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Impact of Processing on the Noncovalent Interactions between Procyanidin and Apple Cell Wall

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ABSTRACT: Procyanidins can bind cell wall material in raw product, and it could be supposed that the same mechanism of retention of procyanidins by apple cell walls takes place in cooked products. To evaluate the influence of cell wall composition and disassembly during cooking on the cell walls' capacity to interact with procyanidins, four cell wall materials differing in their protein contents and physical characteristics were prepared: cell wall with proteins, cell wall devoid of protein, and two processed cell walls differing by their drying method. Protein contents varied from 23 to 99 mg/g and surface areas from 1.26 to 3.16 m^2/g. Apple procyanidins with an average polymerization degree of 8.7 were used. The adsorption of apple procyanidins on solid cell wall material was quantified using the Langmuir isotherm formulation. The protein contents in cell wall material had no effect on procyanidin/cell wall interactions, whereas modification of the cell wall material by boiling, which reduces pectin content, and drying decreased the apparent affinity and increased the apparent saturation levels when constants were expressed relative to cell wall weight. However, boiling and drying increased apparent saturation levels and had no effect on apparent affinity when the same data were expressed per surface units. Isothermal titration calorimetry indicated strong affinity ($K_a = 1.4 \times 10^4$ M^{-1}) between pectins solubilized by boiling and procyanidins. This study higllights the impact of highly methylated pectins and drying, that is, composition and structure of cell wall in the cell wall/procyanidin interactions.

KEYWORDS: *boiling, tannins, porosity, pectins, calorimetry*

INTRODUCTION

The health benefits of fruits and vegetables in the human diet are attributed to their particular levels of micronutrients such as polyphenols and carotenoids. To play a role in the human body these molecules must be released from the food matrix. However, their bioavailability may be low and is still poorly understood,[1] especially for processed food. Something appears to be missing in the knowledge of the role of food matrix in retention phenomena of these molecules, which limits the possibility of linking diet to the potential effects of its components.[2] For procyanidins, which are major phenolics in some fruits, tea, and chocolate, a particularly relevant mechanism in the limitation of their bioavailability is their binding to the extracellular matrix that forms the cell walls of fruits. Interactions between procyanidins and food matrix may have a strong impact on their bioaccessibility and biological properties.

The bioaccessibility and bioavailability of each micronutrient differ greatly. It is widely accepted that bioaccessibility depends on several parameters, including the initial concentration in the food matrix and their chemical structure, as well as chemical interactions with other micronutrients and biomolecules present in the food matrix and the food matrix itself.[3,4] Thus, as a consequence, the most abundant micronutrients in the diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues.[1] However, the lack of bioaccessibility of native molecules does not mean the absence of biological effects.[5,6]

Polyphenols are an abundant class of secondary metabolites characterized by the presence of phenol functions; they comprise the flavonoids, among which are the procyanidins, which are oligo- or polymers of the flavan-3-ols catechin and epicatechin. Polyphenols are present in the vacuoles of plant tissues and must first be extracted to be bioaccessible. The bioaccessibility of constituents such as polyphenols is linked to their extractability or diffusivity.[2] Fruit processing often involves tissue disruption and brings compounds contained within the cellular organelles and cytoplasm in contact with extracellular matrix. During such a process, polyphenols breach the vacuolar frontiers and bind to extracellular cell walls.[7] On raw material disrupted mechanically, it was previously shown that apple cell walls play a major role in regulating the polyphenol composition and concentrations in apple juice and cider,[7−9] in particular by their ability to selectively adsorb procyanidins. Among the different apple polyphenol classes (hydroxycinnamic acids and flavonoids), only procyanidins bound to cell walls.[7] The extraction of procyanidins was largely limited by

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complex formation with cell walls. Oligomeric and polymeric procyanidins have the ability to bind noncovalently on apple cell walls by simple incubation in aqueous buffer.[7,8,10−12] This binding is fast and spontaneous, which means that it will occur whenever tissue degradation happens. The cell wall−procyanidin adsorption mechanism involves weak associations, more precisely, a combination of hydrogen bonds and hydrophobic interactions.[7,8] The amounts of bound procyanidins and their affinity constants for cell walls are strongly influenced by compositional and structural parameters, such as stereochemistry, conformational flexibility, molecular weight, that is, number average degree of polymerization ($\overline{DP}n$), and concentration.[7,8,11] Modification of the physical state of cell walls (i.e., harsh drying) affected most their capacity to bind procyanidins.[10] The apparent affinity constants between procyanidins and model polysaccharides decrease as follows: pectins ≫ xyloglucan > starch > cellulose.[11] Moreover, the procyanidin adsorption to cell walls limited the depolymerization of pectins supposedly induced by pectin lyase. Following the initial extraction of pectins, the NaOH (4 M) treatment used to extract hemicelluloses contains more residual pectins when procyanidins are bound to cell wall.[12] Pectins thus play a major role in procyanidin/cell wall interactions. Questions that remained unanswered concerned the role of the minor protein component of the cell walls as well as varietal origin of the cell walls.

However, questions linked to the impact of the thermal treatment on the cell walls on their capacity to adsorb procyanidins have to be resolved. The modification of dietary fibers and cell walls in plant tissue during cooking has been extensively studied:[13−15] cooking results primarily in pectin solubilization and degradation, accompanied by increased cell walls swelling. The polyphenols in cooked fruits and vegetables have received less attention. Although procyanidins form a significant part of the polyphenols of fruits such as apple,[16] pear,[17] or peach,[18] few studies address their variation after cooking.[16−18] Our initial work on the polyphenolic composition of applesauce showed both that its composition is very close to that of the apple flesh and that procyanidins are retained in the solid component of applesauce.[16] When pears are cooked, procyanidins are retained in the pear tissue, probably by interaction with the cell walls, whereas the other polyphenols are partially leached into the cooking water.[17] This supports the hypothesis that the same mechanisms of retention of polyphenols take place in cooked and raw products and motivated our study on the affinity of procyanidins for cell walls after cooking and their interaction with the pectins solubilized during cooking. We are now interested in identifying the cell wall factors, that is, resulting structural changes or chemical composition during cooking, that may influence their interaction with procyanidins.

In this study the impact of modifying the composition (pectins, proteins) and the surface (drying by solvent exchange or by freeze-drying) of apple cell walls on their interactions with procyanidins was examined. Moreover, the interaction between procyanidins and pectins solubilized during cooking was characterized using isothermal titration calorimetry (ITC).

MATERIAL AND METHODS

Standards and Chemicals. Chlorogenic acid, (+)-catechin, and (−)-epicatechin were obtained from Sigma-Aldrich (Deisenhofen, Germany). 4-Coumaric acid was obtained from Extrasynthese (Lyon, France). Phloridzin was obtained from Fluka (Buchs, Switzerland). Sugar standards were from Fluka. d_3-Methanol was from Acros Organics (Geel, Belgium). $NaBH_4$, *N*-methylimidazole, acetic anhydride, and toluene-α-thiol were from Sigma-Aldrich. Acetonitrile was of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Hexane, methanol, and acetone of chromatographic quality were provided by Fisher Scientific.

Plant Material. Apple fruits (*Malus* × *domestica* Borkh.) from the 'Marie Menard' and 'Petit Jaune' cultivars were harvested at maturity (after starch regression) during the 2000 season in the experimental orchard of the Centre Technique des Productions Cidricoles (Sées, Orne, France). Fruits were mechanically peeled and cored as previously described by Guyot et al.,[19] and cortex tissues were freeze-dried (for polyphenol extraction) or submitted to preparation of cell wall material.

Apple fruits (*Malus* × *domestica* Borkh.) from the 'Ariane' cultivar were harvested at maturity (after starch regression) during the 2007 season in the experimental orchard of INRA in Angers (49, France).

Preparation of Cell Wall Material. Phenol buffer (PB)-insoluble solids from Ariane and Petit Jaune cultivars were prepared according to the method of Renard et al.;[7] the samples were named PB-Ariane and PB-Petit Jaune, respectively. Their final drying was performed by solvent exchange.

Alcohol-insoluble solids (AIS) from Ariane cultivar were prepared according to the methods of Renard et al.[20] and Renard;[17] the sample was named AIS-Ariane. The final drying was performed by solvent exchange.

