Redlich-Peterson, and Toth equations (Freundlich, 1906; Langmuir, 1918; Redlich & Peterson, 1959; Toth, 1971) (Supplementary Table 1). The mathematical and theoretical descriptions of these isothermal adsorption models have been detailed previously, and are, therefore, not introduced here.

The mostly used model has been the Langmuir equation with high coefficients of determination ($R^2 > 0.9$) (Phan et al., 2015; Wu et al., 2011; Gao et al., 2012a; Le Bourvellec et al., 2005a; 2005b; Shi et al., 2015). Le Bourvellec et al. (2005a, 2005b) employed Langmuir equation to study the interactions between apple cell walls and procyanidins. The adsorption data well fitted with the Langmuir equation with $R^2 > 0.96$. Phan et al. (2015) used Langmuir equation to well describe the isothermal adsorption of ferulic acid, gallic acid, chlorogenic acid, catechin, and cyanidin-3-glucoside by cellulose. The apparent adsorption capacity and apparent binding affinity constant were calculated. Catechin (1488 μg/mg cellulose) and ferulic acid (1409 μg/mg cellulose) had the highest apparent adsorption capacity, followed by chlorogenic acid (1060 μg/mg cellulose) and cyanidin-3-glucoside (1109 μg/mg cellulose). Gallic acid had the lowest apparent adsorption capacity (388 μg/mg cellulose) (Phan et al., 2015). This further supports that the molecular structure of polyphenols may greatly impact their interactions with cell wall polysaccharides as discussed in the section 3.2. Cyanidin-3-glucoside had the highest apparent binding affinity constant among all the polyphenols. This may be due to the charge attraction effect between this anthocyanin (positive change) and cellulose (negative charge) at pH 3.4

(Phan et al., 2015). Shi et al. (2015) successfully used both Langmuir and Freundlich equations to model the isothermal adsorption behaviours of rice brans with tea polyphenols. Apart from the adsorption capacity, Freundlich constant (*n*) related to the adsorption heterogeneity can be obtained. The adsorption is homogeneous when $1/n$ is 1, and is heterogeneous when $0 < 1/n < 1$. This approach may be extended to study the impact of polysaccharides and polyphenols on the interactions as they can be very structurally heterogeneous (Table 2). Wu et al. (2011) employed Langmuir, Freundlich, and Redlich-Peterson equations to model the adsorption of tea polyphenols by oat β-glucans. Freundlich equation best described the adsorption process with the highest R^2 (0.979), followed by Redlich-Peterson equation (R^2 = 0.917). Langmuir equation gave the lowest R^2 (0.7734) (Wu et al., 2011). This suggests that multilayer coverage was involved in this adsorption process (Wu et al., 2011). Gao et al. (2012a) used Langmuir, Freundlich, Redlich-Peterson, and Toth equations to describe the adsorption of (−)-epigallocatechin-3-gallate (EGCG) (a tea polyphenol component) by oat β-glucans. Toth equation best described the adsorption process with R^2 of 0.99, which was followed by Redlich-Peterson (R^2 = 0.978), Langmuir (R^2 = 0. 941), and Freundlich (R^2 = 0.887) equations. Fitting of the adsorption data by Toth isothermal model suggested a heterogeneous surface of the oat β-glucans during the process (Gao et al., 2012a). Therefore, the suitability of specific equations appeared to be dependent on the specific interactions and experimental conditions. Various mathematical models with different parameters reflect different aspects of the interactions, and they may be employed together to reflect the adsorption process. The meaning of the parameters of the mathematical isothermal models remains to be better linked to the chemical, physical, and structural aspects of the interactions.

5. Polysaccharide-polyphenol-protein interactions

It is common that polysaccharides, polyphenols, and proteins co-exist in food systems. There is a good body of knowledge of the protein-polyphenol interactions (Le Bourvellec & Renard, 2012; Jakobek, 2015). The presence of cell wall polysaccharides greatly influences the association and precipitate formation between proteins and polyphenols (Gazzola et al., 2012; Mateus et al., 2004; Gonçalves et al., 2011; Oliveira & Pintado, 2015; Soares et al., 2009; Soares et al., 2012) (Table 3). Various analytical methods have been employed to probe the three-component interactions, including scanning ion occlusion sensing (SIOS) (Gazzola et al., 2012), saturation transfer difference-NMR spectroscopy, nephelometry, fluorescence quenching (Gonçalves et al., 2011), HPSEC (high-performance size exclusion chromatography) (Soares et al., 2012) and so on. For example, molecular size distributions of protein before and after the interactions were monitored by HPSEC. The altering molecular size indicated the affinity of polyphenols with polysaccharides (Fig. 2f) (Soares et al., 2012). SIOS was used to study the size and concentration of protein aggregates as affected by polyphenols and polysaccharides (Gazzola et al., 2012).

The presence of polysaccharides tends to reduce the association between protein and polyphenols, depending on the type and composition of each component as well as the experimental conditions (Table 3). Two molecular mechanisms have been proposed to explain the interactions (Fig. 3d and 3e). The disrupted associations between proteins and polyphenols could be due to either the formation of ternary protein-polyphenol-polysaccharide complexes, or the association between polyphenols and polysaccharides in solution which competes for protein interactions (Fig. 3d and 3e).

Polysaccharide type and composition can affect the three-component interactions (Soares et al., 2009 and 2012; Gonçalves et al., 2011; Mateus et al., 2004). The ability of various polysaccharides to inhibit the procyanidin B3-trypsin interactions followed the order of xanthan, polygalacturonic acid, gum arabic, and pectin (Gonçalves et al., 2011). Similarly, xanthan disrupted the association between grape seed procyanidin fractions and bovine serum albumin (BSA) to a greater extent than gum arabic (Mateus et al., 2004). Xanthan and polygalacturonic acid may be able to encapsulate the procyanidins through the formation of a gel-like network (Norton et al., 1984) (Fig. 3e). Different polysaccharides adapt different conformation in solution under different experimental conditions. Gum arabic and pectin may have the conformation that are not able to trap/interact with the procyanidins efficiently (Gonçalves et al., 2011). Soares et al. (2012 and 2009) showed that pectin and polygalacturonic acid formed ternary complexes with salivary proteins/porcine pancreatic α-amylase and condensed tannins, preventing the protein-polyphenol interactions, while arabic gum competed with the protein to bind tannins. Polyphenol structure and composition play important roles in the three-component interactions (Mateus et al., 2004; Oliveira & Pintado, 2015). The inhibitory effect of polysaccharides on protein (BSA)-polyphenol (procyanidins) association decreased with the increasing size of procyanidin fractions (Mateus et al., 2004). Increasing size of procyanidins facilitated their interactions with BSA due to increased number of interaction sites. Protein type and structure play important roles in the three-component interactions (Gazzola et al., 2012). For example, chitinase and thaumatin-like proteins (TLP) interacted with polysaccharides and polyphenols differently in wine systems (Gazzola et al., 2012). Wine polyphenols and polysaccharides little influenced the chitinase aggregation in model wine system, while different TLP isomers had different and decreased

susceptibility to aggregation in the presence of wine polysaccharides and polyphenols (Gazzola et al., 2012).

The presence of protein and polysaccharide may impact the polyphenol bioavailability in human digestive tract (Oliveira & Pintado, 2015). Pectin and β-lactoglobulin (β-LG) formed complexes with cyanidin-3-glucoside and (+)-catechin, increasing their bioavailability in gastrointestinal tract model systems with varying pH and digestive enzymes (Oliveira & Pintado, 2015). It may be expected that the environmental conditions such as pH, temperature, and ionic strength play important roles in the three-component interactions and complexation. How these factors impact the polysaccharide-polyphenol-protein interactions remains to be studied.

6. Food applications

The interactions between cell wall polysaccharides and polyphenols can play important roles in the processing and quality of food and beverage products (Table 4). The food products included strawberry yoghurt, jam, canned pears wine and apple juice (Bindon et al., 2010a and 2010b; Le Bourvellec et al., 2007; Buchweitz et al., 2013; Le Bourvellec et al., 2014; Oliveira et al., 2015).

In wine and fruit juice production, the release of polyphenols from fruits and vegetables into beverages greatly depends on the extents of polyphenol-cell wall interactions. There tends to be a significant loss of polyphenols from the fruits/vegetables during juice production, which remain in the pomace (Le Bourvellec et al., 2007; Bindon et al., 2010a and 2010b). The relevance of grape cell wall polysaccharide-polyphenol interactions to the wine making has been reviewed (Hanlin et al., 2010). For example, 25% and 27% of grape proanthocyanidins were found in wine and marc after fermentation, while 48% of proanthocyanidins remained in the seeds or lees

(Bindon et al., 2010a and 2010b). This could be readily attributed to the retention of proanthocyanidins by the cell walls during vinification (Bindon et al., 2010b) (Fig. 4a). The impact of polyphenol structure on the polyphenol-cell wall polysaccharide interactions has been discussed in a previous section in detail (Table 2). It has been established that larger proanthocyanidins have stronger binding with cell wall polysaccharides (Le Bourvellec et al., 2005b). This preferential selectivity of cell walls for binding polyphenols with specific structure and composition may explain the absence of high molecular weight proanthocyanidins in the wine (Bindon et al., 2010a and 2010b).

Polyphenols in food systems may be degraded due to oxidation and other environmental factors. Association of these susceptible polyphenols with cell wall polysaccharides may reduce their degradation in food systems. Indeed, addition of apple and sugar beet pectins in strawberry jam increased the overall storage stability of the anthocyanins (Buchweitz et al., 2013). The anthocyanin stability of the food systems depended on the type and structure of both the polysaccharides and the anthocyanins. Apple and sugar beet pectins, but no the citrus pectin, enhanced the anthocyanin stability, while the stability of pelargonidin-3-malonylglucoside was not affected by pectin addition (Buchweitz et al., 2013). Indeed, the outcomes of polyphenol-polysaccharide interactions depend on various factors including the types of both polyphenols and polysaccharides as discussed above (Table 2). The pink discoloration of canned pear slices could also be explained by the polyphenol-polysaccharide interactions (Fig. 4b) (Le Bourvellec et al., 2014). The pear procyanidins degraded into the colorant anthocyanidins which bound to the cell walls (Le Bourvellec et al., 2014). The interactions were so strong that successive solvent extractions and enzymatic degradation of cell walls hardly removed the colorant. This led to the

suggestion that co-valent bonding may be involved in this type of associations due to the highly reactive carbocations (Le Bourvellec et al., 2014). Mixing strawberry and yoghurt led to the decreased contents of both protein and polyphenols due to polyphenol-protein interactions (Oliveira et al., 2015). Addition of carrageenans decreased the protein content more, and may be attributed to the protein-polyphenol-polysaccharide interactions as discussed above (Table 2).

The interactions have also been used to mask the astringency of polyphenols (Troszyńska et al., 2010), to produce bioadsorbent for carrying tea polyphenols (Shi et al., 2015), and to create self-assembled micelle system for curcumin delivery (Liu et al., 2013 and 2014). Masking astringency could be due to the increased viscosity as well as the interactions between polysaccharides and polyphenols (Troszyńska et al., 2010). Different polysaccharides had different capacity of masking the astringency of polyphenols. The sequence of the tested polysaccharides followed the order of carboxymethylcellulose > guar gum > xanthan gum > arabic gum. Indeed, the extents of polyphenol-polysaccharide interactions depend greatly on various factors including polysaccharide structure as discussed above (Table 2). The bioavailability of polyphenols tends to be low in humans. Special delivery systems are needed for controlled and targeted releasing (McClements & Li, 2010). Polyphenol-polysaccharide interactions have been employed for targeted delivery of bioactive components (Liu et al., 2013 and 2014; Shi et al., 2015). Octenylsuccinate oat β-glucans was employed to form micelles for the encapsulation of curcumin (a bioactive polyphenol) (Liu et al., 2013 and 2014). This encapsulation greatly increased the stability and water solubility of curcumin in model systems (Fig. 4c), which could be attributed to the curcumin-octenylsuccinate β-glucan interactions (Liu et al., 2013 and 2014). Rice brans were used to load tea polyphenols due to the high adsorption

capacity (Shi et al., 2015). Langmuir and Freundlich models well described the isothermal adsorption of tea polyphenols (Shi et al., 2015). Rice bran is rich in cell wall polysaccharides such as β-glucans as well as lipids and proteins. The binding of tea polyphenols with rice bran could be attributed not only to polysaccharide-polyphenol, but also to protein-polyphenol and lipid-polyphenol interactions (Jakobek et al., 2015). Indeed, cellulase and proteinase as well as defatting treatments significantly reduced the adsorption capacity of rice bran (Shi et al., 2015). Due to the important function of targeted delivery of bioactive molecules, it would be interesting to probe if there is any synergism/antagonism among lipid, protein, and polysaccharide for polyphenol interactions.

