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Impact of Noncovalent Interactions between Apple Condensed Tannins and Cell Walls on Their Transfer from Fruit to Juice: Studies in Model Suspensions and Application

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The adsorption of procyanidins (condensed tannins) on cell-wall material was quantified by bringing into contact solutions of procyanidins and suspensions of cell-wall material. A model was developed on the basis of the Langmuir isotherm formulation and a factorial experimental design. The parameters that influenced the adsorption were the concentration and molecular weight of the procyanidins, the ionic strength of the solution, the temperature, and the apple cell-wall concentration. The model was applied to partitioning of procyanidins from apple between juice and mash. The parameters to be taken into account are the composition of the apples and, specifically, (i) the concentration and molecular weight of the procyanidins, (ii) their acidity and pH as a determinant of the ionic strength, and (iii) their cell-wall content and the temperature at pressing. To estimate the ability of the model to relate procyanidin concentrations in the juice to their concentration in the apple, apples of three varieties of widely different procyanidin compositions were pressed in conditions that prevent oxidation. In these conditions, yields in the juice were >80% for phenolic acids or catechin monomers but <50% for procyanidins, with the lowest rates obtained for the higher polymers in accordance with the model.

KEYWORDS: Polyphenols; procyanidin; polysaccharides; extraction; Langmuir isotherms

INTRODUCTION

Cider apples, especially bitter and bittersweet apples, are characterized by high concentrations of tannins (*1, 2*). Tannins in apple are procyanidins, consisting of oligomers and polymers of catechin units, with >95% (–)-epicatechin, linked to one another by either C4 → C8 or C4 → C6 bonds. Their molecular weights differ between the varieties, and number average degrees of polymerization ($\overline{DP}n$) between 2 and 50 can be observed (*1–3*). These tannins contribute to the organoleptic properties of cider, especially by their astringency and bitter taste (*4*).

Recent studies, carried out at the Unité de Recherches Cidricoles, have highlighted a discrepancy, both quantitative and qualitative, between procyanidin concentrations in the fruits and juices (*2*). Apples rich in high $\overline{DP}n$ procyanidins give juices poor in procyanidins, and these procyanidins have a much lower $\overline{DP}n$, while after extraction, the same high-molecular-weight procyanidins appear soluble in acidic aqueous solution. Procyanidins of high degrees of polymerization could be particularly affected because of their capacity to be selectively adsorbed on cell-wall material and their sensibility to oxidation (*2, 5*). The first step of processing of apple into cider is the crushing and pressing of the fruits, during which polyphenols, polyphenol oxidase, oxygen, and cell walls, initially segregated, come into contact and may react (*5, 6*). Thus, the phenolic composition of apple mush and cider depends upon the initial composition of the fruit and the extraction conditions of the juice (*2*).

Our recent results showed that apple cell walls had the capacity to bind apple procyanidins and that this retention depends upon compositional and structural parameters, such as stereochemistry, conformational flexibility, and molecular weight, and procyanidin concentrations (*5, 7–9*). Besides, we found that changing the cell-wall material by changing its origin or by extraction of pectins and xyloglucans has less effect (*5*) than modifying the physical state of the cell-wall material (*8*). A decrease of the cell-wall-material porosity by harsh drying decreased the apparent affinity between cell-wall material and procyanidins. This drying modified the cell-wall surface, with a marked decrease of the surface area, from 2.15 to 0.52 m^2/g, and the conversion of a porous material to a nonporous material (*8*). The question of the mechanism(s) of the association between procyanidins and cell-wall material was approached by using physicochemical parameters or compounds that have the capacity to inhibit or increase the ionic interaction, hydrophobic interaction, or hydrogen bonding (*7*). In the pH range of 2–7,

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pH has no influence on association (*5, 7*). The electrostatic or ionic interaction does not appear to play any part in the association between procyanidins and cell-wall material. The amount of bound procyanidins increases when the ionic strength increases and decreases with an increasing temperature (*7*). The addition of either a chaotropic agent, such as urea, or a solvent, such as dioxane or ethanol, also resulted in decreased association between procyanidins and cell-wall material (*5, 7*). This indicates that the adsorption mechanism involved the establishment of weak interactions, more precisely hydrogen bonds and hydrophobic interactions (*5, 7*).

We have shown (*5, 8, 9*) that procyanidins/cell-wall association could be adjusted by a type-I isotherm (Langmuir isotherm) (*10, 11*)

$$\mathrm{PPb} = \frac{N_{\max} K_L [\mathrm{PP_f}]}{1 + K_L [\mathrm{PP_f}]} \quad (1)$$

where PPb is the amount of bound procyanidins (expressed in g/g of adsorbent), $[PP_f]$ is the free solute procyanidin concentrations (expressed in g/L) at equilibrium, K_L is an apparent affinity constant (expressed in L/g), and N_{max} is a measure of the adsorbant bound by the sorbent under equilibrium conditions. We have chosen the type-I isotherm formula (Langmuir isotherms) to describe binding of tannins to apple cell walls because it is simple and intuitive, with only two parameters, and because, for all of the curves, a good fitting of the data was obtained (*5, 8, 9*). However, it is only an empirical description and not meant to imply a mechanism, such as described by Langmuir (*10*) for gas adsorption on solid surfaces. Freundlich and Scatchard isotherm formulations were also tested but were less efficient. Both K_L and N_{max} were reported (*5, 8*) (with their confidence intervals) for procyanidin fractions varying by their $\overline{DP}n$ and galloylation and for cell-wall preparations of different varietal origin and treatment (drying and extraction).

The cell-wall material/procyanidin association was thus influenced by a number of chemical and physicochemical factors, which will, in practice, depend upon the initial characteristics of the apples (procyanidins $\overline{DP}n$ and concentration, ionic strength (*5, 7*), and cell-wall content) or the pressing environment (temperature) (*7*).

The aim of this work was to relate the mechanisms and parameters from work on isolated procyanidins and isolated cell walls to actual pressings of apples. For that, in a first step, we will attempt to link the Langmuir parameters to these intrinsic and extrinsic factors to quantify the effects of the various factors on adsorption and to be able to extrapolate the results obtained to all apples. This appeared possible because procyanidins of apples constitute an homogenous series of polymers of (–)-epicatechin and (+)-catechin (*3, 12*), and their physicochemical properties can therefore be expected to be primarily related to their $\overline{DP}n$. This entails establishing a relation between the parameters in the isotherm formulation (eq 1), K_L (affinity of the cell wall), and N_{max} (saturation level), and the factors ($\overline{DP}n$ of the procyanidins, temperature, and ionic strength) that influenced adsorption. This will be done by using the results reported previously (*7, 8*). We will then derive mathematical relations and estimate parameters between several relevant factors and adsorption.

