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Polyphenolic Profiles of Basque Cider Apple Cultivars and Their Technological Properties

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The polyphenolic compositions of 31 Basque cider apple cultivars were determined in pulp, peel, and juice by high-performance liquid chromatography with diode array detection analysis of crude extracts and after thiolysis. Total polyphenols are distributed in a wide concentration range depending on the cultivar. Procyanidins are the class of polyphenols that present major concentrations in apple. Their average degrees of polymerization range from 4 to 8 depending on the cultivar. Apple cultivars were technologically classified into bitter and nonbitter categories using different classification systems obtained by applying several pattern recognition techniques, such as principal component analysis, *K*-nearest neighbors, soft independent modeling of class analogy, partial least-squares, and multilayer feed-forward−artificial neural networks, to apple pulp, peel, or juice data (individual polyphenol concentrations, total procyanidin content, and the average degree of polymerization of procyanidins). Bitter apple cultivars present higher contents of flavan-3-ols and/or dihydrochalcones than nonbitter cultivars. Detailed knowledge of the polyphenolic profile of each apple cultivar affords information about their susceptibility to oxidation, their sensory properties (bitterness, astringency), and their possible influence on the characteristics and quality of the final product (juice, cider) when apples are processed.

KEYWORDS: Polyphenol; apple; cider; bitterness; HPLC; thiolysis; chemometrics; pattern recognition

INTRODUCTION

Several works have been carried out to study the chemical constituents and the technological qualities of different apple cultivars in order to select the most appropriate for the elaboration of ciders, juices, and other apple-derived products (*1*). The main technological properties that apple cultivars used for cidermaking should present are (a) a high juice yield, (b) a medium-high level density and sugar content and a reduced dried extract, (c) a balanced concentration of pectins, polyphenols, and organic acids, (d) a low nitrogen content, and (e) aromas and interesting sensory qualities. Moreover, it is required that apple fruit has a good resistance to manipulation during harvest and transportation. Furthermore, in Asturias and the Basque Country (the main Spanish cidermaking regions), it is desirable that fruit maturation takes place late in order to process the fruits when temperatures are low enough; thus, the fermentative process develops more slowly (*2*).

Apples present a wide diversity of polyphenols classified into several major classes. The flavan-3-ols include monomeric (catechins) and polymeric (procyanidins) forms, mainly constituted by (−)-epicatechin units. Among the hydroxycinnamic acids, 5-caffeoylquinic acid and 4-*p*-coumaroylquinic acid show the highest contents. The major species of the dihydrochalcones are phloretin glucoside and xyloglucoside, being generally considered to be specific to apples. And finally, flavonols and anthocyanins are essentially present in apple peel (*3*). In cider apple cultivars, polyphenol interest is due to the fact that they are responsible for the color and the balance of bitterness to astringency, which defines the "overall mouthfeel" of ciders (*4*). Furthermore, they are implicated in the alcoholic and malolactic fermentations as metabolites, providing cider aroma, and as inhibitors of the microbiological growth, controlling fermentation rates and cider spoilage (*5*). Polyphenols are also involved in the colloidal stability of cider (*6*).

Technological classification of cider apple varieties is commonly based on the total polyphenol content (Folin−Ciocalteu method) and the total acidity of their juices. Following these criteria, apple cultivars are classified in six technological groups: sweet (<3.55 g of sulfuric acid/L, <1.45 g of tannic acid/L), bittersweet (<3.55 g of sulfuric acid/L, >1.45 g of tannic acid/L), semiacid (3.55−4.80 g of sulfuric acid/L, <1.45 g of tannic acid/L), semiacid−bitter (3.55−4.80 g of sulfuric acid/L, >1.45 g of tannic acid/L), acid (>4.80 g of sulfuric acid/L, <1.45 g of tannic acid/L), and acid−bitter (>4.80 g of sulfuric acid/L, >1.45 g of tannic acid/L) (*2*). However, the information obtained by the analysis of these global parameters is limited, because no distinction among the different classes

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of polyphenols and their diverse properties is made. This kind of information is especially interesting when it is taken into account that some polyphenols or classes are those that give certain characteristics to the final product. Thus, hydroxycinnamic acids are precursors of volatile phenols formed during fermentation, which contribute to cider aroma (*7*), 5-caffeoylquinic acid and catechins generate colored products by enzymatic oxidation and coupled oxidation reactions with other polyphenols (*8*), and procyanidins are responsible for cider bitterness and astringency (*9*). 5-Caffeoylquinic acid also contributes to the astringency of apple juices and ciders (*34*).

In addition, these global estimations performed on juice do not provide complete information on the polyphenolic potential of fruit, because an important part of the native compounds is oxidized and adsorbed on apple cell walls when juice is made. Therefore, a precise knowledge of the composition of cider apple cultivars may contribute to a better understanding of their implication in the quality and diversity of apple-derived products, such as cider and apple juice. In this sense, several characterization studies of different dessert apple varieties (*10*) and cider apple cultivars from Spain (*11*), France (*3*), and the United Kingdom (*12*) have been carried out on the basis of their polyphenolic profiles. In this work, the polyphenolic profiles of Basque cider apple cultivars are characterized and their technological properties are related to their composition in polyphenols.

MATERIALS AND METHODS

Reagents and Standards. Methanol (Romil Chemical Ltd., Heidelberg, Germany) was of HPLC grade. Water was purified on a Milli-Q system from Millipore (Bedford, MA). Glacial acetic acid, formic acid, toluene-α-thiol, Folin−Ciocalteu reagent, fuming hydrochloric acid 37%, sodium hydroxide, and potassium hydrogen phthalate (GR volumetric standard) provided by Merck (Darmstadt, Germany) and ascorbic acid provided by Panreac (Barcelona, Spain) were of analytical quality. All solvents used were previously filtered through 0.45 μm nylon membranes (Lida, Kenosha, WI).