7. Nutritional aspects

Cell wall polysaccharides and polyphenols commonly co-exist in food systems and enter into human digestive track. The polysaccharide-polyphenol interactions during digestion may greatly contribute to their health effects (Table 5). Apple pectins and freeze-dried apples rich in polyphenols were fed to rats to study their effects on the plasma lipids and cecal fermentations (Aprikian et al., 2003). Compared with feeding either apple pectins or freeze-dried apple rich in polyphenols, a combination of both was more effective in reducing the concentrations of plasma cholesterol and triglycerides and in increasing the concentrations of short-chain fatty acids from cecal fermentation (Aprikian et al., 2003). This may be due to the interactions between the polyphenols and pectins of apples. Gao et al. (2012b) studied the impact of interaction of barley β-glucan-tea polyphenol on the antioxidant status and glucose metabolism of diabetic rats induced by streptozotocin. Barley β-glucans and tea polyphenols significantly improved various

physiological parameters of blood glucose, serum lipids, and serum antioxidant status of the diabetic rats. The effect of barley β-glucan and tea polyphenol combination was better than that of the individuals (Gao et al., 2012b). It is, therefore, clear that synergistic interactions in improving the diabetic conditions occurred between these two components (Aprikian et al., 2003; Gao et al., 2012b; Wang & Zhu, 2015 and 2016). However, it is not clear if the health benefits were due to the physical associations between polyphenols and polysaccharides. The molecular mechanisms and pathways responsible for these health effects remain to be explored.

The bound polyphenols with cell walls mostly cannot be adsorbed in small intestine and enter into the large intestine for fermentation and metabolism (Saura-Calixto et al., 2010; Snelders et al., 2014; Padayachee et al., 2013). Saura-Calixto et al. (2010) examined the colonic fermentation of non-extractable proanthocyanidins bound with dietary fiber (e.g., cell walls) of carob pod and red grapes *in vitro* (small intestine digestion and colon fermentation model) and *in vivo* (human). The major metabolites of colon fermentation were hydroxyphenylacetic acid, hydroxyphenylvaleric acid and two hydroxyphenylpropionic acid isomers *in vitro* and 3,4-dihydroxyphenyl acetic acid *in vivo* as measured by HPLC-ESI-MS^2 (high performance liquid chromatography-electrospray ionisation-tandem mass spectroscopy) (Saura-Calixto et al., 2010). Difference in the metabolites of the two products (carob pod and red grapes) suggests the role of cell wall polysaccharides in the fermentation process. Indeed, it was shown that the type of cell walls may be a critical factor affecting the associations with polyphenols (Table 2). Snelders et al. (2014) studied the *in vitro* antioxidant activity and fermentability of arabinoxylanoligosaccharides (AXOS) which were feruloylated or mixed with ferulic acids (FA). Both bound and free FA reduced the AXOS fermentation. The bound FA reduced the enzyme

hydrolysis by steric hindrance, while the free FA and metabolites may be antibacterial (Snelders et al., 2014). The structure of AXOS had little impact on the fermentation. 4-vinylguaiacol was the major metabolite of FA and the antioxidant activity decreased during fermentation (Snelders et al., 2014). For the part of the carbohydrates, prebiotic effects due to the production of short chain fatty acids were observed, which was not affected by the presence of FA (Snelders et al., 2014). Nordlund et al. (2012) studied the fermentation of brans and aleurones of rye, wheat, and oat using *in vitro* small intestinal and colon fermentation models. The fermentation depended on the type of the bran/aleurone. Wheat bran had the slowest fermentation, while rye bran and aleurone had the fastest one (Nordlund et al., 2012). The differences in fermentation pattern could be attributed to particle size, solubility, and composition of cell walls. Different types of dietary fiber in cereal brans greatly impacted the fermentation of polyphenols to various extents (Nordlund et al., 2012). Metabolites of ferulic acid and benzoic acid were dominant of the *in vitro* fermentation products of polyphenols in cereal brans. Phenylpropionic acids converted from ferulic acids were the major metabolites of all the brans (Nordlund et al., 2012). Wheat aleurone was possessed by dry-grounding or enzymatic hydrolysis (xylanase/feruloyl esterase) before feeding to diet-induced obese mice, and the urinary metabolites were profiled by non-targeted LC-qTOF-MS (liquid chromatography-quadrupole time-of-flight-mass spectrometry) (Pekkinen et al., 2014). Enzymatic hydrolysis increased the urinary secretion of glycine conjugates and ferulic acid sulfate due to the releasing of bound polyphenols. In contrast, native and ground wheat aleurone gave higher concentrations of microbial metabolites (hydroxyl- and dihydroxyphenylpropionic acids, hippuric acid) as most of the bound polyphenols entered into the colon for fermentation (Pekkinen et al., 2014) (Fig. 5). Rosa et al. (2013) studied the effects

of dry-grinding and enzymatic hydrolysis of wheat aleurone on the formation of metabolites, using an *in vitro* fermentation model of human faecal microbiota. Dry-grinding increased the rate of short-chain fatty acid formation due to the increased surface areas of the aleurone particles, while enzymatic hydrolysis gave a higher concentration of phenylpropionic acids which are the metabolites of free ferulic acids (Rosa et al., 2013). Overall, there is a lack of reports on the relationships between *in vitro*/animal studies and human clinical trials.

Apart from the non-covalent interactions between polyphenols and cell walls, covalently bonded polyphenols with cell wall polysaccharides (e.g., ferulic acid bound to cereal brans) also play important roles during fermentation in human digestive track (Snelders et al., 2014; Nordlund et al., 2012). Dietary fiber has a major impact on the adsorption and bioavailability of natural plant antioxidants in humans (Palafox-Carlos et al., 2011). Even though it is well established that polyphenol-polysaccharide/oligosaccharide complexes may define the metabolism pattern in the colon of humans, the specific health effects of these metabolites from colon fermentation remain to be established. Epidemiological studies showed that a high intake of whole plant foods rich in cell wall polysaccharides and polyphenols is linked with a low occurrence of various chronic diseases (Campbell & Campbell, 2005; Campbell & Jacobson, 2014). Therefore, understanding the interactions between polysaccharides and polyphenols are fundamentally important for human health.

8. Conclusions and future outlook

A range of analytical methods, including microscopy, spectroscopy, calorimetry, and chromatography, have been used to directly and indirectly probe the noncovalent interactions

between cell wall polysaccharides and polyphenols. The outcomes of the interactions between cell wall polysaccharides and polyphenols depend on various factors. These include the composition and structures of both polysaccharides and polyphenols, experimental and environmental conditions, as well as the analytical methods. The noncovalent interactions are mostly driven by hydrophobic contacts and also by hydrogen bonding, though other types of associations such as ionic interactions may also involve in the interactions under certain experimental conditions. Various mathematical models including Langmuir and Freundlich equations well described the isothermal adsorption behaviours of cell wall polysaccharides with polyphenols. The presence of polysaccharides greatly decreased the interactions between polyphenol and protein through competitive reactions or formation of ternary complexes. Cell wall polysaccharide-polyphenol interactions may greatly impact the quality attributes of some food and beverages such as wine and cider. Nutritionally, cell wall bound polyphenols have little adsorption in stomach and small intestine and enter into large intestine for fermentation and metabolism. The importance of the polyphenol-cell wall polysaccharide interactions in human health supports the view that reductionism approach to the study of nutrition should be critically viewed (Campbell & Jacobson, 2014).

In order to better understand the cell wall polysaccharide-polyphenol interactions, the following topics can be explored:

(1) There is extensive knowledge of the interactions between polyphenol and protein (Le Bourvellec & Renard, 2012; Jakobek, 2015). The analytical methods used for polyphenol-protein interactions may be extended to include polyphenol-polysaccharide interactive systems. (2)

Interactions among cell wall polysaccharides, polyphenols, and environmental conditions affecting the binding should be explored. (3) The parameters derived from the isothermal adsorption equations remain to be linked to the physicochemical and structural aspects of the interactions. (4) As the cell walls contain a range of different types of polysaccharides, synergistic/antagonistic interactions between various types of cell wall polysaccharides in binding the polyphenols should be studied. There may be polysaccharide-polysaccharide interactions, affecting the binding. (5) The impact of different types of processing techniques on the cell wall structures and the interactions should be better explored for either maximising or minimising the affinity for different application purposes. (6) The presence of other components such as starch, protein, and lipid on the polyphenol-polysaccharide interactions should be studied as they mostly co-exist in various food systems and different types of food products has different composition. (7) The bound polyphenols with cell walls in the colon in relation to chronic diseases such as cancer, obesity, and diabetes should be studied to biochemically improve our knowledge. Nutritional benefits of the metabolites of polyphenol-cell wall polysaccharide complexes from the large intestine fermentation remain largely unknown.

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Table 1 Methods for characterisation of non-covalent interactions between cell wall polysaccharides and polyphenols

Technique	Cell wall polysaccharides	Polyphenols	Uses	References
Confocal laser scanning microscopy	Cellulose, cell walls of carrot	Anthocyanins of purple carrots	Confocal laser scanning microscopy with fluorescent dye (Congo red) revealed that the localised anthocyanins stacked to the cell wall polysaccharides (Fig. 2a)	Padayachee et al., 2013; 2012a
Isothermal titration calorimetry (ITC)	Apple/citrus pectins	Apple procyanidins	Thermodynamic properties (association constant, stoichiometry, enthalpy change of binding) of cell wall-procyanidin interactions were studied by ITC (Fig. 2d)	Le Bourvellec et al., 2012; Watrelot et al., 2013 and 2014
Saturation transfer difference (STD) NMR spectroscopy	Pectins	Anthocyanins	STD-NMR technique has been used to reveal the dissociation constant (K_d) of the pectin-anthocyanin interactions	Fernandes et al., 2014

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			(Fig. 2b). Protons involved in the pectin-anthocyanin interactions showed STD-NMR signals, while those that were not involved had no signals in the NMR spectrum	
Molecular dynamics simulation	Pectins	Anthocyanins	Computer-based molecular dynamics simulation was employed to study the conformational characteristics of anthocyanins and pectins in solution (Fig. 2c). The solvent-accessible surface area of pectin was obtained	Fernandes et al., 2014
Dynamic light scattering (DLS)	Grape cell walls	Grape seed tannins	DLS was used to monitor the aggregation of tannins with wine polysaccharides. Aggregates with increasing size were formed upon storage. Polysaccharides had little effect on the initial aggregation of tannins, while affecting the development of particle size. The rhamnogalacturonan II dimer facilitated the	Riou et al., 2002

			formation of aggregates with increasing particle size	
UV−vis spectrophotometry	Apple and citrus pectins	Procyanidins	Spectrophotometry (absorbance at 650 nm) was used to monitor the aggregation of pectins and procyanidins due to the interactions	Watrelot et al., 2013 and 2014

Table 2 Noncovalent interactions between cell wall polysaccharides and polyphenols in various food systems