The second step corresponds to the translation of this adsorption equation into a model that can allow for the determination of the juice procyanidin concentrations according to the initial composition of the fruits and the process conditions, such as temperature, using data easily measurable in an actual experiment (procyanidins concentration, cell-wall content, pH, and acidity). Last, the validity and limits of this model were tested. For that, three apple varieties of widely different procyanidin compositions were pressed in conditions that prevent oxidation, to estimate the ability of the model to relate the procyanidin concentration in the juice to the composition of the apple.

MATERIALS AND METHODS

Plant Material. Apple fruits (*Malus domestica* Borkh.) of the Avrolles, Kermerrien, and Jeanne Renard varieties were used for procyanidin preparations, and apple fruits of the Petit Jaune variety were used for the cell-wall preparation. They were harvested at commercial maturity 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 already described (*13*), and cortex tissues were freeze-dried for procyanidin extraction.

Apple fruits of the Kermerrien, Guillevic, and Douce Coët Ligné varieties were used for apple juice preparation. They were harvested at commercial maturity during the 2003 season in the experimental orchard of the Centre Technique des Productions Cidricoles.

Perry pears of the Fausset variety were harvested before commercial maturity on October 10, 2002 in the orchard of Mr. Boisgontier (Orne, France). The fruits were stored at ambient temperature for 12 days and chilled by storage for 2 days at 2 °C before transformation.

Chemicals. Methanol, acetonitrile, and acetone of chromatographic quality were provided by Biosolve (Distribio, Evry, France), and toluene-α-thiol was provided by Merck (Darmstadt, Germany). Hexane (Merck, Darmstadt, Germany) was of analytical quality.

Preparation of Cell-Wall Material. Cell-wall material (CWM) was prepared from Petit Jaune apples devoid of starch (negative visual iodine test) by the phenol/buffer method according to Renard et al. (*5*) A buffer simulating the ionic conditions in apple juice (1.2 mM $CaCl_2$, 2.0 mM $MgCl_2$, 0.5 g/L KCl, 60 mg/L ascorbic acid, and 4 g/L malic acid plus sodium disulfite (antioxidant) at 1 g/L, adjusted to pH 3.5 with 5 M NaOH) was used throughout the procedure (this solution will be called "buffer" in the following paragraphs). Cold apple slices (~100 g) were suspended in chilled buffer (500 mL) plus Triton 100 (2 g/L) and octanol (1 mL) and blended for 6 successive bursts of 15 s in a Braun kitchen blender. The detergent was then washed off with chilled buffer in a cold room (4 °C) on a G3 sintered glass filter until foaming disappeared. The cell walls were then suspended in chilled acetone/water (60:40, v/v) and transferred to a G3 sintered glass filter, still in the cold room. After washing with acetone/water, the excess solvent was removed by aspiration under vacuum and the remaining paste was weighed and suspended in 4 times its weight of phenol for 1 h at room temperature. The saturated phenol solution was removed by extensive washing with buffer on G3 sintered glass (until the phenol smell disappeared). The sample was finally solvent-exchanged in 70% ethanol (3 times), then with 96% ethanol (3 times) and acetone (3 times), and then overnight in an oven at 40 °C.

Extraction and Purification of Apple and Pear Procyanidins. Apple and pear procyanidins were isolated as described in Le Bourvellec et al. (*7*). Procyanidin fractions named Adp 70, Adp 10, and Adp 3 were obtained from Avrolles, Kermerrien, and Jeanne Renard apple varieties with $\overline{DP}n$ of 65.5, 9.8, and 2.5, respectively. The pear procyanidin fraction was named Pdp 35; it was obtained from the Fausset pear variety and had a $\overline{DP}n$ of 34.7.

Preparation of Apple Juices. A total of 2 kg of fruits (varieties Kermerrien, Douce Coët Ligné, and Guillevic) were washed and then crushed, in a Record type 1 C crusher (Blaumeyer, Bouzonville, France). A total of 50 mL of a 20 g/L solution of NaF, corresponding to 0.5 g/kg of fruit, was atomized at the exit of the crusher gradually during the process. The apple pulp was pressed on a Hafico hydraulic press (Fisher, Düsseldorf, Germany) for 5 min at maximum pressure (23 bar). Aliquots of 500 μL of must were collected after pressing for the measurement of phenolic composition, and aliquots of 50 mL of must were collected for the determination of acidity and pH.

Adsorption Experiments. Adsorption experiments were conducted according to the method already described by Renard et al. (*5*). Cell-wall suspension (in 2 mL of a citrate/phosphate buffer at pH 3.8) and procyanidin solution (0.5 mL) were incubated for 1 h in an 8 mL empty Sep-Pack prep column (Interchim, Montluçon, France) equipped with a filter with a pore mean diameter of 20 μm under planetary agitation. After incubation, the solution and the cell-wall/polyphenol complex were separated by filtration under vacuum. Polyphenol adsorption was measured by optical density (OD) at 280 nm and/or thioacidolysis after freeze-drying as described below. The amount of procyanidin adsorbed by the CWM was determined by subtracting the concentration in the supernatant from that of the initial solution, i.e., procyanidin solution prior to mixing with CWM. The variable conditions were temperature (5–35 °C), ionic strength (from 0.01 to 1 M), procyanidin concentration (0.25–20 g/L), and CWM concentration (2–16 g/L). Unless stated, all experiments were carried out using a procyanidin concentration of 1 g/L and a cell-wall concentration of 5 g/L. The effect of the temperature and ionic strength for fraction Adp 70 was studied at a procyanidin concentration of 2.5 g/L. All assays were duplicated.

Analytical. Polyphenols were measured by high-performance liquid chromatography (HPLC) after thioacidolysis as described previously (*14*). The number average degree of polymerization of procyanidins was calculated as the molar ratio of all of the flavan-3-ol units (thioether adducts plus terminal units) to (–)-epicatechin and (+)-catechin corresponding to terminal units, after correction for monomers when present. The HPLC apparatus was a Waters (Milford, MA) system 717 plus autosampler equipped with a cooling module set at 4 °C, a 600 E multisolvent system, a 996 photodiode array detector, and a Millenium 2010 Manager system. The column was a 5 μm, 80 Å, 4 × 250 mm Purospher RP18 end-capped (Merck, Darmstadt, Germany). The solvent system was a gradient of solvent A [aqueous acetic acid, 2.5% (v/v)] and solvent B (acetonitrile): initial, 3% B; 0–5 min, 9% B linear; 5–15 min, 16% B linear; 15–45, 50% B linear, followed by washing and reconditioning the column.