Polyphenol standards were supplied as follows: (+)-catechin, (−)-epicatechin, rutin, phloridzin, 5-caffeoylquinic acid, *p*-coumaric acid, and tannic acid by Sigma-Aldrich Chemie (Steinheim, Germany); hyperoside, isoquercitrin, avicularin, quercitrin, and ideain chloride by Extrasynthèse (Genay, France). (−)-Epicatechin 4*R*-benzylthioether and 4-*p*-coumaroylquinic acid were kindly provided by Dr. Guyot and phloretin 2′-*O*-xyloglucoside and procyanidin B2 by Dr. F. A. Tomás-Barberán and Dr. C. Santos-Buelga, respectively. Stock standard solutions of (+)-catechin, (−)-epicatechin, (−)-epicatechin 4*R*-benzylthioether, rutin, phloridzin, 5-caffeoylquinic acid, *p*-coumaric acid, and tannic acid at a concentration of 1 mg mL^{-1} and hyperoside, isoquercitrin, quercitrin, and ideain at 0.6 mg mL^{-1} were prepared in methanol and stored at 4 °C in darkness. The other standards were prepared in approximate concentrations and used for chromatographic peak identification.

Plant Materials. Pulp and peel from 31 different apple cultivars used in the Basque Country for cidermaking were analyzed (see **Tables 1**−**3**). Apples were harvested in the Experimental Orchards of the Diputación Foral de Gipuzkoa in Hondarribia (Guipúzcoa, Spain) and the Diputación Foral de Bizkaia in Zalla (Vizcaya, Spain) during the 2000 and 2001 seasons.

Apple Powder Preparation. Fruits of the 2000 and 2001 seasons were harvested at maturity, which was tested by the lugol index (*13*). For each variety and season, two or three batches of 10 apple fruits were mechanically peeled and cored and sprayed with an aqueous solution of formic acid 3% in order to avoid polyphenol oxidation. Peels and pulps were immediately frozen in liquid nitrogen, and then they were freeze-dried. An aliquot for each variety was used to determine the fresh/dry matter ratio. The dried tissues were crushed in closed vials to avoid hydration, obtaining a homogeneous powder that was stored at room temperature in a desiccator until analysis. Aliquots of 0.5 g of freeze-dried apple peel or pulp were used for each analysis.

Apple Juice Preparation. Fruits of the 2000 and 2001 seasons were used for making juices. Two or three batches of fruits (1 kg) were constituted for each cultivar. Each batch was milled and pressed to obtain crude juice, using procedures similar to those used by Basque cidermakers (a grinder and a traditional press) but in small scale. A solution of diluted sodium fluoride (50 mL, 1 g/L in water) was added to the apples before pressing in order to avoid oxidation to a certain extent. This added volume was subtracted for yield calculations, and a correcting factor was applied for calculating polyphenol concentrations. Then, crude apple juices were centrifuged (10000 rpm, 15 min) at 4 °C to obtain clear apple juices. Aliquots of centrifuged apple juices were sampled for the determination of polyphenolic profiles by HPLC (2 × 1 mL) and total polyphenol content by the Folin−Ciocalteu method (0.5 mL) and total acidity (40 mL). Aliquots for HPLC analyses were freeze-dried and stored in a desiccator until analysis. Other aliquots were frozen and kept at −20 °C, being defrosted just before analysis.

Analytical Procedures. *Thiolysis and Direct Solvent Extraction and Reversed-Phase HPLC Analysis of Freeze-Dried Samples.* Different aliquots of freeze-dried samples (0.5 g) were submitted to thiolysis as described by Guyot et al. (*14*) and to direct solvent extraction with 30 mL of methanol/water/acetic acid (30:69:1, v/v/v) with ascorbic acid (2 g/L) in an ultrasonic bath during 10 min (*15*). Then, both thiolysis reaction mixtures and crude solvent extracts were filtered through a 0.45 μm PTFE filter (Waters, Milford, MA) prior to injection into the HPLC system.

Chromatographic analyses were performed on a Hewlett-Packard series 1100 system, equipped with a vacuum degasser, a quaternary pump, a thermostated autosampler, a thermostated column compartment, and a DAD, connected to HP ChemStation software. A reversed-phase Nova-Pak C18 (300 × 3.9 mm i.d., 4 μm) column and a Nova-Pak C18 (10 × 3.9 mm i.d., 4 μm) guard column (Waters, Barcelona, Spain) were used. Solvents that constituted the mobile phase were acetic acid/water, 10:90, v/v (A), and methanol (B). The elution conditions applied were as follows: 0−10 min, 0% B isocratic; 10−40 min, linear gradient from 0 to 15% B; 40−60 min, 15% B isocratic; and finally, washing and reconditioning of the column. The flow rate was 0.8 mL min^{-1}, and the injection volume was 50 μL for the crude extracts or 10 μL for the thiolysis media. The chromatographic separation was carried out at 25 °C. Catechins and dihydrochalcones were monitored and quantified at 280 nm, hydroxycinnamic acids at 320 nm, flavonols at 370 nm, and anthocyanins at 530 nm. Polyphenol identification was achieved by comparison of their retention times and their UV−visible spectra with those of the standards that were available. Some other chromatographic peaks were assigned to a particular polyphenol class according to their UV−visible spectra and bibliographic sources. In this sense, those unknown chromatographic peaks that exhibit flavan-3-ol spectra were appointed as CAT-*n*, and those with a spectrum of 5-caffeoylquinic acid as CAA-*n*, of *p*-coumaric as CMA-*n*, of dihydrochalcone as PLD-*n*, of flavonol as QG-*n* and of anthocyanin as CG-*n* (where "*n*" is a number). Quantification was performed by reporting the measured integration areas in the calibration equation of the corresponding standards. Thus, procyanidin B2 and the unknown flavan-3-ols were quantified as (+)-catechin, phloretin 2′-*O*-xyloglucoside and the unknown dihydrochalcones as phloridzin, avicularin and the unknown flavonols as rutin, CAA-*n* species as 5-caffeoylquinic acid, 4-*p*-coumaroylquinic acid and CMA-*n* species as *p*-coumaric acid, and the unknown anthocyanins as ideain.