Modification of Cell Wall Materials by Boiling. The PB-Ariane cell wall materials were immersed in hot buffer (25 g/L, in citrate/phosphate buffer, pH 3.8, ionic strength = 0.1 M) and left for 20 min at boiling (temperature = 100 °C). After boiling, the buffer solution and the cell wall materials were separated by hot filtration under vacuum in a G3 sintered glass filter. The cell wall materials were dried by two modes of drying. First, solvent exchange using 96% ethanol was performed three times and using acetone, three times, and then the samples were dried overnight in an oven at 40 °C. Alternatively, the isolates were freeze-dried (start temperature −20 to 20 °C during 3 days). This gave two experimental samples PB-20 min-100 °C-S (solvent exchange) and PB-20 min-100 °C-FD (freeze-dried). The buffer solutions, containing pectins, were directly dialyzed, to eliminate buffer salts, during 24 h against deionized water in a dialysis tube of theoretical porosity of 12 kDa (Sigma Chemical Co., St. Louis, MO, USA) and freeze-dried. The pectin samples were named Pectin-S and Pectin-FD from cell wall material dried by solvent exchange or freeze-dried, respectively.

Only Pectin-FD was further used to investigate the interaction of pectin with procyanidins using ITC because Pectin-S was not obtained in sufficient quantity.

Extraction and Purification of Apple Procyanidins. Hexane, methanol, and aqueous acetone (40:60, v/v) extracts of apple polyphenols were obtained by successive solvent extractions of 'Marie Menard' freeze-dried pulp.[21] Hexane and methanol extracts were discarded as they did not contain the required procyanidin fraction with a number average degree of polymerization around 10.[12] Aqueous acetone extracts containing procyanidins were pooled and concentrated on a rotary evaporator prior to freeze-drying.

The freeze-dried aqueous acetone extracts were injected on a 20 × 5 cm column of LiChrospher 100 RP-18 (12 μm) (Merck, Darmstadt, Germany) and purified as described in Renard et al.[7]

Scanning Electron Microscopy. The cell wall material samples (PB-Ariane, PB-Petit Jaune, AIS-Ariane, PB-20 min-100 °C-S, and PB-20 min-100 °C-FD) were mounted on specimen stubs with double-sided, carbon-conductive, adhesive tape and covered with 20 nm gold layers by ion sputtering (Balzers SCD 004 Sputter Coater, Balzers, Bal Tec. AG, Fürstentum, Lichtenstein). Samples were examined by a Philips XL30 (Philips, Eindhoven, The Netherlands) scanning electron microscope operated at an accelerating voltage of 10 kV and an intensity of 26 μA.

Surface Area. Surface area was determined from nitrogen adsorption isotherms, using a Micromeritics AZAP 2010 system and

Table 1. Composition and Surface Area (BET) of the Different Apple Flesh Cell Wall Materials[a]

		composition (mg/g dry matter)									
cell wall material	BET (m^2/g)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	MeOH (DM)	Pr
AIS-Ariane	2.64	9	10	57	58	13	63	257	256	32 (68)	99
PB-Petit Jaune	2.15	12	9	113	59	18	54	303	217	29 (73)	23
PB-Ariane	2.35	12	10	63	62	15	69	294	319	32 (56)	32
PB-20 min-100 °C-FD	1.26	8	9	49	55	14	57	266	216	17 (45)	32
PB-20 min-100 °C-S	3.16	11	14	60	78	19	71	299	209	31 (81)	32
Pectin-S		8	2	141	13	0,4	47	12	578	71 (67)	
Pectin-FD		10	3	100	29	2	47	22	671	76 (63)	
SDM		0.4	0.4	7.2	1.8	0.5	2.7	6.5	18.4	0.9 (3.0)	

[a]Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Gal A, galacturonic acid; MeOH, methanol; DM, degree of methylation; Pr, protein; SDM, standard deviation of the mean, n = 14.

monitored by an AZAP 2010 version 5.01 (Micromeritics, Norcross, GA, USA). Samples were degassed prior to generation of a Brunauer–Emmett–Teller (BET) isotherm using nitrogen at −196 °C.

Adsorption Experiments. Adsorption experiments were conducted according to the methods described by Renard et al.[7] and Le Bourvellec et al.[8] The cell wall material suspension (12.5 mg in 2 mL) and the polyphenol solution (0.5 mL) were incubated in an 8 mL empty Sep-Pack preparative column (Interchim, Montluçon, France) equipped with a sinter of porosity 20 μm under planetary stirring. After incubation, the solution and the cell wall material/polyphenol complexes were separated by filtration under vacuum. The content of the free polyphenols was measured by OD at 280 nm and/or HPLC following thioacidolysis after freeze-drying as described under Analysis. The amount of polyphenol adsorbed by the cell wall materials was determined by subtracting the amount in the supernatant from that of the initial polyphenol solution. All experiments were carried out at 25 °C, using a citrate/phosphate buffer, pH 3.8, ionic strength = 0.1 M with an incubation time of 1 h and polyphenol concentrations varying from 0.25 to 16 g/L. All assays were carried out in duplicate.

The experimental data were fitted to a Langmuir[22] formula, which describes the amount of bound solute (procyanidins) PP_b (in g/g of adsorbent) as a function of the free solute (procyanidins) concentration $[PP_F]$ at equilibrium.

$$PP_b = \frac{N_{max} K_L [PP_F]}{1 + K_L [PP_F]} \quad (1)$$

where K_L is an apparent affinity constant (expressed in L/g) and N_{max} is the total amount of available binding site or apparent saturation level (expressed in g/g adsorbent).

ITC Experiment. A VP-ITC instrument (MicroCal, GE Healthcare, Chalfont St. Giles, UK) was used to measure enthalpy changes associated with pectin/procyanidin interactions at 298 K. Pectin-FD and procyanidin fractions were dissolved in the same buffer, that is, 0.1 M citrate phosphate at pH 3.8. All solutions were degassed prior to the measurements. In a typical experiment, Pectin-FD solution (3 mM, galacturonic acid equivalent) was placed in the 1.448 mL sample cell of the calorimeter and procyanidin solution (3 mM, epicatechin equivalent) was loaded into the injection syringe. Procyanidin solution was titrated into the sample cell as a sequence of 30 injections of 10 μL aliquots. The duration of each injection was 20 s, and the time delay between successive injections was 5 min. The contents of the sample cell were stirred throughout the experiment at 307 rpm to ensure thorough mixing. The reference cell was filled with deionized water.

Raw data obtained as a plot of heat flow (μcal s^{-1}) against time were then integrated peak-by-peak and normalized to obtain a plot of observed enthalpy change per mole of injectant (ΔH, kcal mol^{-1}) against the molar ratio (procyanidin/pectin). Peak integration was performed using Microcal Origin 7.0 (Microcal Software, GE Healthcar). The experimental data were fitted to a theoretical titration curve using Microcal Origin, with ΔH (enthalpy change), K_a (association constant), and n (number of binding sites per molecule) as adjustable parameters, from the relationship

$$Q_i = \frac{nP_t \Delta H V_0}{2}\left[1 + \frac{A_t}{nP_t} + \frac{1}{nK_a P_t} - \sqrt{\left(1 + \frac{A_t}{nP_t} + \frac{1}{nK_a P_t}\right)^2 - 4\frac{A_t}{nP_t}}\right] \quad (2)$$

where P_t is the total pectin concentration, A_t is the total concentration of the ligand, V_0 is the volume of the cell, and Q_i is the total heat released for injection i. ΔG values and entropy contributions can be then determined from the standard equation

$$\Delta G = -RT \ln K_a = \Delta H - T\Delta S \quad (3)$$

where ΔG, ΔH, and ΔS are the changes in, respectively, Gibbs free energy, enthalpy, and entropy of binding, T is the absolute temperature, R = 8.32 J mol^{-1} K^{-1}, and K_a is the association constant.

Control experiments included the titration of procyanidin fraction into buffer and were subtracted from titration experiments.

Analysis. Polyphenols were measured by high-performance liquid chromatography (HPLC)/diode array detection (DAD) after thioacidolysis as described by Guyot et al.[21] and Le Bourvellec et al.[16] The DPn of procyanidins was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (+)-catechin corresponding to terminal units.