Cell-wall contents were quantified in Kermerrien, Guillevic, and Douce Coët Ligné as ethanol-insoluble solids after freeze-drying, according to Renard (*15*). The juice acidity and density were measured according to Le Quéré et al. (*16*).

Data Analysis. Data were fitted by the general linear model (GLM) using the Statgraphics software plus version 5.1 (Manugistics, Rockville, MD) to explain the quantity adsorbed by the selected independent variables and to determine the simple and quadratic effects as well as the effects of the interaction. The nonlinear model was fitted using the Statgraphics software plus version 5.1 (Manugistics, Rockville, MD).

Data were the mean of two replicate runs. Standard deviations (SDs) of a run were calculated, for each set of experimental conditions, by pooling the estimate of run variance according to Box et al. (*17*). The confidence interval ($p < 0.05$) was then calculated with the standard error (SD of the mean), with the degree of freedom for the determination being the sum of individual degrees of freedom.

RESULTS AND DISCUSSION

Plant Material. CWM and polyphenol characteristics were previously described in refs *7–9*. The phenol/buffer method (*5*) was chosen to obtain cell walls devoid of procyanidins and with very low protein content. The CWM was a typical apple CWM, rich in pectin, cellulose, and xyloglucans; this preparation developed a surface area [Brunauer–Emmett–Teller (BET)] of $\cong 2 m^2/g$.

In the three procyanidin fractions purified from apple parenchyma (Adp 3, Adp 10, and Adp 70) and the fraction purified from pear juice (Pdp 35), (–)-epicatechin was always the predominant constitutive unit, accounting for more than 95% of the total units for all fractions (*3, 12*). (+)-Catechin, only present as terminal units, accounted for 0–3% of the total units for all fractions. The main difference between these four fractions was their molecular size

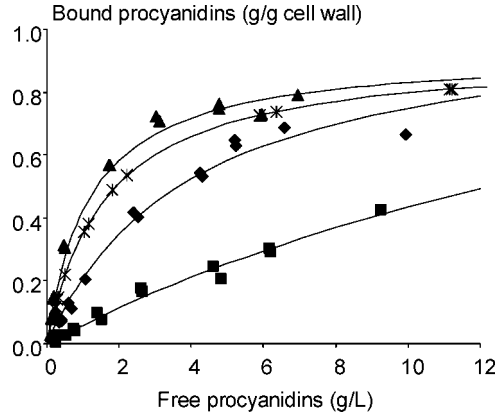

Figure 1. Binding isotherms for apple CWMs and procyanidins at pH 3.8 and 25 °C. The lines are the corresponding Langmuir adsorption isotherms: (▲) Adp 70, (∗) Pdp 35, (♦) Adp 10, and (■) Adp 3.

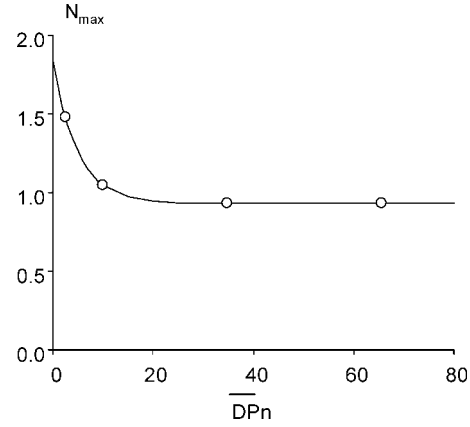

Figure 2. N_{max} evolution as a function of the n of procyanidins, $\overline{DP}n$ at pH 3.8 and 25 °C: (○) N_{max} and (–) predicted value (eq 2).

(i.e., $\overline{DP}n$), with their average molecular weight varying from 0.7 to 19 kDa. In all purified procyanidins, a distribution of molecular weights was observed.

Binding Isotherms. Binding isotherms were obtained for the four polyphenol fractions of $\overline{DP}n$ 70, 35, 10, and 3 at 25 °C and in 0.1 M citrate buffer at pH 3.8. Isotherms were described using the Langmuir formulation, and the parameters of the Langmuir equation (K_L and N_{max}) were calculated for each isotherm (*8*). For all of the curves, satisfactory fitting of the data was obtained with the Langmuir isotherm formula. As shown in our earlier studies (*8*) (**Figure 1**), the amount of bound procyanidins increased with the concentration and $\overline{DP}n$. At low concentrations, the binding of the procyanidins was almost total for the highly polymerized fractions, i.e., Adp 70 and Pdp 35, while most of the procyanidins remained in solution for the fraction that contained low $\overline{DP}n$ oligomers, i.e., Adp 3. For Adp 70 and Pdp 35 fractions, at high procyanidin concentrations, the curves plateaued, indicating a saturation of the CWM. This saturation level could be very high; for Adp 70 and Pdp 35 fractions, levels up to 70% by weight of the initial CWM were obtained in the concentration range used. However, whereas for Adp 70 and Pdp 35, the calculated saturation level was validated on the isotherms, for Adp 3 and Adp 10, this saturation level was not reached in the feasible concentration range and the estimate had a high level of incertitude (*8*).

N_{max} Modeling. The apparent plateau level N_{max} (**Figure 2**) decreased as $\overline{DP}n$ increased; the value obtained for $\overline{DP}n = 3$ was much higher than that obtained for $\overline{DP}n = 10$, with itself