Total Polyphenol Content by Folin−Ciocalteu Method. Estimation of the global polyphenol content in apple juices was performed according to the Folin−Ciocalteu method adapted from Singleton and Rossi (*16*). Centrifuged juice aliquots (0.5 mL) were diluted 20-fold in methanol/2.5% aqueous acetic acid (10:90, v/v). Folin−Ciocalteu reagent (0.25 mL) was added to a 0.5 mL of the diluted cider solution. The mixture was homogenized with a vortex and, after 3 min for allowing the reaction to take place, 1 mL of Na_2CO_3 (200 g/L) and 3.25 or 8.25 mL of ultrapure water were added, depending on the cultivar polyphenol content, and homogenized. Then, the mixture was incubated for 10 min at 70 °C. Once it had cooled at room temperature, it was homogenized and its absorbance was measured at 700 nm with

Table 1. Concentrations (Milligrams per Kilogram of Apple) of Flavan-3-ols, Hydroxycinnamic Acids, Dihydrochalcones, and Flavonoids in Apple Pulps (2000 and 2001 Seasons)[a]

		flavan-3-ols				hydroxycinnamic acids				dihydrochalcones				flavonols			
variety[b]		CAT	EC	PB2	CAT-2	CQA	CAA-1	CMA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QCI
AG	mean	10	65	74	8	158	19	1.0	16.7	2	2.4	9	10.8	nd	0.21	0.4	0.5
	SD	3	16	6	2	49	1	0.2	0.5	1	0.6	4	0.5		0.03	0.2	0.3
BK	mean	16	59	66	7	150	7.5	0.4	4.5	2	3	6	10	nd	0.35	0.48	1.12
	SD	9	16	5	2	52	0.8	0.1	0.4	1	2	2	2		0.02	0.05	0.06
ER	mean	12.3	70	64	7.5	191	18	nd	21.6	6.1	3.3	45	18.2	nd	nd	nd	0.50
	SD	0.7	5	6	0.5	15	1		0.7	0.9	0.3	2	0.7				0.07
GG	mean	1.9	136	164	16.2	267	10.4	nd	4.4	1.57	1.52	30	4.6	nd	0.23	0.258	1.7
	SD	0.3	6	7	0.7	7	0.9		0.3	0.07	0.07	3	0.5		0.04	0.009	0.2
GK	mean	17	111	120	11.80	347	13.3	0.7	8.7	8	4	53	27	nd	0.42	1.0	1.72
	SD	7	20	9	0.07	37	0.2	0.2	0.4	7	2	1	4		0.09	0.8	0.07
GM	mean	23	165	149	14	587	37	nd	42	2.8	2.8	29	12.8	nd	0.58	0.78	1.06
	SD	4	20	23	1	61	4		1	0.4	0.2	1	0.2		0.09	0.04	0.05
GZ	mean	17	54	59	6	233	26	1.1	23	3	2.6	11	7.36	nd	0.50	0.5	1.1
	SD	5	21	21	1	30	9	0.6	10	3	0.7	10	0.03		0.09	0.1	0.3
IB	mean	18	79	96	10	103	25	2	23	3.1	3	22	14	nd	0.38	0.6	2
	SD	2	11	25	3	51	9	1	7	0.1	1	5	5		0.05	0.2	1
LR	mean	30	379	529	46	2420	46	1.7	64	nd	10	47	159	0.88	3.2	6.0	4.8
	SD	4	49	62	4	20	6	0.8	5		2	3	18	0.08	0.1	0.4	0.4
MK	mean	1.2	227	324	27.4	444	20	nd	16.3	2.0	2.9	53	14	nd	0.83	1.56	3.9
	SD	0.2	8	9	0.8	15	1		0.9	0.3	0.2	3	1		0.05	0.09	0.4
MN111	mean	17	60	59	7	161	21.8	0.52	19	2	2.0	7.7	6.83	nd	nd	0.3	0.4
	SD	2	9	11	2	6	0.5	0.06	5	1	0.8	0.6	0.04			0.1	0.1
MNEM7	mean	38	145	143	13	155	10	nd	1.0	0.8	2	7.5	14	nd	0.19	0.59	3
	SD	3	33	37	3	81	7		0.7	0.2	1	0.9	6		0.01	0.04	2
MX1	mean	41	204	209	20	369	33	0.5	24	7.9	3	49	22	nd	0.46	1.1	4.4
	SD	17	63	55	5	28	8		7	1.0	1	6	5		0.09	0.4	0.9
MX10	mean	407	770	512	43	231	17.5	nd	16	7	5.9	67	29	nd	0.81	0.7	5.9
	SD	48	28	15	1	12	0.7		1	1	0.3	5	3		0.02	0.1	0.4
MX11	mean	20	50	45	5.01	439	17.3	nd	8	2.1	1.8	27.3	15.9	nd	0.35	0.49	1.7
	SD	4	5	5	0.04	40	0.7		1	0.3	0.1	0.8	0.7		0.05	0.08	0.1
MX2	mean	36	416	465	37	626	10	nd	4.1	0.59	nd	9.1	6	nd	0.17466	0.43	2.3
	SD	8	26	70	6	71	1		0.9	0.04		0.2	2		0.00009	0.04	0.2
MX3	mean	62	213	146	13.1	673	50	nd	63	4.5	4.2	44	32	nd	0.42	1.1	1.9
	SD	9	22	20	0.9	18	4		8	0.9	0.4	5	3		0.08	0.2	0.3
MX4	mean	63	154	160	15	481	21	nd	14	0.94	1.71	12	10.1	0.47	0.7	0.83	3.8
	SD	6	11	12	1	40	3		1	0.03	0.06	1	0.7	0.03	0.1	0.09	0.5
MZ	mean	31	215	189	16.3	733	37	0.9	49	3.47	3.4	32.0	18	nd	0.8	1.3	1.6
	SD	9	22	8	0.1	13	2	0.1	2	0.09	0.3	0.7	3		0.2	0.6	0.1
PK	mean	100	177	100	10.4	160.7	64	4	85	11	17	45	54	nd	0.4	1.3	0.9
	SD	11	5	4	0.3	0.4	1	4	16	6	10	15	9		0.2	0.2	0.4
PL	mean	58.56	79	58	6	373	6	0.20	5.0	2.3	2.5	8.0	7.1	nd	0.55	0.6	1.5
	SD	0.02	17	5	1	2	1	0.09	0.9	0.8	0.6	0.5	0.4		0.06	0.2	0.4
PT	mean	50	92	80	8.0	564	46.78	0.88	66	5.5	3.66	37	16.67	nd	0.8	1.1	4
	SD	13	9	15	0.9	136	0.05	0.05	2	0.2	0.02	4	0.08		0.1	0.5	1
TT	mean	23	65	65	7	264	25	0.96	14	1.4	2.3	8.7	10.1	nd	0.45	0.8	4.37
	SD	5	9	4	1	47	14	0.01	3	0.2	0.4	0.3	0.2		0.02	0.1	0.03
TX	mean	7	77	98	9.3	199	14	0.815	17.1	2	1.9	16	12	nd	0.4	0.9	2
	SD	9	3	7	0.5	195	3	0.003	0.1	2	0.6	13	7		0.2	0.6	1
UG	mean	114	199	116	10.6	179.8	56	1.6	100	9	15	47	51	nd	0.32	1.0	0.6
	SD	1	4	18	0.9	0.2	3	0.2	29	3	6	21	7		0.07	0.4	0.1
UGS	mean	40	288	201	18.5	375	19	nd	16	1.6	1.2	16	11	nd	2.0	0.9	4
	SD	18	26	32	0.4	19	2		4	0.3	0.1	6	4		0.3	0.5	2
UH	mean	6	50	55	5.4	189	13	0.8	12.2	2.65	1.6	18	11.5	nd	0.32	0.7	1.9
	SD	2	3	12	0.6	4	1	0.3	0.9	0.04	0.2	3	0.6		0.05	0.2	0.5
UM	mean	9	41	40	5	420	19	nd	12	1.7	2.0	15	13	0.4	0.3	1.2	2
	SD	7	24	17	3	117	4		4	0.4	0.4	3	4		0.2	0.5	1
UR	mean	54	134	110	11	462	15	nd	10	6	2.3	29.8	11	nd	nd	0.77	2.1
	SD	15	37	28	3	149	2		5	3	0.8	0.9	3			0.05	0.8
URZ	mean	58	145	124	12	533	19.1	0.204	13	6	2.2	34	11.6	nd	nd	0.7	1.73
	SD	17	35	26	2	121	0.2	0.008	5	2	0.5	3	1.0			0.2	0.05
UT	mean	14	107	111	10.6	190	13	nd	8.2	5.0	2.3	29	10	nd	0.21	nd	1.27
	SD	2	7	8	0.6	11	1		0.8	0.8	0.4	2	1		0.02		0.03