Polysaccharide type	Polyphenol type	Reaction conditions	Targeted parameter	Major findings	References
Cellulose	24 polyphenols including gallotannins and ellagitannins	Solid state	Amount of bound polyphenols on cellulose thin layer chromatography	The affinity of cellulose with polyphenols is in positive relationship with the molecular weight, their hydrophobicity, and number of galloyl groups. Ellagitannins had much lower affinity with cellulose than gallotannins. Hydrophobic interactions predominated	Tang et al., 2003
Bacterial cellulose--pectin	Purple carrot juice concentrate containing	4 °C, up to 14 days, pH 4.0	Concentrations of anthocyanins by HPLC	Anthocyanins bound to both pectin and cellulose, and higher	Padayachee

composite and cellulose	non-acylated and acylated anthocyanins			amount of pectins in the composite facilitated the adsorption. Non-acylated and acylated anthocyanins had similar adsorption patterns. A two-stage adsorption pattern was observed. The initial stage was fast with 13--18% of anthocyanins absorbed. The second stage was slow and gradual	et al., 2012a
Bacterial cellulose--pectin composite and cellulose	Ferulic acid, chlorogenic acid, and caffeic acid of purple carrot juice concentrate	4 °C, up to 14 days	Concentrations of phenolic acids by HPLC	Phenolic acids bound to both pectin and cellulose composites to various extents with caffeic acid having the maximum adsorption and ferulic acid the	Padayachee et al., 2012b

				minimum. Cellulose had a higher initial adsorption within 1 h, and all the polysaccharide composites showed similar extents of adsorption after a few days	
Carrot plant cell walls and a bacterial cellulose--pectin model	Anthocyanins, phenolic acids	*In vitro* gastric (pH 2.0, 1 h, 37 °C) and small intestinal (pH 5.7 for 30 min before pH 7.0 for 2 h, 37 °C) digestion	Released polyphenols from the digestion were quantified by HPLC	The majority of polyphenols were bound to cell wall materials. Simulated gastric and small intestinal digestion hardly released the bound polyphenols from the cell wall materials (< 2% of polyphenols released). These bound but not released polyphenols would reach the	Padayachee et al., 2013

		models		colon for fermentation	
Bacterial cellulose	Ferulic acid, gallic acid, chlorogenic acid, (+/−)-catechin, cyanidin-3-glucoside	4 °C, up to 24 h	Concentrations of polyphenols in supernatant by UV-spectrophotometry	All the polyphenols bound to cellulose up to 60% (w/w) of the cellulose weight. Langmuir binding isotherms fit the adsorption data well with $R^2 > 0.92$. The native charges of the polyphenols had little effect on the binding to the cellulose	Phan et al., 2015
Bacterial cellulose	Cyanidin-3-glucoside, ferulic acid, (+/−)-catechin	Temperature (4--37°C), pH 3--7, NaCl	The amounts of bound polyphenols quantified by spectrophotometry	pH was the most influential on the binding between cellulose and polyphenols, followed by the temperature. NaCl	Phan et al., 2016

		(0--100 mM), 2 h		concentration had little influence. Cyanidin-3-glucoside is the most sensitive to the experimental conditions and catechin is the least. A second-order polynomial equation was employed to describe the interactions between cellulose and polyphenols as affected by the reaction conditions	
Citrus pectin	Anthocyanins (cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside)	pH 1.5, 4.0	Saturation transfer difference (STD)-NMR spectroscopy and computer molecular dynamics simulation	A weak interaction between anthocyanins (hemiketal form) and pectins was noted. Delphinidin-3-*O*-glucoside with three OH groups had stronger	Fernandes et al., 2014

			was used to study the interactions	binding to pectin than cyanidin-3-*O*-glucoside. Acidic forms of anthocyanins (flavylium cation) had a stronger affinity with pectin	
Blueberry pectins with different solubility	Anthocyanins and blueberry juice	pH 2.0--4.5, 4 °C, 18 h	Concentrations of anthocyanins bound to pectins measured by a spectrophotometry-based method	All the anthocyanins bound to blueberry pectins. The binding was dependent on the pH and pectin-type. The lowest binding was at pH 4.5. Water soluble pectin had the lowest binding of anthocyanins. Chelator (EDTA) soluble and sodium carbonate soluble pectins had higher bindings at pH 2.0--3.6 and pH 3.6--4.5, respectively. Ionic	Lin et al., 2016

				interactions between pectins (carboxyl group) and anthocyanins (flavylium cations) and anthocyanin molecular stacking were suggested as two major mechanisms of binding	
Apple cell walls	Apple polyphenols	25°C, 5−120 min, pH 2.4−6, polyphenol concentrations up to 10 g/L	Concentrations of polyphenols bound to cell walls by HPLC	Hydroxycinnamic acids and epicatechin had no binding to cell walls, while procyanidins bound fast (up to 0.6 g per g cell walls). Increasing initial concentrations and size of procyanidins increased the binding. The binding was completely disrupted by urea (8 M) or acetone and water	Renard et al., 2001

				mixture. Binding of procyanidins inhibited the enzymatic hydrolysis of the cell walls	
Apple cell wall (native and modified)	Procyanidins of grape seed and pear	2.5--90 min, pH 2.2--7, temperature 5--35 °C, ionic strength (0.01 to 1 M), alcohol concentration (0−97%), urea (1−6 M)	Procyanidin concentration was quantified by HPLC after thioacidolysis	Increasing size, galloylation, and portion of (+)-catechin of procyanidins increased the binding by cell walls. The pH (2.2--7) had little effect on the binding, while the presence of urea, dioxane, and ethanol decreased it. Increasing ionic strength and decreasing temperature increased the binding, suggesting the interactions were modified by hydrogen bonds and	Le Bourvellec et al., 2004, 2005a; 2005b

				hydrophobic interactions. Langmuir isotherm adsorption model well described the adsorption of procyanidins by cell walls. Increasing procyanidin concentration increased the amount of adsorbed procyanidins with decreased proportion of procyanidins bound. Decreasing the cell wall porosity (by drying) increased the saturation level and binding per unit surface. Different types of cell wall polysaccharides (pectin, xyloglucan, and cellulose)	

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				showed different binding capacity with procyanidins. Pectin had the highest binding capacity, while cellulose and xyloglucan bound procyanidins weakly. Apparent saturation levels of cellulose and xyloglucan were higher than that of pectin	
Apple cell walls	Procyanidins of apple and pear	pH 3.8, 1 h, 5--35°C	Composition of procyanidins was analysed by HPLC	The effect of ionic strength, temperature, concentrations of polyphenols and cell walls, and types of procyanidins on the interactions were studied and modelled. The model was related to the separation of procyanidins	Le Bourvellec et al., 2007

				from apple into juice as shown in Table 3	
Apple cell wall	Apple proanthocyanidins in native and oxidised forms	1 h, 25 °C, pH 3.8	Polysaccharide extractability from apple cell wall	Polyphenols decreased the yield of pectin and the solubility of highly methylated pectins, and reduced the depolymerisation of pectins by pectin lyase. Oxidation of proanthocyanidins increased the cell wall binding. Binding of oxidised proanthocyanidins by pectins increased the yield of hemicellulose using 4 M NaOH	Le Bourvellec et al., 2009
Apple cell walls as affected by boiling and	Apple procyanidins	pH 3.8, ionic strength 0.1	Unbound proanthocyanidins measured by HPLC,	The presence of protein in the cell walls had little effect on the procyanidin-cell wall	Le Bourvellec et al., 2012

drying		M, 1 h	isothermal titration calorimetry	interactions. Boiling and drying increased the apparent saturation levels and had no effect on the apparent affinity of cell walls per surface unit. Isothermal titration calorimetry analysis of the interactions between solubilized pectins and procyanidins indicated strong interactions (Fig. 2d)	
Apple cell walls and pectins, raw and cooked onion cell walls	Quercetin	37°C, pH 6.5, 2 h	Concentration of quercetin in supernatant after centrifugation (1, 000 g, 5 min) was reflected by the *in vitro*	The reduced antioxidant activity of the supernatant containing quercetin may reflect the binding of this polyphenol with apple/onion cell walls and pectins as well as its oxidation	Sun-Waterhouse et al., 2008a; 2008b

			antioxidant activity		
Apple and citrus cell wall pectins differing in molecular structure	Apple procyanidins differing molecular size (DP = 9 and 30)	pH 3.8, ionic strength at 0.1 mol/L	Thermodynamics of pectin-procyanidin interactions were probed by isothermal titration calorimetry; aggregation and turbidity were monitored by UV−visible spectroscopy	Procyanidins with larger size (DP = 30) had stronger binding. Apple and citrus pectins interacted differently with procyanidins as reflected by different absorbance maxima and stoichiometry. Large procyanidins interacted strongly with highly methylated pectins. Interactions between methylated homogalacturonans and procyanidins (DP = 30) were most hydrophobic. The presence of neutral sugar side chains in	Watrelot et al., 2013 and 2014

				pectins (e.g., rhamnogalacturonans) strongly limited their interactions with procyanidins	
Cell walls of grape skin and flesh	Proanthocyanidins (PA) of grape skin, seed, and flesh	1 h, 32 ^{o}C	Composition of proanthocyanidins after centrifugation was analysed by gel-permeation chromatography and phloroglucinolysis	Increasing molecular size of proanthocyanidins increased the binding between flesh cell walls and proanthocyanidins. Seed-derived proanthocyanidins was preferred in the interactions. Interactions between cell walls and proanthocyanidins of skin did not follow this pattern, suggesting the role of cell wall structure on the interactions	Bindon et al., 2010b and 2010a

Cabernet Sauvignon grape skin and flesh cell walls	Cabernet Sauvignon grape skin proanthocyanidins	1 h, 32 ^{o}C	Unbound proanthocyanidins quantified by phloroglucinolysis and gel permeation chromatography	Degree of polymerization of proanthocyanidins increased up to 33 after grape veraison. Binding of skin cell walls with proanthocyanidins decreased after veraison. In colorless Cabernet Sauvignon grape mutants, proanthocyanidins of high molar mass had a higher binding capacity to the skin cell walls	Bindon & Kennedy, 2011
Wine polymeric material	Wine anthocyanins	4 °C, up to 66 h, dialysis membrane was employed	Amount of unbound anthocyanins measured by HPLC	Anthocyanins with coumaroyl and acetyl moieties had higher affinity with wine polymeric material than non-acylated anthocyanins. Hydrophobic	Gonçalves et al., 2012

				interactions predominated	
Fractions of grape skin cell walls	Grape skin procyanidins	1 h, 27 ^{o}C	Unbound proanthocyanidins measured by phloroglucinolysis	Grape skin cell walls were selectively fractionated by different types of solvents. A large portion of cell wall-bound proanthocyanidins (54%) was with the chelator-soluble fractions (pectins). Hemicellulosic fractions had a high binding with the proanthocyanidins. The lignocellulosic residue had much reduced interactions with proanthocyanidins. All the fractions except for the lignocellulosic residue better	Ruiz-Garcia et al., 2014

				bound the proanthocyanidins of high molecular mass	
Insoluble grape skin cell walls	Six different commercial enological tannins	Room temperature, 90 min, pH 3.6	Concentrations of tannins bound to cell walls analysed by HPSEC and phloroglucinolysis	The highest binding of one tannin was 61%. The molecule size of tannins than the degree of galloylation was more related to the binding. Oxidised tannins had a strong binding to the cell walls	Bautista-Ortín et al., 2014
Grape skin cell walls	Anthocyanins and tannins of grape	Model system: pH 3.6, 90 min, room temperature; grape	Concentrations of anthocyanins and tannins analysed by RP-HPLC and HPSEC, respectively	The presence of anthocyanins facilitates the extraction of tannins from the grape skins and seeds. The adsorptions of anthocyanins and tannins by cell walls are competitive	Bautista-Ortín et al., 2016

		vinification			
Barley and oat β-glucans	21 vanillin-inspired phenolic derivatives	Equilibrium dialysis assays at pH 7, up to 36 h, 37 ^{o}C	The amounts of phenolics bound to β-glucans	Glucosides of phenolics had little binding with β-glucans. Phenolics with a hydroxyl group in para-position to a CHO group had the strongest binding, while additional functional groups reduced the binding. Enzyme degradation of β-glucans reduced the binding capacity	Simonsen et al., 2009
Oat β-Glucan	Tea polyphenols	Temperature (20−60 °C), pH (3−7), PBS buffer	Equilibrium dialysis assay was used and the tea polyphenol concentration was	The adsorption of tea polyphenols by oat β-glucans was optimised through response surface methodology.	Wu et al., 2011

		concentration (0.05−0.5 M),	measured by spectrophotometry	The highest adsorption was 134.55 μg/mg at pH 5.6, PBS (phosphate-buffered saline) buffer concentration of 0.13 M, and temperature of 40 °C. Freundlich isotherm adsorption model best described the equilibrium data	
Soluble oat β-glucans	Tea polyphenols, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)- epigallocatechin gallate, (−)-gallocatechin gallate,	pH 5.56 PBS solution (0.13 M), 40 °C, 16 h in dialysis bag	Content of polyphenols bound to β-glucans	(−)-Epigallocatechin gallate (EGCG) had the largest capacity and efficiency of adsorption by β-glucans. Response surface methodology analysis showed that the reaction conditions for the maximum adsorption were concentration of EGCG (0.7	Gao et al., 2012a

	(−)-catechin			mg/mL), pH 5.8, concentration of PBS (0.10 M), and temperature of 50 °C. The isotherm adsorption of EGCG can be best described by Toth model as compared with Langmuir, Redlich-Peterson, and Freundlich Models. Individual polyphenols with galloyl group are better adsorbed by the β-glucans	
Soluble oat β-glucans	Thirty-six polyphenols with diverse molecular structures	pH 5.56, 40 °C, 16 h. Ultrafiltration was employed	Concentrations of polyphenols bound to β-glucans	Impact of polyphenol structure on the binding properties of β-glucans was studied. Presence of three or less hydroxyl groups	Wang et al., 2013

				facilitated the binding while more hydroxyl groups (> 3) decreased the binding interactions. Adsorption capacity of flavonoid isomers followed the order of flavonol (the highest binding), flavone, flavanone, and isoflavone (the least binding). Methoxy and methyl groups on phenolic acids decreased their adsorption onto β-glucans. Gallic acid esterification decreased the binding while catechin galloylation increased it.	

