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1 **Interactions between heterogeneous cell walls and two procyanidins:**  
2 **Insights from the effects of chemical composition and physical**  
3 **structure**

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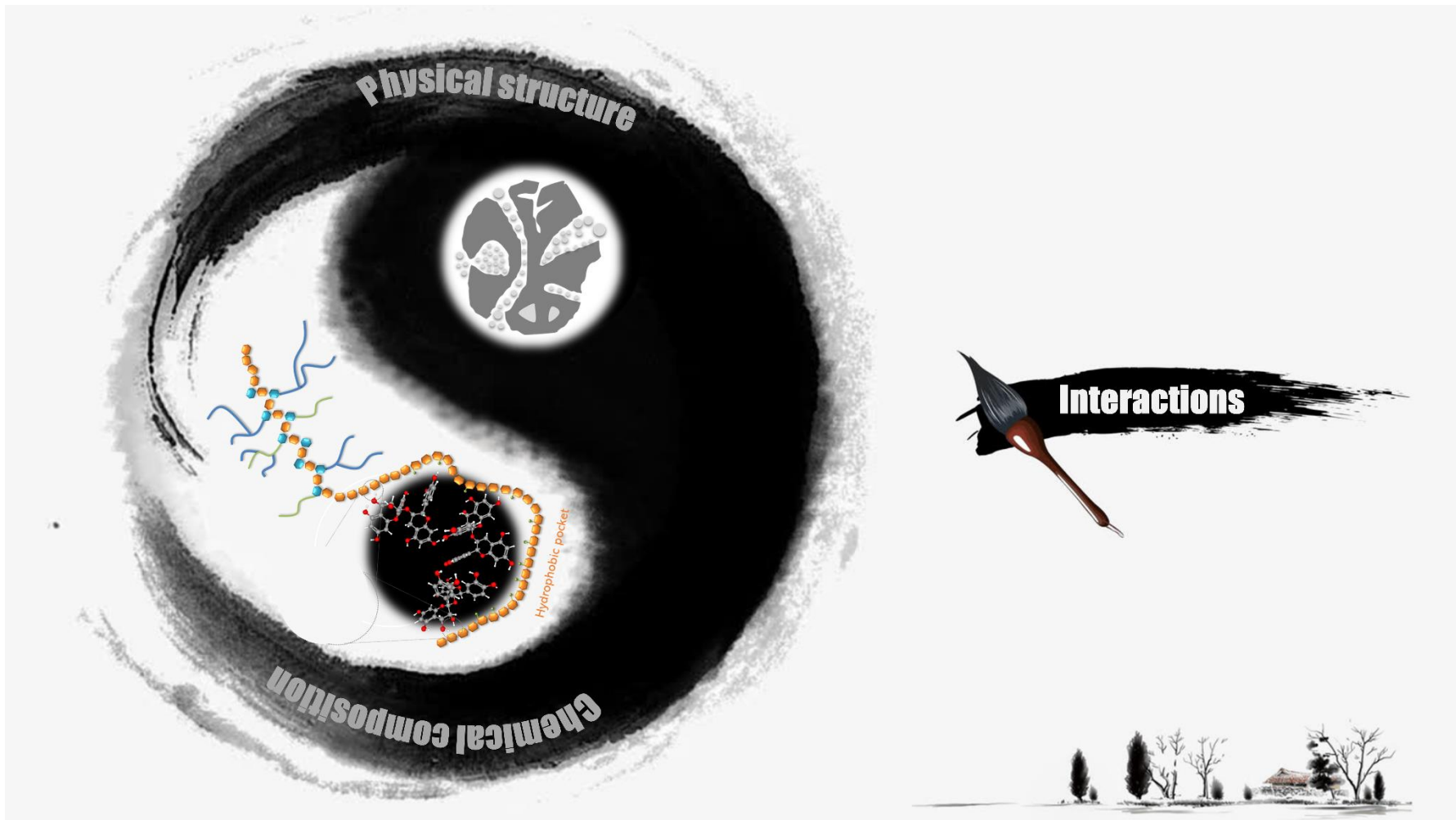
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21

## Abstract

22 Cell wall polysaccharides (CWPs) and phenolic substances, e.g., procyanidins,  
23 widely co-exist in fruit and vegetables and interact in complex patterns during  
24 chewing, food processing and *in vivo* digestion, impacting the food physicochemical  
25 and nutritional qualities. Interactions were characterized between two procyanidins  
26 and heterogeneous CWPs (four native and twelve modified) from apple, beet and  
27 kiwifruit presenting various chemical compositions and physical structures.  
28 ATR-FTIR discriminated the complexes from the initial purified procyanidins and  
29 CWPs. Langmuir isotherms and ITC indicated that native CWPs, from all botanical  
30 origins, had a higher affinity for procyanidins than the modified ones, which were all  
31 poorer in pectins. The CWPs that interact more with procyanidins were characterized  
32 by their high pectin content and linearity, and high porosity. Increasing the molecular  
33 size of procyanidins increased their complexation with CWPs. This work is an  
34 important guide to the encapsulation and controlled release of active compounds and  
35 the subsequent respective digestive behavior and human health.

36 **Keywords:** Polyphenols, Condensed tannins, Polysaccharides, Adsorptions, Porosity,  
37 Composition

### 38 Abbreviations:

39  $\overline{DP}_n$ : number-average degree of polymerization of procyanidins; DM; degree of  
40 methylation; AIS, Alcohol Insoluble Solids; ATR-FTIR, Attenuated Total Reflectance  
41 Fourier Transform Infrared Spectroscopy; Isothermal Titration Calorimetry, ITC.

## 42 1. Introduction

43 Cell walls and polyphenols are important components of the dietary fiber  
44 complexes in plant-based foods. They coexist in plants in a separated cell  
45 compartment (Renard, Watrelot, & Le Bourvellec, 2017). The interactions between  
46 cell walls and polyphenols may occur during chewing, pre-consumption food  
47 processing and subsequent digestion, which modify their structure and composition,  
48 thereby affecting their bioefficacy or modulating gut microbiota (Loo, Howell, Chan,  
49 Zhang, & Ng, 2020). There seems to be a lack of understanding of the interactions  
50 between the different components in the food matrix. This may limit the possibility of  
51 linking the specific components to the potential effects of diet, particularly the role of  
52 macromolecular polyphenols such as procyanidins. These procyanidins can  
53 spontaneously and quickly bind to the cell walls through hydrogen bonding or  
54 hydrophobic interaction (Liu, Le Bourvellec, & Renard, 2020; Renard, Baron, Guyot,  
55 & Drilleau, 2001; Renard et al., 2017). However, the knowledge of the influence of  
56 different types of cell walls (plant origin or processing modification) on the  
57 interactions is still lacking.

58 Plant cell walls are the extracellular matrices surrounding plant cells, which  
59 determine the texture of plant-based food (Carpita & Gibeaut, 1993). The dominant  
60 thin, hydrophilic and highly hydrated type I cell wall of fruit and vegetables (Waldron,  
61 Smith, Parr, Ng, & Parker, 1997) is one of the major source of dietary fiber in human  
62 food. It consists of a network of cellulose microfibrils, tethered by hemicelluloses

63 such as xyloglucans, xylans and mannans, embedded in an amorphous matrix  
64 constituted mostly of pectins. These cell walls are very diverse, depending on the  
65 composition, size distribution, shape, charge, extractability and combination of their  
66 constituent components. They are susceptible to physiochemical transformation  
67 reactions during food processing, thereby changing their structure and leading to  
68 changes in their functional characteristics. For example, textural alterations related to  
69 changes in cell wall porosity impact affinity for procyanidins (Le Bourvellec, Bouchet,  
70 & Renard, 2005). In addition, the presence of polyphenols can also change the  
71 functional properties of cell wall polysaccharides. For example, feruloylated  
72 arabinans can inhibit the softening of radishes during thermal processing (Li, Liu, Tu,  
73 Li, & Yan, 2019). The polysaccharide-polyphenol aggregation may decrease the  
74 viscosity of a polysaccharide solution (Tudorache & Bordenave, 2019). Therefore, the  
75 application of these interactions could improve the development of functional  
76 polysaccharides in the food industry.

77 Polyphenols are broadly distributed in fruit and vegetables, and have a positive  
78 effect on human health. Some unesterified phenolic acids and glucosylated  
79 monomeric polyphenols are easily absorbed in the upper digestive tract, while  
80 macromolecular polyphenols, e.g., procyanidins, are poorly absorbable (Santhakumar,  
81 Battino, & Alvarez-Suarez, 2018; Saura-Calixto & Pérez-Jiménez, 2018) and reach  
82 the colon. However, many studies have shown that the cell wall-polyphenol  
83 complexes can improve the metabolization of polyphenols in the colon (Le  
84 Bourvellec et al., 2019; Loo et al., 2020; Phan et al., 2020; Tarko & Duda-Chodak,

85 [2020](#)). Microbiological enzymes in the colon can promote the metabolism of the  
86 polyphenols from the complexes, and further bioprocess them from a non-absorbable  
87 form to a bioavailable one. Simultaneously, the interactions between polyphenols and  
88 cell walls and commensal microorganisms during the digestion process can adjust the  
89 balance between the growth of beneficial bacteria and pathogenic microorganisms  
90 ([Dobson et al., 2019](#)). A comprehensive understanding of the cell  
91 wall-macromolecular polyphenol interactions may enable food manufacturers to take  
92 full advantage of these beneficial effects.

