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Variability of the Polyphenolic Composition of Cider Apple (*Malus domestica*) Fruits and Juices

SYLVAIN GUYOT,* NATHALIE MARNET, PHILIPPE SANONER,† AND JEAN-FRANÇOIS DRILLEAU

Unité de Recherches Cidricoles, Biotransformation des Fruits et Légumes, Institut National de la Recherche Agronomique, B.P. 35327, 35653 Le Rheu Cedex, France

Five French cider apple varieties were compared on the basis of their detailed polyphenol profile in the cortex and in the juices. Among the factors studied, variety was the most important variability factor in fruits, whereas polyphenol profiles showed an overall stability from one year to another, and a limited decrease of polyphenol concentration was observed during the starch regression period of fruit maturation. In juices, procyanidins remained the preponderant polyphenol class with concentrations up to 2.4 g/L even in centrifuged juices. Compared to the fruits, the average degree of polymerization of procyanidins was significantly reduced in the juice. Centrifugation of the crude juice had only minor effects on the polyphenol composition. For one variety, highly polymerized procyanidins with average degrees of polymerization of 25 were shown to be soluble in the centrifuged juice at a concentration of close to 1.2 g/L. Oxygenation of the juices during processing resulted in a significant decrease of all classes of native polyphenols. Catechins and procyanidins were particularly affected by oxidation, whereas caffeoylquinic acid was partly preserved. The transfer of polyphenols after pressing was maximal for dihydrochalcones and minimal for procyanidins with extraction yield values close to 80 and 30%, respectively.

KEYWORDS: ***Malus domestica*; cultivars; maturity; procyanidin; phenolic acid; dihydrochalcone; oxidation; process**

INTRODUCTION

Polyphenols are important secondary metabolites in cider apple fruits that are involved in essential organoleptic criteria such as color, bitterness, astringency, and colloidal stability of cider (*1*). In addition, some phenolics are precursors of cider aromas (i.e. volatile phenols) (*2*). During cider making, polyphenols may also influence important technologic steps such as clarification or fermentation. For example, tannins may act as inhibitors of pectic enzymes involved in the clarification process (*3*). Polyphenols are also powerful antioxidants with a wide range of biological activities. Recently, it was shown that some particular phenolics of alcoholic ciders (i.e., caffeic acid, quercetin, and phloretin) are metabolized or excreted by humans (*4*).

Apple phenolics show a great diversity of structures and can be classified into several major classes. The flavanols (FA) are subdivided into catechins (CAT) and procyanidins (PCA). The catechins are predominantly (−)-epicatechin (EC) and low concentrations of (+)-catechin (CT). Procyanidins are oligomeric and polymeric catechins, (−)-epicatechin being always the major constitutive unit. They correspond to the apple tannin fraction and may be found in highly polymerized forms in some cider varieties (*5*). They are the main polyphenols in dessert apples (*6*), and their concentration in cider apple is generally higher (*5*). The hydroxycinnamic acid class (HCA) includes mainly caffeoylquinic acid (CQA) and *p*-coumaroylquinic acid (*p*CoQA). Among commonly consumed fruits, the dihydrochalcones class (DHC) is specific to apples (*7*). Phloridzin (PLZ) and phloretin xyloglucoside (XPL) are the two major molecules of this class. Finally, flavonols and anthocyanins are essentially present in apple peel.

In apples, polyphenol concentrations show a great variability according to the variety (*8*−*10*), and this is enhanced when cider varieties are considered (*5*, *11*). Other factors such as fruit maturity (*12*, *13*), fruit season (*14*), light exposure (*15*), and storage conditions (*16*) contribute to the variability of phenolic concentrations in apples. However, most of these results concern dessert apples and do not take into account procyanidins, which are the major phenolic compounds even in dessert varieties (*6*). In the present paper, the thiolysis−reversed phase HPLC method was used to characterize the polyphenolic profile of apple fruit cortex and apple juices. The method was efficient to assay procyanidins in crude samples (*17*). Moreover, it gives access to the nature of the catechin constitutive units of procyanidins and to their average degree of polymerization ($\overline{DP}n$), the latter

* Author to whom correspondence should be addressed [telephone 33 (0)2 23 48 52 09; fax 33 (0)2 23 48 52 10; e-mail guyot@rennes.inra.fr).
† Present address: Coopérative Elle & Vire, Département Ingrédients Bioactifs, B.P. 2, 50890 Condé sur Vire, France.

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being strongly related to associative properties of procyanidins, which are responsible for the astringency of ciders (*1*).