				o-Coumaric acid bound to β-glucans more than *m*- and *p*-coumaric acids	
Crude fiber of the flesh of mango, pineapple, papaya, guava, and wheat	Methanol extracts of the plant foods	pH 2.5, room temperature, up to 2 h	Total phenolic content and *in vitro* antioxidant activity in the supernatant of the reaction system	Wheat dietary fiber reduced the total phenolic content up to 38% and antioxidant activity up to 48%, while the fiber of the fruits reduced the total phenolic contents up to 25% and antioxidant activity up to 22%. Mango fiber gave the greatest reduction among the fruit fibers	Quirós-Sauceda et al., 2014

Table 3 Interactions among cell wall polysaccharides, proteins, and polyphenols

Polysaccharide type	Polyphenol type	Protein type	Reaction conditions	Major findings	References
Xanthan (XG), gum arabic (GA), pectin	Procyanidin fractions from grape seeds	Bovine serum albumin (BSA)	pH 5.0, procyanidin concentration of 0.1 g/L	All the polysaccharides disrupted the interactions and aggregation of BSA and various fractions of procyanidins. XG had the highest effect and GA had the least. The inhibitory effect of polysaccharides decreased with the increasing size of procyanidin fractions	Mateus et al., 2004
Gum arabic, pectins, β-cyclodextrin	Condensed tannins of grape seed	Porcine pancreatic α-amylase	pH 5.0	Nephelometry, fluorescence quenching, and dynamic light scattering methods were used to study the interactions. These carbohydrates reduced the precipitation due to α-amylase-tannin complexation. Different fractions of tannins with different molecular sizes were used. It was suggested that	Soares et al., 2009

				β-cyclodextrin and gum arabic competed with α-amylase for tannin association, while pectins interacted with both tannin and α-amylase to form three-component complexes (Fig. 3d)	
Pectin (PC), polygalacturonic acid (PA), xanthan gum (XG), gum arabic (GA),	Procyanidin B3	Trypsin	Molar ratio of trypsin to procyanidin B3 at 1:30, the concentration ranges of PC, PA, XG, and GA were 2−10, 0.2−1.0, 0.001−0.09, 0.2−1.6 g/L, respectively	Various techniques including saturation transfer difference-NMR spectroscopy, nephelometry, and fluorescence quenching were used to study the interactions. All the carbohydrates disrupted the interactions between procyanidin B3 and trypsin through competitive reactions (Fig. 3d). The ability to inhibit the procyanidin B3-trypsin interactions/aggregation followed the order of XG > PA > GA > PC. The ionic feature of carbohydrates and the ability to encapsulate procyanidins contributed to the inhibitory effects	Gonçalves et al., 2011

				(Fig. 3e)	
Arabic gum (AG), polygalacturonic acid (PGA), and pectin	Condensed tannins of grape seed	Salivary proteins	~20°C, polysaccharides and tannins were mixed for 30 min before salivary proteins was added and mixed for 50 min	Polysaccharides effectively inhibited protein-tannin precipitation. Pectin was the most efficient and PGA was the least. Pectin and PGA formed complexes together with proteins and polyphenols (Fig. 3d), while AG and protein competed to bind the tannins. The hydrophobic and hydrophilic interactions were involved in interactions	Soares et al., 2012
Chardonnay wine polysaccharides	Chardonnay wine polyphenols	Chitinase and thaumatin-like proteins (TLP) of Chardonnay	70°C for 1 h followed by 25°C for 15 h	Scanning ion occlusion sensing (SIOS) was used to study the size and concentration of protein aggregates as affected by the presence of polyphenols and polysaccharides. Chitinase had the highest tendency to form aggregates with the largest	Gazzola et al., 2012

		wine		particles. Polyphenols and polysaccharides had little impact on the chitinase aggregation in the simulated wine conditions. TLP isoforms had different susceptibility to aggregation. The presence of polyphenols and polysaccharides decreased the aggregation of some TLP isoforms	
Pectin	(+)-Catechin and cyanidin-3-glucoside	β-Lactoglobulin (β-LG)	*in vitro* models with gastrointestinal tract conditions: 1, mouth digestion model: α-amylase solution at	In mouth model, the presence of pectin and β-LG decreased the free cyanidin-3-glucoside by 23%, while the presence of pectin decreased that by 73%, suggesting the formation of pectin-β-LG complexes. In gastric model, the presence of pectin and β-LG gave a higher cyanidin-3-glucoside content than the pectin alone. In intestinal digestion model, the presence of pectin and β-LG decreased cyanidin-3-glucoside content much less than the pectin alone. The presence of pectin had a similar	Oliveira & Pintado, 2015

			37°C, 1 min; 2, gastric digestion model: pH 2, 37°C, 1 h, pepsin; 3, intestinal digestion model: pH 6, 2 h, 37°C, pancreatin	effect on catechin than the presence of both pectin and β-LG	

Table 4 Applications of polyphenol-cell wall polysaccharide interactions in food systems

Food systems	Interactions	Implications for food products	Reference
Apple juice production	Apple cell wall-procyanidins	Pressing of apple for juice production was related to the cell wall-procyanidin interactions. The pressing conditions for the yields of polyphenols in juice agreed with that from the models. The interactions between cell walls and polyphenols explained the loss of the latter in the pomace during juice production	Le Bourvellec et al., 2007
Grape wine	Grape cell walls−grape proanthocyanidins (PA)	During vinification, 25% and 27% of grape PA were found in wine and marc after fermentation, while 48% of PA remained in the seeds or lees. This may be explained by the binding interactions of cell walls and PA. PA with high molecular weight was not detected in the wine. It may be removed due to the binding with cell walls	Bindon et al., 2010a and 2010b
Stabilisation	Strawberry	Strawberry extracts were mixed with citrus and apple (low	Buchweitz

of strawberry anthocyanins	anthocyanins-different pectins	esterified amidated, low and high methyl esterified) and sugar beet pectins at pH 3. Apple and sugar beet pectins enhanced the anthocyanin stability, and citrus pectin had little effect. Amidation and esterification of pectins had little effect. Stability of pelargonidin-3-malonylglucoside was not affected by pectin addition. The stability of anthocyanins by pectins may be partially attributed to their binding interactions	et al., 2013
Pink discoloration of canned pears (Fig. 4b)	Procyanidin/anthocyanin−cell wall of pears	Procyanidins of canned pear slices partially degraded into anthocyanidins, contributing to the pink color development. The colorant strongly bound to the cell walls as revealed by successive solvent extractions and cell wall enzymatic degradation. Apart from the non-covalent bonding between polyphenols and cell walls, covalent bonding was suggested as the pink colorant was very resistant to extraction	Le Bourvellec et al., 2014
Strawberry	Carrageenan-strawberry	Mixing strawberry and yoghurt led to the decreasing in both protein and polyphenol contents. The presence of carrageenan	Oliveira

yoghurt	polyphenol-β-lactoglobulin	decreased the protein content more. This indicated the formation of carrageenan-yoghurt protein complexes	et al., 2015
Masking the astringency of polyphenols	Guar, xanthan, arabic gums, carboxymethylcellulose (CMC); tannic acid, polyphenolic extracts of chokeberry, green tea, walnut	All the polysaccharides above the critical concentrations reduced the polyphenol astringency. CMC had the greatest reduction which was followed by guar gum, xanthan gum, and arabic gum	Troszyńska et al., 2010
Delivery system for curcumin (Fig. 4c)	Octenylsuccinate oat β‑glucan−curcumin	Octenylsuccinate oat β-glucan self-assembled into spherical micelles. The presence of these micelles enhanced the water solubility of curcumin. Response surface methodology revealed that the maximum loading of curcumin in the micelles was 4.21 μg/mg. Solubilised curcumin interacted with the micelles most through hydrophobic interactions to form an amorphous complexes	Liu et al., 2013 and 2014
Bioadsorbent	Rice bran	Langmuir and Freundlich models well described the isothermal	Shi et al.,

for tea polyphenols		adsorption of tea polyphenols by defatted rice bran. Cellulase and proteinase as well as defatting treatments greatly decreased the adsorption capacity of the rice bran	2015

Table 5 Nutritional aspects of polyphenol-cell wall polysaccharide interactions

Property	Polysaccharide type	Polyphenol type	Experiment	Major findings	Reference
Cecal fermentations and lipid metabolism	Apple pectin	Apple concentrate rich in polyphenols	Rats was fed with apple pectin, apple concentrate rich in polyphenols, or their combinations	Combination diet had positive effects on large intestine fermentations and lipid metabolism of rats, and the effects were higher than those of either apple pectin or apple concentrate rich in polyphenols. This suggested the interactions between apple polyphenols and apple pectins	Aprikian et al., 2003

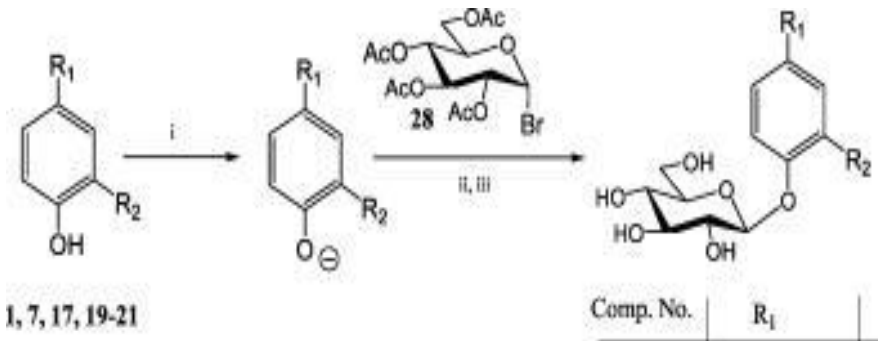
Metabolism of proanthocyanidins in colon model	Dietary fiber of carob pod and red grapes	Proanthocyanidins of carob pod and red grapes	*In vitro* small intestine digestion and colonic fermentation model was used. Human studies were also employed	The metabolites of proanthocyanidins in both *in vitro* model and humans were two isomers of hydroxyphenylpropionic acid, hydroxyphenylacetic acid, and hydroxyphenylvaleric acid. Difference in the metabolite profiles of the two types of materials was noted	Saura-Calixto et al., 2010
Metabolism of cereal bran and aleurone	Cell walls of rye, wheat, and oat brans	Polyphenols of rye, wheat, and oat brans	*In vitro* small intestinal and colon fermentation models	Phenylpropionic acid from ferulic acid was the major metabolites of all the brans. Wheat aleurone fraction gave the highest amount of phenolic metabolites. The	Nordlund et al., 2012

				fermentation of wheat bran was the slowest, while that of rye bran and aleurone was the fastest with the greatest extent. Acetate was the most dominated among the short-chain fatty acids from the fermentation	
Antidiabetic	Barley β-glucan	Tea Polyphenols	Diabetic rat induced by streptozotocin was orally fed with diets containing barley β-glucan, tea polyphenols, or their combinations	Combination diet had a better antidiabetic effect than the individuals in improving the antioxidant status and glucose metabolism in diabetic rats. This indicated the synergistic interactions between barley β-glucans and tea	Gao et al., 2012b

				polyphenols	
Metabolism of wheat aleurone	Wheat aleurone	Polyphenols of wheat aleurone	*In vitro* fermentation model with human faecal microbiota was used. Metabolites of both cell wall polysaccharides and polyphenols were monitored	Wheat aleurone was treated by dry grinding or enzymatic hydrolysis (xylanase/feruloyl esterase). Enzymatic hydrolysis of wheat aleurone gave a higher formation rate and concentration of the metabolites of ferulic acid (phenylpropionic acids), and had little effects on the formation of short-chain fatty acids. Dry grinding increased the formation rate of short-	Rosa et al., 2013

				chain fatty acids by increasing the surface area of the particles	
Metabolism of wheat aleurone	Wheat aleurone	Polyphenols of wheat aleurone	Urinary metabolites of diet-induced obese mice fed with wheat aleurone were profiled. Wheat aleurone was processed by dry-grinding or enzymatic hydrolysis (xylanase/feruloyl esterase)	Enzymatic hydrolysis of wheat aleurone released the bound polyphenols into free forms and increased the excretion of glycine conjugates and ferulic acid sulfate. Native and ground wheat aleurone gave higher amounts of metabolites from microbial fermentation in the large intestine (hydroxyl- and dihydroxyphenylpropionic acids, hippuric acid)	Pekkinen et al., 2014