[a] CQA, caffeoylquinic acid; CAA-1, unknown hydroxycinnamic acid with caffeic acid UV spectra; CAT, (+)-catechin; CAT-2, unknown flavan-3-ol; CMA-2, unknown hydroxycinnamic acid with *p*-coumaric acid UV spectra; EC, (–)-epicatechin; HYP, hyperoside; IQC, isoquercitrin; PB2, procyanidin B2; PCQ, *p*-coumaroylquinic acid; PLD-1, hydroxyphloretin diglycoside; PLD-2, hydroxyphloretin monoglycoside; PLG, phloridzin; PLXG, phloretin 2′-*O*-xyloglucoside; QCI, quercitrin; QG-1, unknown quercetin glycoside; nd, not detected; t, traces; SD, standard deviation. [b] AG, Azpuru Garratza; BK, Bost Kantoi; ER, Errezila; GG, Gazigorri; GK, Goikoetxea; GM, Geza Miña; GZ, Gazilokia; IB, Ibarra; LR, Larrabetzu; MK, Moko; MN111, Manttoni 111; MNEM7, Manttoni EM7; MX1, Mendexa 1; MX10, Mendexa 10; MX11, Mendexa 11; MX2, Mendexa 3; MX3, Mendexa 2; MX4, Mendexa 4; MZ, Mozoloa; PK, Piko; PL, Palazio; PT, Patzuloa; TT, Txistu; TX, Txalaka; UG, Ugarte; UGS, Urdai Goika Santutxu; UH, Urtebi Haundia; UM, Udare Marroi; UR, Urdin; URZ, Urdin Zalla; UT, Urtebi Txiki.

Table 2. Concentrations (Milligrams per Kilogram of Apple) of Flavan-3-ols, Hydroxycinnamic Acids, Dihydrochalcones, an Flavonols in Apple Peels (2000 and 2001 Seasons)[a]