Neutral sugars, galacturonic acids, and methanol were analyzed as described by Renard and Ginies.[23]

Nitrogen was analyzed according to the Kjeldahl method. Protein was calculated as N × 6.25.[24]

The molecular weight distribution of polysaccharides was determined using a HPLC system involving a Jasco LC-NET II/ADC interface, a Jasco PU-2080 plus intelligent HPLC pump, a Jasco RI-2031 plus intelligent RI detector, and a degasser and controlled by ChromNav software (Jasco, Tokyo, Japan). Separations were achieved using two columns in series, a (300 × 8.0 mm i.d.) Shodex OH-pack SB-802 HQ column (Showa Denko Europe, Munich, Germany) and a (300 × 7.8 mm i.d.) TSK-Gel PWXL column (Tosohaas, Stuttgart, Germany) at 35 °C in combination with a guard column (40 × 6.0 mm i.d.) TSK-Gel PWXL (Tosohaas). Solutions (20 μL) of the extracts (10 mg/mL) were injected and eluted with 0.4 M sodium acetate buffer, pH 3.6, at 0.8 mL/min. The retention time of dextran T40 was 11.1 min, and the one of dextran T500 was 15.2 min.

Statistical Analysis. Results are presented as mean values, and the reproducibility of the results was expressed as pooled standard deviation. Pooled standard deviations were calculated for each series of replicates using the sum of individual variances balanced by the individual degrees of freedom.[25]

Analysis of variance (ANOVA) by Fisher 's test (F) was used to compare the means and performed using the Excelstat package of Microsoft Excel. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of Cell Wall Material. Table 1 summarizes the compositions of the different apple cell wall materials (CWM).

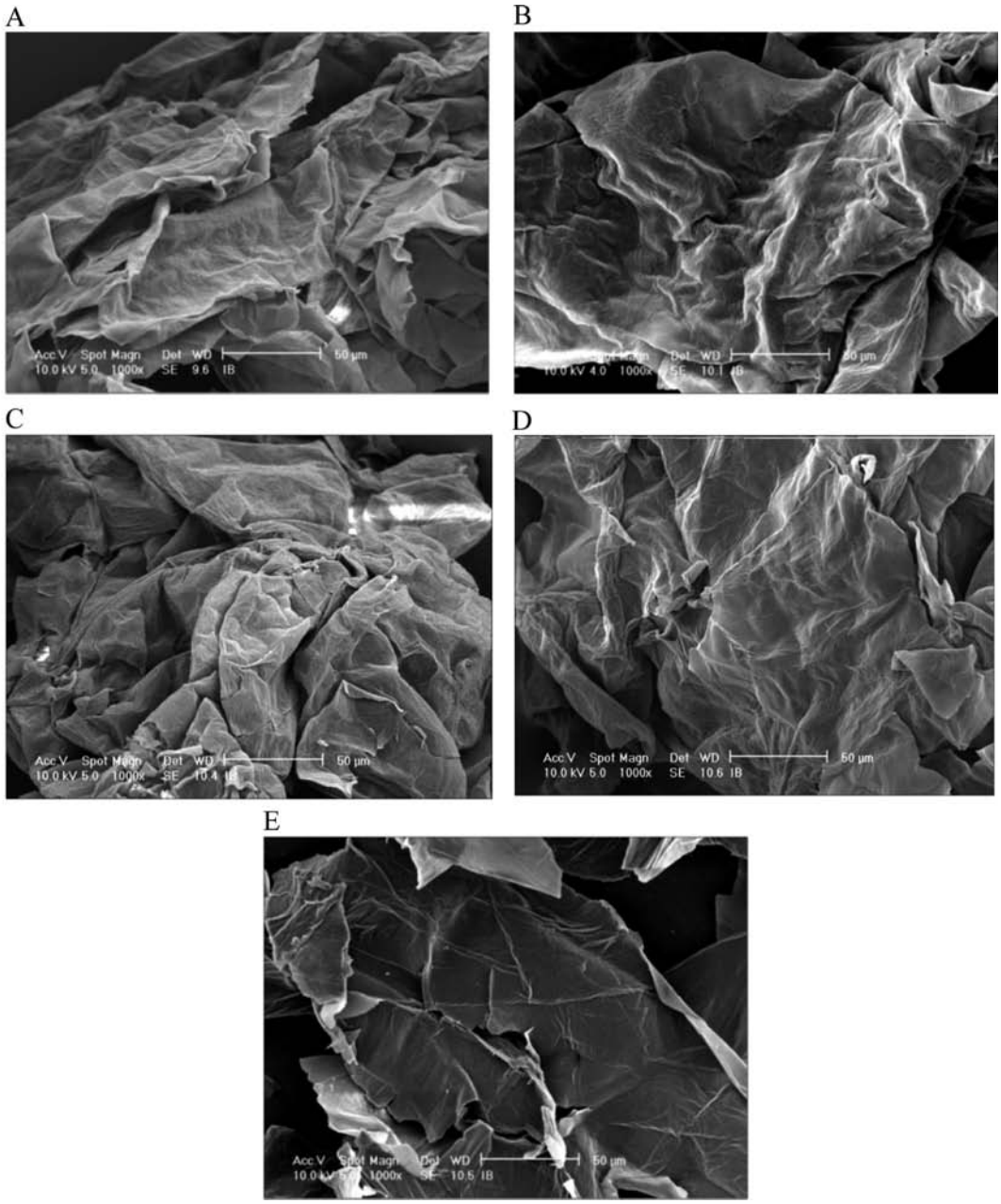

Figure 1. Scanning Electron Microscopy (SEM) of AIS-Ariane (A), PB-Ariane (B), PB-Petit Jaune (C), PB-20 min-100 °C-S (D), and PB-20 min-100 °C-FD (E).

Two methods were used to obtain CWM containing different protein concentrations. The phenol−buffer method[7,26] was chosen to obtain cell walls devoid of procyanidins and with very low protein content. The phenol treatment removes protein,[27] and an aqueous acetone treatment removes polyphenols.[7] Procyanidins could not be detected by HPLC after thioacidolysis and the cell wall materials were white. Low amounts of protein were detected, 23 mg/g (2.6%) and 32 mg/g (3.9%) for PB-Petit Jaune and PB-Ariane, respectively, which was consistent with previous results.[7,8,26] In comparison, the protein content of cell walls prepared from Ariane apples by the ethanol-insoluble method was significantly higher. AIS-Ariane contains 99 mg/g protein (13.1%). These results were within the range of the literature values for CWM prepared according to the ethanol-insoluble method.[20,26] Renard et al.[20] have shown that cell walls prepared from Golden Delicious apples by the ethanol-insoluble method contain 50−100 mg/g protein. AIS-Ariane thus contained 3−4 times as much protein as the PB samples.

The sugar compositions of the different cell wall preparations were very close (Table 1) and consistent with the literature.[8,16,20,26,28,29] They reflected the macromolecular composition of the apple cell wall:[8,16,20,26,28,29] cellulose, highly methylated pectins relatively rich in xylogalacturonans, fucogalactoxyloglucan, and mannan. The two main sugars were glucose (from 257 mg/g to 303 mg/g) and galacturonic acid (from 217 mg/g to 319 mg/g). Glucose came essentially from cellulose.[20] Pectic substances were present in high amounts as shown by the high galacturonic acid content and were highly methylated (from 56 to 68%). In previous work,[20,26,30] degrees of esterification from 63 to 75% were obtained in the AIS preparations from apple. Besides glucose

and galacturonic acid, the neutral sugars arabinose (from 57 to 113 mg/g), xylose (from 58 to 62 mg/g), and galactose (from 54 to 69 mg/g) predominated. The one notable difference was a higher arabinose content in the cell walls from Petit Jaune, almost twice that present in the cell walls from Ariane.

The surface area, determined by N_2 binding isotherms, varied from 2.15 to 2.64 m^2/g for the three "raw" cell walls. The specific surface areas were consistent with previous results.[7,11] Surface area is related to porosity: the more porous the structure is, the higher the surface area. Panels A, B, and C of Figure 1 show the SEM observations of AIS-Ariane, PB-Ariane, and PB-Petit Jaune. The CWM were formed by a network of empty cells with thick walls and irregular shape. No difference in visualization was obtained between cell walls whether they had been prepared after phenol buffer or alcohol.