Fermentation by human gut microbiota	Arabinoxylan-oligosaccharides (AXOS)	Ferulic acid (FA) covalently and non-covalently bound to AXOS	AXOS with FA was fermented *in vitro* by cultured human colon microbiota	FA in both bound and free forms reduced the fermentation. *In vitro* antioxidant activity decreased as the fermentation metabolised both the bound and free FA	Snelders et al., 2014



Comp. No.	R_1	R_2
22	CHO	OCH_3
23	$COOC_2H_3$	OCH_3
24	COCH3	OCH_3
25	$COOCH_3$	OCH_3
26	CH2OH	H
27	CHO	OCH3

Figure 1a Phenolic glucosides 22-27 from chemical synthesis: (i) NaOH, H_2O, 15 min, <10 ^oC; (ii) acetone, 24 h, room temperature; (iii) MeOH, MeONa/MeOH, 1−2 h, room temperature (Simonsen et al., 2009) (Reprinted with permission from the publisher)

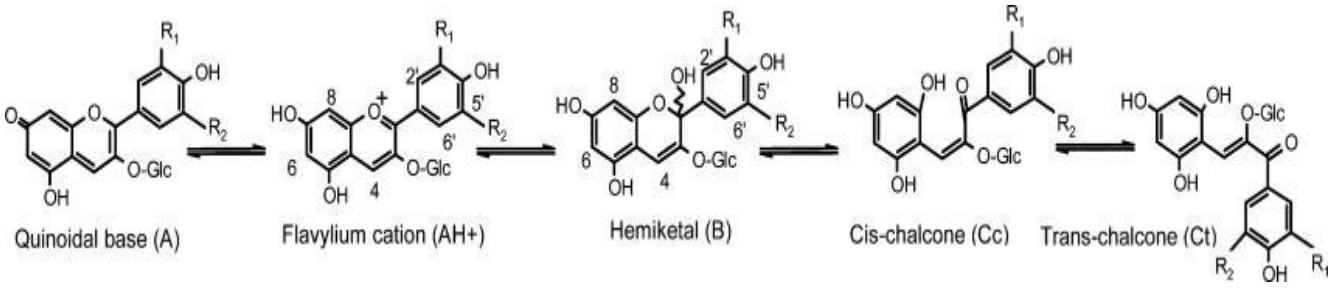


Figure 1b Equilibrium forms of anthocyanins in an acidic medium; the anthocyanin is cyanidin-3-O-glucoside when R1 = OH, R2 = H, and is delphinidin-3-O-glucoside when R1 = R2 = OH (Fernandes et al., 2014) (Reprinted with permission from the publisher)

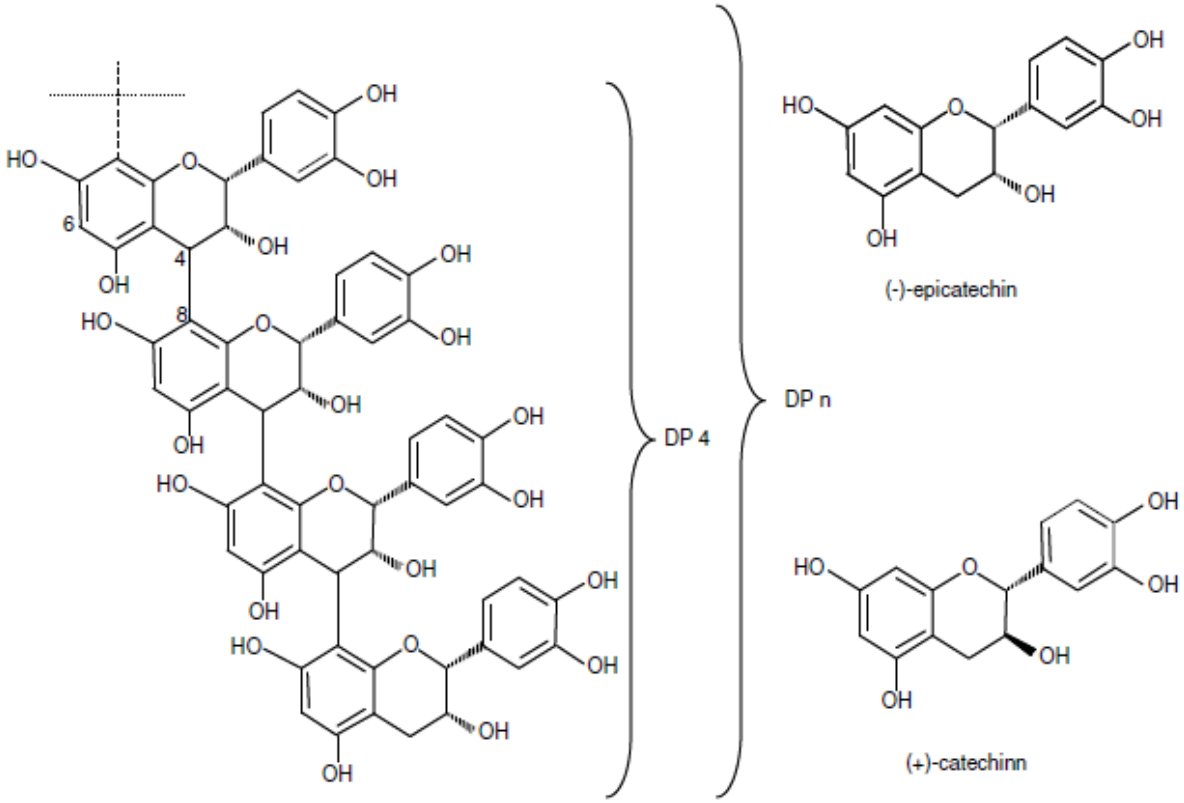


Figure 1c Chemical structure of procyanidins ((−)-epicatechin-based) and the flavan-3-ols units (Le Bourvellec et al., 2009) (Reprinted with permission from the publisher)

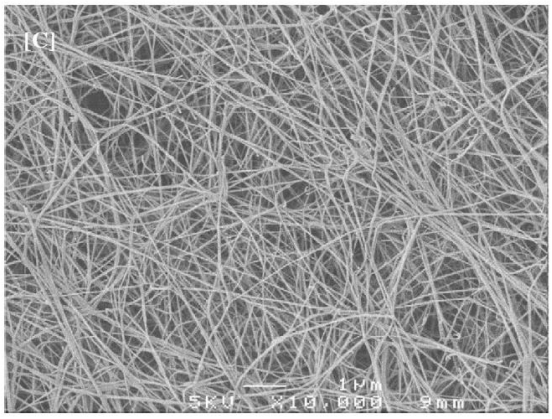


Figure 1d SEM (scanning electron microscopy) photograph of purified bacterial cellulose by NaOH (0.5 M), resembling plant cell wall cellulose (Phan et al., 2015) (Reprinted with permission from the publisher)

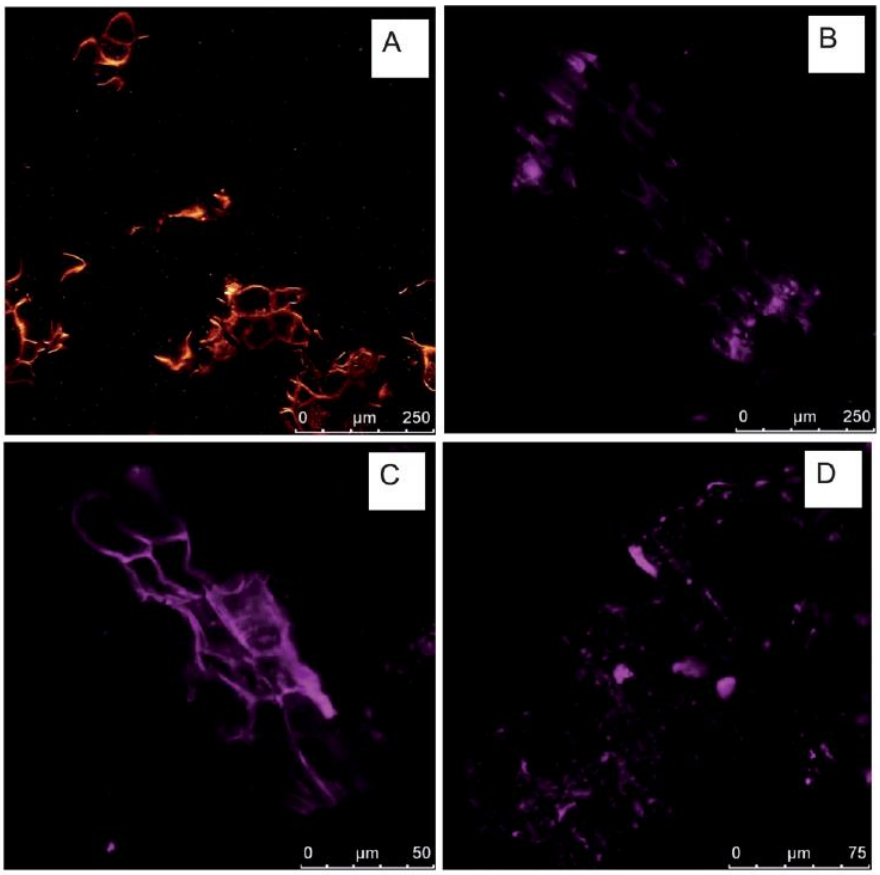


Figure 2a Confocal laser scanning microscopy of plant cell wall material of a blanched purple carrot puree; A, plant cell materials stained by congo red (0.02%); B and C, auto-fluorescence (purple) of anthocyanins bound to cell walls before *in vitro* gastric and small intestine digestion; D, auto-fluorescence (purple) of anthocyanins bound to cell walls after *in vitro* gastric and small intestine digestion (Padayachee et al., 2013) (Reprinted with permission from the publisher)

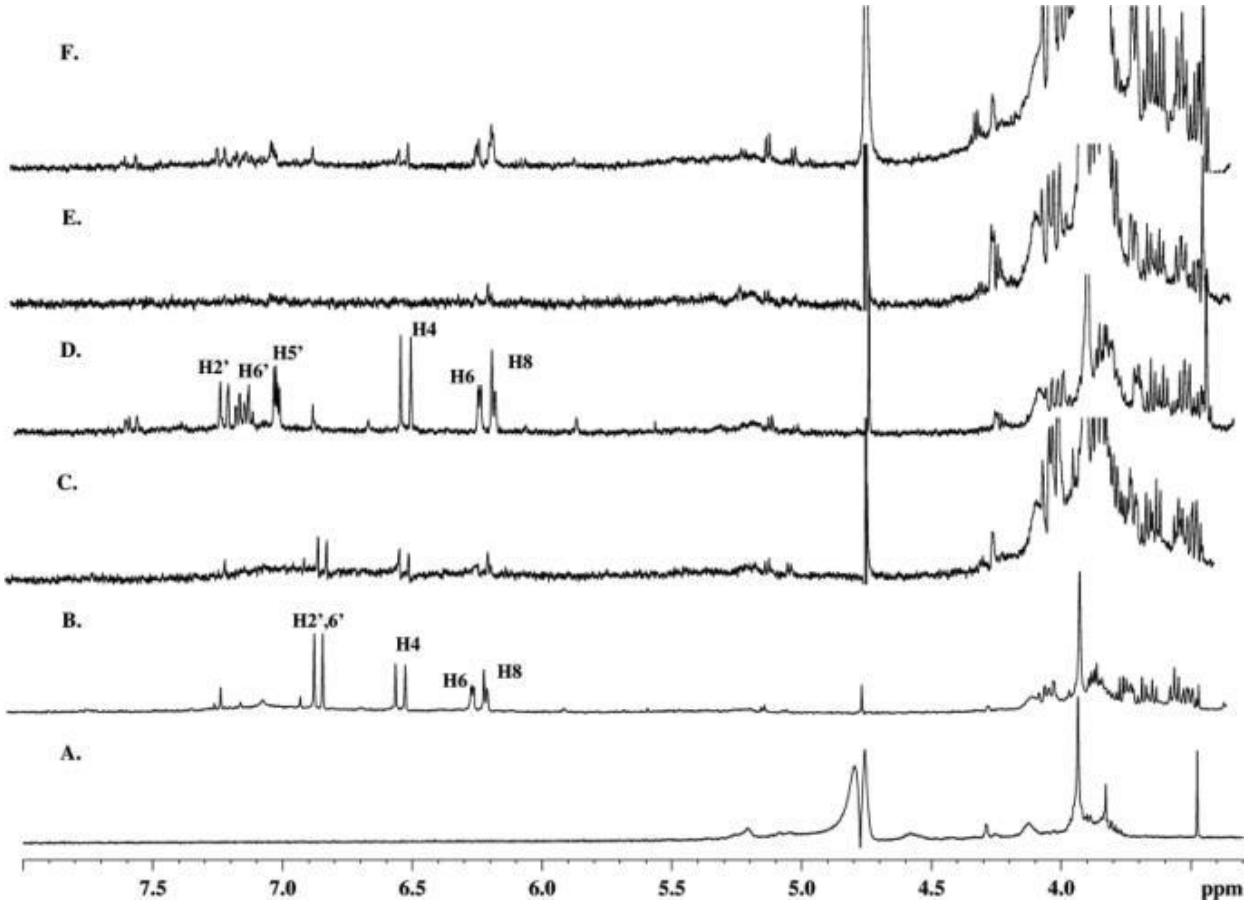


Figure 2b ^{1}H NMR spectra obtained in $D^{2}O$/DMSO (5%) at pH 4.0 and 313 K; A, pectin (4 μM); B, mixtures of pectin (4 μM) and delphinidin-3-*O*-glucoside (700 μM) or D (with cyanidin-3-*O*-glucoside); spectra C and E are the corresponding STD spectra of B and D, respectively; F is the STD spectrum of cyanidin-3-*O*-glucoside−pectin mixtures at a higher molar ratio of anthocyanin to pectin (Fernandes et al., 2014) (Reprinted with permission from the publisher)