		flavan-3-ols				hydroxycinnamic acids					dihydrochalcones				flavonols							snthocyanins				
variety[b]		CAT	EC	PB2	CAT-2	CQA	CAA-1	CMA-2	CAA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QG-2	QG-3	AVI	QCI	IDE	CG-1	CG-2	CG-3	CG-4
AG	mean	1.9	24	26	3.6	16	3.5	0.8	0.6	1.7	1.9	4	9	16	31	6	15	2	nd	17	10.8	0.11	nd	nd	nd	nd
	SD	0.5	5	2	0.3	5	0.1	0.2	0.2	0.2	1.0	2	5	3	2	4	2	2		2	0.6	0.04				
BK	mean	2.7	14	16	2.0	15	0.9	0.1	0.23	0.3	1.4	3	5	10	19	6.3	9.8	0.89	nd	12.1	6	nd	nd	nd	nd	nd
	SD	0.9	2	3	0.6	1	0.1		0.02	0.2	0.5	2	2	3	3	0.8	0.8	0.08		0.2	1					
ER	mean	3.0	16	16	2.2	17	3.1	0.26	nd	0.8	11	18	19	48	19	3.3	6.2	0.74	nd	10	4.9	nd	nd	nd	nd	nd
	SD	0.9	6	5	0.3	2	0.4	0.08		0.3	4	6	4	13	3	0.4	0.4	0.08		1	0.8					
GG	mean	3.4	32	32	3.4	21	1.8	nd	1.0	0.24	2.9	3.4	18	16	28	7	13	3.3	2.0	18	7.5	2.5	0.11	0.05	0.10	0.07
	SD	0.6	8	8	0.5	4	0.5		0.1	0.05	0.8	0.7	2	3	5	1	2	0.5	0.3	3	0.7	0.4	0.03	0.02	0.03	0.02
GK	mean	6.5	49	41	4.7	38	4	0.27	0.81	1.1	5.7	21.1	21	107	44	8	12	1.4	nd	19	14	7	0.3	nd	0.06	0.023
	SD	0.6	6	4	0.3	14	1	0.01	0.04	0.1	0.4	0.4	5	25	14	2	2	0.3		2	2	4	0.2		0.04	0.004
GM	mean	8	81	53	5.9	54	7.8	0.65	nd	4.6	6.7	12.5	20	57	54	12	23	2.0	0.39	45	24	0.036	nd	nd	nd	nd
	SD	1	17	10	0.7	9	0.9	0.06		0.4	0.4	1.0	2	6	9	2	2	0.2	0.06	4	2	0.005				
GZ	mean	2.1	18	23	2.8	17	7	0.67	0.76	2	3	8	8	19	35.2	13	15.9	4	3	14.0	8	0.5	0.012	nd	nd	nd
	SD	0.6	1	7	0.5	5	2	0.09	0.04	1	2	4	7	10	0.1	1	0.1	1	1	0.3	1	0.2	0.003			
IB	mean	2	29	34.2	5	4	4	0.90	0.27	2.2	4	5	16	21	32	10	19	4	3	20.5	14.6	0.8	nd	nd	nd	nd
	SD	2	7	0.2	1	3	3	0.05	0.04	0.6	2	1	2	6	18	4	5	2	1	0.9	0.4	0.1				
LR	mean	29	196	252	25.4	593	17	1.43	1.9	17.6	11.3	56	56.0	378	60	21	33	2.3	nd	67	17	25	0.8	nd	0.63	0.56
	SD	3	5	8	0.8	10	1	0.02	0.7	0.5	0.2	6	0.3	34	9	2	4	0.2		12	3	2	0.1		0.01	0.03
MK	mean	0.6	39	54	5.4	42	3.9	0.26	nd	1.3	4	3.8	22	20.3	54	9	14.0	6	4.0	21	6.4	6	0.23	nd	0.22	0.21
	SD	0.2	10	10	0.6	4	0.2	0.05		0.2	1	0.4	2	0.3	11	1	0.7	1	0.9	3	0.6	1	0.06		0.06	0.05
MN111	mean	2.1	11.7	10	1.7	13	4.4	0.52	0.553	1.18	1.7	7	4	10	19	6	9	2	2	11	3.7	0.11	nd	nd	nd	nd
	SD	0.9	0.8	3	0.1	4	0.4	0.03	0.004	0.01	0.5	4	2	2	11	4	5	1	1	3	0.9	0.03				
MNEM7	mean	5	51.2	63	6.8	7	1.2	nd	1.5	nd	1.35	2.3	6.2	13	20	7	11	1.1	0.11	18	10	nd	nd	nd	nd	nd
	SD	3	0.7	9	0.8	2	0.4		0.4		0.02	0.3	0.9	5	11	3	2	0.3	0.02	6	2					
MX1	mean	9	95	95	9	16	10	0.7	3.5	4	12.4	27	33	78	11	5	8	0.72	nd	15	8	nd	nd	nd	nd	nd
	SD	6	37	20	2	5	6	0.2	0.7	2	0.6	5	1	2	3	1	2	0.05		1	2					
MX10	mean	41	302	109	14	4.8	2.9	0.56	2.0	3.0	11.5	14.5	60	81	18	16	nd	0.65	0.13	18	10	0.8	nd	0.08	nd	nd
	SD	3	27	13	2	0.7	0.2	0.03	0.1	0.2	0.6	0.5	8	9	2	3		0.06	0.02	2	1	0.2		0.01		
MX11	mean	8	28	31	4.0	130	7	0.35	nd	2	5	12	29	98	43	13	20.9	1.7	nd	27	13	0.8	nd	nd	nd	nd
	SD	3	9	8	0.7	25	1	0.09		1	2	2	5	15	9	3	0.8	0.3		5	2	0.4				
MX2	mean	4	94	121	11.0	75	2.4	nd	0.90	0.508	1.9	3	8.6	11	11	4	5	0.4	nd	13	10	0.2	nd	nd	nd	nd
	SD	2	6	2	0.1	8	0.4		0.05	0.005	0.5	1	0.8	2	8	3	2	0.3		4	4	0.2				
MX3	mean	13	152	104	12.3	139	22	1.9	1.7	17	4.4	11	23	76	32	8.0	18	1.3	nd	26	21	2.4	0.05	nd	nd	nd
	SD	2	4	3	0.4	7	1	0.2	0.3	2	0.3	1	2	10	3	0.5	2	0.2		5	2	0.2	0.01			
MX4	mean	3.7	67.4	65	6.4	46	5.7	nd	3.7	2.1	1.2	0.6	14	6.9	41	25	16	1.7	0.09	19	25	1.5	0.035	nd	nd	nd
	SD	0.4	0.8	2	0.2	2	0.2		0.6	0.1	0.3	0.1	2	0.8	7	3	2	0.1	0.02	2	4	0.4	0.005			
MZ	mean	7	76	62	7	66	7	0.8	0.3	6.1	4.7	10.91	23	73	58	16	25	2.25	0.4	47	27	0.094	nd	nd	nd	nd
	SD	3	21	20	2	2	3	0.2	0.1	0.1	0.1	0.02	2	12	14	3	4	0.09	0.1	7	3	0.005				
PK	mean	18.9	77.3	33.0	5.1	7.2	6.5	2.6	0.39	10.2	5.4	15.7	23.0	111.3	27.3	7.4	13.3	0.9	nd	24.8	6.8	8.5	0.297	nd	0.10	0.057
	SD	7.6	18.0	8.3	0.6	1.6	1.0	0.7	0.09	5.9	0.9	5.4	1.7	2.6	5.8	0.8	1.4	0.2		4.5	1.1	1.4	0.005		0.02	0.005
PL	mean	11.3	71	53	5.8	37	1.4	nd	0.41	0.40	2.1	2.658	11.2	15	16	8.0	12	0.80	nd	18	4.3	0.4	nd	nd	nd	nd
	SD	0.8	5	6	0.5	14	0.6		0.06	0.05	0.3	0.008	1.0	2	3	0.2	1	0.05		2	0.5	0.1				
PT	mean	7.8	41	41	4.6	37	6	0.6	0.24	5	4	7	23	42	31	17	17	1.4	0.28	26	10	0.09	0.04	nd	nd	nd
	SD	0.4	14	4	0.6	8	4	0.1	0.07	1	2	2	8	11	21	10	9	0.7	0.05	12	4	0.03	0.01			
TT	mean	5	34	36.5	4.5	33	6	0.6	2	2.63	2.6	5	10.3	15	19	6	12	0.9	nd	14	6	4	0.18	nd	0.03	nd
	SD	2	7	0.6	0.4	4	2	0.1	1	0.06	0.2	1	0.8	2	3	1	2	0.2		3	2	2	0.05		0.02	
TX	mean	0.7	14	23	3	13	3.1	0.40	0.8	1.1	3	4	7	16	32	8	14	1.3	0.12	12	9	0.04	nd	nd	nd	nd
	SD	0.6	2	9	1	10	0.9	0.04	0.1	0.6	2	3	7	13	18	1	3	0.4	0.02	2	1	0.01				
UG	mean	15	68	28	4.4	6	8	2.2	0.32	9	4	12	20	105	18	6	11	0.6	nd	19	6	5	0.17	nd	0.05	0.04
	SD	2	14	5	0.9	1	4	0.8	0.04	4	2	5	10	25	12	3	5	0.4		10	3	2	0.08		0.02	0.01