Apple polyphenols are present in the whole of the fruit, the main fraction being found in the cortex zone (*18*). When apples are processed into juices, polyphenols are involved in specific physicochemical interactions with the solid part of the fruits, especially the apple cell-wall material (*19*). During pressing, apple procyanidins may associate with cell-wall insoluble polysaccharides and be partly retained on the pomace. In addition, polyphenols undergo biochemical and chemical alterations due to enzymatically catalyzed oxidation by polyphenol oxidase (*8*). The oxidation process takes place when apples are crushed and pressed. The phenolic substrates of polyphenol oxidase, mainly CQA and catechins, come in contact with the enzyme in the presence of oxygen. First, *o*-diphenolic substrates are converted into their corresponding *o*-quinone form. *o*-Quinones are very reactive species, which immediately react according to two main ways. On the one hand, being highly electrophile, *o*-quinones may be involved in coupling reactions with nucleophilic sites of polyphenols and with other nucleophiles that are present in the medium. On the other hand, they may also form reactive semiquinone radicals. These reactions lead to the formation of coupling products (*20*). *o*-Quinones are also powerful oxidants able to convert *o*-diphenolic structures into quinones by an oxidation−reduction mechanism. For example, hydroxycinnamic *o*-quinones oxidize catechins into catechin *o*-quinones and are reduced back to their original form (*21*). These association and oxidation phenomena might thus deeply modify the polyphenol profile between the fruit and the juice. Several studies have been published concerning the polyphenolic composition of apple juices related to various processing conditions (*22*−*26*), and the effect of oxidation on the polyphenol profiles has been discussed (*22*, *27*). However, data are scarce about polyphenol extraction from the fruits to the juice particularly concerning the procyanidin class.

The purpose of this study was to estimate the polyphenol extraction yields during processing of apple to must in the cider-making process and to assess the variability of polyphenol concentrations in French cider apple fruits and must according both to fruit-related factors (variety, harvest year, and maturity) and process-related factors (centrifugation and oxidation).

MATERIALS AND METHODS

Plant Materials. The fruits of five apple cultivars (*Malus domestica* var. Avrolles, Bedan, Kermerrien, Dous Möen, and Petit Jaune) corresponding to the major cider cultivars grown in France were harvested during three seasons (1996, 1997, and 1998) in the experimental orchard of the CTPC (Sées, Orne, France). For the 1998 season, three ripeness stages in the September−October period were considered. Ripeness stages corresponded to three stages of starch regression (0, 50, and 100%). Starch regression percentages were evaluated visually using the starch regression test measuring the starch−iodine complex color on a cross section of an apple (*28*). Only the 100% regression state (complete maturity) was considered for fruits of the 1996 and 1997 seasons. About 30 kg of apple fruits of each variety and each maturity state was harvested. Fruits were manually calibrated, and only the predominant size was used for further operations.

Apple Powder Preparation. For each variety and each maturity stage, three batches of 10 fruits were constituted. Fruits were mechanically peeled and cored using a Kali apparatus (Kali France) as already described (*18*). Only the cortex zone, which constituted the main part of the fruit, was considered for the preparation of the powders because this tissue could be used for polyphenol analysis. Tissues were frozen and freeze-dried, and an aliquot of each batch was used to determine the fresh/dry matter ratio. Then, homogeneous powders were obtained by crushing the dried tissues in closed vials to avoid hydration. Powders were kept in a desiccator and in darkness until analysis.

Apple Juice Preparation. Only fruits of the 1998 growing season were considered for juice preparation. For each variation factor (i.e., variety, maturity state, and oxidation state), three batches of fruits (1 kg) were constituted. Each batch was milled by a Record crusher (type 10, Blaumeyer, France); then, the milled material was pressed on a small laboratory high-pressure press (model HP5, 5 L, Hafico, Fischer and Co., Dusseldorf, Germany) to obtain the crude juice. The hydraulic pressure was set at 400 bar, corresponding to an effective pressure of 24 kg/cm^2 on the plant material. A solution of diluted sodium fluoride (50 mL, 1 g/L in water) was added to the apple pulp before pressing to obtain juices without oxidation. Concurrently, oxidized juices were obtained by replacing the sodium fluoride solution with pure water. This added volume was subtracted for the yield calculations, and a correcting factor was applied for calculations of polyphenol concentrations. For the making of oxidized juices, the temperature of fresh raw materials was controlled by storing the fruits at 20 °C for 2 h before crushing and pressing. Then, oxidation of the slices was carried out for 15 min in air atmosphere; after pressing of the slices, oxidation of the juices was continued for 15 min at 20 °C under air agitation. Then, crude apple juices were centrifuged (6000*g* for 15 min) to obtain clear apple juices; juice yields were measured. Aliquots (2 × 500 μL) of centrifuged and crude juices were freeze-dried in 5 mL vials. Freeze-dried samples were kept in a desiccator until analysis.

Thiolysis, Methanol Extraction, and Reversed Phase HPLC Analysis of Freeze-Dried Samples. Freeze-dried samples of the 1998 seasons corresponding to apple juices and apple powders were directly [apples powders of the 1996 and 1997 seasons were first submitted to a solvent extraction procedure using methanol and aqueous acetone (*5*); then thiolyses were performed on those solvent extracts] submitted to the thiolysis reaction in methanol according to the previously published method (*17*). Then, the thiolysis reaction media were analyzed by reversed phase HPLC (*17*). The thiolysis reaction leads to the depolymerization of procyanidin structures by converting the flavanol extender units into their carbocations and the lower units into monomeric flavanols. Immediately, the carbocations combine with toluene-α-thiol, leading to the formation of the thioether adducts (*29*). By making the distinction between terminal and extension units, HPLC analysis of thiolysis media allows the determination of the nature and the proportion of the constitutive units of procyanidins (*30*, *31*). Moreover, it gives access to the calculation of their average degree of polymerization ($\overline{\mathrm{DP}}n$). In addition, procyanidin concentrations are obtained by summing all catechin equivalents assayed on the thiolysis chromatogram after subtraction of the amounts of native monomeric catechins assayed on the chromatogram without thiolysis.