Incubating the cell walls in hot buffer to mimic cooking is expected to result in pectin solubilization and extraction. Pectins can be extracted from CWM with cold and/or hot water, buffer, or chelator solutions.[12,17,31−34] The extraction yields of CWM after boiling (100 °C, 20 min, citrate/phosphate buffer, pH 3.8) were very close, 10.3% for preparation of PB-20 min-100 °C-FD and 10.6% for PB-20 min-100 °C-S. These extraction yields were high compared with those reported by Stevens and Selvendran,[31] but low compared to those of Renard and Thibault.[33] Stevens and Selvendran[31] reported yields of 6.9% for the extraction of soluble pectins using hot water (80 °C, pH 5) from cell walls of Cox's Orange Pippin apples. Renard and Thibault[33] obtained yields of 31.2% for soluble pectin extraction using hot water (80 °C, pH 6.5) from cell walls of Golden Delicious apples. The differences observed could be due to the conditions used. The β-elimination reaction, which is an important nonenzymatic pectin degradation reaction during thermal processing, is promoted by high methoxy ester content, increasing temperature, increasing pH, and the presence of monovalent salts.[35] The balance of higher temperature, lower pH, and chelating properties of citrate can thus explain the yields obtained here.

The composition (Table 1) of these pectins, that is, pectin-S and pectin-FD, solubilized after boiling were close to those reported for hot-water-soluble pectins,[31,33] with high degrees of methylation (around 65%) and high galacturonic acid content (around 600 mg/g), although they differed for their neutral sugar compositions, particularly arabinose and xylose. The two pectin extracts were chromatographed by size exclusion chromatography; a major peak with a retention time next to the one of dextran T500 indicated the predominance of high molecular weight pectins (Figure 2). The predominance of a high molecular weight pectins is often observed for hot-water-extracted pectins or pectins solubilized during the early stages of cooking.[17] The cooking procedure used here extracted typical apple pectins, with high galacturonic acid content, high degree of methylation, and high molecular weight.

Conversely, in the insoluble cell walls (PB-20 min-100 °C-FD and PB-20 min-100 °C-S) boiling at 100 °C during 20 min caused a decrease of the sugars characteristic of pectins, that is, galacturonic acid, rhamnose, arabinose, and galactose (Table 1). The mode of drying the CWM affects the specific surface areas and causes a definitive modification of the cell wall surface.[36] Drying by freeze-drying caused a marked decrease of the surface area, from 2.35 to 1.26 m^2/g, and a conversion to a nonporous material, as well as a modification of the electron microscopy images. After freeze-drying, cracks were observed at the surface of the CWM, and freeze-drying resulted in a compaction and collapse of the network (Figure 1B,E). These results are consistent with those of Le Bourvellec and Renard[10] on drying of CWM from apple. Drying is known to modify physical properties of dietary fibers by inducing collapse of CWM[13] and, in particular, collapse of β-glucans[29] (i.e., cellulose and xyloglucans) and pectins.[37] It can change surface area and reduce macroporosity.[38] Indeed, the BET isotherms (data not shown) indicated conversion from a macroporous structure (PB-Ariane) to a nonporous structure (PB-20 min-100 °C-FD). However, drying by solvent exchange caused a marked increase of the surface area, from 2.35 to 3.16 m^2/g (Table 1), which resulted from expansion of the tissues when pectins were removed. There was no difference in electron microscopy images between CWM after boiling, which were dried by solvent exchange, and the initial CWM (Figure 1B,D). Weightman et al.[39] have shown that depectinization of fiber, prepared from pea hulls by the alcohol-insoluble solid method, results in an expansion of the tissues and an increase of the porosity. The two cell walls obtained after boiling shared chemical characteristics but markedly differed in terms of physical characteristics.

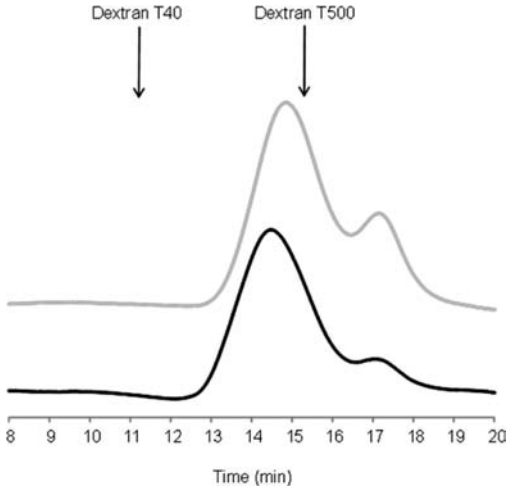

Figure 2. Chromatography of the pectins extracted after boiling (20 min, 100 °C) of PB-Ariane on two columns in series, a Shodex OH-pack SB-802 HQ column and a TSK-Gel PWXL column (eluted with 0.4 M sodium acetate buffer, pH 3.6): gray line, Pectin-S; black line, Pectin-FD.

Polyphenol Characterization. The apple variety Marie Ménard was chosen to obtain a purified fraction of degree of polymerization around 10.[12] The water−acetone extract of apple polyphenols was obtained by successive solvent extraction of the freeze-dried parenchyma. The water−acetone extract, fraction DP9, obtained after methanol elimination of sugars, acids, and polyphenols of low molecular weight, contained 73% of flavan-3-ols composed mainly of polymerized procyanidins[19,21] (Table 2). Procyanidins represented 93% of polyphenols present in fraction DP9. Their respective $\overline{DP}n$ was 8.7. (−)-Epicatechin was always the predominant constitutive unit, accounting for >95% of total units.[19] Fraction DP9 also contained traces of monomeric flavan-3-ols ((−)-epicatechin), hydroxycinnamic acids (chlorogenic acid and *p*-coumaroylquinic acid), and dihydrochalcones (phloridzin and phloretin-2-xyloglucoside).

Binding Isotherm Formulation. During our previous works,[7,8,10,11] we have seen that the shape of the binding isotherms corresponded to a type I isotherm, also called a

Table 2. Composition (Milligrams per Gram Dry Matter) of Apple Procyanidin Fraction DP9[a]

	flavan-3-ols				DHC		HA	
fraction	CAT	EC	PCA	$\overline{DP}n$	XPL[b]	PLZ	CA	PCQ[c]
DP9	0	4.4	680.1	8.7	0.5	2.2	39.9	0.3
SD		0.07	14.58	0.47	0.01	0.02	0.83	0.02

[a]DHC, dihydrochalcones; HA, hydroxycinnamic acid; CAT, (+)-catechin; EC, (−)-epicatechin; PCA, procyanidins; $\overline{DP}n$, number average degree of polymerization of flavan-3-ols (catechins + procyanidins); XPL, phloretin-2-xyloglucoside; PLZ, phloridzin; CA, chlorogenic acid; PCQ, *p*-coumaroylquinic acid; SD, standard deviation, $n = 2$. [b]Quantified as phloridzin. [c]Quantified as *p*-coumaric acid.

Langmuir isotherm.[22,40] This description was chosen because for all the curves a good fit of the data was obtained with $r^2 > 0.9$. However, it is only an empirical description, not meant to imply a mechanism such as described by Langmuir[22] for gas adsorption on solid surfaces. Indeed, most of Langmuir's underlying hypotheses are not verified. In particular, the binding is not totally reversible, so that only apparent association constants can be determined.[10]

Effect of Protein Content. The binding isotherms for AIS-Ariane, PB-Ariane, and PB-Petit Jaune were adjusted according to the Langmuir isotherm (i.e., as a function of the free procyanidin concentrations). For all of the curves (data not shown) a good fit of the data was obtained with this description ($r^2 > 0.9$), and the isotherms obtained were very similar to those observed previously[8,10,11] with procyanidins of similar $\overline{DP}n$. The calculated apparent constants K_L and N_{max} are given in Table 3.