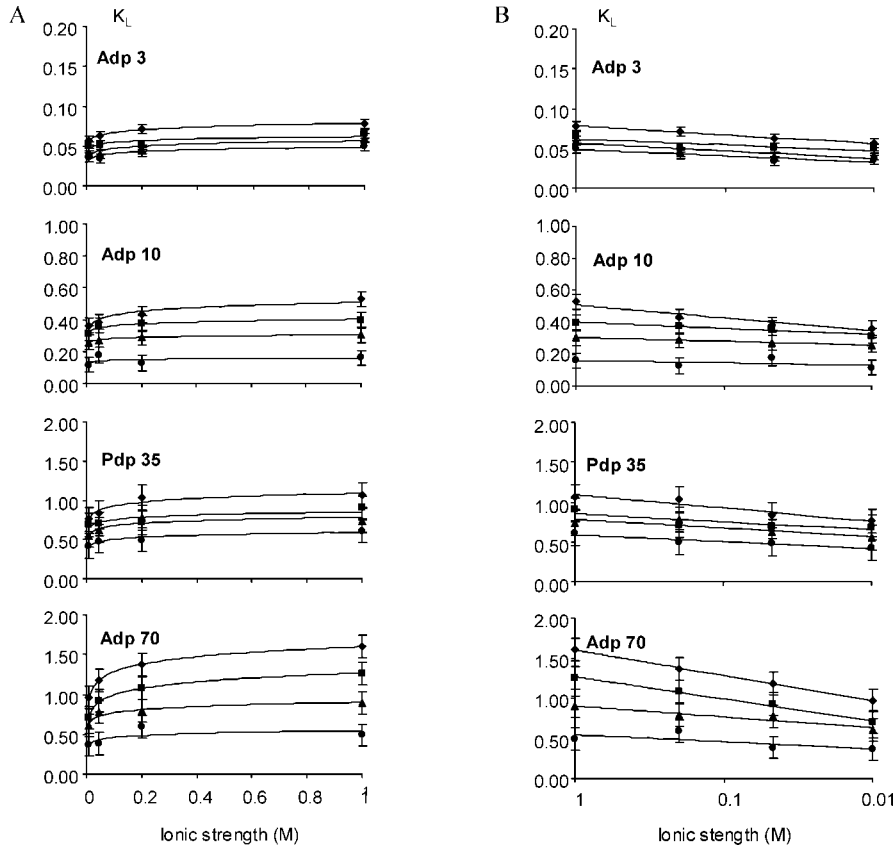

Figure 3. K_L evolution as function of the ionic strength for different temperatures and procyanidins. (**A**) Normal scale and (**B**) logarithmic scale: (♦) 5 °C, (■) 15 °C, (▲) 25 °C, and (●) 35 °C.

being higher than those obtained for $\overline{DP}n = 35$ and 70, which were similar. These data were fitted by an exponential equation

$$N_{max} = Am \cdot \exp(Cm \cdot \overline{DP}n) + Bm \quad (2)$$

Am, Bm, and Cm values (Am = 0.91; Bm = 0.93; and Cm = −0.20) were obtained by minimizing the sum of the squared differences between the calculated and experimental data using the Solveur of Excel. The shape of that curve (eq 2) gave a sharp decrease followed by a quasi-horizontal asymptote, which was reached for $\overline{DP}n \cong 25$.

In the rest of the work, we have assumed that N_{max} was independent of environmental conditions in the range of temperature and ionic strength explored. Indeed, Renard et al. (*19*) showed that wheat bran properties were not affected by ionic strength. In addition, we considered that temperature did not modify the saturation level. A possible modification by temperature cannot be excluded, but it would then be translated, in our model, by an affinity change. The values of N_{max} obtained for the various polyphenol fractions with complete Langmuir isotherms for a temperature (25 °C) and an ionic strength (0.1 M) (**Figure 1**) were used for the other conditions of temperature and ionic strength.

***K*L Modeling.** In the same manner as we had used the binding isotherms of ref *8* to connect the variation of N_{max} to the $\overline{DP}n$ of procyanidins, the evolution of the apparent association constant K_L between apple CWM and procyanidins (*8*) could be calculated under various conditions. The binding of procyanidins for apple CWM was previously shown to be independent of pH but to vary with the temperature and ionic strength in addition to $\overline{DP}n$ (*7*). This was studied using a factorial experimental design with four temperatures (5, 15, 25, and 35 °C), four ionic strengths (0.01, 0.047, 0.2, and 1 M), of course $\overline{DP}n$ (3, 10, 35, and 70), and one concentration. Assuming that N_{max} is independent of environmental conditions, the intensity of binding at any given point can be converted to K_L values using eq 1 and gives eq 3

$$K_L = \frac{PPb}{[PP_f](N_{max} - PPb)} \quad (3)$$

For each $\overline{DP}n$, we could thus calculate K_L for the various values of temperature and ionic strength by replacing N_{max} by its value determined in the preceding paragraph.

Figure 3A represents the evolution of K_L with the ionic strength for four temperatures (5, 15, 25, and 35 °C) and for each procyanidin fraction. When adsorption was carried out with monomers, no binding was observed (*5*) and $K_L = 0$. The variable $\overline{DP}n$ was thus replaced by ($\overline{DP}n$-1), which will be thereafter noted $\overline{DP}n^*$. K_L increased with the ionic strength. When a logarithmic scale was used for the ionic strength (**Figure 3B**), K_L increased linearly. The variable "ionic strength" was thus replaced by its logarithm, noted IS*.

Statistical Analysis. The results were first analyzed with the GLM of the Statgraphics software plus. It expressed K_L according to $\overline{DP}n^*$, temperature, ionic strength (IS*), their quadratic expression, and the interactions between these three factors. The $\overline{DP}n^*$ appears to be highly significant with both linear ($F = 568$; $p < 0.0001$) and quadratic ($F = 155$; $p < 0.0001$) effects and two interactions with temperature ($F = 91$; $p < 0.0001$) and ionic strength ($F = 32$; $p < 0.0001$). An interaction between these three parameters was also significant but with a lower probability: $F = 6.5$; $p < 0.012$. Neither temperature nor ionic strength had significant effects independent of the $\overline{DP}n^*$.

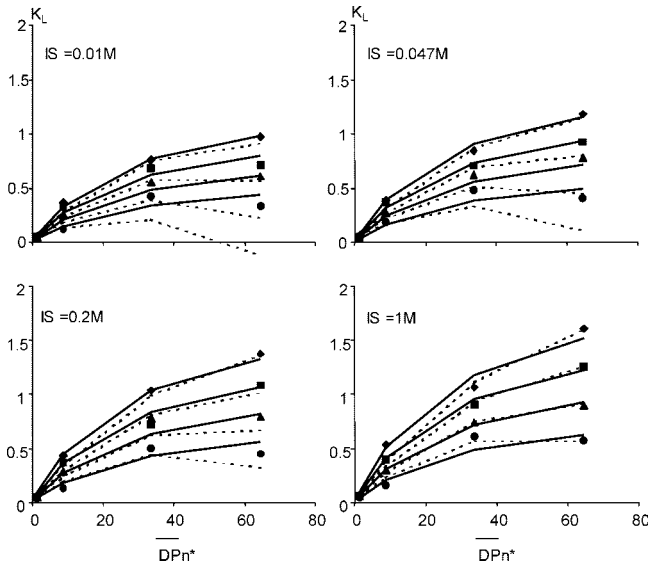

Figure 4. K_L evolution as function of $\overline{DP}n^*$ for different temperatures and ionic strengths and a comparison of K_L observed and fitted either by GLM (eq 4) or the nonlinear model (eq 10). K_L observed: (♦) 5 °C, (■) 15 °C, (▲) 25 °C, and (●) 35 °C. (- - -) Predicted value by the GLM (eq 4) and (–) predicted value by the nonlinear model (eq 10).