After thiolysis, no distinction can be made between native catechins and catechins coming from the terminal units of procyanidins. For this reason, methanol extractions of polyphenols from freeze-dried samples without thiolysis were also performed to separately assay native catechins [i.e., (−)-epicatechin and (+)-catechin] by reversed phase HPLC (*17*). These chromatograms were also used to quantify procyanidin B2 individually.

Phenolic compounds that were not flavanols (i.e., hydroxycinnamic acids and dihydrochalcones) were assayed on thiolysis chromatograms as previously described (*17*).

HPLC peaks were identified on chromatograms according to their retention times and their UV−visible spectra by comparison with standards. Caffeoylquinic acid, (+)-catechin, (−)-epicatechin, hyperoside, and phloridzin were commercially available (Sigma-Aldrich). *p*-Coumaroylquinic acid was purified from a commercial cider (*5*). As already mentioned in previous work (*6*, *18*), (−)-epicatechin benzylthioether and procyanidin B2 were kindly furnished by J. M. Souquet (UMR, SPO, INRA, Montpellier, France). Phloretin xyloglucoside identification was already performed in a previous work (*5*), and its quantification was expressed in phloridzin equivalents. For each compound, the quantification was performed by reporting the measured integration area in the calibration equation of the corresponding standard. Integrations were performed at 280 nm for catechin monomers, procyanidin B2, thioether adduct, and dihydrochalcones, at 320 nm for hydoxycinnamic compounds, or at 350 nm for flavonols.

Table 1. Concentrations[a] (Milligrams per Kilograms of Fresh Weight) of Phenolic Compounds in the Cortex of Five French Cider Apple Varieties at Maturity[b]

variety[c]	CT[d]		EC[d]		B2[d]		PCA[d]		CQA[d]		*p*CoQA[d]		XPL[d]		PLZ[d]		TOT[d]	
AV	t[e]		t		19	*2*	2750	*244*	191	*22*	119	*10*	90	*29*	31	*6*	3182	*297*
BD	150	*34*	497	*29*	374	*21*	1910	*148*	689	*53*	150	*5*	15	*4*	32	*3*	3441	*223*
DM	124	*33*	376	*72*	235	*18*	1557	*201*	953	*178*	107	*17*	24	*4*	19	*3*	3161	*433*
KE	47	*6*	615	*162*	589	*62*	3280	*370*	993	*133*	99	*19*	41	*8*	51	*9*	5065	*550*
PJ	24	*4*	124	*32*	181	*40*	1300	*104*	395	*69*	28	*5*	12	*2*	16	*4*	1899	*186*

[a] Mean of three harvest years (1996, 1997, and 1998) considering three replicates for each year. Italic values correspond to standard deviation ($n = 9$). [b] Complete starch regression. [c] AV, Avrolles; DM, Dous Möen; PJ, Petit Jaune; KE, Kermerrien; BD: Bedan. [d] CT, (+)-catechin; EC, (−)-epicatechin; B2, procyanidin B2; PCA, total procyanidins including B2; CQA, caffeoylquinic acid; *p*CoQA, *p*-coumaroylquinic acid; XPL, phloretin xyloglucoside; PLZ, phloridzin; TOT, total polyphenols. [e] t, traces.

Apple powders of the 1996 and 1997 seasons were first submitted to a solvent extraction procedure using sequentially hexane, methanol, and aqueous acetone 40:60 (v/v) according to an already described procedure (*5*, *18*); then thiolyses were performed on the methanolic and aqueous acetone extracts after removal of the solvent and freeze-drying.

Statistical Analysis. Standard deviation calculations, *t* tests, and Wilcoxon signed rank tests, with significance levels of $\alpha = 0.05$, and two-way ANOVA were performed on the original data using SigmaStat (Jandel Corp.) software.

RESULTS AND DISCUSSION

Varietal and Seasonal Variability in Apple Cortex. Apples from five varieties were harvested during three seasons to follow the variability of the polyphenolic composition from one year to another. **Table 1** shows data corresponding to the mean of each compound concentration over the three years. The data confirm the varietal diversity that was already reported (*5*) for French cider varieties. Considering the variety and the season as factors of variability, two-way analysis of variance showed that variety was mainly involved in variability ($130 < F < 194$; $P < 0.0001$, depending on the considered phenolic compound), whereas year accounted for much less ($7 < F < 37$; $P < 0.0001$, depending on the considered phenolic compound). Nevertheless, the year effect was significant for all compounds ($P < 0.0001$), but we cannot exclude a methodological artifact: thiolysis−HPLC were performed on apple extracts for the 1996 and 1997 seasons but directly on crude apple powders for the 1998 season. Finally, as already observed for dessert apples (*14*), it could be concluded that an overall stability of the polyphenol composition was observed for each variety from one year to another.