Table 3. Apparent Langmuir Parameters for Binding Isotherms of the Different Apple Procyanidin DP9 Fraction/Cell Wall Material Combinations[a]

	DP9			
cell wall material	K_L (L/g)	N_{max} (g/g)	K_L (L/m^2)	N_{max} (g/m^2)
AIS-Ariane	0.45	0.59	0.17	0.22
PB-Petit Jaune	0.38	0.62	0.17	0.29
PB-Ariane	0.43	0.61	0.18	0.26
PB-20 min-100 °C-FD	0.21	0.67	0.17	0.53
PB-20 min-100 °C-S	0.22	0.79	0.07	0.25

[a]K_L, apparent affinity constant; N_{max}, apparent saturation level.

Changing the CWM by changing its varietal origin or its protein content (Tables 3 and 4) had no impact on the binding; apparent affinities and apparent saturation levels were the same for the different CWM, that is, AIS-Ariane, PB-Ariane, and PB-Petit Jaune, whether the results were expressed per gram of absorbent or per surface. Moreover, for fixed initial concentrations (procyanidins $\overline{DP}n$ 8.7 at 1 g/L and CWM at 5 g/L) (Table 4), the amount of retained procyanidins was not significantly different (Table 5) for the CWM used. Around 50% of the fraction was retained by the CWM. Le Bourvellec et al.[8] found for an initial fraction of $\overline{DP}n$ 9.8 at 1 g/L that 56% of the fraction was retained by CWM from the Petit Jaune variety.

Table 4. Binding of Fraction DP9 on Cell Wall Material: Quantification and Characterization[a]

		bound		free
cell wall material	initial solution $\overline{DP}n$	% total	$\overline{DP}n$	$\overline{DP}n$
AIS-ariane	8.7	52	12.7	4.9
PB-Petit Jaune	8.7	50	13.2	6.1
PB-Ariane	8.7	50	12.7	5.1
PB-20 min-100 °C-FD	8.7	44	15.9	4.7
PB-20 min-100 °C-S	8.7	43	13.4	5.2
SD	0.47	0.50	0.91	0.23

[a]Initial polyphenol concentration, 1 g/L; CWM concentration, 5 g/L; buffer citrate/phosphate, pH 3.8; ionic strength, 0.1 M; 25 °C; incubation time, 1 h; $\overline{DP}n$, number average degree of polymerization of flavan-3-ols (catechins + procyanidins); SD, standard deviation, $n = 5$.

Table 5. Fishers' *F* Values and *P* Values Associated with ANOVAs (Type of Cell Wall Material) Performed on Procyanidins: Percentage of Binding, Polymerization Degree of Bound Procyanidins, and Polymerization Degree of Unbound Procyanidins[a]

	type of cell wall material	
variable	*F*	*P*
percentage of binding	24.53	0.002
$\overline{DP}n$ bound	1.07	0.46
$\overline{DP}n$ unbound	1.37	0.36

[a]$\overline{DP}n$, number average degree of polymerization of flavan-3-ols (catechins + procyanidins).

A selective partition of the procyanidins between the soluble phase and the insoluble material was observed: the $\overline{DP}n$ of the bound procyanidins (Table 4) was higher than those of the initial fraction and of the free procyanidins.[7,8] No significant differences (Table 5) were observed in terms of $\overline{DP}n$ of the bound or unbound procyanidins between the protein-rich cell wall AIS-Ariane and other extracts tested. The interactions between the cell walls and the procyanidins were not influenced by protein content of the cell walls.

Effect of Processing and Drying. The binding isotherms for PB-Ariane, PB-20 min-100 °C-S, and PB-20 min-100 °C-FD were adjusted according to the Langmuir isotherm (i.e., as a function of the free procyanidin concentrations). For all of the curves (data not shown) a good fit of the data was obtained with this description ($r^2 > 0.9$). The calculated apparent constants K_L and N_{max} are given in Table 3.

When the data were expressed per gram of adsorbant, PB-Ariane, PB-20 min-100 °C-S, and PB-20 min-100 °C-FD differed by their apparent affinities and saturation levels with differences higher than a factor 2 overall for apparent affinities (Table 3). The adsorption properties of never-dried cell walls in apples should therefore also be close to those calculated here. However, apparent saturation levels for PB-20 min-100 °C-S and PB-20 min-100 °C-FD were close to but higher than those determined with PB-Ariane. When the data were calculated as a function of the amount of procyanidins bound per surface area of the adsorbant, the comparisons between PB-Ariane, PB-20 min-100 °C-S, and PB-20 min-100 °C-FD changed. Apparent affinities per m^2 were the same for PB-20 min-100 °C-FD and

PB-Ariane, and apparent saturation levels increased markedly after boiling. However, in the case of PB-20 min-100 °C-S the apparent affinity per m^2 decreased markedly after boiling and the apparent saturation level was the same. Three things must be kept in mind at this point: (1) the surface areas given here were measured dry and might be quite different in an aqueous medium; (2) procyanidins are by nature multidentate ligands able to bind simultaneously to more than one point of the macromolecule[41] and here interact with polysaccharides, themselves composed of a number of units presenting some conformational mobility; and (3) formation of multilayers seems likely to occur, given the sizes of the procyanidins molecules and the amounts bound.

Moreover, for fixed initial concentrations (procyanidins $\overline{DP}n$ 8.7 at 1 g/L and CWM at 5 g/L) (Table 4), the amount of retained procyanidins was significantly (Table 5) lower for PB-20 min-100 °C-S and PB-20 min-100 °C-FD than for PB-Ariane. Around 50% of the initial procyanidin fraction was retained by the CWM PB-Ariane, whereas only 44% was retained for PB-20 min-100 °C-FD and 43% was retained for PB-20 min-100 °C-S. A selective partition of the procyanidins between the soluble phase and the insoluble material was also observed between "raw" and boiled CWM: the $\overline{DP}n$ of the bound procyanidins (Table 4) was higher than those of the initial fraction and of the free procyanidins.[7,8] However, whereas differences in amount of adsorbed procyanidins were observed between PB-Ariane and boiled and dry CWM, that is, PB-20 min-100 °C-FD and PB-20 min-100 °C-S (Table 4), no significant differences between the $\overline{DP}n$ of bound and unbound procyanidins (Table 5) were observed. The decrease of apparent affinity or amount of procyanidins bound to the cell wall did not change the selective partition of the procyanidins between the soluble phase and the insoluble CWM, and the ability of CWM to selectively adsorb highly polymerized procyanidins was the same. In the cases of PB-20 min-100 °C-FD and PB-20 min-100 °C-S a decrease in the adsorption of procyanidins of all molecular sizes occurred.

Discussion. The impact of protein content of the CWM has been studied using two CWM with the same chemical characteristics but different protein contents. The interactions between the cell walls and the procyanidins were not influenced by protein content of the cell walls, and the mechanisms thus were confirmed to involve interactions between procyanidins and polysaccharide components of the cell walls.

The impact of boiling of the CWM has been studied using three different CWM: an initial CWM used as a control and the same CWM modified by boiling at 100 °C during 20 min to eliminate the highly methylated pectins (Table 1) and then dried by solvent exchange or by freeze-drying. Processing by boiling had an impact on the binding properties of the CWM. Apparent affinity values obtained with cell walls after boiling were lower than those of PB-Ariane (Tables 3 and 4). This could be ascribed to elimination of highly methylated pectin and to drying, that is, modification of porosity after drying using solvent exchange or freeze-drying. Boiling of the CWM followed by drying by solvent exchange (the same drying as the control PB-Ariane) highlighted the importance of pectin in the procyanidins/cell wall material interactions and confirmed what had been supposed previously, namely, a more marked affinity of the procyanidins for pectins than for the other cell wall polysaccharides and, in particular, for highly methylated pectins.[7,11,12] The increase of the saturation level in the case of PB-20 min-100 °C-S could be due to an increase of the surface area, from 2.35 m^2/g for PB-Ariane to 3.16 m^2/g for PB-20 min-100 °C-S (Table 1). The increase in cell wall porosity after soluble pectin elimination and drying by solvent exchange may enhance the encapsulation of procyanidins within a more open network.[11] This may also favor the formation of interactions by hydrogen bonds or hydrophobic interactions with the constitutive polysaccharides of the cell wall such as residual pectin and hemicellulose.[11] In the case of PB-20 min-100 °C-FD the increase of saturation level together with a reduction of surface area, from 2.35 m^2/g for PB-Ariane to 1.26 m^2/g for PB-20 min-100 °C-FD (Table 1), would be contradictory if the adsorption was a simple surface phenomenon. This may be due to three mechanisms: (1) the existence of a cooperative mechanism;[7,10] (2) a self-association of procyanidins;[10,42,43] and (3) a facilitation due to cell wall material architecture[10] of the autoassociation of procyanidins.