Equation 4 summarizes the empirical model obtained by general linear regression after elimination of the nonsignificant effects:

$$K_L = a(\overline{DP}n)^2 + b\overline{DP}n^* + c\overline{DP}n^* \cdot T + d\overline{DP}n^* \cdot IS^* + e\overline{DP}n^* \cdot T \cdot IS^* + f \quad (4)$$

The value obtained after fitting were $a = -0.00025$ (±0.00002), $b = 0.04300$ (±0.00151), $c = -0.00054$ (±0.00003), $d = 0.00545$ (±0.00060), $e = -0.00011$ (±0.00003), and $f = 0.03361$ (±0.01471). Constants are given with their $p > 0.95$ confidence interval, estimated by general linear regression. Both the quadratic effect of $\overline{DP}n^*$ and its interaction with the other factors, especially temperature, can be visualized in **Figure 4**. The general shape of the curves confirmed the existence of a quadratic effect of $\overline{DP}n^*$, but the curves presented a horizontal asymptote, the height of which varied according to the temperature and ionic strength.

This statistical analysis modeled the effect of $\overline{DP}n^*$, temperature, and ionic strength (IS*) by a second-order polynome, explaining 94.6% of the variability of K_L. The standard error of the estimates showed the standard deviation of the residuals to be 0.090. However, polynomial models present two disadvantages. First, it is not easy to ascribe a biochemical meaning to the second-order terms. Second, the evolution of K_L at the boundaries of the model was not well-defined; for example, K_L would decrease for high $\overline{DP}n$, contrary to observation (*5*).

***K*L as a Nonlinear Function of $\overline{DP}n$, Temperature, and Ionic Strength.** We therefore tried an alternative nonlinear model, whose coefficients would be easier to understand (biochemically) and will be more stable at the boundaries. The relation between K_L and $\overline{DP}n^*$ can be considered as an increasing continuous function, which tends toward an asymptotic value. The increase could be explained by the concomitant increase in the number of aromatic and ortho-diphenol groups able to bind to the cell wall (*5*, *7*–*9*), and the asymptotic evolution could be due to steric hindrance in procyanidins and/or the possibility of intramolecular organization.

We chose to fit this observed evolution by a hyperbolic empiric equation:

$$K_L = Ak\frac{\overline{DP}n^*}{Bk + \overline{DP}n^*} \quad (5)$$

This equation accounted for the general shape of the curves and was consistent with the quadratic effects of $\overline{DP}n^*$ (**Figure 4**). Equation 5 has two parameters Ak and Bk, with Ak corresponding to the asymptote value of this hyperbolic curve and Bk corresponding to the value of $\overline{DP}n^*$, for which K_L reached Ak/2.

A first trial of fitting of eq 5 to the observed data indicated that Bk values were rather stable (between 20 and 30) in the temperature and ionic strength range (**Figure 4**). Giving a fixed value to Bk both limits the number of parameters and led to a reasonable fitting. A fixed value of Bk means that the effect of temperature and ionic strength concern only the asymptote Ak. To link Ak to these conditions, we temporarily assigned a value of 25 to Bk. With Bk fixed, we could fit the value of K_L to eq 5 with the Solveur of Excel and calculate Ak.

Ak varied linearly (I in **Figure 5**) with IS* and could be expressed for each temperature by the relation

$$Ak = A \cdot IS^* + B \quad (6)$$

The slope (*A*) and intercept of the lines (*B*) varied linearly with the temperature (II in **Figure 5**) and could be therefore described as

$$A = C \cdot T + D \quad (7)$$

$$B = E \cdot T + F \quad (8)$$

Combining eqs 6–8 yields the following relation:

$$Ak = C \cdot T \cdot IS^* + D \cdot IS^* + E \cdot T + F \quad (9)$$

All of the interaction terms that were significant in the statistic treatment were found in this new equation, except the quadratic

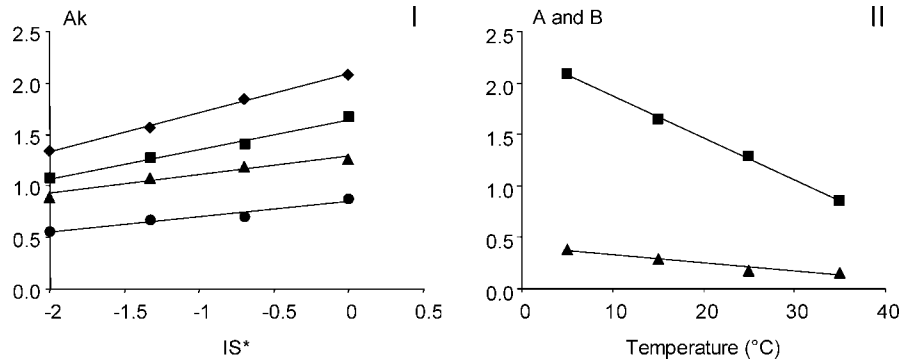

Figure 5. Modeling the evolution of K_L with the environmental conditions. (I) Ak evolution as function of the temperature and ionic strength. (II) *A* and *B* evolution as function of the temperature. For I, (♦) 5 °C, (■) 15 °C, (▲) 25 °C, and (●) 35 °C. For II, (▲) *A* and (■) *B*.

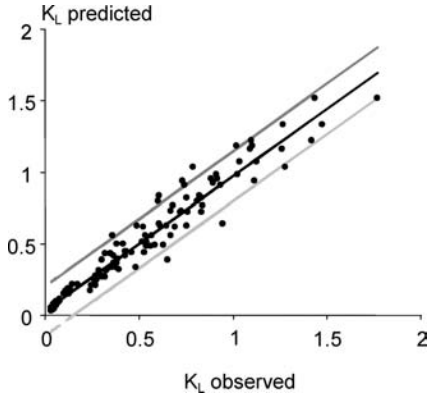

Figure 6. Validation of the modeling of the affinity constant of apple procyanidins for apple CWM as a function of the temperature, ionic strength, and procyanidin degree of polymerization: K_L predicted values versus observed values; (●) K_L, (gray line) higher prevision limit, (black line) regression, and (light gray line) lower prevision limit.