The classification of cider apple varieties on the basis of the total polyphenol concentration obtained by Sanoner et al. (*5*) was confirmed (**Table 1**). Procyanidins (PCA) were the main phenolic compounds for all apple varieties, accounting for 49−86% of total polyphenols depending on the variety. As was already shown with regard to dessert apples (*6*), procyanidin B2 accounted for 1−20% of total procyanidins in cider apples, showing that the dimeric form does not represent the main procyanidin fraction. Otherwise, varieties showed significant levels of caffeoylquinic acid (CQA) and (−)-epicatechin (EC) as already observed (*5*), and the unusual polyphenol profile of Avrolles (quasi absence of monomeric catechins, low level of CQA, and high level of highly polymerized procyanidins) was confirmed.

Table 2 shows qualitative parameters such as average degree of polymerization of flavan-3-ols ($\overline{DP}n$) and concentration ratios between the main compounds in each polyphenol class.

These parameters were stable over the three years of harvest. The Avrolles variety confirmed its unusual polyphenol composition, showing the lower CQA/*p*CoQA and PL/XPL ratios and the exceptionally high polymerization state of the procyanidins.

Table 2. Qualitative Criteria[a] for the Characterization of the Polyphenol Profile in the Cortex of Five French Cider Apple Varieties at Maturity[b]

	variety									
phenolic	AV		BD		DM		KE		PJ	
$\overline{DP}n$ FA	45.5	*10.5*	2.5	*0.1*	2.5	*0.1*	3.4	*0.2*	4	*0.1*
FA/HCA	8.90	*0.64*[c]	3.05	*0.10*	1.96	*0.19*	3.45	*0.23*	3.49	*0.54*
EC/CT			3.46	*0.84*	3.08	*0.31*	13.01	*2.54*	5.02	*0.86*
CQA/*p*CoQA	1.60	*0.10*	4.60	*0.26*	8.90	*0.97*	9.82	*0.59*	14.33	*0.99*
PLZ/XPL	0.36	*0.06*	1.95	*0.20*	0.70	*0.08*	1.13	*0.13*	1.13	*0.17*

[a] Mean of three harvest years (1996, 1997, and 1998). [b] Complete starch regression. [c] Italic values corresponded to standard deviation ($n = 9$).

The highest CQA/*p*CoQA and EC/CAT ratios were observed for the Petit Jaune and Kermerrien varieties, respectively. These criteria may influence the technological behavior of these varieties during processing. For example, highly polymerized procyanidins in the Avrolles variety may be responsible of browning inhibition by associating with polyphenol oxidase (PPO) (*32*). A low CQA/*p*CoQA ratio in the variety might also contribute to a low sensitivity to enzymatic browning: Previous studies have shown evidence of competitive inhibition of PPO by *p*-coumaric acid (*33*); therefore, *p*-coumaroylquinic acid (*p*CoQA) might also be a competitive inhibitor of the catecholase activity of PPO, whereas CQA is its main substrate. A final factor of the low sensitivity to browning of Avrolles was the absence of monomeric catechins as catechins significantly contribute to the generation of intensely colored pigments when they are oxidized (*8*).

On the whole, it can be concluded that the polyphenol profile of the cider apple varieties was stable from one year to another and might serve as a biochemical tool to characterize the variety.

Variability Related to Maturity in Apple Cortex. Three maturity stages of apple fruits were chosen in the starch regression period of fruit maturation. This period is of great importance for the quality of processed fruits because it corresponded to the conversion of starch to glucose, fructose, and saccharose, which is related to the quality of the juice for processing.

The polyphenol profiles of the cortex of the five cider apple varieties at different maturity stages are presented in **Table 3**.

Although the overall polyphenol profiles were not largely modified, total polyphenol concentrations in fresh matter significantly decreased during the maturation period. Nevertheless, this decrease was weak for Avrolles and Dous Möen (7 and 5%, respectively, between the extreme states) and more marked for Bedan, Kermerrien, and Petit Jaune (15, 13, and 18%, respectively). Considering the variety and the maturity as factors of variability, two-way ANOVA analysis showed that this lowering was significant for all phenolic compounds individually ($5.7 < F < 14$, $P < 0.0001$, depending on the

Table 3. Polyphenol Composition (Milligrams per Kilogram of Fresh Weight) and $\overline{DP}n$[a] in the Cortex of Cider Apples for Different Varieties at Different Maturity States (1998 Season)