Table 2. (Continued)

		flavan-3-ols				hydroxycinnamic acids					dihydrochalcones				flavonols						anthocyanins			
variety[b]		CAT	EC	PB2	CAT-2	CQA	CAA-1	CMA-2	CAA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QG-2	QG-3	AVI	QCI	IDE	CG-1	CG-2	CG-3	CG-4
UGS	mean	10	140	87.3	9.2	48	5.0	0.610	0.63	2.9	3.2	2.9	14	13	15	8	16	3	1.2	26	12	nd	nd	nd	nd	nd
	SD	2	27	0.8	0.6	10	0.3	0.008	0.01	0.3	0.9	0.4	4	3	5	2	5	1	0.6	9	5					
UH	mean	0.9	14	22	2.8	17	4	0.391	0.9	1.4	3	5.71	10	22	31	9	15	1.09	0.049	15	10	0.048	nd	nd	nd	nd
	SD	0.2	5	1	0.2	8	2	0.009	0.4	0.3	2	0.09	3	7	6	3	3	0.09	0.004	4	3	0.008				
UM	mean	1.9	12	16	3	53	5.9	0.23	0.7	2.0	7	25	7	41	3	1.8	4	0.3	nd	9	2	0.08	nd	nd	nd	nd
	SD	0.7	4	2	1	5	0.4	0.01	0.1	0.4	5	17	5	35	2	0.7	1	0.1		3	1	0.04				
UR	mean	5	33	41	5	46	4	nd	2	1	4.4	6	17	34	22	6	10	0.9	0.2	11.8	9	3.0	0.10	nd	nd	nd
	SD	4	16	27	3	15	2		1	1	0.6	1	6	11	13	2	2	0.3		0.2	2	0.1	0.01			
URZ	mean	6	51	61	6	79	6	0.3	3.389	2	6	9	19	29	6	1.9	3.7	0.33	nd	6.2	4.2	0.7	0.036	nd	nd	nd
	SD	3	7	23	2	31	1	0.1	0.008	1	2	4	2	2	1	0.1	0.5	0.06		0.6	0.1	0.7	0.002			
UT	mean	2.0	34	30	3.3	13	3.2	0.39	0.76	1.0	4.6	11	12	35	28	8.1	11.6	0.8	nd	13.7	11	0.055	nd	nd	nd	nd
	SD	0.1	2	3	0.1	1	0.2	0.06	0.08	0.1	0.7	2	1	3	5	0.7	0.5	0.1		0.2	1	0.008				

[a,b] Abbreviations: See **Table 1**; AVI, avicularin; CAA-2, unknown hydroxycinnamic acid with caffeic acid UV spectra; CG-1, CG-2, CG-3, CG-4, unknown anthocyanins; IDE, ideain; QG-2, QG-3, unknown flavonols.

a Shimadzu UV-260 spectrophotometer (Kyoto, Japan) against a blank [0.5 mL of methanol/acetic acid 2.5% (10:90) plus reagents] in the reference cell. Quantification was achieved by reporting the absorbances in the calibration curve of tannic acid used as standard polyphenol.