Adsorption of one epicatechin unit on a binding site could facilitate the positioning of the rest of the molecule on the CWM, thus favoring contact between other units of the procyanidins and binding sites located in the vicinity.[7,44] This cooperative mechanism may enhance the amount of bound procyanidins.

Procyanidins are multidentate molecules that can autoassociate.[10,42,43] It has been previously shown in our experimental conditions,[10,43] that procyanidins are prone to autoassociation and, particularly, ones of intermediate degree of polymerization, that is, procyanidins with $\overline{DP}n$ around 10 had the strongest ability to autoassociate.

The architectural structure of PB-20 min-100 °C-FD (Figure 1E) could explain the high apparent saturation level observed: the low porosity and the smoothness of the CWM would facilitate autoassociation of procyanidins.

As a consequence of cooperative mechanism, autoassociation of procyanidins, and architectural structure of PB-20 min-100 °C-FD, formation of multilayers at the surface of the CWM is possible and even probable, given the amounts bound.[10]

Elimination of highly methylated pectins after boiling decreased the apparent affinity of cell walls for procyanidins, but the effects on saturation level were less clear as drying also modified the cell wall porosity.

Presentation of the apparent affinities and saturation levels as a function of the amount of procyanidins bound per surface area of the adsorbent changed the comparisons between PB-Ariane, PB-20 min-100 °C-S, and PB-20 min-100 °C-FD. Apparent affinities per m^2 were the same for PB-20 min-100 °C-FD and PB-Ariane, and apparent saturation levels increased markedly after boiling. In the case of PB-20 min-100 °C-S apparent affinity per m^2 decreased markedly after boiling and apparent saturation level was the same. However, the calculated surface areas given here were measured dry and might be quite different in an aqueous medium. The reduction of porosity after freeze-drying was consecutive to a collapse of the structure of the cell wall,[13,29,38] which could itself be accompanied by a change of conformation of constitutive cell wall polysaccharides. The formation of multilayers seems to be facilitated in the case of PB-20 min-100 °C-FD due to the structure of the CWM, that is, a nonporous material. Le Bourvellec and Renard[10] have shown that the modification of the physical state of cell walls (i.e., harsh drying) affects the most their capacity to interact with procyanidins. In that study, modifying the CWM by harsh drying increased the saturation level[10] by facilitating the autoassociation of the polyphenols. Reduction of the

apparent affinity of PB-20 min-100 °C−S could also be explained by an elimination of highly methylated pectins.[7,11,12]

The expression of the apparent affinity and apparent saturation level per gram of absorbent highlights the impact of the pectins in the cell wall/procyanidin interactions, whereas the expression of constant per m^2 of absorbent demonstrated the impact of the drying, that is, the collapse of the CWM.

ITC Experiment. Insofar as we have demonstrated strong interactions between procyanidins and pectins,[7,11,12] characterization of these interactions was done using ITC.

ITC is a powerful direct method to study the interactions between polyphenols and macromolecules.[41] It enables the determination of the thermodynamic parameters (stoichiometry, association constant, and enthalpy of binding) of polyphenol/macromolecule interactions[45−48] in one direct measurement.

The titration of 3 mM (galacturonic acid equivalent) Pectin-FD by 3 mM (epicatechin equivalent) fraction DP9 is shown in Figure 3. Each peak corresponds to the heat change associated with injection of aliquot of procyanidin fraction DP9 solution into the calorimeter cell containing the Pectin-FD solution. A negative heat flow represents an exothermic change, and a positive heat flow represents an endothermic change. A decrease in exothermic peaks upon procyanidins addition was observed (Figure 3). As the procyanidin concentration increased, the number of available binding sites on the Pectin-FD decreased; hence, the exothermic contribution to the enthalpy changes associated with binding was decreased. All of the binding sites of Pectin-FD were saturated, and the addition of more procyanidins led to a plateau. The interaction between procyanidins and pectins thus corresponded also to a classical ligand interaction with the presence of a limited number of binding sites for the procyanidin on the pectin macromolecule.

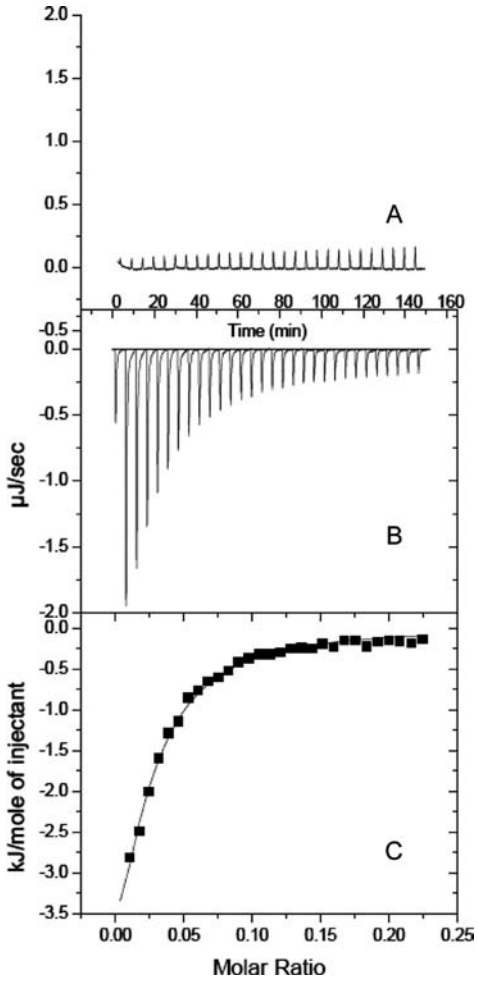

Figure 3. (A) Blank experiment for injection of fraction DP9 3 mM (epicatechin equivalent) in buffer (pH 3.8, FI 0.1 M); (B) raw data plot of heat flow against time for the titration of 3 mM (epicatechin equivalent) fraction DP9 into 3 mM (galacturonic acid equivalent) Pectin-FD; (C) integration of peak areas and normalization yields a plot of molar enthalpy change against procyanidin/pectin ratio. The one-site fit curve is displayed as a thin line. Experiments were done in duplicate.

From the titration curve, thermodynamic parameters were determined. The stoichiometry (n) of the binding was calculated to be 0.0236 (±0.006), suggesting that approximately 1 mol equivalent of flavan-3-ol monomer binds to 42 mol equivalents of galacturonic acid. The stoichiometry (n) was lower than that observed for tannin/poly(L-proline) interactions[47,49] or tannin/bovine serum albumin or tannin/gelatin interactions[46] (Table 6). This is probably due to steric hindrance phenomena;[47,49] the n value is limited by the size of the tannin molecule because a larger molecule can occupy a greater number of binding sites on pectin.[46] Interactions with association constant $K_a > 10^4\ M^{-1}$ are considered to be of high affinity.[50] Thus, the association constant ($K_a = 1.4 \times 10^4\ M^{-1} \pm 3.1 \times 10^3$) observed in our study suggested strong interaction between procyanidin fraction DP9 and Pectin-FD. However, this binding constant is at least 2 orders of magnitude lower than that observed for tannin/gelatin interactions,[46] or tannin/poly(L-proline) interactions[47,49] (Table 6) but 1 order of magnitude greater than tannin/bovine serum albumin interactions[46] (Table 6). The associations between procyanidins and CWM could be related to a tanning effect as in the case of polyphenol/protein interactions.[41] This tanning effect is a function of both the presence of functional groups able to form hydrogen bonds and hydrophobic interactions with macromolecules and of the molecular weight, that is, the size of the polyphenol.[41] The larger polyphenols have been reported to bind more strongly with CWM.[7,8,10,11] However, Frazier et al.,[46] Poncet-Legrand et al.,[47] and McRae et al.[49] have used procyanidin fractions with lower $\overline{DP}n$ than that used here (Table 6). De Freitas and Mateus[51] have shown that the esterification of (−)-epicatechin with gallic acid at the C(3) hydroxyl function in the dimer B_2 increased its capacity to form insoluble complexes with proline-rich protein. They explained this by the fact that the (−)-epicatechin gallate molecule has a well-exposed galloyl function which enables binding to several sites. The galloylation of some flavan-3-ol units of the grape seed tannins used by Frazier et al.,[46] Poncet-Legrand et al.[47] and McRae et al.[49] compare to apple procyanidins without galloylated unit might contribute to the differences observed here. Moreover, they[51] have also shown that (+)-catechin had a higher tannin specific activity for proline-rich protein than (−)-epicatechin. It is well-known that grape seed procyanidins contain (+)-catechin as extension unit and terminal unit[8,52] whereas apple procyanidins contain predominantly (−)-epicatechin with (+)-catechin only as terminal unit,[19,21] which might play a role. Fletcher et al.[53] have shown by NMR that polymers related to (+)-catechin would form a right-handed helix, whereas polymers related to (−)-epicatechin would form a left-handed helix. The difference observed here could also be