93 Cell wall-procyanidin interactions can be mediated by their morphology,  
94 chemical composition and molecular architecture, that is, their porosity, the  
95 characteristics of their constitutive pectins, such as side-chains and branching ratios,  
96 molar mass, degree of esterification, functional groups, and conformation ([Liu et al.,  
97 2020; Liu, Renard, Rolland-Sabaté, & Le Bourvellec, 2021](#)). Cell walls with high  
98 pectin contents have higher affinities for procyanidins, as in the case of pear tissues  
99 where the affinity decreases in the order: parenchyma cells > mesocarp (i.e., flesh)  
100 hypanthium > stone cells > epidermis ([Brahem, Renard, Bureau, Watrelot, & Le  
101 Bourvellec, 2019](#)). Cell walls are complex and composed of heterogeneous polymers,  
102 of which, pectins have the highest affinity for procyanidins ([Le Bourvellec & Renard,  
103 2005; Le Bourvellec, Watrelot, Ginies, Imbert, & Renard, 2012](#)). Moreover, high  
104 affinities are typically observed between highly methylated pectins and highly  
105 polymerized procyanidins ([Watrelot, Le Bourvellec, Imbert, & Renard, 2013](#)).  
106 Concerning branching of pectins, the general rule can be summarized as follows: the

107 more linear the structure and the less branching areas of pectins in the cell walls, the  
108 better their association with procyanidins (P. A. R. Fernandes et al., 2020; Liu, Renard,  
109 Rolland-Sabaté, & Le Bourvellec, 2021; Watrelot, Le Bourvellec, Imberty, & Renard,  
110 2014). This result also applies to the interaction with anthocyanins (Koh, Xu, &  
111 Wicker, 2020). For polyphenols, highly polymeric procyanidins with more hydroxyl  
112 and aryl rings bind more tightly to cell walls (Bindon, Smith, & Kennedy, 2010;  
113 Renard et al., 2017; Tang, Covington, & Hancock, 2003). However, what happens  
114 when these influencing factors are placed in the same cell wall-procyanidin  
115 interaction system? Do they act as antagonists or synergists? This still remains to be  
116 elucidated.

117 Although the interactions between the cell walls and procyanidins have been the  
118 focus of previous research (Brahem et al., 2019; Le Bourvellec et al., 2005; Le  
119 Bourvellec, Guyot, & Renard, 2004; Le Bourvellec & Renard, 2005; Renard et al.,  
120 2001), to date, there does not appear to be systematic interaction studies which  
121 investigate the impact of botanical origins, processing and maturity modifications and  
122 evolutions of cell walls in the establishment of cell wall-procyanidin associations. We  
123 are now interested in identifying the cell wall factors, that is, their spread and  
124 diversity of structures and composition, that may influence their interactions with  
125 procyanidins. In the current work, we examined the capacity of sixteen cell walls,  
126 from apple, beet and kiwifruit at two maturity stages (ripe and overripe) differing in  
127 both their physical and chemical characteristics to adsorb procyanidins. Interactions  
128 were quantified via Langmuir isotherms, as well as isothermal titration calorimetry to



129 measure thermodynamic changes caused by non-covalent binding. A better  
130 understanding of the interactions between plant cell wall polysaccharides and  
131 polyphenols (especially procyanidins, important macromolecular antioxidants) is  
132 crucial for the development of plant-based food industry.

## 133 **2. Material and methods**

### 134 **2.1. Standard**

135 Sugar standards (arabinose, mannose, fucose, xylose, rhamnose, and galactose)  
136 and phloridzin were purchased from Fluka (Buchs, Switzerland). Methanol-d<sub>3</sub> was  
137 from Acros Organics (Geel, Belgium). 4-Coumaric acid was provided from Extra  
138 synthese (Lyon, France). All other reagents and solvents were of analytical grade.

### 139 **2.2. Procyanidins extraction, purification and analysis**

140 Procyanidins of DP12 and DP39 were prepared from apple fruits (*Malus ×*  
141 *domestica* Borkh.) of the ‘Marie Menard’ and ‘Avrolles’ cider cultivars, respectively,  
142 as described in [Liu, Renard, Rolland-Sabaté, Bureau, & Le Bourvellec \(2021\)](#). Briefly,  
143 this included extraction by aqueous acetone from the freeze-dried apple powders after  
144 washing by hexane and methanol, purification on a LiChrospher 100 RP-18 (12 µm,  
145 Merck, Darmstadt, Germany) column, concentration and storage under vacuum at  
146 -80 °C.

147 Purified procyanidins were characterized by high-performance liquid  
148 chromatography with diode array detection (with/without thioacidolysis) on a

149 Shimadzu Prominence system (Kyoto, Japan) as described in principle by [Guyot,](#)  
150 [Marnet, Sanoner, & Drilleau \(2001\)](#). The separation condition were as described by  
151 [Le Bourvellec et al. \(2011\)](#).

### 152 **2.3. Preparation and characterization of cell wall polysaccharides**

153 Sixteen cell walls were prepared as described by [Liu, Renard, Rolland-Sabaté,](#)  
154 [Bureau, et al. \(2021\)](#). In brief, Alcohol-Insoluble Solids (AIS) were first prepared  
155 from apple, beet and kiwifruit (ripe and overripe) parenchyma, and named native  
156 apple cell wall (ACN), native beet cell wall (BCN), native kiwifruit cell wall ripe  
157 (KCRN), native kiwifruit cell wall overripe (KCON). Then the four native cell walls  
158 modified by heating in a citrate-phosphate solution (0.1 mol/L) at pH 2.0, 3.5 or 6.0,  
159 and the insoluble cell wall residues were retained; the extracted pectins have been  
160 used in another study ([Liu, Renard, Rolland-Sabaté, & Le Bourvellec, 2021](#)). The  
161 modified insoluble cell wall residues were used for the following experiments, i.e.,  
162 apple cell walls modified at pH 2.0/3.5/6.0 (named AC2/3/6, respectively), beet cell  
163 walls modified at pH 2.0/3.5/6.0 (named BC2/3/6, respectively), kiwifruit cell walls  
164 ripe modified at pH 2.0/3.5/6.0 (named KCR2/3/6, respectively) and kiwifruit cell  
165 walls overripe modified at pH 2.0/3.5/6.0 (named KCO2/3/6, respectively).

166 Cell wall compositions were analyzed as described by [Liu, Renard,](#)  
167 [Rolland-Sabaté, Bureau, et al. \(2021\)](#). Approximately 10 mg of cell walls were  
168 prehydrolyzed with 72% sulfuric acid (250 µL) for 1 h at room temperature ([Saeman,](#)  
169 [Moore, Mitchell, & Millett, 1954](#)). Neutral sugars were analyzed as alditol acetates

170 (Englyst, Wiggins, & Cummings, 1982) by GC-FID HP 5890 Series II (Agilent, Inc.,  
171 Palo Alto, USA). Galacturonic acid was measured by the meta-hydroxyl-diphenyl  
172 assay using a spectrophotometer (V-530 Jasco, Tokyo, Japan). The methanol content  
173 was measured by stable isotope dilution assay using headspace-GC-MS (QP2010  
174 Shimadzu, Kyoto, Japan) after saponification. The acetic acid content was measured  
175 by the acetic acid assay kit (K-ACET, ACS Manual Format, Megazyme International,  
176 Ireland). Ferulic acid was released by saponification according to the method of  
177 Micard, Renard, & Thibault (1994) by spectrophotometry (V-530Jasco, Tokyo,  
178 Japan).

179 Brunauer-Emmett-Teller (BET) isotherms (Brunauer, Emmett, & Teller, 1938)  
180 were used to determine the surface area of the cell walls by nitrogen adsorption  
181 isotherms at -196 °C using the Micromeritics AZAP 2010 system and monitored by  
182 AZAP 2010 version 5.01 (Micromeritics, Norcross, GA, USA).

183 The water-binding capacity was measured by filtration method using  
184 approximately 250 mg (dry weight) of cell walls left to soak with 10 mL of water  
185 containing NaN<sub>3</sub> (1 g/l) for 1 h at room temperature, then filtered and dried (103°C,  
186 overnight). The water binding capacity (WBC) was calculated as follows:

$$187 \text{ WBC (g/g)} = (M2 - M3) / (M3 - M1) \times 100 \% . \quad (\text{Eq. 1})$$

188 With M1: weight of filter, M2 total weight after filtration and M3 after drying. Each  
189 sample was analyzed in triplicate.

#### 190 **2.4. Procyanidin-cell wall interactions**

#### 191 2.4.1. Binding isotherm methodology

192 The experiments were performed (duplicate) at 25 °C using buffer  
193 (citrate/phosphate system, pH 3.8, ionic strength 0.1 mol/L) according to [Renard et al.](#)  
194 [\(2001\)](#). The procyanidin solution (from 0.25 to 12 g/L) and the cell wall suspension (5  
195 mg/mL) were incubated under agitation in 8 mL empty Sep-pack preparation columns.  
196 After incubation, the free procyanidins solution and the cell wall-procyanidin  
197 complexes were isolated by filtering (20 µm porosity). The free procyanidins content  
198 was measured by spectroscopy at 280 nm (JASCO V-730 UV-visible  
199 spectrophotometer, Tokyo, Japan). Adsorbed procyanidins were measured by  
200 deducting the amount in the supernatant from that in the initial procyanidin solution.  
201 The average degree of polymerization of procyanidins ( $\overline{DP}_n$ ) was calculated as the  
202 molar ratio of all flavan-3-ol units (thioether adduct plus terminal units minus  
203 (+)-catechin and (-)-epicatechin naturally present in the samples and determined by  
204 analysis of the samples by HPLC-DAD with and without thiolysis) to (+)-catechin  
205 and (-)-epicatechin corresponding to the terminal units minus (+)-catechin and  
206 (-)-epicatechin naturally present in the samples and determined by analysis of the  
207 samples by HPLC-DAD with and without thiolysis.

208 The binding isotherms were interpreted according to the type-I Langmuir  
209 approach ([Renard et al., 2001](#)), which expresses bound solute (procyanidins)  $PP_b$  (in  
210 g/g of adsorbent) as a function of the free solute (procyanidins) concentration  $[PP_f]$  at  
211 equilibrium.

212 
$$PP_b = \frac{N_{\max}K_L[PP_f]}{1+K_L[PP_f]} \quad (\text{Eq. 2})$$

213 where  $N_{\max}$  is the total amount of available binding sites (expressed in g/g  
214 adsorbent) and  $K_L$  is an apparent affinity constant (in L/g).