Total Acidity and pH of Apple Juices. Apple juice total acidity was determined by a potentiometric titration. An aliquot of apple juice (40 mL) was placed in a glass cell, as well as 40 mL of water that had been previously boiled and cooled at room temperature. An aqueous solution of NaOH (0.1 M) was used as titrator, once it had been standardized with potassium hydrogen phthalate. The automated system used to perform the potentiometric titration was developed by Cazallas et al. (*17*), using a $Ag-AgCl_{(s)}$ reference electrode and a glass electrode. Titrator additions were carried out with an automatic buret Metrohm Dosimat 725. The whole system was controlled by the software POSPETR (*17*). Analyses were performed at 25 °C. The titration equivalence point was calculated by considering titrator added volume and the potential measurements in each addition using the software POTCAL (*18*). Total acidity results were expressed in grams of sulfuric acid per liter of juice. Apple juice pH values were also measured with a Mettler Toledo MP-125 pH-meter (Greifensee, Switzerland).

Data Analysis and Chemometric Procedures. Certain samples of the 2000 and 2001 seasons were used for the development of classification rules of apple cultivars in the technological groups (bitter and nonbitter), described by Alonso-Salces et al. (*19*). Data analysis and predictions with the mentioned decision rules were performed on the concentration of individual polyphenols determined by high-performance liquid chromatography with diode array detection (HPLC-DAD), the total concentration of procyanidins, and the average degree of polymerization of procyanidins (DPn). Each sample was represented in the multidimensional space by a data vector, which is an assembly of the 27 features in peel, the 18 features in pulp, and the 19 features in juice. Data vectors were analyzed using chemometric procedures that have been described in the literature (*20*), such as cluster analysis (CA), principal component analysis (PCA), *K*-nearest neighbors (KNN), soft independent modeling of class analogy (SIMCA), partial least-squares (PLS), and multilayer feed-forward−artificial neural networks (MLF-ANN). Statistical and chemometric data analyses were performed by means of the statistical software packages Statgraphics (*21*), Parvus (*22*), SPSS (*23*), and The Unscrumbler (*24*). Bitterness predictions of apple cultivars for which this information was not known or was confusing were performed, to achieve an accurate technological classification of the Basque cider apple varieties studied.

RESULTS

Polyphenolic profiles of cider apple cultivars in pulp, peel, and juice for the 2000 and 2001 seasons were characterized by HPLC-DAD, the analytical data being summarized in **Tables 1−3**. For apple juices (17 cultivars of the 2000 season and 27 cultivars of the 2001 season), their total polyphenol content, total acidity, and pH were determined (**Table 4**).

Preliminary Statistical Data Treatment. In a first approach, an analysis of variance was performed on each apple material (pulp, peel, juice) data matrix, made up of individual polyphenol concentrations, total procyanidin contents, and DPn (seasons 2000 and 2001). Most variables were not significantly different except for some features that were present in very low concentrations (<2% of total polyphenol content). Moreover, box and whisker plots of these features confirmed that they were not totally discriminant between the two seasons. These differences observed in some features are likely due to the influence on fruit composition of certain factors such as the weather, the nutrient status of the soil, and other environmental factors (*6*). Therefore, they were considered as part of the possible variability that apple compositions could present among seasons. CA and PCA were carried out on the data of each apple material, but no natural groupings of the samples due to the harvest season were detected in pulp and peel. However, in juice, two partially overlapped groups were observed. Apple pulp and peel com-

Table 3. Concentrations (Milligrams per Liter) of Flavan-3-ols, Hydroxycinnamic Acids, Dihydrochalcones, and Flavonols in Apple Juice (2000 and 2001 Seasons)[a]