variety	maturity	CT		EC		B2		PCA		$\overline{DP}n$		CQA		*p*CoQA		XPL		PLZ		total[b]	
AV	0	0	*0*[c]	0	*0*	30	*16*	3114	*386*	34.9	*2.2*	240	*25*	158	*20*	124	*6*	41	*5*	3676	*433*
	50	0	*0*	0	*0*	20	*2*	2894	*94*	37.8	*1.7*	226	*21*	132	*16*	111	*2*	33	*2*	3395	*124*
	100	0	*0*	0	*0*	19	*2*	2922	*237*	36.7	*3.0*	210	*5*	127	*9*	114	*10*	37	*4*	3410	*244*
BD	0	297	*35*	539	*33*	319	*9*	2242	*536*	2.23	*0.1*	933	*175*	175	*31*	15	*4*	34	*5*	4236	*686*
	50	149	*37*	540	*33*	338	*13*	1840	*233*	2.29	*0.1*	690	*35*	155	*8*	23	*16*	38	*10*	3435	*200*
	100	146	*54*	521	*12*	371	*25*	2023	*68*	2.37	*0.0*	728	*32*	153	*1*	17	*3*	34	*2*	3622	*59*
DM	0	161	*14*	424	*23*	245	*33*	1906	*231*	2.58	*0.1*	809	*199*	108	*22*	32	*3*	20	*2*	3460	*68*
	50	141	*37*	429	*39*	282	*95*	1770	*270*	2.64	*0.1*	952	*103*	115	*10*	31	*1*	23	*2*	3461	*404*
	100	154	*9*	439	*21*	240	*15*	1586	*88*	2.42	*0.0*	939	*49*	114	*10*	27	*1*	20	*2*	3280	*167*
KE	0	43	*6*	517	*24*	608	*84*	4043	*134*	3.39	*0.0*	1176	*54*	132	*3*	54	*1*	68	*5*	6033	*152*
	50	51	*2*	576	*12*	620	*46*	3648	*371*	3.13	*0.1*	1005	*69*	108	*9*	41	*1*	49	*2*	5479	*436*
	100	43	*2*	574	*21*	640	*35*	3450	*453*	3.20	*0.0*	1014	*136*	96	*21*	45	*8*	51	*13*	5273	*624*
PJ	0	30	*4*	103	*4*	170	*14*	1476	*53*	4.07	*0.2*	476	*28*	39	*2*	17	*1*	17	*2*	2159	*75*
	50	27	*2*	97	*5*	147	*12*	1109	*92*	4.01	*0.1*	371	*28*	26	*2*	12	*1*	13	*1*	1656	*121*
	100	22	*3*	97	*20*	146	*11*	1228	*47*	4.12	*0.1*	376	*29*	27	*2*	13	*2*	15	*3*	1778	*61*

[a] $\overline{DP}n$, average degree of polymerization of flavanols (catechins + procyanidins). [b] Total polyphenols. [c] Italic values correspond to standard deviation ($n = 3$).

Table 4. Polyphenol Composition (Milligrams per Liter) and $\overline{DP}n$[a] in the Juice[a] of Cider Apples for Different Varieties at Different Maturity States

variety	maturity	CT		EC		B2		PCA		$\overline{DP}n$		CQA		*p*CoQA		XPL		PLZ		total[b]	
AV	0	nd		nd		nd		1815	*16*[c]	34.1	*2.6*	266	*9*	205	*11*	178	*1*	84	*3*	2570	*35*
	100	nd		nd		nd		1328	*27*	26.3	*2.5*	228	*12*	171	*4*	145	*7*	54	*3*	1942	*18*
BD	0	203	*23*	362	*31*	233	*29*	908	*84*	1.72	*0.0*	717	*30*	192	*3*	23	*2*	37	*1*	2443	*150*
	100	222	*8*	338	*3*	231	*18*	907	*109*	1.97	*0.0*	632	*25*	169	*6*	17	*4*	31	*1*	2316	*115*
DM	0	180	*8*	363	*16*	221	*19*	780	*71*	1.81	*0.0*	948	*38*	153	*7*	45	*4*	26	*4*	2495	*132*
	100	162	*11*	324	*22*	231	*23*	872	*19*	2.15	*0.1*	793	*26*	125	*4*	41	*4*	20	*1*	2337	*82*
KE	0	52	*2*	389	*28*	459	*52*	2265	*183*	2.66	*0.1*	1055	*32*	124	*5*	55	*5*	67	*5*	4007	*200*
	100	43	*6*	445	*7*	516	*36*	2376	*10*	2.77	*0.0*	1014	*16*	103	*2*	45	*1*	45	*2*	4071	*20*
PJ	0	nd		87	*12*	139	*8*	471	*34*	2.42	*0.1*	399	*14*	36	*1*	25	*1*	18	*1*	1036	*38*
	100	12	*1*	74	*2*	nd		661	*27*	3.01	*0.1*	403	*9*	31	*1*	20	*1*	14	*1*	1207	*30*

[a] Except for the OX lines, values correspond to nonoxidized and noncentrifuged juices. $\overline{DP}n$, average degree of polymerization of flavanols (catechins + procyanidins); OX, oxidized and noncentrifuged juices. [b] Total polyphenols. [c] Italic values correspond to standard deviation ($n = 3$).

considered compound) with the exception of (−)-epicatechin concentrations ($F = 1.2$, $P = 0.3$), which remained stable during the starch regression period. Finally, the contribution of maturity to the polyphenol variability in apples was weak in comparison to the variability arising from the variety ($94 < F < 134$, $P < 0.0001$, depending on the considered phenolic compound).

The results were wholly consistent with previous studies on polyphenol concentration according to the maturity of apples, showing that polyphenol accumulation was completed during fruit development before the maturation period (*12*, *15*).