#### 215 **2.4.2. Isothermal Titration Calorimetry (ITC)**

216 The thermodynamic parameters changes caused by the cell wall-procyanidin  
217 interactions were measured by ITC using TAM III microcalorimeter (TA instruments,  
218 USA) as describe by [Liu, Renard, Rolland-Sabaté, & Le Bourvellec \(2021\)](#). In brief,  
219 purified procyanidins (30 mmol/L in (-)-epicatechin equivalent) and cell walls (ca. 8  
220 mg) were dissolved and suspended, respectively, in the same citrate/phosphate buffer  
221 pH 3.8, ionic strength 0.1 mol/L. The procyanidin was titrated into the sample cell by  
222 50 injections of 5  $\mu$ L; each injection lasted 5 s, with separating delay of 900 s for  
223 return to horizontal baseline. The content of the sample cell was stirred throughout the  
224 experiment at 90 rev/min. Blanks (titration of procyanidin fractions into  
225 citrate/phosphate buffer) are deducted from sample titration experiments. Experiments  
226 were performed in duplicates. All errors shown in the paper are based on the accuracy  
227 of the data fitting.

#### 228 **2.4.3. ATR-FTIR spectra**

229 The cell wall samples retained by filtration after the binding isotherm experiment  
230 were further analyzed by ATR-FTIR. Cell wall-procyanidin complexes were selected  
231 at four concentrations in binding isotherm experiment, i.e., the initial procyanidin  
232 concentrations added were 0.25, 1, 6 and 12 g/l. The ATR-FTIR spectra of cell walls,

233 procyanidins and freeze-dried complexes were recorded in triplicate (16 scans per  
234 spectrum, 4000 to 600  $\text{cm}^{-1}$ ) using a Tensor 27 FT-IR spectrometer (Brucker Optics,  
235 Wissembourg, France) equipped with a single-reflectance horizontal ATR cell  
236 (Golden Gate equipped with diamond crystal, Bruker Optics). Spectra were analyzed  
237 and controlled by OPUS software Version 5.0 as described by [Liu, Renard,](#)  
238 [Rolland-Sabaté, Bureau, et al. \(2021\)](#).

## 239 **2.5. Statistical analysis**

240 Results were expressed as mean values, and their reproducibility presented as the  
241 pooled standard deviation (Pooled SD) ([Box, Hunter, & Hunter, 1978](#)). MATLAB 7.5  
242 software (Mathworks Inc., Natick, MA, USA) with SAISIR package ([Cordella &](#)  
243 [Bertrand, 2014](#)) was used for pre-processing (baseline correction and standard normal  
244 variate) and Principal Component Analysis (PCA). Calculated and observed Langmuir  
245 curves were fitted by minimizing the sum of the square of the difference between  
246 observed and calculated values to obtain the Langmuir parameters ( $K_L$  and  $N_{\max}$ )  
247 using the solver package of Microsoft Excel. Moreover, the confidence intervals  
248 ( $P > 0.95$ ) of the Langmuir formula's constants were calculated using the Marquardt  
249 estimation method of the non-linear regression package of XLSTAT (version 2020.1.1,  
250 Addtionsoft SARL, Paris, France). Heatmap was performed with Python 3.5 software  
251 using the Seaborn package ([Waskom, 2014](#)).

## 252 **3. Results**

### 253 **3.1. Composition and structure of the macromolecules and cell wall complexes**

### 254 **3.1.1. Procyanidin fractions**

255 The two apple varieties, i.e., ‘Marie Ménéard’ and ‘Avrolles’, were selected to  
256 obtain two procyanidins with different degree of polymerization (Le Bourvellec,  
257 Guyot, & Renard, 2009). The purified extracts contained about 850 mg/g of phenolic  
258 compounds, mainly procyanidins plus traces of other phenolic compounds  
259 (Supplementary Table 1). ‘Marie Ménéard’ and ‘Avrolles’ procyanidins were composed  
260 of more than 99 % (-)-epicatechin units and differed by their degree of polymerization  
261 ( $\overline{DP}_n = 12$  and 39, respectively). All the results were in accordance with (Le  
262 Bourvellec et al., 2012; Liu, Renard, Rolland-Sabaté, & Le Bourvellec, 2021).

### 263 **3.1.2. Cell wall polysaccharides**

264 Detailed chemical compositions of the cell walls are available in our previous  
265 paper (Liu, Renard, Rolland-Sabaté, Bureau, et al., 2021) and the sugar ratios based  
266 on the sugar content, specific surface area and water binding capacity for cell walls  
267 were calculated (Table 1). A large diversity was obtained on two major parameters,  
268 namely (i) the nature and levels of cell wall polymers and (ii) physical characteristics,  
269 which further varied independently in this series of cell walls. Concerning the  
270 chemical composition and physical structure, apple cell walls were characterized by  
271 high xylose content, signaling presence of xylogalacturonans and  
272 fucogalactoxyloglucans (Le Bourvellec et al., 2004; Renard, Voragen, Thibault, &  
273 Pilnik, 1990, 1991), with intermediate specific surface area and relatively high  
274 water-binding capacity. Only beet cell walls contained detectable ferulic acids. They

275 also had the highest content of pectic neutral sugar side-chains, notably arabinans, and  
276 the highest acetyl contents, with a high specific surface area. Kiwifruit cell walls  
277 appeared to be the richest in homogalacturonans (conservely, contained pectins with  
278 the highest linearity), with the lowest side-chain, arabinose/galactose ratio and acetyl  
279 content, with a relatively low specific surface area.

280 All these characteristics were further modulated after processing at different pH  
281 values. For example, at pH 2.0, arabinan or galactan side-chains in the cell walls were  
282 lost due to acid hydrolysis, while galacturonic acid and DM were retained to the  
283 greatest extent possible.  $\beta$ -elimination reduced the content of galacturonic acid and  
284 esterification (DM and Ac.A) in the cell walls after modification at pH 6.0. Pectin  
285 depolymerization was least significant and structural modification modest (both acid  
286 hydrolysis and  $\beta$ -elimination) at pH 3.5. Different pH modifications enhanced or  
287 reduced, to varying degrees, the porosity/specific surface area and water-binding  
288 capacity of the cell walls. The ferulic acid cross-linked to the cell wall was present  
289 only in the beet cell wall.

290 Therefore, a large diversity of structural features was obtained in the cell wall set  
291 for structure linearity, branching degree and length of side-chains, arabinose/galactose  
292 ratio, degree of methylation and acetylation, specific surface area and water-binding  
293 capacity. In addition, these factors, which were assigned to the cell wall to influence  
294 their interactions, showed varying degrees of importance.

### 295 **3.2. Global characterization by ATR-FTIR spectroscopy**



296 ATR-FTIR can be used to detect changes in the main components of cell wall  
297 polysaccharides as well as their modification by other components (Liu, Renard,  
298 Bureau, & Bourvellec, 2021). On the principal component analysis, the first two  
299 principal components (PC1 48.5 % and PC2 22.7 %) explained 71.2% of the total  
300 variance (Fig. 1A). Cell walls, cell wall-procyanidin DP12/39 complexes and  
301 procyanidins were separated into three distinct groups. Within the cell  
302 wall-procyanidin group, complexes constituted two distinct subgroups: cell wall  
303 associated with a low procyanidin concentration (0.25 g/l and 1 g/l) and a high  
304 procyanidin concentration (6 g/l and 12 g/l). A gradation of the groups was observed  
305 along the PC1 from the left to the right with the increasing of procyanidin  
306 concentration.

307 No clear discrimination according to botanical origins, processing conditions or  
308 procyanidin sizes was obtained. The loadings on PC1 and PC2 revealed similar  
309 patterns and some relevant wavenumbers (Fig. 1B). Along the PC1 axis, the samples  
310 were distributed owing to the presence or absence of procyanidins (Fig. 1A). PC1  
311 separated cell walls on the left bottom, cell wall-procyanidin complexes in the middle  
312 and purified procyanidins on the right bottom according to the presence of linked  
313 procyanidins or/and purified cell wall/procyanidin complexes. The negative loadings  
314 of PC1 were characterized by the peaks at 1740 and 1015  $\text{cm}^{-1}$  which could be due to  
315 pectic compounds. Positive loadings for PC1 concerned wavenumbers mostly  
316 characteristic of procyanidins (1604, 1519, 1440, 1284 and 1196  $\text{cm}^{-1}$ ), which  
317 progressively increased from 0.25 g/l to 12 g/l. PC2 allowed discrimination between

318 apple/beet cell wall-procyanidin complexes and kiwifruit cell wall-procyanidin  
319 complexes in the middle of plot (Fig. 1A).

### 320 **3.3. Binding isotherms**

321 The binding isotherms of all cell wall-procyanidin complexes (DP12 and DP39)  
322 were obtained by placing the suspended cell walls in contact with procyanidin  
323 solutions. The isotherms are illustrated by the Langmuir formulations (Eq. (2)) in Fig.  
324 2 and the Langmuir parameters ( $K_L$  and  $N_{max}$ ) were calculated (Table 2), both in the  
325 form of per g or per  $m^2$  of adsorbent. The data were fitted satisfactorily ( $r^2 > 0.85$ )  
326 using the Langmuir isotherm formula. The amount of adsorbed procyanidins  
327 increased with their concentration and finally reached a plateau at high concentrations  
328 indicating cell wall saturation (Fig. 2).

#### 329 **3.3.1. Interactions with Procyanidins of DP 12**

330 Isotherm curves varied depending on the composition and the structure of the cell  
331 walls (Fig. 2). For the isothermal adsorption curve of procyanidin DP12, beet cell  
332 walls had the highest saturation level (plateau of the curve) of bound procyanidins and  
333 the highest affinity (slope of the curve) together with apple cell walls. Moreover, after  
334 processing, two distinct changes could be noted between chemical compositions and  
335 physical surface morphology for different cell walls: ACs, BC2/3 and KCOs cell  
336 walls were characterized by a decrease of bound procyanidin after modification. This  
337 may be attributed to the loss of pectins from the native cell walls. By contrast, affinity  
338 increased for KCOs and BC6, which may be due to an increase of their specific

339 surface area after processing, thus increasing their adsorption capacity for the  
340 procyanidin DP12. The Langmuir constants ( $K_L$  and  $N_{max}$ ) obtained after fitting  
341 confirmed these results (Table 2A).