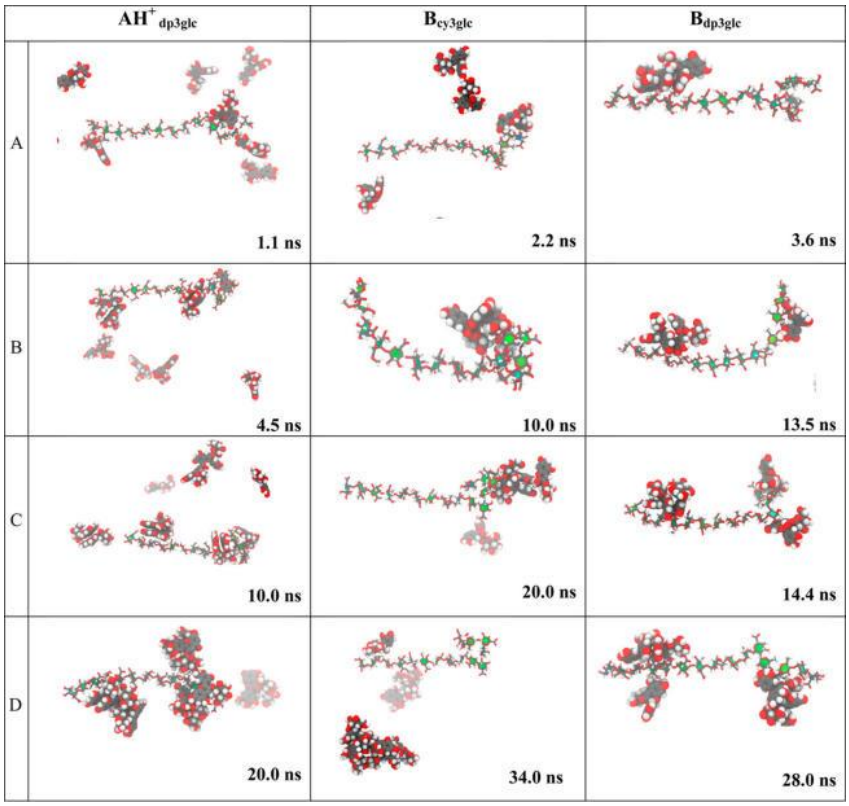


Figure 2c Representative geometries of anthocyanin-pectin systems along the course of each Molecular Dynamics simulation; cy3glc, cyanidin-3-O-glucoside; dp3glc, delphinidin-3-*O*-glucoside; AH^+, flavylium cation form of anthocyanin; B, hemiketal form of anthocyanin; the pectin and anthocyanin are described as sticks and with van der waals interactions; each panel represents an expansion of the total anthocyanin-pectin system (Fernandes et al., 2014) (Reprinted with permission from the publisher)

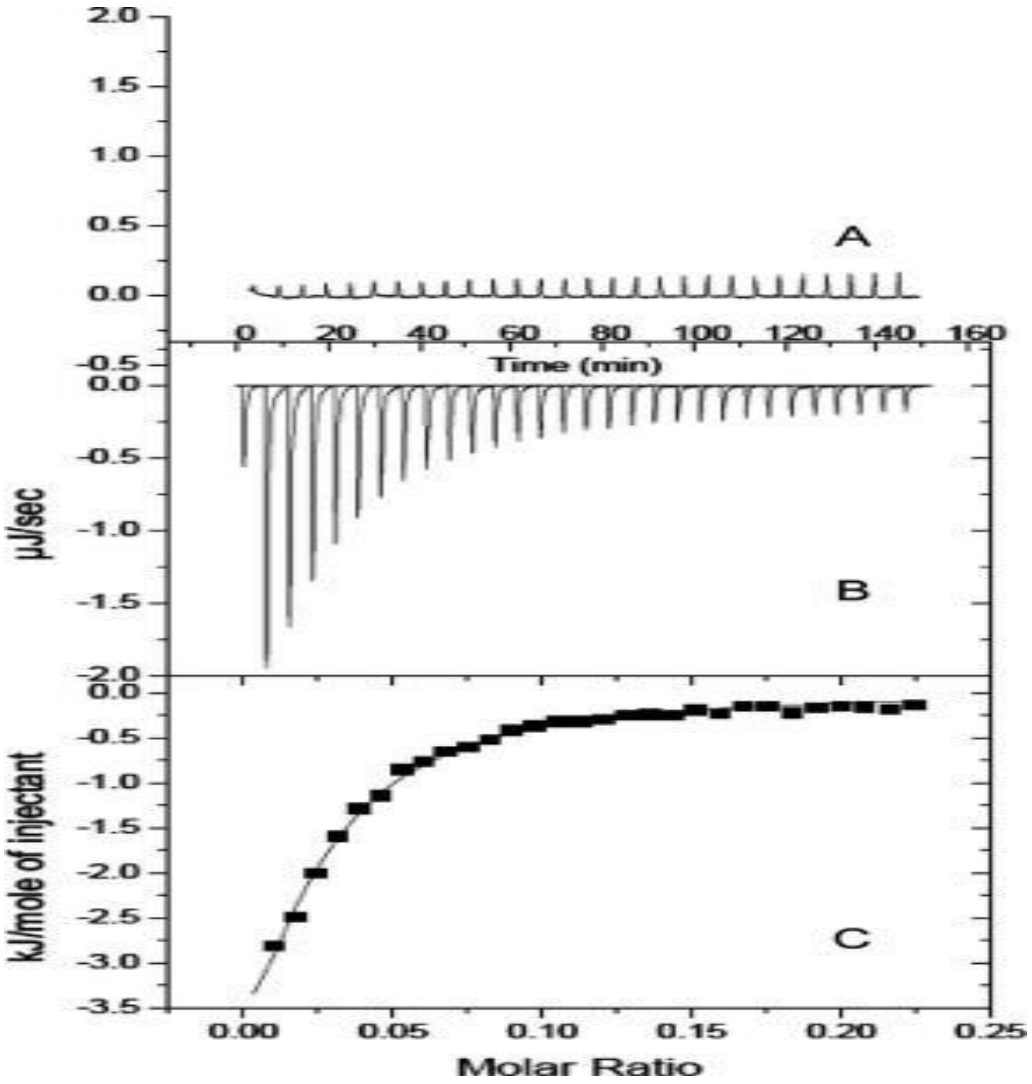


Figure 2d Isothermal titration calorimetry of procyanidin and apple cell wall interactions; A, blank injection of procyanidin fraction (DP 9) in buffer; B, raw plot of heat flow against time for the titration of procyanidin fraction (DP 9) into pectin solution; C, plot of enthalpy change against procyanidin/pectin ratio from integration of peak areas and normalization, the fitting curve is the thin curved line (Le Bourvellec et al., 2012) (Reprinted with permission from the publisher)

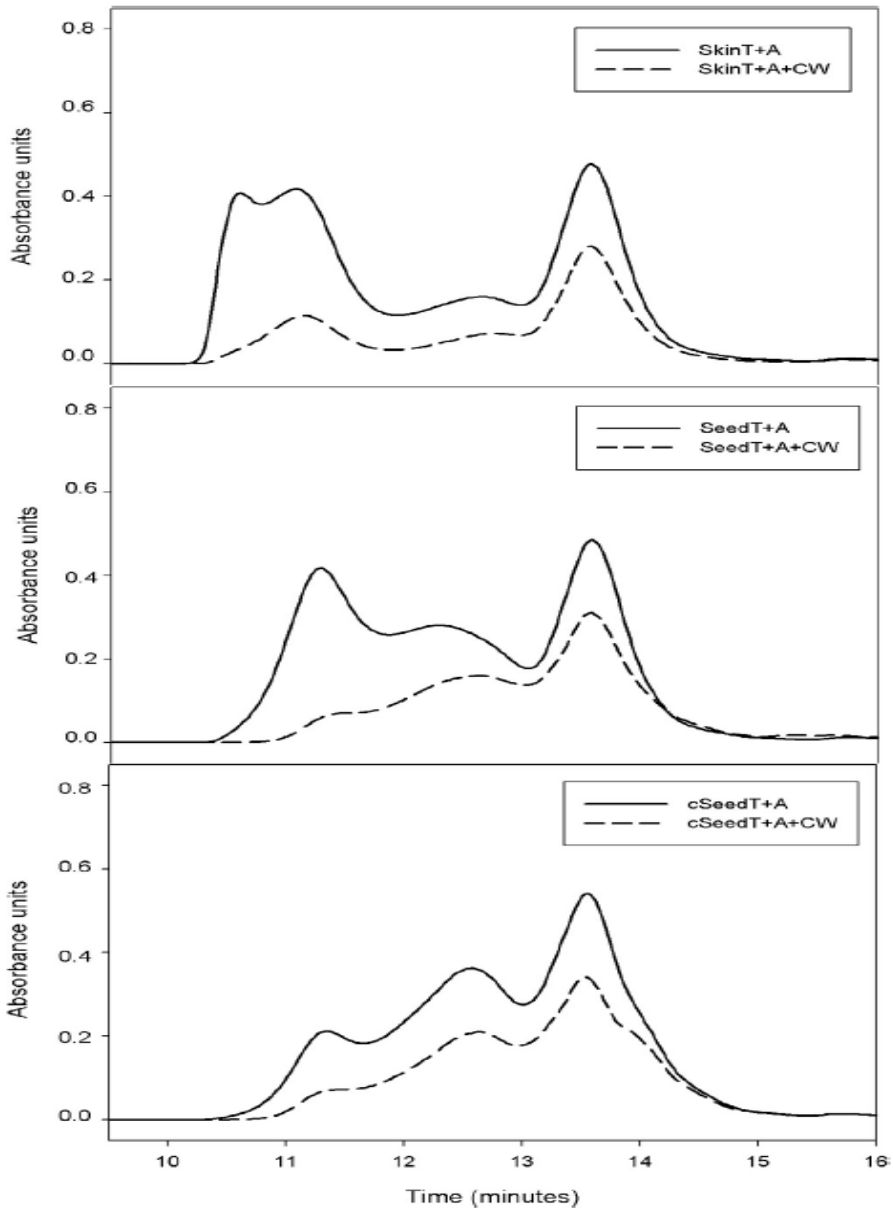


Figure 2e High-performance size-exclusion chromatography of anthocyanins (A) and tannins (T) of grape seeds/skins before and after interactions with cell walls (CW) in suspension (Bautista-Ortín et al., 2016) (Reprinted with permission from the publisher)