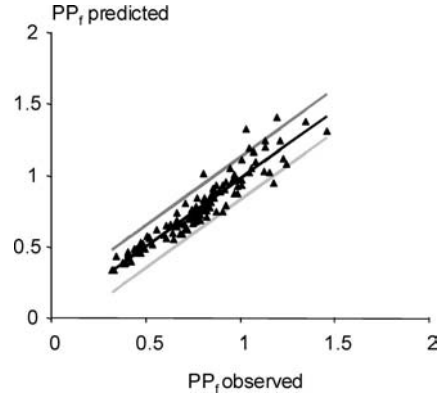

Figure 7. Validation of the modeling of the binding of apple procyanidins to apple CWM as a function of the temperature, ionic strength, and procyanidin degree of polymerization: free procyanidin concentration (PP_f) predicted values versus observed values; (▲) PP_f, (gray line) higher prevision limit, (black line) regression, and (light gray line) lower prevision limit.

effect of $\overline{DP}n^*$, which was taken into account by the nonlinear form of the equation.

Final Function of K_L and Parameter Estimation. We now had the equation to describe K_L as a function of the environmental conditions (temperature and ionic strength) and $\overline{DP}n^*$:

$$K_L = [C \cdot T \cdot IS^* + D \cdot IS^* + E \cdot T + F]\frac{\overline{DP}n^*}{Bk + \overline{DP}n^*} \quad (10)$$

The numerical values of the parameters (including Bk) were then determined by minimizing the sum of the squares of differences between experimental data (four temperatures × four ionic strengths × four $\overline{DP}n$ values, in duplicate, i.e., 128 points) and those predicted from the model using the nonlineary regression of the Statgraphics software plus. The value obtained after fitting were $C = -0.008$ (±0.002), $D = 0.429$ (±0.052), $E = -0.043$ (±0.003), $F = 2.404$ (±0.116), and $Bk = 28.755$ (±2.989).

This model explained 94.8% (R^2 statistic) of K_L variability. The adjusted R^2 statistic, which is more correct to compare models having different explanatory variables, is 94.6%. The standard deviation of the residuals is 0.091. There was no notable improvement in statistical quality between the nonlinear and linear models. However, the nonlinear model provided a better evolution of K_L at the boundaries of the system (**Figure 4**), in particular avoiding the sharp decrease in K_L observed with the generalized linear model at high $\overline{DP}n$ values. The representation (**Figure 6**) of the K_L predicted values versus K_L observed values gives a first validation of the elaborated model of K_L. We obtained an estimate of the affinity with a variation of approximately 10% of the maximum value.

Calculating PP_f. Our aim was to predict the concentration in procyanidins in the juice by knowing the concentration in the apple and the experimental conditions. Equation 1, of which we clarified the parameters K_L and N_{max}, connected the quantity of procyanidins bound in grams per gram of CWM to the concentration of free procyanidins in the solution. Therefore, we first had to convert it to an equation giving the procyanidin concentration in solution as a function of measurable quantities, i.e., the original procyanidin and CWM contents in the apple.

The initial total procyanidin concentration ([Tot]) in g/L in the medium is the sum of the free procyanidin concentration ([PP_f]) in g/L and the amount of bound procyanidins corresponding to 1 L of suspension. This amount can be expressed as a function of the quantity of procyanidins bound to 1 g of CWM (PPb) in g/g, and the CWM ([CWM]) contained in 1 L of the suspension in g/L

$$[Tot] = PPb[CWM] + [PP_f] \quad (11)$$

or after rearrangement

$$PPb = \frac{[Tot] - [PP_f]}{[CWM]} \quad (12)$$

Combining this equation (eq 12) with eq 1, which links PPb and [PP_f] with the parameters K_L and N_{max}, yields the following relation:

$$\frac{[Tot] - [PP_f]}{[CWM]} = \frac{N_{max} \cdot K_L[PP_f]}{1 + K_L[PP_f]} \quad (13)$$

Expressing eq 13 according to [PP_f], we obtained

$$-K_L[PP_f]^2 + [K_L([Tot] - [CWM]N_{max}) - 1][PP_f] + [Tot] = 0 \quad (14)$$

Equation 14 was an equation of the second degree, whose only possible root was given by eq 15:

$$[PP_f] = \frac{(K_L([Tot] - [CWM]N_{max}) - 1) + \sqrt{(K_L([Tot] - [CWM]N_{max}) - 1)^2 + 4K_L[Tot]}}{2K_L} \quad (15)$$

A parameter of eq 15 that we had not encountered before was [CWM].

Figure 7 represents the PP_f predicted values versus PP_f observed values in the previous experiments. The coefficient of correlation is 0.951, which indicates a strong relation between the two variables; the slope is 0.95.

Effect of Variation of the CWM Concentration. As a first step in evaluating the robustness of the model, we measured the impact of a variation of the CWM concentration on free procyanidin concentrations [PP_f] for a constant total procyanidin concentrations. This is represented in **Figure 8** for an initial concentration of procyanidins of 4 g/L. Increasing the CWM concentration led to a reduced free procyanidin concentration. The evolution of the concentration of free procyanidins differed for the different procyanidin fractions. In the case of the lowest $\overline{DP}n$ fraction (Adp 3), the reduction was almost linear. On the other hand, in the case of the fractions of medium and high $\overline{DP}n$ (Adp 10 and Adp 70), the free procyanidin concentrations

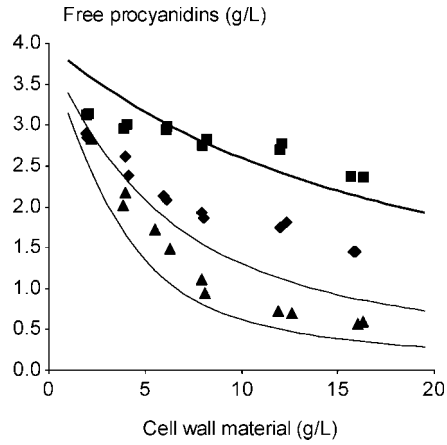

Figure 8. Binding of apple procyanidins to apple cell walls: free procyanidin concentration (for an initial procyanidin concentration of 4 g/L) as function of the CWM concentration at pH 3.8 and 25 °C. (▲) Adp 70, (♦) Adp 10, and (■) Adp 3.

decreased linearly up to a value of concentration in the CWM of 7.5 g/L before inflecting and then plateaued (Adp 70). Whichever fraction was tested, there were always some free procyanidins, despite the increased CWM concentration. The binding of procyanidins on the CWM was not total, and there was thus an equilibrium between the free procyanidins and the bound procyanidins.