342 The different chemical compositions and physical surface morphologies observed  
343 between botanical origins and after processing had an impact on both apparent affinity  
344 ( $K_L$ ) and apparent saturation level ( $N_{max}$ ). ACN, BC3, KCRN, KCR6 and KCON/2/3  
345 had close  $K_L$  values between 0.21 L/g and 0.29 L/g, while their apparent saturation  
346 level ( $N_{max}$ ) ranged from 0.39 to 0.75 g/g. These could be due to two contrasting  
347 observations: (i) either all their features were at an intermediate level, e.g., ACN with  
348 moderate pectin linearity (homogalacturonan chains), neutral sugar side-chains and  
349 specific surface area; or (ii) they combined features at the two extremes, e.g., KCON  
350 had the highest pectin linearity and the lowest specific surface area. BC6 with the  
351 highest specific surface area (16.7 m<sup>2</sup>/g) had the highest affinity ( $K_L=0.54$  L/g). This  
352 may be due to the fact that the morphology of large specific surface area favors the  
353 stacking of intermediate-size procyanidins. Conversely, AC2/3/6 and KCR2 had  
354 relatively low affinity ( $\leq 0.17$  L/g) and high saturation level ( $\geq 0.70$  g/g), which may  
355 be explained by the fact that they had at least two of the following features, namely  
356 relatively low pectin linearity, low specific surface area, low degree of methylation or  
357 high xylose contents.

358 The apparent Langmuir parameters were also converted as a function of the  
359 amount of procyanidins bound per cell wall surface area (Table 2B). This expression

360 had most impact on the relative ranking of cell walls which had a larger specific  
361 surface area. An increase of cell wall surface area was accompanied by a decrease of  
362  $K_L$  and  $N_{max}$  related to cell walls with lower surface areas so to their physical  
363 characteristics.

364 [Table 2B](#) also provides cell wall-procyanidin interaction characteristics (the  
365 amount of bound procyanidins and  $\overline{DP}n$  of free procyanidins). The average amount  
366 of bound procyanidins varied between 0.061 g/g cell wall (AC6) and 0.091 g/g (BC6),  
367 corresponding to 32 % and 53 % of the initial added procyanidins. Different levels of  
368 bound procyanidins were found for each cell wall. The binding levels of native cell  
369 walls could be ranked: ACN  $\approx$  BCN  $>$  KCRN  $>$  KCON. For AC2/3/6 and BC2/3,  
370 the amount of bound procyanidins decreased after pH modifications, while conversely  
371 for BC6, KCR3/6 and KCO2/3/6 it increased. Cell walls with higher specific surface  
372 area had a higher affinity for procyanidin DP12. The initial procyanidin fractions  
373 were more polymerized ( $\overline{DP}n = 12$ ) than the free procyanidins remaining in the  
374 supernatant ( $3.7 < \overline{DP}n < 4.5$ ) after interaction. Therefore, all cell walls were  
375 selective for highly polymerized procyanidins.

### 376 **3.3.2. Interactions with Procyanidins of DP 39**

377 The binding isotherms for cell walls and procyanidin DP 39 are presented in [Fig.](#)  
378 [2](#). Compared to procyanidin DP 12, the amount of bound procyanidins were higher  
379 for the highly polymerized fractions (DP 39). Binding isotherms demonstrated the key  
380 role of the  $\overline{DP}n$  in modulating the interactions between cell walls and procyanidins.

381 The calculated apparent constants are given in [Table 2](#). For ACs, BCN/2/3, and KCO3,  
382 higher apparent affinities ( $K_L$ ) were obtained when  $\overline{DPn}$  increased. However, for  
383 BC6, KCRs and KCON/2/6, lower apparent affinities ( $K_L$ ) were obtained when  $\overline{DPn}$   
384 increased. In these cell walls, high content of neutral sugar side-chains, both low  
385 degree of methylation and specific surface area may limit their affinity. Their  
386 apparent saturation levels ( $N_{max}$ ) were higher than those of procyanidins of low  $\overline{DPn}$   
387 ([Table 2](#)). These binding isotherms did not reach a stable plateau ([Fig. 2](#)), probably  
388 due to a lack of data in the high concentration range, i.e., above the limit of  
389 procyanidin solubility.

390 The average amount of bound procyanidins varied between 0.084 g/g cell wall  
391 (KCR2) and 0.148 g/g (ACN), corresponding to 46 % and 61 % of the initial used  
392 procyanidins ([Table 2B](#)). The binding levels of native cell walls decreased in the  
393 following order: ACN > BCN > KCON > KCRN. For AC2/3/6, BC2/3/6 and KCR2,  
394 the amount of bound procyanidins decreased after pH modifications, while it  
395 increased for KCR3/6 and KCO2/3/6. The initial procyanidin fractions were more  
396 polymerized ( $\overline{DPn} = 39$ ) than the free procyanidins remaining in the supernatant  
397 ( $12.4 < \overline{DPn} < 21.9$ ). This again indicated that all cell walls were selective for highly  
398 polymerized procyanidins.

### 399 **3.4. Isothermal titration calorimetry**

#### 400 **3.4.1. Interactions with Procyanidins of DP12**

401 Thermodynamic parameters from ITC titration of cell walls by procyanidins

402 DP12 are shown in [Table 3](#). Typical thermograms were obtained for all cell walls (ca.  
403 8 mg) titrated by procyanidin DP12 (30 mmol/L epicatechin equivalent) with strong  
404 exothermic peaks (data not shown). Stoichiometry (defined as ratio of  
405 epicatechin/galacturonic acid) was fixed at 0.1 for all cell walls (1 molecule of  
406 epicatechin bound 10 units of galacturonic acid) using a one-site model. This value  
407 was determined from previous studies ([Brahem et al., 2019](#)).

408 The association constant ranged between  $1.0 \times 10^2 \text{ M}^{-1}$  and  $3.2 \times 10^3 \text{ M}^{-1}$  and  
409 the top three highest affinities decreased in the following order: ACN > KCRN >  
410 KCON ([Table 3](#)). ACN with a low (Ara+Gal)/Rha ratio (9.5), both medium Gal  
411 A/(Rha+Ara+Gal) ratio (1.2) and specific surface area ( $5.4 \text{ m}^2/\text{g}$ ), and a high DM  
412 (82 %) ([Table 1](#)) had the highest affinity for procyanidin DP12, showing that a  
413 combination of positive factors contributed to its high interactions. Similarly, KCRN  
414 and KCON had a high pectin linearity (homogalacturonan chains) and a low degree of  
415 branching, and they also contained a high affinity component, i.e., kiwifruit pectins as  
416 shown by [Liu, Renard, Rolland-Sabaté, Bureau, et al. \(2021\)](#). For ACN/2/6, BC6,  
417 KCRN and KCON/6, analysis of the thermodynamic contributions ( $\Delta G = \Delta H - T\Delta S$ )  
418 related to the exothermic reactions indicated a strong entropy contribution ( $-T\Delta S$  from  
419  $-18$  to  $-9 \text{ kJ/mol}$ ) showing that the interactions were mostly driven by entropy. This  
420 indicated that the binding system was mostly driven by hydrophobic interactions. By  
421 contrast, the enthalpy contributions were high for AC3, BCN/2/3, KCR2/3/6 and  
422 KCO2/3 ( $\Delta H$  from  $-46$  to  $-12 \text{ kJ/mol}$ ) indicating that the interactions were mostly  
423 driven by hydrogen bonds.

### 424 **3.4.2. Interactions with procyanidins of DP 39**

425 Titration of all cell walls by procyanidins DP39 showed complex curves  
426 characterized by strong exothermic peaks. Thermodynamic parameters are shown in  
427 [Table 3](#). To avoid over-fitting, the stoichiometry (n) was also fixed at 0.1 for all cell  
428 walls to allow fit and determination of association parameters.

429 The association constant  $K_a$  between cell walls and procyanidins DP39 ranged  
430 from  $1.0 \times 10^2 \text{ M}^{-1}$  to  $1.6 \times 10^3 \text{ M}^{-1}$  in the decreasing order: KCO2/6 > KCR6 >  
431 ACN > AC3 > KCR2  $\approx$  AC2 > KCRN/3  $\approx$  KCON/3 > AC6  $\approx$  BCN/2/3/6. The affinity  
432 of the beet cell walls was generally low. Regarding the interactions between all cell  
433 walls and procyanidins DP39, contribution of enthalpy ( $\Delta H$  from -4 to -26 kJ/mol)  
434 related to the exothermic reactions indicated that the interactions were mostly driven  
435 by hydrogen bonds due to the high number of hydroxyl groups in procyanidins DP39.

## 436 **4. Discussion**

### 437 **4.1. Comparison of spectroscopy, calorimetry and binding isotherm methods**

438 Using ATR-FTIR, isothermal titration calorimetry and binding isotherm, cell  
439 walls with different levels of porosity, pectin linearity (homogalacturonan chains),  
440 side-chain abundance, and degree of esterification were shown to interact with  
441 procyanidins (DP12 and DP39). The spectra of procyanidins DP12 and DP39 were  
442 close, as well as for other procyanidin fractions, e.g., pear procyanidins ([Brahem et al.,](#)  
443 [2019](#)). Therefore, the following characteristic spectra can be used to distinguish  
444 between initial cell walls and cell wall-procyanidin complexes. Bands at 1604, 1519

445 and  $1440\text{ cm}^{-1}$  can be attributed to C=C and C-C stretching bond vibrations in typical  
446 aromatic rings (Alfaro-Viquez, Esquivel-Alvarado, Madrigal-Carballo, Krueger, &  
447 Reed, 2020; Edelmann, Diewok, Schuster, & Lendl, 2001). Bands at 1284 and 1196  
448  $\text{cm}^{-1}$  can be assigned to phenolic OH and C-O group deformation vibrations and  
449 bending vibrations (Velasco et al., 2014).