Polyphenol Composition of Nonoxidized and Noncentrifuged Cider Apple Juices (Table 4). The total polyphenol concentration in crude juices was in the range of 1−4 g/L, which was in concordance with previous data on the total polyphenol level in French (*34*) and English (*35*) cider apple juices. Procyanidins represented 32−70% of total polyphenols depending on the variety, with concentrations close to 2 g/L in some juices. Although they corresponded to highly polymerized forms ($\overline{DP}n$ close to 25, see **Table 5**), procyanidins were present in high concentration in the juice of the Avrolles variety, showing that even in a highly polymerized state, procyanidins can be significantly extracted when apples are transformed into crude juices. For the other varieties, the oligomeric forms were preponderant, the dimeric procyanidin B2 accounting for 20−30% of total procyanidins. Absent in the juice of Avrolles, monomeric catechins accounted for 8−24% of total polyphenols in the other varieties: the larger proportion was observed for the Bedan and Dous Möen varieties. (−)-Epicatechin was the major compound of this class; however, in the Bedan variety, (+)-catechin represented up to 40% of total catechins. Hydroxycinnamic acids accounted for 20−42% of total polyphenols, CQA being always predominant. However, the ratio between CQA and *p*CoQA was variety dependent (see **Table 5**). Phloridzin and phloretin xyloglucoside were the two major dihydrochalcones. This class accounted for 2−3% of total polyphenols excepted for the Avrolles variety, for which the contribution reached 10%, corresponding to concentrations up to 260 mg/L.

Table 5. Qualitative Criteria for the Characterization of the Polyphenol Profile in Nonoxidized and Noncentrifuged Juices of Five French Cider Apple Varieties at Maturity[a]

	variety									
phenolic	AV		BD		DM		KE		PJ	
$\overline{DP}n$ FA	26.3	*2.5*	2.0	*0.03*	2.1	*0.02*	2.8	*0.05*	3.0	*0.05*
FA/HCA	3.34	*0.21*	1.85	*0.07*	1.48	*0.01*	2.54	*0.05*	1.72	*0.03*
EC/CT			1.53	*0.04*	2.00	*0.01*	10.44	*1.27*	6.08	*0.97*
CQA/*p*CoQA	1.33	*0.04*	3.74	*0.09*	6.34	*0.17*	9.88	*0.06*	13.05	*0.08*
PLZ/XPL	0.37	*0.00*	1.91	*0.38*	0.49	*0.06*	1.00	*0.06*	0.7	*0.05*

[a] Complete starch regression. Italic values correspond to standard deviation ($n = 3$).

Table 6. Results of the Wilcoxon Signed Rank Test (with $P < 0.05$) To Evaluate the Effect of Centrifugation on Nonoxidized Apple Juices

	median before centrifugation (mg/L)	median after centrifugation (mg/L)	significance	*P* value
FA	1387	1349	yes	0.0060
$\overline{DP}n$ FA	2.55[b]	2.50[b]	yes	<0.0001
CAT	476	492	no	0.386
HCA	859	819	no	0.0641
DHC	64.5	68.0	yes	0.0133

[a] $N = 30$ corresponding to five varieties, two maturity states, and three replicates. [b] $\overline{DP}n$ units.

From a qualitative point of view, polyphenol profiles of the juices (**Table 5**) were also strongly modified when they were compared with the cortex (**Table 2**). The main variation involved procyanidins. Thus, the $\overline{DP}n$ of flavanols (i.e., catechins + procyanidins) is significantly reduced in the juice ($P < 0.0001$), and this was particularly evident for the Avrolles variety. Moreover, the FA/HCA ratio was significantly reduced in the juices ($P < 0.0001$). These observations attested to the retention of the polymerized procyanidins in the pomace due to their partial but selective adsorption on apple cell-wall materials (*19*). Other qualitative criteria such as the CQA/*p*CoQA, PLZ/XPL, and EC/CT ratios were weakly modified (**Table 5**).

Effect of Maturity on the Polyphenolic Composition of the Juice (Table 4). No marked effect of the maturity of the fruits on the total polyphenol concentration of the juice was observed except for the Avrolles variety, which showed a strong decrease (35%) of the procyanidin concentration in the juice coming from ripe fruits. Although the polymerization states of procyanidins did not differ with maturity in the flesh (**Table 3**), the $\overline{DP}n$ of procyanidins in the juice coming from ripe Avrolles apples was 26.3 (**Table 4**), whereas it was 34.1 for juices coming from the unripe fruits (**Table 4**), showing that maturity selectively influenced the extraction of highly polymerized procyanidins.

Effect of Centrifugation of the Nonoxidized Juices. The polyphenol profiles were compared before and after centrifugation (**Table 6**). A significant difference was observed for flavanols. Their concentrations as well as their $\overline{DP}n$ values were slightly but significantly lower in centrifuged apple juices. This difference may be explained by the removal of a small part of the procyanidins because of their adsorption on cellular and cell-wall particles, which are in suspension in the crude juices. Adsorption of procyanidins on apple cell-wall polysaccharides was recently shown to be more effective for the more polymerized procyanidins (*19*). As a whole, the procyanidin concentration remained very high in centrifuged nonoxidized apple juices, in the range of 560-2360 mg/L depending on the variety. Even Avrolles juices showed PCA concentrations up to 1250 mg/L with $\overline{DP}n$ values close to 25, showing that procyanidins can be soluble in cider apple juices even in highly polymerized forms.

No significant difference was observed for catechins and hydroxycinnamic derivatives. These compounds are small molecules that might not be involved in associations with crude juice particles. Dihydrochalcones were slightly but significantly affected by centrifugation (**Table 6**). However, the *p* value of the Wilcoxon test was relatively high (**Table 6**) and, as far as we know, no argument could be advanced to explain this observation.