The adjustment of the experimental data by curves from eq 15 showed similar shapes but also some discrepancies. For $\overline{DP}n = 70$ and 10, the concentration of free procyanidins was systematically higher than predicted by the model. For $\overline{DP}n = 3$, the slopes differed. However, it must be kept in mind at this point that there was a margin of error for the initial calculation of K_L and N_{max} (*8*), in particular for the N_{max} of $\overline{DP}n = 3$, for which there was very little inflection in the isotherm.

Application to Experimental Juice Extraction: Estimation of the Extraction Characteristic Parameters. To use this model, we must first translate the amount of polyphenols in the fruit (in g/kg of fresh weight) to get the concentration (in g/L) of polyphenol in the juice contained in that fruit.

Estimation of the Volume of Juice by Kilograms of Apples. The first question to be considered to apply this model to the apple process is how much juice is in an apple? For this estimation, an apple may be regarded as a sponge (with the CWM, skin, pips, and carpelles as insoluble solid material) filled with a liquid (the juice). This "liquid" compartment is then considered as homogeneous. Under this hypothesis, this liquid has the same soluble solids (dry matter) content/water content as that of juice extracted by direct pressing.

The determination of the dry matter content of the apple and the corresponding juice is then used to calculate the liquid compartment of the fruit. The theoretical total juice in 1 kg of apples, V_{juice} (in L/kg) could be calculated using eq 16

$$V_{juice} = \frac{1000(1 - DM_{apple})}{(1 - DM_{juice})DJ} \quad (16)$$

with DM_{apple} = dry matter of apple, and DM_{juice} = dry matter of juice, and DJ = density of the juice in kg/m^3.

Estimation of the Theoretical Initial Concentration of Polyphenols. The concentration in any solute in apple in g/kg ($[PP]_{apple}$) could thus be transformed into theoretical concentrations in the juice present in this apple. The initial total procyanidin concentration [Tot] in g/L could be calculated as

$$[Tot] = \frac{[PP]_{apple}}{V_{juice}} \quad (17)$$

Estimation of the Ionic Strength of the Juice. To estimate the ionic strength, we considered that apple juice is a potassium malate buffer. Malic acid is the major acid in apple juice, with a minor amount of citric and ascorbic acids. Potassium is by far the major cation in apple juice (*20*), and bivalent cations (Ca^{2+}, Mg^{2+}, etc.) could be neglected because their concentrations are at least 100-fold less than that of potassium. With this simplification, the ionic strength could be estimated using pH and acidity (pK_a values of malic acid: $pK_{a_1} = 3.46$ and $pK_{a_2} = 5.13$).

Polyphenols Transfer from Apple to Juice. Six batches of apples (three varieties, with two batches of each) of different phenolic composition, including procyanidin molecular weight and juice acidities, were pressed. Oxidation was prevented by the addition of NaF, an inhibitor of polyphenoloxidase (*21*). The polyphenol compositions of apples and juices were measured, as well as CWM contents, dry matter content of apple and juice density, pH, and acidity of the juice. The apple composition and conditions under which the pressings were performed are summarized in **Tables 1–3**.

Procyanidins. The various cider apple varieties presented different procyanidin concentrations and characteristics. The Kermerrien variety was the richest, with a concentration of about 5.5 g/kg, whereas the varieties Douce Coët Ligné and Guillevic had approximately half this concentration (**Table 2**). This was in agreement with the varietal diversity that was already reported (*1*, *2*). The initial total procyanidins concentration in theoretical juice in the apple [Tot] thus varied from 2 to 7.5 g/L.

The composition of the juices confirmed the partitioning variations and the selective distribution of the procyanidins according to the $\overline{DP}n$ already observed (*5*, *7*). The Guillevic variety, which had a $\overline{DP}n$ of 50, gave a juice practically devoid of procyanidins (0.2–0.3 g/L), whereas the Kermerrien variety with $\overline{DP}n$ of 6.7 gave a juice containing 2.8–2.9 g/L of procyanidins. The $\overline{DP}n$ of the free procyanidins was lower than in the apple (**Table 2**).

Most of the procyanidins were indeed retained by the CWM. We observed a retention of 93–50% (extraction rates of 7–50%) depending upon the varieties, with the least retained being those with the lowest $\overline{DP}n$ (**Table 2**). This was lower than calculated (binding of 86–94% of the procyanidins), especially for Douce Coët Ligné and Kermerrien. These varieties were characterized by low $\overline{DP}n$ procyanidins. Guyot et al. (*2*) found average retention rates of procyanidins of ca. 70%. Three factors could explain this difference. In the development of our model, we have used purified CWM, for which all CWM surfaces were accessible to the procyanidins. This was not true for the crushed apples; the coarse particles still contained intact cells, so that all CWM areas would not be accessible to the procyanidins. Also, in the suspension experiments, we allowed enough time for equilibrium to be reached. Pressing took only a few minutes, and although we had planned some contact time, we were not at equilibrium. This may be even enhanced by the limited diffusion in the coarse particles obtained by crushing. Therefore, during pressing, procyanidin/cell-wall equilibrium was not reached, because of the lack of cell-wall accessibility and time for equilibration. A second point was that we had used dried CWM, and we have seen (*8*) that drying had a strong impact on the binding affinity, i.e., K_L and capacity, i.e., N_{max}; the harsher the drying, the lower the K_L and higher N_{max}. Extrapolating backwards from the difference between harsh and soft

Table 1. Data Used for Modeling of the Juice Procyanidin Concentrations[a]

variety	[CWM] (g/kg of FW)	$[PP]_{apple}$ (g/kg of FW)	juice dry matter (kg/kg)	apple dry matter (kg/kg)	juice density (kg/m^3)	pH	acidity (mmol of H^+/L)	ionic strength (M)	temperature (°C)
Douce Coët Ligné 1	28.5	1.88	0.15	0.20	1061	4.40	19.2	0.033	14.5
Douce Coët Ligné 2	25.9	1.86	0.15	0.19	1059	4.33	20.4	0.032	14.7
Guillevic 1	23.3	3.34	0.13	0.18	1052	3.90	46.4	0.043	15.5
Guillevic 2	24.4	2.94	0.13	0.18	1051	3.84	47.5	0.041	15.5
Kermerrien 1	28.3	5.47	0.14	0.17	1055	4.43	20.9	0.038	14.6
Kermerrien 2	29.8	6.64	0.14	0.18	1053	4.41	20.0	0.035	14.8

[a] FW = fresh weight, [CWM] = CWM concentrations, and $[PP]_{apple}$ = apple procyanidin concentrations.