450 Binding isotherms of cell wall-procyanidin interactions were well described by  
451 previous research as type I isotherms (the so-called Langmuir isotherms), e.g., apple,  
452 pear and grape cell walls (Bindon, Bacic, & Kennedy, 2012; Brahem et al., 2019; Le  
453 Bourvellec et al., 2004; Le Bourvellec & Renard, 2005; Renard et al., 2001). However,  
454 this fit is an empirical description and is not equivalent to the same mechanism  
455 described by Langmuir (Langmuir, 1918) for the adsorption of gases on solid surfaces.  
456 This method can provide some adsorption parameters and behaviors. The amount of  
457 procyanidins (DP12 and 39) bound to each cell wall ranged from 61 mg/g cell wall to  
458 148 mg/g cell wall, with an apparent affinity constant range of 0.11 to 0.60 L/g. Their  
459 interactions detected by ITC seem to be exothermic, and driven by both entropy and  
460 enthalpy contributions, which are caused by hydrophobic interactions (or changes in  
461 solvation and conformation) and hydrogen bonds, respectively (Leavitt & Freire, 2001;  
462 Liu et al., 2020; Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007). The order of  
463 magnitudes of their affinity constant ( $K_a$ ) ranged from  $10^2$  to  $10^3\text{ M}^{-1}$ . These three  
464 methods are complementary: (i) rapid and sensitive to detect the presence of  
465 procyanidins in complexes (ATR-FTIR); (ii) allowing determination of the binding  
466 parameters, e.g., bound amount,  $K_L$  and  $N_{\max}$  (binding isotherms); (iii) enabling



467 access to thermodynamic (enthalpy/enthalpy) changes, i.e., mechanism, and  
468  $K_a$ /affinity (ITC).

469 The above indicators are the main evaluation parameters indicating the strength  
470 of the interactions. The effect of different cell wall composition/structure on the  
471 interactions was assessed by PCA and correlation analysis. The PCA results are  
472 presented in [Supplementary Fig. 1](#). PC1 and PC2 explained 52% of the total variance  
473 of cell wall characteristics and their interaction with procyanidin DP12 and DP39.  
474 According to [Supplementary Fig. 1](#), some interaction parameters were distributed  
475 separately for different procyanidins, implying that the mechanisms of interaction  
476 may be different. This was also reflected in the correlation analysis ([Fig. 3](#)). To be  
477 specific, the linearity of pectin was correlated with  $K_a$  (by ITC) for procyanidin DP12  
478 and 39. This suggested a significant contribution of homogalacturonan content to the  
479 affinity. In addition, porosity/surface area (by BET) showed correlation with bound  
480 procyanidin DP12 and their  $K_L$  (DP12, binding isotherms) and moderate correlation  
481 with bound procyanidin DP39 and their  $K_L$  (DP39, binding isotherms). This inferred  
482 that low polymerized procyanidins are more readily bound by porous cell walls,  
483 whereas high polymerized procyanidins may be restricted. Although side-chain  
484 abundance and ferulic acid content showed some correlation with  $K_L$ , these factors are  
485 detrimental to the interactions. This implied that porosity was probably antagonistic to  
486 side-chain abundance and ferulic acid content, but that porosity played a dominant  
487 role in cell wall-procyanidin interactions.

## 488 4.2. Pectin content and linearity in cell walls

489 Firstly, during heat treatment at different pH, cell walls are modified so that a  
490 portion of the soluble polysaccharides are extracted giving extractable pectins. The  
491 second point to note is that irrespective of the pectin content of the cell wall starting  
492 material, the main goal of our study was to determine the effect of the chemical  
493 composition and physical structure of the cell wall on their binding properties for  
494 procyanidins. This is because the changes in cell wall adsorption of procyanidins  
495 resulting from pectin extraction are already reflected in cell wall modifications. That  
496 is, not only the composition changes, but also the porosity of the cell wall increases  
497 (i.e., enables more internal cavities) or decreases (i.e., cell wall collapse or shrinkage)  
498 with the difference in the original structure or the treatment conditions.

499 The higher affinities for procyanidins DP12/39 were obtained for mostly  
500 unmodified cell walls, with both higher  $K_a$  and more bound procyanidins. In contrary,  
501 lower affinities were observed for most modified cell walls. This confirms the  
502 findings of [Ruiz-Garcia et al. \(2014\)](#) who suggested that the removal of pectin  
503 significantly reduces the adsorption of proanthocyanidins by cell wall residues,  
504 however, despite pectin elimination the cell wall still have and affinity for  
505 procyanidins ([Le Bourvellec et al., 2012](#)). Native cell walls (A/B/KC/KOCN)  
506 exhibited the highest extractable pectin contents, while the modified cell walls, to  
507 varying degrees, lost extractable pectins (loss ratio pH 6.0 > 3.5 > 2.0). Extractable  
508 pectins have a high binding capacity and affinity for procyanidins ([Le Bourvellec et](#)

509 [al., 2005, 2012; Liu et al., 2020; Ruiz-Garcia et al., 2014](#)). Pectin content and linearity  
510 did not make a significant difference to the binding capacity, i.e.,  $K_L$  obtained using  
511 binding isotherms, of different cell walls with procyanidins, as other factors are also  
512 involved in the regulation of their interactions. The affinity  $K_a$  (obtained using ITC) of  
513 beet cell walls was relatively lower than that of apple and kiwifruit cell walls. This  
514 may be due to their complex arabinan side-chain structures and the presence of ferulic  
515 acid covalently linked to arabinans that limit interactions ([P. A. R. Fernandes et al.,](#)  
516 [2020; Liu, Renard, Rolland-Sabaté, & Le Bourvellec, 2021; Watrelot et al., 2014](#)).  
517 Binding isotherms appear to be more sensitive than ITC to factors influencing  
518 interactions, as they could take into account the physical aspects of the binding.

519 Different conditions of processing or treatments can also significantly influence  
520 interactions. For example, pH 2.0 modification caused removal of most of the neutral  
521 sugar side-chains while degrees of methylation remained high, thus increasing the  
522 linearity of pectin and homogalacturonan content with high DM in the A/B/KC/KOC2  
523 cell walls. This structure probably caused AC2 and BC2 to bind more procyanidins by  
524 stacking than after the other pH treatments, obtained by binding isotherms. However,  
525 this trend was not evident for the affinity  $K_a$  results obtained by ITC. For kiwifruit cell  
526 walls, no relevant pattern was found, probably due to the fact that pH modification did  
527 not drastically affect the linearity of pectins insofar as the initial kiwifruit pectins are  
528 already linear with low side-chain content, and the other physical factors (e.g.,  
529 porosity) could also combine to influence this result ([Liu et al., 2020](#)).

### 530 4.3. Surface area/porosity

531 BC6, with the lowest pectin linearity and DM, and the highest side-chains and  
532 branching ratios, could be expected to have the lowest binding capacity and affinity to  
533 procyanidins. This was not the case, however, as it had a relatively high binding  
534 capacity and affinity for the procyanidin DP12. This may be attributed to the fact that  
535 BC6 had the highest specific surface area ( $16.7 \text{ m}^2/\text{g}$ ), i.e., the highest porosity, of all  
536 cell walls. Solvent exchange drying increased the porosity of the cell walls, which  
537 could allow encapsulation of the procyanidins in a more open conformation (Le  
538 Bourvellec et al., 2012). The remodeling and loosening of the grape cell wall due to  
539 ripening also increased the porosity of cell walls, leading to an increase in the  
540 adsorption of proanthocyanidins (Bindon et al., 2012). The porosity of cell walls of  
541 different tissues also varies considerably, for example, the stone cells of pears are  
542 secondary cell walls with a dense structure and less porosity, and therefore have a  
543 lower affinity for procyanidins (Brahem et al., 2019).

544 Despite the high porosity of BC6, its binding capacity and affinity for  
545 procyanidin DP39 was not better than that for DP12. This could be attributed not only  
546 to its high branching ratio preventing the entry of larger molecules of procyanidin  
547 DP39, but also to the size and type of pore. In general, the limiting pore size of the  
548 cell wall is about 5 nm (equivalent to the size of DP34) (Carpita, Sabularse,  
549 Montezinos, & Delmer, 1979), while the pores may have slits, interstices, spherical,  
550 cylindrical, and conical forms (Liu et al., 2020). These two factors may conjointly  
551 modulate the interaction between cell walls with different porosity and procyanidins

552 with different sizes. The water-binding capacity of the cell walls, regulated by  
553 porosity levels and cell wall components (Klaassen & Trindade, 2020; Paudel, Boom,  
554 van Haaren, Siccama, & van der Sman, 2016), may influence their capacity to adsorb  
555 procyanidins, but no causal link can be established between the two at this point.  
556 Notedly, the specific surface areas given here are measured as dry matter and may  
557 differ in aqueous media, thus the next important work should be to focus on the wet  
558 porosity.

#### 559 **4.4. Substitution of the galacturonic acids**

560 Highly methylated pectin has already been demonstrated to have a high affinity  
561 with highly polymerized procyanidins (Liu et al., 2020; Watrelot et al., 2013).  
562 However, this result may be counterbalanced by other factors in the cell wall. The cell  
563 walls modified at pH 6.0, i.e., AC/BC/KCR/KCO6, had the lowest pectin DM in each  
564 species due to  $\beta$ -elimination. The binding capacity and affinity of these cell walls for  
565 procyanidins were not always the least (Tables 2 and 3). Other cell wall components,  
566 e.g., cellulose and hemicelluloses, as well as their physical characteristic like porosity,  
567 may combine to influence the final interactions.

568 Beet cell walls had the highest acetic acid content, but it appears not to be a  
569 positive factor for interactions. This may be related to a reduction in potential binding  
570 sites. Likewise, anthocyanins bind more to low-esterified beet and citrus pectins than  
571 to highly esterified pectins (A. Fernandes et al., 2020; Larsen, Buerschaper, Schieber,  
572 & Weber, 2019). The reduction in acetic acid increased the number of hydroxyl  
573 groups available on the surface, while alleviating the hindrance of groups at adjacent

574 positions on the binding surface, therefore potentially facilitating the accumulation of  
575 procyanidins on the cell wall surface.