Effect of Oxidation of the Juice. Oxidized and nonoxidized apple juices were compared on the basis of their polyphenol compositions (**Figure 1**). Oxidation resulted in a significant decrease of all polyphenol classes. However, the intensity of this decrease differed strongly depending on the class and the variety as was previously shown (*35*).

Catechins were highly reduced in oxidized juices, disappearing almost completely for the Petit Jaune and Bedan varieties. These compounds [i.e., (−)-epicatechin and (+)-catechin] are good substrates of polyphenol oxidase (*8*); moreover, they are largely oxidized by the coupled oxidation mechanism with CQA quinone (*21*, *36*). They are also involved in the formation of addition products with quinones (*20*). Several hypotheses can be proposed to explain the drastic reduction of catechins in the juice of the Bedan variety. First, PPO activity might be high in this variety. Second, the concentrations and proportions of the other polyphenol classes might interfere with the oxidation process. For example, the PCA concentration in the Bedan variety was largely lower than that in the Kermerrien variety. Therefore, for the Bedan variety, catechins might be a preferential nucleophilic target for the electrophilic quinones, leading finally to the formation of addition products. In the Kermerrien variety, the PCA, which are more abundant, might be a competitive target for electrophilic attacks of *o*-quinones, thus preserving catechins from complete disappearance in the oxidized juice.

Procyanidin concentrations in the juices were also strongly reduced by oxidation (**Figure 1**). The decrease varied by a 4−9-fold factor depending on the variety. Moreover, the presented data might be underestimated in oxidized juices because of a methodological bias. The thiolysis−HPLC method permitted oligomeric and polymeric procyanidins to be assayed by converting them into monomeric derivatives corresponding to the catechin units of the native procyanidin structure. During preparation of the oxidized juices, procyanidins may be converted into quinones, which further react leading to structurally modified procyanidins. Therefore, procyanidins may become partially resistant to thiolysis. Then, the thiolysis−HPLC method can theoretically give access only to the assay of the catechin units, which have not been structurally modified by the oxidation reactions. For this reason, the calculated $\overline{DP}n$ of procyanidins was not given as it may largely be altered because of oxidation.

The decrease of procyanidin concentrations might also be due to interactions with apple solid material during crushing of apples in oxidative condition. At this moment, oxidation of procyanidins has already occurred and modified partly their whole structure. These new oxidized and polymerized structures might have their interaction capacity enhanced compared to the native procyanidins, and a greater proportion of the procyanidin fraction would thus be retained on the pomace.

The concentrations of hydroxycinnammic acids were significantly reduced in oxidized juices, by a 1.5−4.5-fold factor depending on the varieties (**Figure 1**). However, the decrease was lower than that of catechins or procyanidins. This observation suggested the involvement of a coupled oxidation mechanism between CQA *o*-quinones and other polyphenols. CQA is the preferential substrate for polyphenol oxidase, but the resulting *o*-quinone is largely converted back to CQA, whereas molecules such as catechins and procyanidins are oxidized into quinones (*37*, *38*). This mechanism probably contributed to a partial protection of caffeoylquinic acid from its oxidation during apple processing.

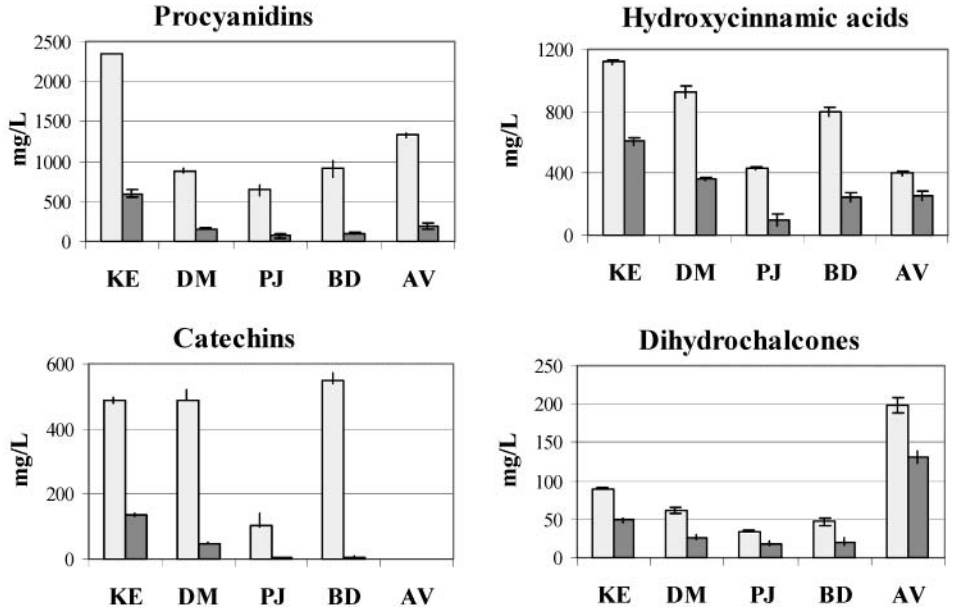

Figure 1. Effect of oxidation on the polyphenol profile of the juice (noncentrifuged and made with 100% maturity apples): (light shading) nonoxidized juice; (heavy shading) oxidized juice.