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Table 6. Thermodynamic Parameters for the Interaction of Procyanidins with Different Macromolecules

procyanidins	macromolecules	n	K_a (10^4 M^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	$-T\Delta S$ (kJ mol^{-1})	reference
$\overline{DP}n$ 8.7	pectin	0.0236 ± 0.006	1.4 ± 0.31	−23.5 ± 0.6	−6.6 ± 3.1	−16.9 ± 3.6	
$\overline{DP}n$ 4.8 and %gall[a] 23.7%	poly(L-proline)	1.8 ± 0.03	70 ± 25	−33.7	−51.3 ± 1.3	17.6	McRae et al.[49]
$\overline{DP}n$ 3.8 and %gall[a] 14%	poly(L-proline)	6.0 ± 0.1	34.3 ± 0.6	−31.6 ± 0.4	−11.2 ± 0.2	−20.4 ± 0.2	Poncet-Legrand et al.[47]
grape seed tannins[b]	gelatin	35	33	−31.5	−38.0	6.5	Frazier et al.[46]
grape seed tannins[b]	BSA[c]	7	0.15	−18.1	−102	8.4	Frazier et al.[46]

[a]Percentage of galloylated constitutives units. [b]Grape seed tannins were composed of procyanidins and galloylated procyanidins ranging from dimers to pentamers.[49] [c]Bovine serum albumin.

due to formation of a kink, due to the presence of (+)-catechin as extention unit, in the grape seed tannin polymer that might permit an open and flexible conformation facilitating the formation of hydrogen bonds and hydrophobic interactions. The difference in stoichiometry and binding constant observed could be due to a well-exposed galloyl function, which enables binding to several sites, and to a more open and flexible structure of grape seed procyanidins compared to apple procyanidins. The differences may also be due to structural and conformational characteristics of the binding macromolecules used,[41] that is, pectin and protein.

The association constant K_a corresponds to a free energy of binding (ΔG) of −23.5 ± 0.6 kJ mol^{-1}. Analysis of the thermodynamic contributions ($\Delta G = \Delta H - T\Delta S$) indicated a limited favorable enthalpy contribution (ΔH = −6.6 ± 3.1 kJ mol^{-1}) related to the exothermic interaction, but a strong entropy contribution ($-T\Delta S$ = −16.9 ± 3.6 kJ mol^{-1}), indicating that the interaction was mostly driven by entropy. Poncet-Legrand et al.[47] have also shown that the interactions between procyanidin-rich fraction (DP4) and poly(L-proline) were entropy-driven. The explanation for the entropically driven reaction has been proposed to be the hydration effect during the complex formation, resulting in the release of water molecules from the complex interface due to hydrophobic interactions.[47] The positive value of entropy thus implied a role of hydrophobic interaction in the formation of the complex.

This is the first time that interactions between procyanidins and pectins have been studied by ITC. In solution, procyanidins show significant affinity for pectins, one of the main components of cell walls. Work is underway by ITC to define the main features of pectin structures that influence interactions with procyanidins.

Disruption of the natural matrix during processing and interaction between procyanidins and cell walls may have a strong influence in the release, in the bioavailability, and in the biological activity of procyanidins. This study showed that despite elimination of pectins, after boiling, the cell wall still had an affinity for procyanidins that may protect them by encapsulation in the matrix[4] during their transit through the digestive system to be metabolized in the colon. This work also showed that the capacity of cell walls to interact with tannins is not related to their protein content, but appears to be linked to their physical state and or pectin content.

It is becoming increasingly clear that the colonic metabolites of procyanidins are carriers of biological activities.[54] These metabolites are produced during fermentation by the colonic microflora of native molecules and are strongly absorbed. The biological effects of procyanidins are largely due not to the molecules themselves but to their degradation products in the colon. Procyanidins associated with cell wall or pectins are not bioavailable in the human upper intestine and reach the colon, where they become fermentable substrates for bacterial microflora.[2] Native procyanidins of high degrees of polymerization more significantly inhibited the fermentation of apple fiber than smaller molecules. However, the relative proportions of short-chain fatty acids are not affected.[55] The role of plant cell walls in the bioaccessibility and fermentation of polyphenols is a poorly documented area. It seems that it can both limit their bioaccessibility in the upper digestive tract, protecting them until they reach the colon, and then act as a nutrient for colonic microorganisms responsible for the conversion of procyanidins into active metabolites. Future research on the health benefits of polyphenols requires a complete understanding of the mechanisms of diffusibility, absorption, bioavailability, metabolism, and excretion.

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Notes

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REFERENCES

(1) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S−242S.

(2) Palafox-Carlos, H.; Ayala-Zavala, J. F.; Gonzalez-Aguilar, G. A. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *J. Food Sci.* **2011**, *76* (1), R6−R15.

(3) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130* (8), 2073S−2085S.

(4) Parada, J.; Aguilera, J. M. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* **2007**, *72*, R21−R32.

(5) Saura-Calixto, F.; Perez-Jimenez, J.; Tourino, S.; Serrano, J.; Fuguet, E.; Torres, J. L.; Goni, I. Proanthocyanidin metabolites associated with dietary fibre from in vitro colonic fermentation and proanthocyanidin metabolites in human plasma. *Mol. Nutr. Food Res.* **2010**, *54*, 939−946.