#### 576 **4.5. Degree of polymerization of procyanidins**

577 Generally, the higher the degree of polymerization of procyanidins, the stronger  
578 the interaction with cell walls. For example, the cell walls have higher binding  
579 capacities and affinities for the procyanidin DP39 than for DP12 (Table 2). This may  
580 be due to the higher number of hydroxyl groups and aryl rings, allowing a higher  
581 number of hydrogen bonds and hydrophobic interaction sites. However, there were  
582 two trends in the affinity of cell walls for procyanidin DP39. AC3, BCN, KCR2/6 and  
583 KCO2 had a higher affinity for DP39 than DP12 by ITC, but ACN/2/6, BC2/3/6,  
584 KCRN/3 and KCON/3/6 had a lower affinity for DP39 than DP12 by ITC. It is likely  
585 that both porosity and chemical composition were responsible for this result. As  
586 discussed in the previous section on porosity, cell walls such as KCR/ON (ca. 1 m<sup>2</sup>/g)  
587 had a very low porosity and larger procyanidins might not easily enter inside their  
588 internal pores. On the other hand, BC2/3/6 had high neutral sugar side-chain  
589 branching ratio, together with high ferulic acid content (Table 1). Therefore, this  
590 structure might cross-link the side-chains and hindered the available binding sites  
591 limiting their interaction with procyanidin DP39. Moreover, the change in  
592 entropy/enthalpy also explains this result, as only hydrogen bonding drives the  
593 interaction with procyanidin DP39, while both hydrophobic interactions and hydrogen  
594 bonds for DP12.

595 Notably, the natural cell wall architecture and organization are also a very

596 important factor, i.e., cell wall matrix interactions (Varner & Lin, 1989). For example,  
597 natural pectins can also strongly interact with other cell wall components, such as  
598 cellulose, which may limit their potential for interactions with other biomolecules  
599 (Broxterman & Schols, 2018). Similarly, the presence of many other covalent or  
600 non-covalent interactions such as interactions between cellulose, xyloglucan/xylan  
601 and RG-I side-chains, may also limit the exposure of binding sites (Ralet et al., 2016;  
602 Zykwinska, Ralet, Garnier, & Thibault, 2005). However, the extent to which these  
603 interactions may vary in different plant cell walls may also be an important factor in  
604 their interaction with polyphenols. Therefore, future work will require a more precise  
605 identification of the architecture and conformation of the cell wall.

## 606 **5. Conclusions**

607 Cell walls from apple, beet and kiwifruit have different levels of binding  
608 selectivity depending on their chemical composition and physical structure. For  
609 extractable polysaccharides (soluble state), e.g., pectins, the linearity and degree of  
610 methylation were important, but for native and modified cell walls (insoluble state),  
611 porosity appears to be a major factor. Binding isotherms play an essential role in the  
612 study of physical adsorption. The ranking of factors affecting cell wall selectivity  
613 were, for those which favor interactions, high porosity (including the size and type)  
614 and pectin linearity and homogalacturonan content (as synergists), while high xylose,  
615 ferulic acid and acetic acid contents, and pectin branching were detrimental (as  
616 antagonists). Further work is needed to confirm the role of wet porosity (i.e., in  
617 suspension) of cell walls.

618 The cell wall structure is generally altered during food processing and digestion.  
619 Each cell wall has its own unique chemical composition, molecular architecture and  
620 physical structure, and has common and specific responses to processing and  
621 digestion. All these factors interact with each other to impact the interactions.  
622 Understanding the relations between the chemical and physical factors remains a huge  
623 challenge, and more work is needed to clarify the mechanisms involved and internal  
624 relationships. Systematic studies of interactions between biomacromolecules allows to  
625 better establish a bridge between food processing and the binding/retention of  
626 bioactive substances in food industry.

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#### 631 **Conflicts of interest**

632 The authors declare no conflicts of interest.

#### 633 **CRedit authorship contribution statement**

634 **Xuwei Liu:** Investigation, Formal analysis, Data curation, Writing - original draft.

635 **Catherine M. G. C. Renard:** Conceptualization, Supervision, Funding acquisition,

636 Project administration, Validation, Writing - review & editing. **Sylvie Bureau:**

637 Investigation, Software, Supervision, Writing - review & editing. **Carine Le**

638 **Bourvellec:** Conceptualization, Supervision, Funding acquisition, Project



639 administration, Validation, Writing - review & editing.

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854

**Table 1.** Characteristic chemical content, sugar ratios, specific surface area and water binding capacity of the different cell wall components from apple, beet and kiwifruit.

Samples	Gal A/ (Rha+Ara+Gal)	Gal A/Rha	(Ara+Gal)/Rha	Ara/Gal	Xyl/Man	FA* (mg/g)	DM* (%)	Ac.A* (mg/g)	BET (m <sup>2</sup> /g)	WBC (g/g)
ACN	1.2	12.4	9.5	1.2	4.9		82	21	5.4	7.8
AC2	1.6	13.0	7.3	0.5	4.9		86	19	1.1	8.5
AC3	0.9	9.1	9.3	1.1	5.1		89	19	1.5	5.9
AC6	0.7	8.9	11.7	1.2	5.0		60	18	2.6	3.9
BCN	0.7	12.3	16.0	4.2	0.7	7.0	65	45	4.5	3.7
BC2	0.9	12.1	12.5	2.4	0.8	7.7	58	41	6.3	7.7
BC3	0.5	8.5	16.3	4.0	0.8	9.6	72	39	2.4	4.2
BC6	0.4	8.9	19.7	4.3	0.8	8.8	42	32	16.7	6.0
KCRN	2.7	28.7	9.5	0.2	2.5		67	11	1.3	5.6
KCR2	2.3	26.5	10.7	0.2	2.6		62	12	0.5	8.0
KCR3	1.5	18.2	11.0	0.3	2.9		70	11	5.9	6.0
KCR6	1.1	15.6	13.4	0.2	2.6		69	11	7.0	6.6
KCON	4.1	38.4	8.4	0.4	2.9		72	11	0.3	4.6
KCO2	2.4	33.4	12.9	0.2	2.6		58	13	1.3	6.9
KCO3	2.2	30.4	13.0	0.3	2.6		65	11	11.4	5.9
KCO6	1.6	18.2	10.4	0.3	2.4		55	11	0.6	4.0
<i>Pooled SD</i>	<i>0.1</i>	<i>1.9</i>	<i>0.9</i>	<i>0.03</i>	<i>0.1</i>	<i>0.2</i>	<i>4.1</i>	<i>0.8</i>	-	<i>1.2</i>

855 Ratios are calculated using the yields of neutral sugar expressed in mol%. Ratios Gal A/(Rha+Ara+Gal) is characteristic for linearity of pectin. Gal A/Rha for contribution of  
856 homogalacturonans to pectin. (Ara+Gal)/Rha for branching of RG-I. Ara/Gal for the proportion of arabinans/galactans. Xyl/Man for contribution of mannans to  
857 hemicelluloses. Gal A: galacturonic acid, Rha: rhamnose, Ara: arabinose, Gal: galactose, Man: mannose, Xyl: xylose. DM: degree of methylation. FA and Ac.A: ferulic acid  
858 and acetic acid content, respectively. BET and WBC are characteristic for specific surface area and water-binding capacity, respectively. AC: apple cell wall, BC: beet cell  
859 wall, KC: kiwifruit cell wall, pH values-: 2: pH 2.0, 3: pH 3.5, 6: pH 6.0. Maturity-: R: -Ripe, O: -Overripe. Pooled SD: pooled standard deviation. \* data adapted from (Liu,  
860 Renard, Rolland-Sabaté, Bureau, et al., 2021).

861 **Table 2.** Binding isotherms between cell walls and procyanidins DP12 and 39: A) Apparent Langmuir parameters for binding isotherms of different cell walls with varying concentrations of  
 862 procyanidin DP12 and DP39, and B) Procyanidin retention and free procyanidins characteristics at 1 g/L of procyanidins and 5 g/L of cell walls.

A										
	Procyanidin DP12					Procyanidin DP39				
	$K_L$ (L/g)	$N_{max}$ (g/g)	$K_L$ (L/m <sup>2</sup> )	$N_{max}$ (g/m <sup>2</sup> )	$R^2$	$K_L$ (L/g)	$N_{max}$ (g/g)	$K_L$ (L/m <sup>2</sup> )	$N_{max}$ (g/m <sup>2</sup> )	$R^2$
ACN	0.25±0.09	0.75±0.13	0.05±0.02	0.14±0.02	0.91	0.6±0.10	1.18±0.07	0.11±0.02	0.22±0.01	0.96
AC2	0.17±0.03	0.75±0.08	0.15±0.03	0.68±0.07	0.98	0.46±0.09	1.23±0.09	0.42±0.08	1.12±0.08	0.96
AC3	0.16±0.04	0.85±0.12	0.11±0.03	0.57±0.08	0.96	0.36±0.05	1.34±0.07	0.24±0.03	0.89±0.05	0.99
AC6	0.11±0.03	0.76±0.12	0.04±0.01	0.29±0.06	0.97	0.21±0.05	1.56±0.17	0.08±0.02	0.60±0.07	0.97
BCN	0.38±0.06	0.75±0.05	0.08±0.01	0.17±0.01	0.97	0.46±0.10	0.91±0.07	0.10±0.03	0.20±0.02	0.95
BC2	0.33±0.06	0.75±0.05	0.06±0.01	0.12±0.01	0.97	0.37±0.06	1.32±0.09	0.06±0.01	0.21±0.02	0.97
BC3	0.28±0.05	0.65±0.05	0.12±0.02	0.27±0.02	0.97	0.34±0.05	1.09±0.06	0.14±0.03	0.45±0.04	0.98
BC6	0.54±0.05	0.51±0.01	0.03±0.01	0.03±0.01	0.99	0.29±0.05	1.43±0.12	0.02±0.01	0.09±0.01	0.98
KCRN	0.25±0.07	0.49±0.06	0.19±0.05	0.38±0.05	0.93	0.21±0.06	1.73±0.25	0.16±0.05	1.33±0.19	0.95