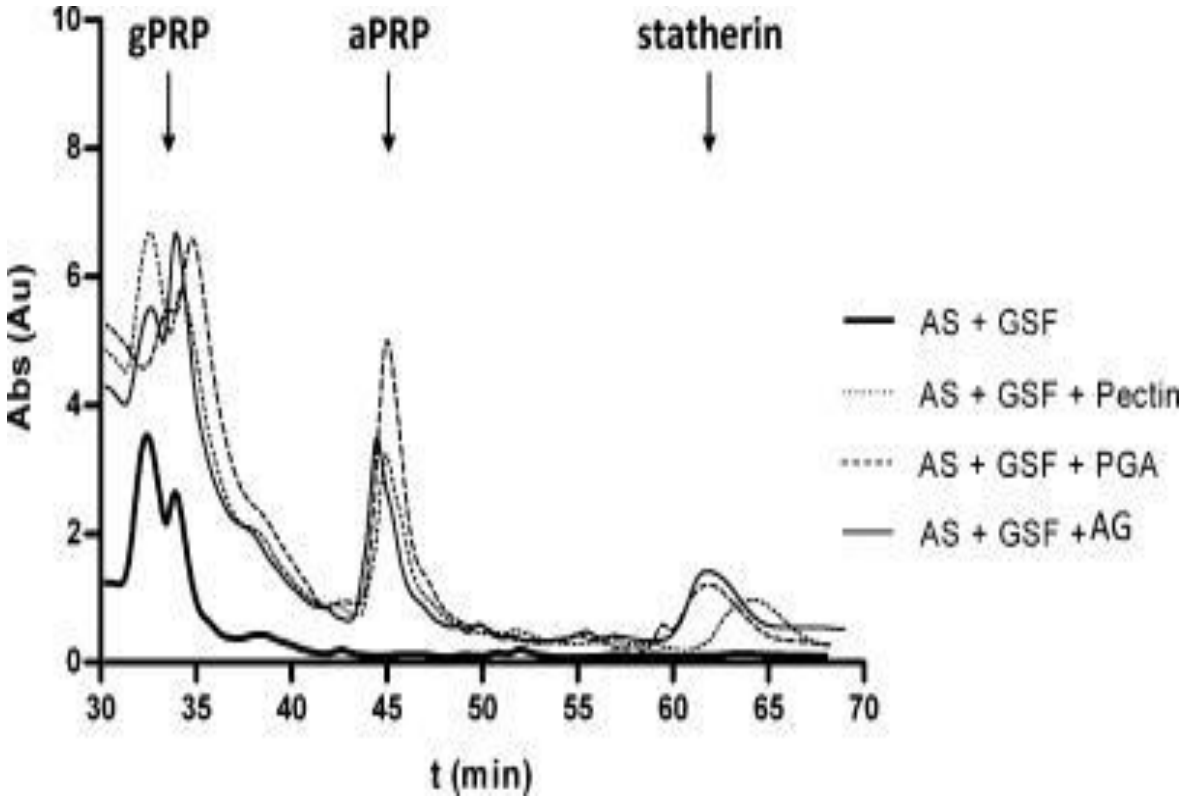


Figure 2f Parts of RP-HPLC (UV-vis detector) chromatograms of acidic saliva (AS) solution after the interactions with GSF in the absence and presence of some polysaccharides; AG, arabic gum; GSF, grape seed fraction (condensed tannins); PGA, polygalacturonic acid; gPRP, aPRP, and statherin represent different family regions of salivary proteins (Soares et al., 2012) (Reprinted with permission from the publisher)

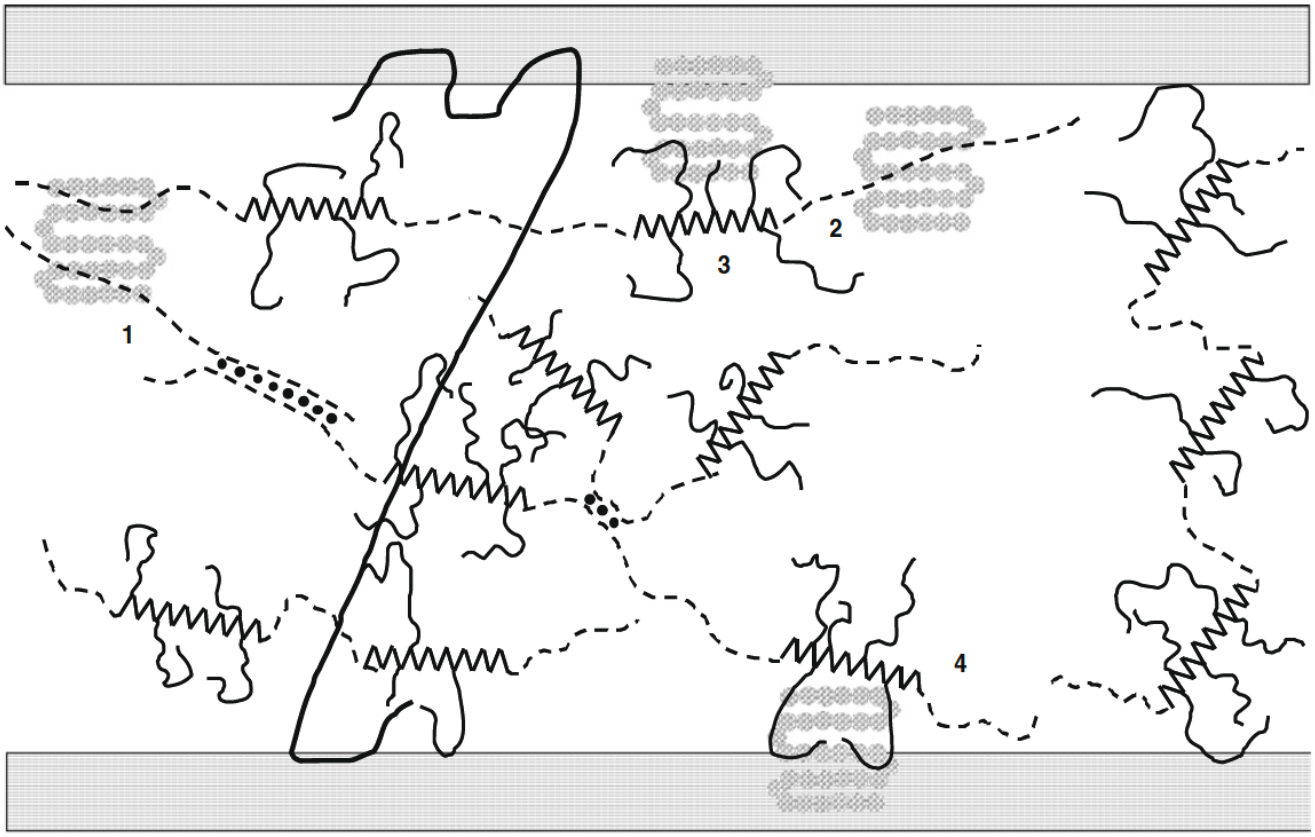


Figure 3a Possible binding sites of procyanidins with cell wall pectins. Shaded solid bands represents cellulose microfibrils, jagged line, rhamnogalacturonan; thin solid line, neutral sugar side chain; dotted line, homogalacturonan; thick solid line, xyloglucan; grey shaded chain, procyanidin. (1) highly methylated pectins soluble in chelating agent; (2) smooth regions of pectins degraded by pectin lyase; (3) hairy regions of pectins loose in cell walls and released by pectin lyase; (4) Reinforced interactions between cellulose and pectin neutral side chains (Le Bourvellec et al., 2009) (Reprinted with permission from the publisher)

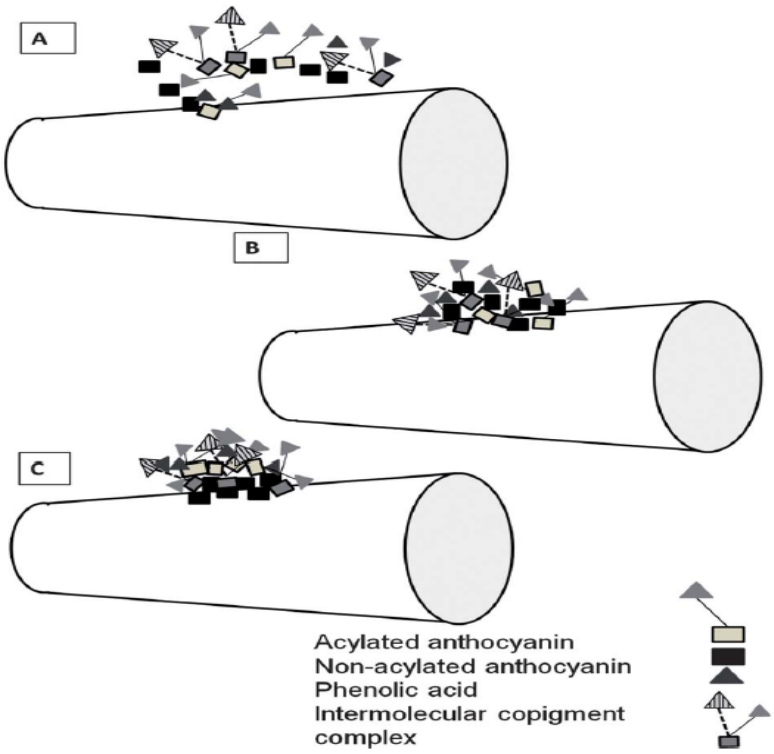


Figure 3b Proposed mechanism of "staining" cellulose microfibrils by anthocyanins and phenolic acids. (A) under-ordered adsorption of anthocyanins and phenolic acids onto the cellulose surface; (B) random agglomeration of anthocyanins and phenolic acids; (C) stacked and stabilised clusters of anthocyanins and phenolic acids, acylated anthocyanins have greater exposure to the solvent (Padayachee et al., 2013) (Reprinted with permission from the publisher)

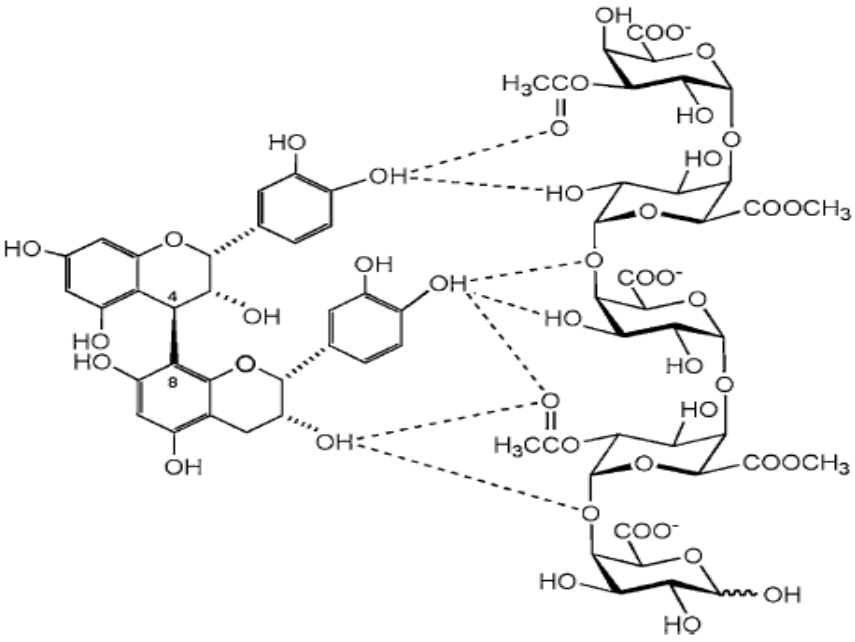


Figure 3c Possible hydrogen bonding between tannins and homogalacturonans grape during vinification (Hanlin et al., 2010) (Reprinted with permission from the publisher)

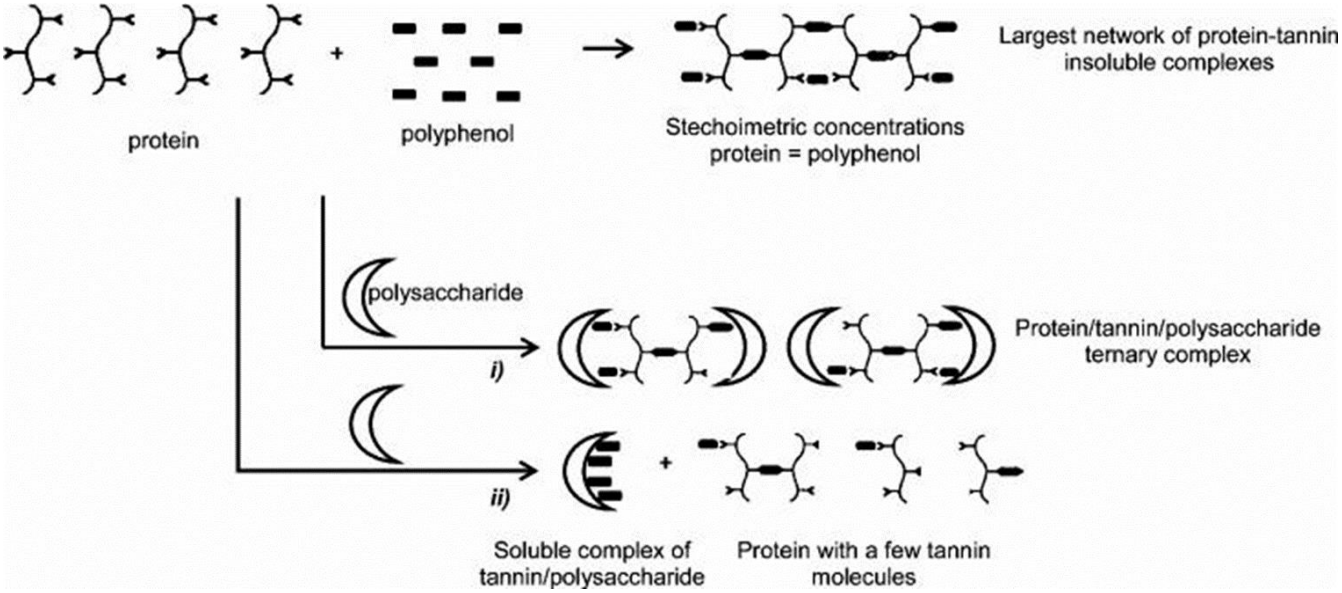


Figure 3d Two proposed mechanisms (i and ii) for the inhibition of the tannin-protein aggregation by polysaccharides (Soares et al.. 2009) (Reprinted with permission from the publisher)