(6) Saura-Calixto, F. Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *J. Agric. Food Chem.* **2011**, *59*, 43−49.
(7) Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau, J. F. Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* **2001**, *29*, 115−125.
(8) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part I. Effect of some environmental parameters. *Biochim. Biophys. Acta* **2004**, *1672* (3), 192−202.
(9) Le Bourvellec, C.; Le Quere, J. M.; Renard, C. M. G. C. Impact of noncovalent interactions between apple condensed tannins and cell walls on their transfer from fruit to juice: Studies in model suspensions and application. *J. Agric. Food Chem.* **2007**, *55*, 7896−7904.
(10) Le Bourvellec, C.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part II: Quantification and impact of cell wall drying. *Biochim. Biophys. Acta* **2005**, *1725*, 1−9.
(11) Le Bourvellec, C.; Bouchet, B.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochim. Biophys. Acta* **2005**, *1725*, 10−18.
(12) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Interactions between apple (*Malus* × *domestica* Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydr. Polym.* **2009**, *75*, 251−261.
(13) Kunzek, H.; Muller, S.; Vetter, S.; Godeck, R. The significance of physico chemical properties of plant cell wall materials for the development of innovative food products. *Eur. Food Res. Technol.* **2002**, *214*, 361−376.
(14) Colin-Henrion, M.; Mehinagic, E.; Renard, C. M. G. C.; Richomme, P.; Jourjon, F. From apple to applesauce: processing effects on dietary fibres and cell wall polysaccharides. *Food Chem.* **2009**, *117*, 254−260.
(15) Binner, S.; Jardine, W. G.; Renard, C. M. G. C.; Jarvis, M. C. Cell wall modifications during cooking of potatoes and sweet potatoes. *J. Sci. Food Agric.* **2000**, *80*, 216−218.
(16) Le Bourvellec, C.; Bouzerzour, K.; Ginies, C.; Regis, S.; Ple, Y.; Renard, C. M. G. C. Phenolic and polysaccharidic composition of applesauce is close to that of apple flesh. *J. Food Compos. Anal.* **2011**, *24*, 537−547.
(17) Renard, C. M. G. C. Effects of conventional boiling on the polyphenols and cell walls of pears. *J. Sci. Food Agric.* **2005**, *85*, 310−318.
(18) Asami, D. K.; Hong, Y. J.; Barrett, D. M.; Mitchell, A. E. Processing-induced changes in total phenolics and procyanidins in clingstone peaches. *J. Sci. Food Agric.* **2003**, *83*, 56−63.
(19) Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J. F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). *J. Agric. Food Chem.* **1998**, *46*, 1698−1705.
(20) Renard, C. M. G. C.; Voragen, A. G. J.; Thibault, J. F.; Pilnik, W. Studies on apple protopectin: I. Extraction of insoluble pectin by chemical means. *Carbohydr. Polym.* **1990**, *12*, 9−25.
(21) Guyot, S.; Marnet, N.; Drilleau, J. F. Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. *J. Agric. Food Chem.* **2001**, *49*, 14−20.
(22) Langmuir, I. The adsorption of gases on plane surfaces of glass, mica and platinum. *J. Am. Chem. Soc.* **1918**, *40*, 1361−1403.
(23) Renard, C. M. G. C.; Ginies, C. Comparison of the cell wall composition for flesh and skin from five different plums. *Food Chem.* **2009**, *114*, 1042−1049.
(24) Hach, C. C.; Brayton, S. V.; Kopelove, A. B. A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. *J. Agric. Food Chem.* **1985**, *33*, 1117−1123.
(25) Box, G. E. P.; Hunter, W. G.; Hunter, J. S. *Statistics for Experimenters, An Introduction to Design, Data Analysis and Model Building*; John Wiley and Sons: New York, 1978.
(26) Renard, C. M. G. C. Variability in cell wall preparations: quantification and comparison of common methods. *Carbohydr. Polym.* **2005**, *60*, 515−522.
(27) Selvendran, R. R.; Oneill, M. A. Isolation and analysis of cell walls from plant material. *Methods Biochem. Anal.* **1987**, *32*, 25−153.
(28) van der Sluis, A. A.; Dekker, M.; de Jager, A.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *J. Agric. Food Chem.* **2001**, *49*, 3606−3613.
(29) Massiot, P.; Renard, C. M. G. C. Composition, physico-chemical properties and enzymatic degradation of fibres prepared from different tissues of apple. *Lebensm. Wiss. -Technol.* **1997**, *30*, 800−806.
(30) Voragen, G. J.; Heutink, R.; Pilnik, W. Solubilization of apple cell walls with polysaccharide-degrading enzymes. *J. Appl. Biochem.* **1980**, *2*, 452−468.
(31) Stevens, B. J. H.; Selvendran, R. R. Structural features of cell-wall polymers of the apple. *Carbohydr. Res.* **1984**, *135* (1), 155−166.
(32) Selvendran, R. R. Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *J. Cell. Sci. Suppl.* **1985**, 51−88.
(33) Renard, C.; Thibault, J. F. Structure and properties of apple and sugar-beet pectins extracted by chelating agents. *Carbohydr. Res.* **1993**, *244* (1), 99−114.
(34) Massiot, P.; Baron, A.; Drilleau, J. F. Characterization and enzymatic hydrolysis of cell-wall polysaccharides from different tissue zones of apple. *Carbohydr. Polym.* **1994**, *25* (3), 145−154.
(35) Keijbets, M.; Pilnik, W. β-Elimination of pectin in the presence of anions and cations. *Carbohydr. Res.* **1974**, *33*, 359−362.
(36) Popielarska-Konieczna, M.; Bohdanowicz, J.; Starnawska, E. Extracellular matrix of plant callus tissue visualized by ESEM and SEM. *Protoplasma* **2010**, *247*, 121−125.
(37) Femenia, A.; Garcia-Pascual, P.; Simal, S.; Rossello, C. Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller. *Carbohydr. Polym.* **2003**, *51*, 397−405.
(38) Guillon, F.; Auffret, A.; Robertson, J. A.; Thibault, J. F.; Barry, J. L. Relationships between physical characteristics of sugar-beet fibre and its fermentability by human faecal flora. *Carbohydr. Polym.* **1998**, *37*, 185−197.
(39) Weightman, R. M.; Renard, C. M. G. C.; Gallant, D. J.; Thibault, J. F. Structure and properties of the polysaccharides from pea hulls−II; Modification of the composition and physico-chemical properties of pea hulls by chemical extraction of the constituent polysaccharides. *Carbohydr. Polym.* **1995**, *26* (2), 121−128.
(40) Brunauer, S.; Deming, L. S.; Deming, W. E.; Teller, E. On a theory of the van der Waals adsorption of gases. *J. Am. Chem. Soc.* **1940**, *62*, 1723−1732.
(41) Le Bourvellec, C.; Renard, C. M. G. C. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 213−248.
(42) Riou, V.; Vernhet, A.; Doco, T.; Moutounet, M. Aggregation of grape seed tannins in model wine − effect of wine polysaccharides. *Food Hydrocolloids* **2002**, *16*, 17−23.
(43) Poncet-Legrand, C.; Cartalade, D.; Putaux, J. L.; Cheynier, W.; Vernhet, A. Flavan-3-ol aggregation in model ethanolic solutions: Incidence of polyphenol structure, concentration, ethanol content, and ionic strength. *Langmuir* **2003**, *19*, 10563−10572.
(44) Baxter, N. J.; Lilley, T. H.; Haslam, E.; Williamson, M. P. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry* **1997**, *36*, 5566−5577.
(45) Kriz, Z.; Koca, J.; Imberty, A.; Charlot, A.; Auzely-Velty, R. Investigation of the complexation of (+)-catechin by β-cyclodextrin by a combination of NMR, microcalorimetry and molecular modeling techniques. *Org. Biomol. Chem.* **2003**, *1*, 2590−2595.

(46) Frazier, R. A.; Deaville, E. R.; Green, R. J.; Stringano, E.; Willoughby, I.; Plant, J.; Mueller-Harvey, I. Interactions of tea tannins and condensed tannins with proteins. *J. Pharm. Biomed. Anal.* **2010**, *51*, 490−495.
(47) Poncet-Legrand, C.; Gautier, C.; Cheynier, V.; Imberty, A. Interactions between flavan-3-ols and poly(L-proline) studied by isothermal titration calorimetry: effect of the tannin structure. *J. Agric. Food Chem.* **2007**, *55*, 9235−9240.
(48) Patel, A. R.; Seijen-ten-Hoorn, J.; Velikov, K. P. Colloidal complexes from associated water soluble cellulose derivative (methylcellulose) and green tea polyphenol (epigallocatechin gallate). *J. Colloid Interface Sci.* **2011**, *364*, 317−323.
(49) McRae, J. M.; Falconer, R. J.; Kennedy, J. A. Thermodynamics of grape and wine tannin interaction with polyproline: implications for red wine astringency. *J. Agric. Food Chem.* **2010**, *58*, 12510−12518.
(50) Turnbull, W. B.; Daranas, A. H. On the value of *c*: can low affinity systems be studied by isothermal titration calorimetry? *J. Am. Chem. Soc.* **2003**, *125*, 14859−14866.
(51) de Freitas, V.; Mateus, N. Structural features of procyanidin interactions with salivary proteins. *J. Agric. Food Chem.* **2001**, *49*, 940−945.
(52) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.
(53) Fletcher, A. C.; Porter, L. J.; Haslam, E. Hindered rotation and helical structures in natural procyanidins. *J. Chem. Soc., Chem. Commun.* **1976**, 627−629.
(54) Williamson, G.; Clifford, M. N. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br. J. Nutr.* **2010**, *104*, S48−S66.
(55) Bazzocco, S.; Mattila, I.; Guyot, S.; Renard, C. M. G. C.; Aura, A. M. Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *Eur. J. Nutr.* **2008**, *47*, 442−452.