KCR2	0.17±0.07	0.6±0.13	0.34±0.14	1.20±0.26	0.89	0.13±0.04	2.18±0.42	0.26±0.08	4.36±0.84	0.97
KCR3	0.35±0.05	0.6±0.03	0.06±0.01	0.10±0.01	0.98	0.3±0.08	1.47±0.17	0.05±0.02	0.25±0.03	0.95
KCR6	0.25±0.14	0.58±0.13	0.04±0.02	0.08±0.02	0.89	0.23±0.06	1.15±0.12	0.03±0.01	0.16±0.02	0.96
KCON	0.21±0.05	0.49±0.05	0.70±0.17	1.63±0.17	0.96	0.19±0.05	1.17±0.14	0.63±0.17	3.90±0.47	0.97
KCO2	0.29±0.12	0.37±0.06	0.22±0.09	0.28±0.04	0.85	0.15±0.03	1.36±0.17	0.12±0.02	1.05±0.13	0.98
KCO3	0.24±0.09	0.44±0.07	0.02±0.01	0.04±0.01	0.89	0.41±0.06	0.85±0.05	0.04±0.01	0.07±0.01	0.98
KCO6	0.45±0.09	0.29±0.02	0.75±0.15	0.48±0.03	0.95	0.34±0.08	1.11±0.07	0.57±0.12	1.88±0.2	0.95

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**B**

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	Procyanidin DP12			Procyanidin DP39		
	Bound procyanidins (g/g CW)	% of initial PCA	$\overline{DP}n$ of Free PCA	Bound procyanidins (g/g CW)	% of initial PCA	$\overline{DP}n$ of Free PCA
ACN	0.083	44%	3.7	0.148	61%	15.7
AC2	0.078	40%	4.5	0.120	70%	20.8
AC3	0.075	36%	4.4	0.113	59%	15.1
AC6	0.061	32%	4.5	0.104	55%	16.4
BCN	0.085	51%	3.8	0.116	59%	20.7
BC2	0.086	45%	3.8	0.110	55%	16.4

BC3	0.067	42%	4.2	0.111	55%	12.4
BC6	0.091	53%	4.2	0.108	54%	15.7
KCRN	0.071	38%	4.4	0.091	47%	19.7
KCR2	0.069	37%	4.1	0.084	46%	16.7
KCR3	0.083	41%	4.3	0.100	57%	21.9
KCR6	0.077	41%	4.4	0.112	64%	21.5
KCON	0.063	35%	4.5	0.104	57%	20.8
KCO2	0.077	42%	4.3	0.104	48%	14.1
KCO3	0.090	47%	4.2	0.126	69%	14.4
KCO6	0.071	40%	4.3	0.114	61%	18.5
<i>Pooled SD</i>	<i>0.002</i>	<i>0.01</i>	<i>0.2</i>	<i>0.003</i>	<i>0.02</i>	<i>1.0</i>

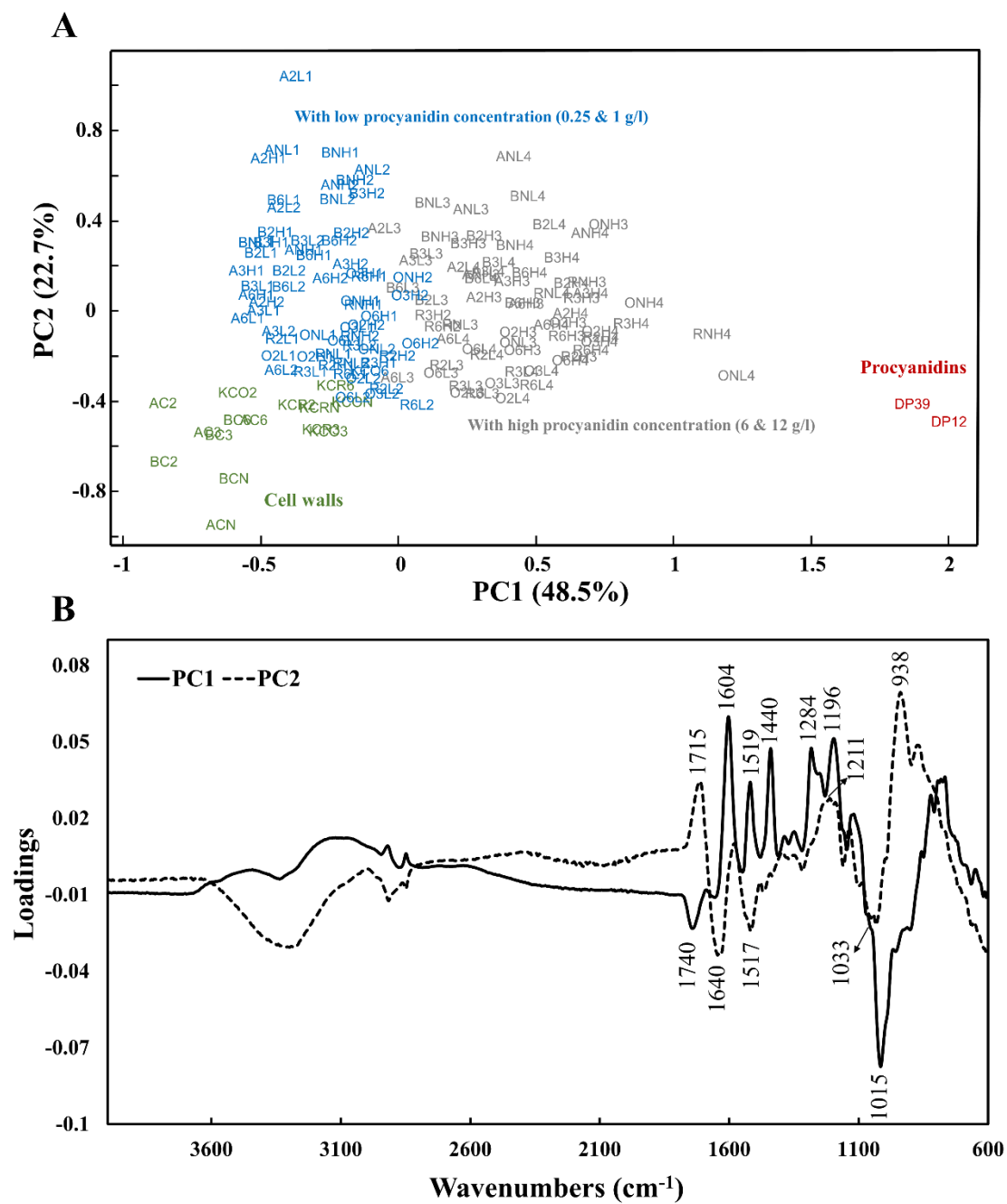
863 Average of duplicates for each; uncertainty on the parameters of the Langmuir isotherms in Table 2A was calculated using the Marquardt estimation approach, precision on the analytical results  
864 in Table 2B using pooled standard deviation..  $\overline{DP}_n$ : number-average degree of polymerization of procyanidins,  $K_L$ : apparent affinity constant,  $N_{max}$ : apparent saturation level. AC: apple cell  
865 wall, BC: beet cell wall, KC: kiwifruit cell wall, pH values-: 2: pH 2.0, 3: pH 3.5, 6: pH 6.0. Maturity-: R: -Ripe, O: -Overripe. Pooled SD: pooled standard deviation.

866 **Table 3.** Thermodynamic parameters of interactions between cell walls and procyanidins DP12 and DP39 (30 mM (-)-epicatechin equivalent) measured by Isothermal Titration  
 867 Microcalorimetry (ITC).

	n	Procyanidin DP12					Procyanidin DP39				
		$K_a$ ( $M^{-1}$ )	$\Delta H$ (kJ/mol)	$\Delta S$ (J/mol/K)	$\Delta G$ (kJ/mol)	-T $\Delta S$ (kJ/mol)	$K_a$ ( $M^{-1}$ )	$\Delta H$ (kJ/mol)	$\Delta S$ (J/mol/K)	$\Delta G$ (kJ/mol)	-T $\Delta S$ (kJ/mol)
ACN	0.1	3180	-1.38	62.44	-19.99	-18.62	1087	-4.36	43.50	-17.33	-12.97
AC2	0.1	1056	-7.42	33.01	-17.26	-9.84	752	-8.13	27.79	-16.42	-8.29
AC3	0.1	375	-11.96	9.16	-14.69	-2.73	932	-8.20	29.33	-16.95	-8.75
AC6	0.1	1166	-7.05	35.05	-17.51	-10.45	275	-15.19	-4.24	-13.92	1.27
BCN	0.1	179	-14.20	-4.50	-12.86	1.34	251	-12.34	4.57	-13.70	-1.36
BC2	0.1	395	-11.00	12.81	-14.82	-3.82	191	-16.19	-10.64	-13.02	3.17
BC3	0.1	208	-22.17	-29.98	-13.23	8.94	119	-33.72	-73.35	-11.85	21.87
BC6	0.1	1690	-6.25	40.85	-18.43	-12.18	212	-22.01	-29.28	-13.28	8.73
KCRN	0.1	2848	-7.46	41.11	-19.72	-12.26	451	-18.39	-15.29	-15.15	3.24
KCR2	0.1	693	-22.09	-19.71	-16.22	5.87	717	-18.52	-7.45	-16.30	2.22
KCR3	0.1	840	-32.74	-53.81	-16.69	16.05	444	-26.48	-38.12	-15.11	11.37
KCR6	0.1	951	-46.09	-97.56	-17.00	29.09	1334	-12.61	17.53	-17.84	-5.23
KCON	0.1	2273	-8.49	35.80	-19.16	-10.68	526	-12.75	9.33	-15.53	-2.78
KCO2	0.1	895	-19.41	-8.59	-16.85	2.56	1589	-4.07	47.60	-18.27	-14.21
KCO3	0.1	1188	-28.99	-38.37	-17.55	11.44	562	-22.73	-23.61	-15.69	7.04
KCO6	0.1	2180	-0.82	61.18	-19.06	-18.24	1527	-6.62	38.73	-18.17	-11.55
<i>Pooled SD</i>	-	<i>256</i>	<i>1.1</i>	<i>2.1</i>	<i>0.3</i>	<i>0.6</i>	<i>104</i>	<i>0.8</i>	<i>2.1</i>	<i>0.2</i>	<i>0.6</i>