Table 2. Retention of Procyanidins during Apple Juice Pressing: Concentrations in the Apple, in Its Liquid Fraction ([Tot]), and in the Expressed Juice [PP_f] and Relationship between Measured and Calculated Juice Procyanidin Concentrations and Retention by Interactions with Cell Walls[a]

variety	$[PP]_{apple}$ (g/kg of FW)	[Tot] (g/L)	$\overline{DP}$n apple	$\overline{DP}$n juice	[PP_f] (g/L)		retained procyanidins (%)	
					measured	calculated	measured	calculated
Douce Coët Ligné 1	1.88	2.10	5.5	2.1	0.79	0.30	62	86
Douce Coët Ligné 2	1.86	2.07	5.0	2.1	0.79	0.34	62	84
Guillevic 1	3.34	3.73	50.2		0.24	0.23	93	94
Guillevic 2	2.94	3.26	49.7		0.31	0.19	91	94
Kermerrien 1	5.47	6.00	6.7	3.0	2.91	0.84	51	87
Kermerrien 2	6.64	7.34	6.7	2.9	2.89	1.02	61	87

[a] FW = fresh weight, $[PP]_{apple}$ = apple procyanidin concentrations, [Tot] = concentrations in the liquid fraction of the apple, and [PP_f] = procyanidin concentrations in the expressed juice.

Table 3. Polyphenols Extraction from Apple to Juice: Concentrations in the Liquid Fraction of the Apple and Expressed Juice and Their Ratios

variety	CQA			pCQ			CAT			EPI			PLZ		
	[Tot] (g/L)	[juice] (g/L)	extracted ratio (%)	[Tot] (g/L)	[juice] (g/L)	extracted ratio (%)	[Tot] (g/L)	[juice] (g/L)	extracted ratio (%)	[Tot] (g/L)	[juice] (g/L)	extracted ratio (%)	[Tot] (g/L)	[juice] (g/L)	extracted ratio (%)
Douce Coët Ligné 1	0.94	0.94	100	0.08	0.08	108	0.13	0.11	85	0.40	0.36	90	0.14	0.03	22
Douce Coët Ligné 2	0.94	0.78	84	0.11	0.08	76	0.12	0.14	112	0.37	0.37	99	0.09	0.03	33
Guillevic 1	0.18	0.14	74	0.09	0.09	96							0.04	0.02	39
Guillevic 2	0.15	0.10	69	0.09	0.02	26							0.04	0.02	39
Kermerrien 1	1.18	1.15	98	0.12	0.13	113	0.07	0.09	125	0.62	0.55	89	0.14	0.04	29
Kermerrien 2	1.35	1.00	74	0.19	0.12	65	0.07	0.08	116	0.65	0.48	73	0.11	0.04	37

[a] CQA = caffeoylquinic acid, [Tot] = concentrations in the liquid fraction of the apple, [juice] = concentrations in the expressed juice, pCQ = paracoumaroylquinic acid, CAT = catechin, EPI = epicatechin, and PLZ = phloridzin.

drying, we suppose that never dried cell walls would be characterized by a higher K_L and a lower N_{max}. Finally, a fact that we have not been able to take satisfactorily into account is the polydispersity of the procyanidins both in the purified fractions and even more in the apples (*5*, *18*). Using a number average mean DP gives a bias in the relative weight of the high and low DP fractions, while we have seen that K_L and N_{max} vary more for low than high DP. We have seen (**Figure 8**) that the model overestimated the quantity of procyanidins bound on the CWM.

The model gives a potential of the apple CWM for binding of procyanidins, but this potential is not reached because of the physical state of the walls or intensity of destructuration of the cells.

Other Polyphenols. The concentration data and the extraction ratio between juices and apple are presented for each polyphenol class and apple variety (**Table 3**). The compositions of the apples were again consistent with previous data (*1*, *2*). Hydroxycinnamic acids were the main polyphenols in the apples and their juices after the procyanidins (**Tables 2** and **3**), with caffeoylquinic acid always being predominant. Absent in the juice of the Guillevic variety, monomeric catechins were the second polyphenolic class after hydroxycinnamic acids for juices of the other varieties. (–)-Epicatechin was the major compound of this class. Dihydrochalcones (here represented by phloridzin) were also less represented in apple juice (*2*).

The ratios of extraction varied according to the polyphenol class. Hydroxycinnamic acids and catechins, all present in apple flesh (*3*), were highly transferred (ratios of 83, 81, 109, and 88%). When oxidation was inhibited, the transfer was quasi-quantitative for polyphenols other than procyanidins and the calculation of the "theoretical juice" volume and composition appeared correct. Only one-third of phloridzin, mainly present in the pips (*3*), was extracted; this might be explained by its sequestration in these resistant tissues and a too short extraction to allow for its diffusion. Guyot et al. (*2*) had obtained extraction yields of 80% for the dihydrochalcones (phloridzin) and 50% for the catechins. The differences observed could be due to pressing conditions, in particular, a more stringent inhibition of oxidation and a shorter contact time, and the way of expressing the results. We calculated concentration ratios, whereas they expressed their results in yields of extraction.

Conclusion. Our previous work (*5*, *7*–*9*) enabled us to better understand the extraction or rather the nonextraction of the procyanidins from apple to juice, as noted by Guyot et al. (*2*). Starting from reconstituted suspensions using purified CWM and procyanidins, we have elaborated a model based on Langmuir isotherms that explained 60–90% of the loss in procyanidins between apples and their juice.

Discrepancies observed between the calculated and experimental results may be explained by noting that the model is based on the total accessibility of the dry cell wall and an

average degree of polymerization, while wet cell walls only partially accessible and a wide procyanidin size distribution exist in apples (*5*, *21*). A great advantage of this model is that it allowed us to identify parameters of influence (temperature and cell-wall content) and simulate their effect. However, there still remains validation, notably verifying the impact of parameters of influence and introducing the impact of oxidation.

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