Dihydrochalcones were also reduced in oxidized juices but only by a 1.4−2.3-fold factor depending on the variety. Dihydrochalcones (i.e., phloridzin and phloretin xyloglucoside) are substrates of the monophenolase (cresolase) activity of PPO, but its catalytic power (V_m/K_m) is much lower than the catecholase activity (*8*). Moreover, because they are wholly preserved in the oxidized juices, results also suggested that dihydrochalcones are not the preferred target for the coupling reaction with *o*-quinones and are not quantitatively involved in the formation of oxidation derivatives.

In addition to native polyphenols, which are discussed in the present work, oxidized juices may also contain oxidation products resulting from coupling reactions.

Extraction Yields of Polyphenols from the Fruits to the Juice. An overall consistency was observed by comparing the phenolic compositions of fruit cortex (**Table 3**) and those of the juices (**Table 4**) coming from the same fruit batches (1998 season): Polyphenol-rich varieties are those leading to juices containing more phenolics, and this observation was also true considering each class of phenolic compound.

The comparison between fruits and juices was limited in the present work because significant parts of the fruits (i.e., the peel, the core, and the seeds) were not considered for their polyphenol content. Only polyphenols in the cortex have been assayed. Nevertheless, previous work (*18*) has shown that polyphenols of the cortex accounted for 65% of total polyphenols of the fruits and that the cortex tissues were shown to correspond to ~84% of the fresh matter of the fruit (*39*). Moreover, the absence of flavonols in all analyzed juices was an indication of the poor extractability of polyphenols from the peel in the present conditions of juice preparation. Although we were conscious of this limitation, it was very informative to have an estimation of the extraction rate of polyphenols from the cortex to the corresponding juice when fruits are processed. The extraction yields were calculated according to the formula

$$\text{yield (\%)} = \frac{[\text{Pj}] \times R}{[\text{Pc}] \times d} \times 100$$

where [Pj] is the concentration of the considered polyphenol in the juice (mg/L), [Pc] is the concentration of the considered polyphenol in the cortex (mg/kg), R, is the extraction yield of the juice (kg of juice/kg of fresh fruits), and *d* is the density of the juice (kg/L).

Table 7. Polyphenol Extraction Yields from the Flesh to Crude Juice[a]

	CAT	PCA	HCA	DHC	TOT
extraction yields (%)	50.8	31.9	65.1	79.0	41.7
confidence interval (± %) at 95%	*5.0*	*4.12*	*7.4*	*9.0*	*3.2*

[a] $n = 15$ for each polyphenol class.

The yields of polyphenol extraction in nonoxidized and noncentrifuged juices are presented for each polyphenol class as a mean of all studied varieties (**Table 7**). The extraction yields were close to 42% for total polyphenols, showing that a large proportion of the polyphenol of the fruits remained in the pomace. The extraction yields varied strongly according to the phenolic class. Hydroxycinnamic acids and dihydrochalcones showed the higher extraction yield values (65 and 80%, respectively), whereas catechins were extracted for a half. For procyanidins, the yield was significantly lower, close to 32%. The lower extraction of procyanidins may result from the association of the procyanidins with the solid part of the fruits, particularly cell-wall materials when fruits are crushed and pressed as was previously shown in a model solution (*19*). Moreover, the selectivity of this adsorption phenomenon as a function of the polymerization state of PCA was confirmed because the calculated $\overline{\text{DP}}n$ value in the fruits was higher than the corresponding $\overline{\text{DP}}n$ in the nonoxidized juices (**Tables 2** and **5**) for all varieties. These results confirmed that solid parts of the fruits, mainly apple cell wall, act as a selective barrier for the polyphenols when fruits are processed to juices.

To conclude, the presented data clearly give evidence of the diversity of polyphenol profiles in cider apple fruits as well as in the corresponding juices according to the variety. Juice aeration had a major effect on the polyphenol profile of the juice. Oxidation of polyphenols in the juice leads to the formation of new phenolic compounds not investigated in the present study. It must be emphasized that extreme aeration conditions were used in our experiment in order to enhance the effect of this factor. Probably, juices do not undergo so large

an aeration in the cider industry when large volumes of fruits are processed. In the limit of our investigations, other factors such as harvest year, fruit maturity, and centrifugation of the juice did not strongly affect the phenolic composition.

As a whole, from the fruit to the juice, polyphenol profiles undergo significant qualitative and quantitative alterations. Procyanidins were particularly affected because of their sensibility to oxidation and their capacity to be selectively adsorbed on cell-wall materials. These mechanisms may have a strong influence on the quality of French ciders.

ABBREVIATIONS USED

AV, Avrolles; DM, Dous Möen; PJ, Petit Jaune; KE, Kermerrien; BD, Bedan; CT, (+)-catechin; EC, (−)-epicatechin; CAT, total catechins (i.e., CT + EC); B2, procyanidin B2; PCA, procyanidins; FA, flavanols (i.e., catechins + procyanidins); $\overline{DP}n$, average degree of polymerization; CQA, caffeoylquinic acid; *p*CoQA, *p*-coumaroylquinic acid; XPL, phloretin xyloglucoside; PLZ, phloridzin.

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