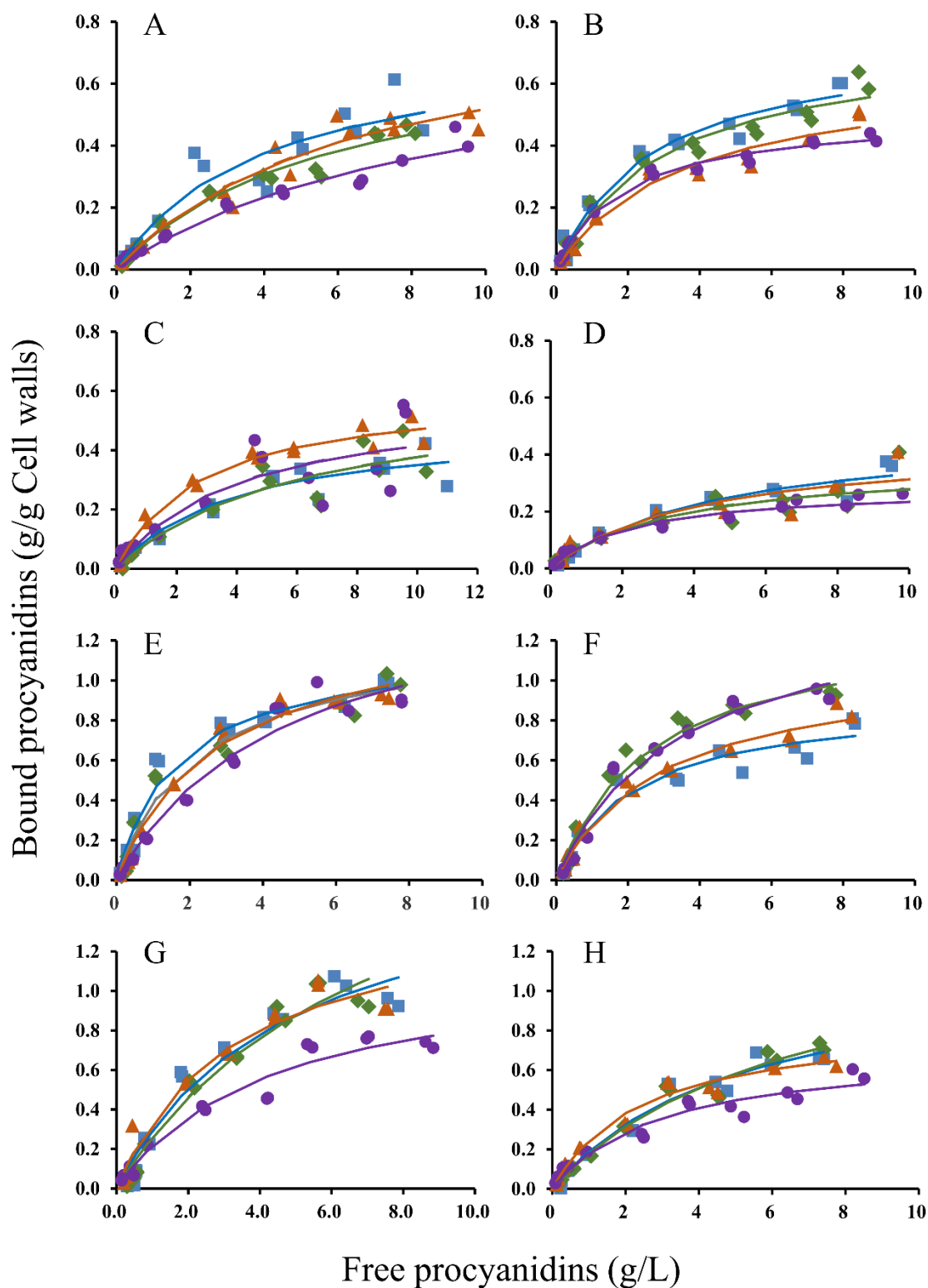
868 Average of duplicates for each.  $\overline{DP}n$ : number-average degree of polymerization of procyanidins,  $K_a$ : affinity level, n: stoichiometry,  $\Delta H$ : enthalpy,  $\Delta G$ : free enthalpy,  $\Delta S$ : entropy. AC: apple cell  
 869 wall, BC: beet cell wall, KC: kiwifruit cell wall, pH values: 2: pH 2.0, 3: pH 3.5, 6: pH 6.0. Maturity: R: -Ripe, O: -Overripe.

870



871

872 **Fig. 1.** Principal component analysis of infrared spectra on cell walls, procyanidins and their complexes. A)   
 873 Sample map; B) Loading profile of components PC1 and PC2 in the range of 4000 - 600 cm<sup>-1</sup>. A(C): apple cell   
 874 wall, B(C): beet cell wall, (KC)R: kiwifruit cell wall (ripe), (KC)O: kiwifruit cell wall (overripe), L/H1: 0.25 g/l   
 875 DP12/39, L/H2: 1 g/l DP12/39, L/H3: 6 g/l DP12/39, L/H4: 12 g/l DP12/39, N: native, pH values-: 2: pH 2.0, 3:   
 876 pH 3.5, 6: pH 6.0. Maturity-: R: -Ripe, O: -Overripe.

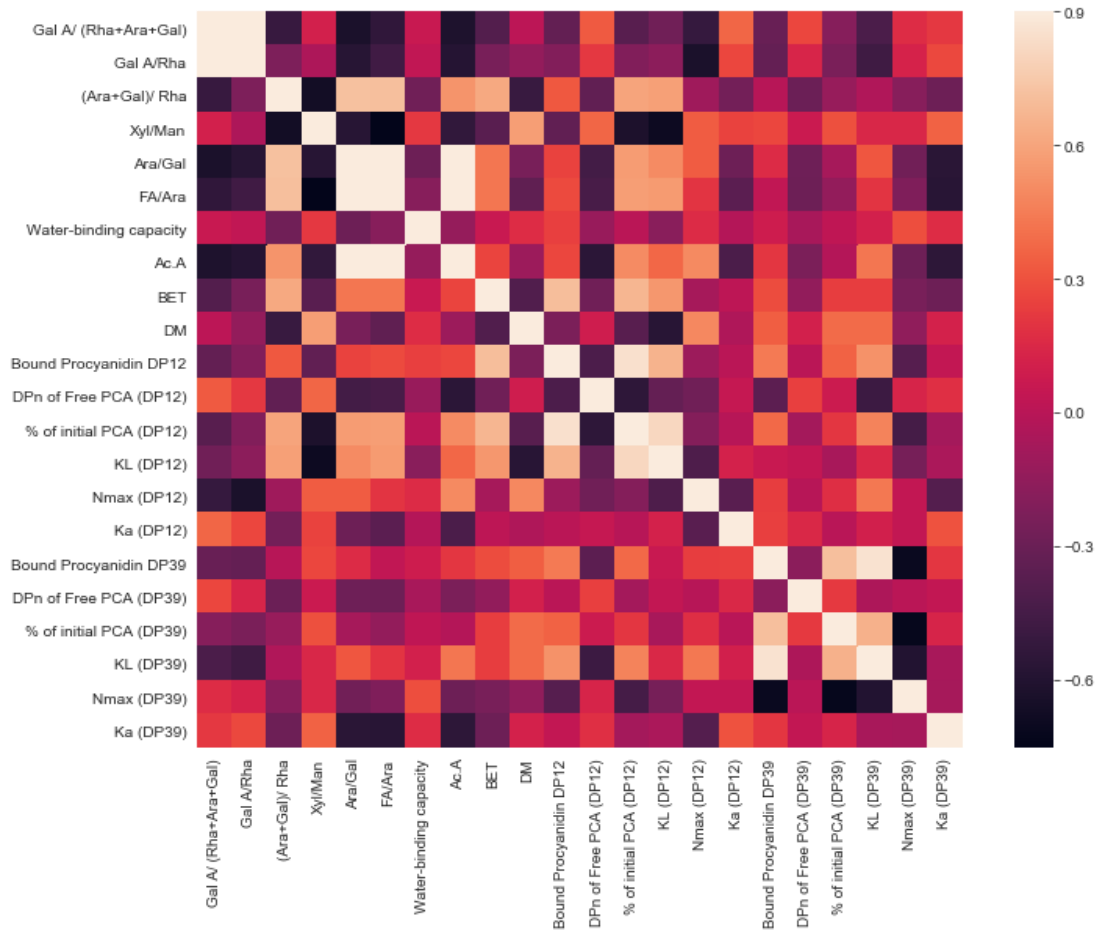


877

878 **Fig. 2.** Binding isotherms for cell walls and procyanidins at pH 3.8, ionic strength 0.1 mol/L, 25 °C. A:  
 879 experimental curve for ACs-DP12 complexes, B: experimental curve for BCs-DP12 complexes, C: experimental  
 880 curve for KCRs-DP12 complexes, D: experimental curve for KCOs-DP12 complexes, E: experimental curve for  
 881 ACs-DP39 complexes, F: experimental curve for BCs-DP39 complexes, G: experimental curve for KCRs-DP39  
 882 complexes, H: experimental curve for KCOs-DP39 complexes. The points and lines are the corresponding  
 883 Langmuir adsorption isotherms for which the calculated parameters are given in Table 2 for different cell walls: ■



884 and — Native cell walls, ◆ and — Cell walls modified at pH 2.0, ▲ and — Cell walls modified at pH 3.5, ●  
 885 and — Cell walls modified at pH 6.0.



886

887 **Figure 3.** Correlation matrix heatmap between carbohydrate compositions and structural characteristics  
 888 of cell walls and binding properties after interaction with procyanidins. Ratios Gal A/(Rha+Ara+Gal) is  
 889 characteristic for linearity of pectin. Gal A/Rha for contribution of homogalacturonans to pectin.  
 890 (Ara+Gal)/Rha for branching of RG-I. Ara/Gal for the proportion of arabinans/galactans. Xyl/Man for  
 891 contribution of mannans to hemicelluloses. DM: degree of methylation. FA and Ac.A: ferulic acid and  
 892 acetic acid content, respectively. BET and WBC are characteristic for specific surface area and  
 893 water-binding capacity, respectively.