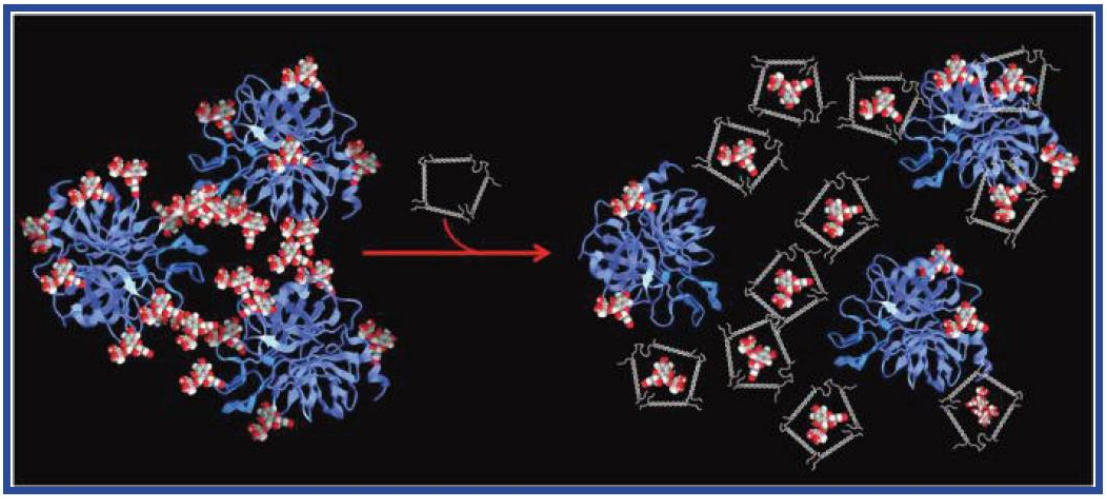


Figure 3e Model on the disruptive effect of xanthan polysaccharide on the procyanidin B3-trypsin association through competition; gray square, blue ribbon, and spheres represent xanthan polysaccharide, trypsin, and procyanidin B3, respectively (Gonçalves et al., 2011) (Reprinted with permission from the publisher)

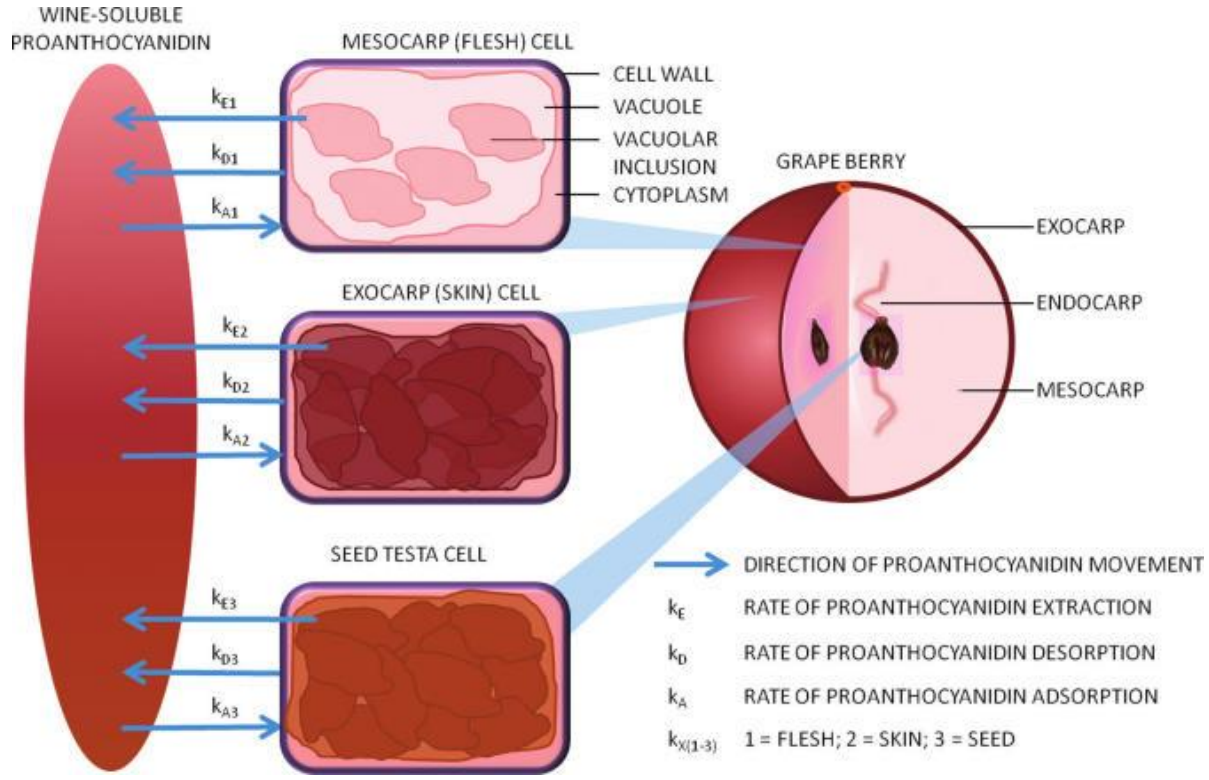


Figure 4a Model of proanthocyanidin extraction, adsorption, and desorption during grape vinification (Bindon et al., 2010b) (Reprinted with permission from the publisher)

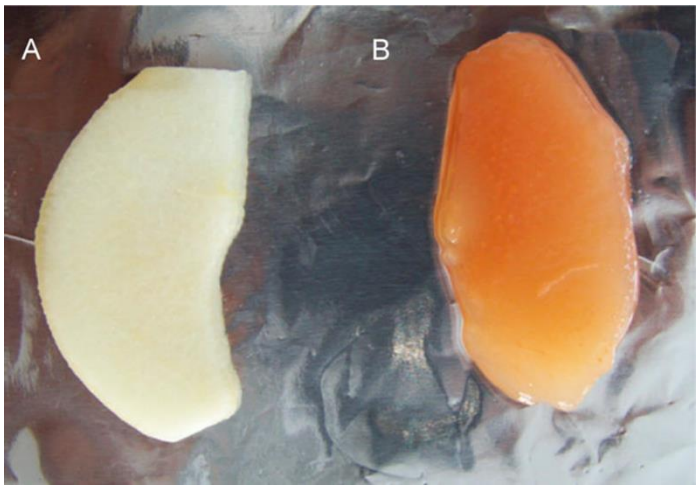


Figure 4b Fresh (A) and pink canned (B) pear slice (Le Bourvellec et al., 2013) (Reprinted with permission from the publisher)

Figure 4c Curcumin in the solution in the absence (right bottle) and presence of octenylsuccinate oat β-glucan (OSG) micelles (2.5 mg/mL) (left bottle) (Liu et al., 2013) (Reprinted with permission from the publisher)

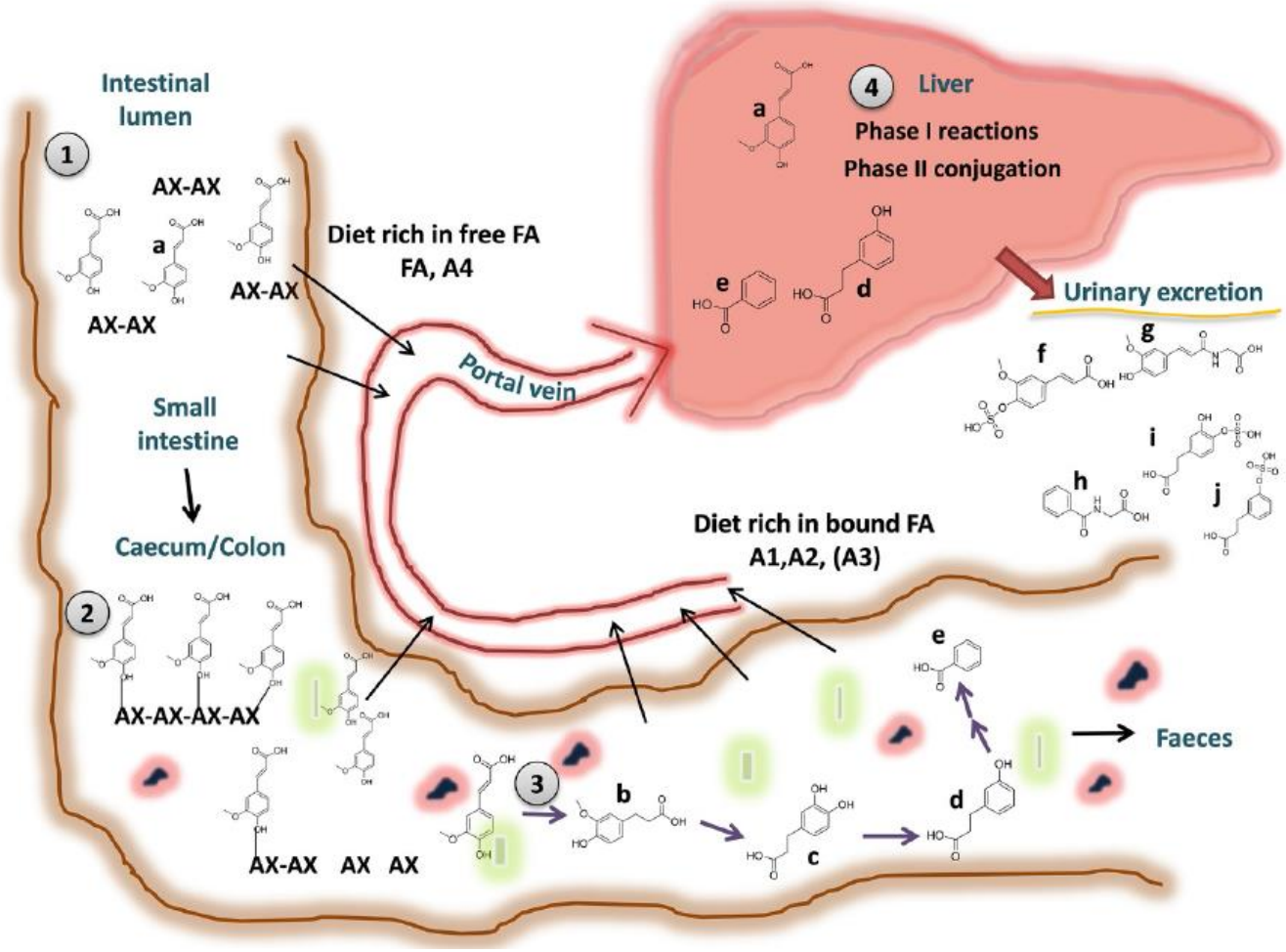


Figure 5 Schematic illustration of the metabolism of ferulic acids (FA) in wheat aleurone as affected by the cell wall polysaccharide (AX: arabinoxylan)-ferulic acid association. Free FA (a) is easily absorbed to portal vein from stomach and upper regions intestine. In contrast, bound FA with AX enter the large intestine for microbial fermentation. Diet rich in free FA was treated by enzymatic hydrolysis (xylanase and feruloyl esterase), diet A3 was treated with xylanase and A4 was treated with xylanase and feruloyl esterase; A1 is of intact wheat aleurone and A2 diet was ground (Pekkinen et al., 2014) (Open access and copyright free)