		flavan-3-ols				hydroxycinnamic acids					dihydrochalcones				flavonols			
variety[b]		CAT	EC	PB2	CAT-2	CQA	CAA-1	CMA-2	CAA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QCI
AG	mean	13	80	77	5.8	280	15	3.0	2.6	30.9	1.06	1.3	21	25	0.8	0.30	0.6	0.716
	SD	5	17	19	0.6	40	2	0.4	0.5	0.7	0.09	0.2	5	5	0.4	0.04	0.6	0.006
BK	mean	19	46	51	4	209	7.4	1.4	1.04	12	1.1	0.8	15	17	1.2	0.6	0.6	0.88
	SD	13	21	19	2	69	0.6	0.3	0.06	4	0.2	0.1	6	7	0.8	0.2	0.3	0.04
ER	mean	10.6	47	66	nd	320	nd	nd	3.6	30	5.6	1.49	65	25	1.5	0.38	nd	0.69
	SD	0.6	2	1		23			0.4	1	0.2	0.07	3	1	0.2	0.02		0.03
GG	mean	3.3	197	234	17	445	nd	nd	1.5	9.6	nd	nd	49	11.1	1.1	0.55	0.17	1.7
	SD	0.3	23	24	2	32			0.1	0.5			4	0.7	0.1	0.05	0.02	0.2
GK	mean	19	106	119.8	8	478	14	nd	1.9	16.9	4	1.2	78	29	1.0	0.6	1	1.63
	SD	7	9	0.8	2	17	1		0.8	0.5	1	0.1	10	10	0.5	0.2	1	0.04
GM	mean	23.0	145	130	8	734	nd	nd	nd	81	1.9	nd	32.4	11	1.05	0.87	nd	1.3
	SD	0.7	5	4	1	43				2	0.2		0.3	1	0.01	0.05		0.2
GZ	mean	20	39	59	5.7	323	15.7	3	1.6	49	1.5	1.7	17	22	1.1	0.9	0.7	1.26
	SD	14	28	24	0.3	17	0.8	1	0.2	10	0.1	0.2	10	3	0.4	0.2	0.4	0.09
IB	mean	24	81	115	7.9	172	23	6.5	3	39	3	1.0	41	16	0.8	0.6	0.8	2
	SD	4	6	10	0.4	86	17	0.8	2	10	3	0.3	3	2	0.2	0.2	0.6	1
MK	mean	4	286	388	25	654	nd	nd	2	21	nd	nd	67	18	1.5	1.1	nd	3
	SD	12	167	242	14	275			1	11			31	2	0.7	0.6		2
MN111	mean	22	49	47	4.8	265	18.2	2.3	3.7	36	1.2	0.7	24	20	0.52	0.30	0.74	0.48
	SD	2	5	8	0.1	18	0.6	0.3	0.2	2	0.1	0.2	2	2	0.01	0.02	0.07	0.08
MNEM7	mean	29	142	126	10	226	11.1	nd	6.0	5	0.640	0.8	16	14	0.67	0.45	0.8	1.8
	SD	7	20	13	3	26	0.8		1.0	3	0.003	0.5	2	1	0.05	0.04	0.1	0.7
MX10	mean	407	822	550	48	410	nd	nd	7.6	27.6	nd	1.6	137	33	1.0	1.676	1.67	6.5
	SD	32	28	18	1	5			0.4	0.3		0.2	9	2	0.1	0.009	0.06	0.3
MX11	mean	21	49	70	nd	832	nd	nd	4.9	19	3.1	1.51	66	42	1.61	0.98	nd	1.8
	SD	2	7	6		59			0.5	2	0.4	0.05	6	4	0.09	0.05		0.1
MX2	mean	20	422	447	29	959	nd	nd	4.2	9	nd	nd	20	20	0.63	0.50	nd	3.7
	SD	1	20	29	3	63			0.1	1			1	2	0.05	0.03		0.1
MX3	mean	42	234	162	nd	1099	nd	nd	6.5	214	8	3.2	90	56	1.9	1.06	nd	1.65
	SD	4	9	15		24			0.4	17	1	0.5	4	5	0.2	0.09		0.05
MX4	mean	48	156	152	14	638	nd	nd	6.5	20.3	nd	nd	16	16	4.0	2.3	2.4	5.4
	SD	6	13	17	2	97			0.8	0.9			1	1	0.3	0.3	0.2	0.4
MZ	mean	29	126	103	7	697	16	3.50	0.62	67	1.87	1.01	39	17	0.55	0.841	1.4	1.155
	SD	10	21	33	1	31	1	0.04	0.06	10	0.07	0.03	3	6	0.07	0.005	0.2	0.008
PK	mean	108	174	109	5.9	229	44	9	3	214	5	3.3	82	92	1.0	0.8	2.0	0.65
	SD	25	29	44	0.9	27	23	6	1	95	2	0.3	33	5	0.4	0.1	0.1	0.08
PL	mean	70	88	55	3.55	544	8	nd	2.2	10	1.20	nd	16.9	15	0.5	0.9	0.3	0.5
	SD	15	16	13	0.02	74	1		0.2	3	0.04		0.2	6	0.3	0.2	0.3	0.1
PT	mean	37	82	80	7	740	31.6	2.6	1.6	119	2.4	1.2	45	17	1.2	1.4	1	2.8
	SD	3	5	13	2	113	0.7	0.5	0.2	14	0.9	0.1	12	3	0.9	0.3	1	0.2
TT	mean	28	67	71	6.1	469	22.2	2.6	5.3	34	1.0	1.70	26	35	0.7	0.56	1.2	2.0
	SD	5	18	29	0.8	55	0.5	0.3	0.4	5	0.1	0.06	2	4	0.1	0.04	0.1	0.1
TX	mean	10	75	105	8	186	15	1.7	1.5	22	nd	0.7	23	17	1.0	0.6	0.7	1.4
	SD	12	11	33	3	135	1	0.5	0.1	5			15	5	0.6	0.1	0.7	0.4
UG	mean	96	149	74	5	177	36	8	1.3	188	4	2.3	65	78	0.8	0.6	1.5	0.75
	SD	23	20	23	1	62	19	3	0.1	72	3	0.8	36	21	0.3	0.2	0.2	0.09
UGS	mean	56	521	360	26	888	nd	nd	7	40	nd	nd	43	20	1.9	4.1	0.512	4.5
	SD	8	45	37	4	72			1	3			4	2	0.3	0.5	0.009	0.5
UH	mean	5	55	90	3	225	9.0	3.7	1.504	22	1	0.782	18	12	0.9	0.5	0.8	1.4
	SD	2	12	36	9	176	0.7	0.5	0.005	1	1	0.006	11	2	0.2	0.2	0.3	0.9
UM	mean	11.9	62	64	nd	586	nd	nd	2.9	21.3	2.1	1.05	20	20	0.56	0.67	1.3	1.6
	SD	0.8	10	9		54			0.5	0.8	0.2	0.06	3	2	0.05	0.07	0.2	0.1
UR	mean	41	125	94	9.1	529	18	1.0	5	18	2.7	0.9	41	21	1.0	0.37	0.8	1.5
	SD	26	23	41	0.2	195	1	0.1	2	8	0.5	0.2	6	10	0.4	0.05	0.9	0.9
URZ	mean	33.8	113	70	6	570	20	1.8	6.3	29	3	0.88	48	21	0.34	0.3	1.2	1.54
	SD	0.6	22	1	2	49	1	0.3	0.9	10	2	0.06	13	4	0.09	0.1	0.1	0.01
UT	mean	12	84	81	nd	243	nd	0.92	1.7	17	4.5	1.04	31	15	1.4	0.53	0.37	1.0
	SD	2	1	1		9		0.05	0.1	2	0.3	0.06	3	1	0.1	0.06	0.06	0.1

[a,b] Abbreviations: See **Tables 1** and **2**.

positions were not significantly different between seasons, so the differences observed in apple juices were due to the slightly different methods used each season to make juice. Hence, juice data included the variability introduced by the juice elaboration procedure.

After this preliminary study, the complete data matrices of peel, pulp, and juice with all of the cider apple cultivars studied were considered, and their technological characterization was performed by classifying them as bitter or nonbitter on the basis of their polyphenolic profiles. From the traditional classification of apple cultivars in technological groups, based on total acidity and total polyphenol content (Folin−Ciocalteu method) of the monovarietal apple juices, the bitterness of certain cultivars was established (**Table 4**). The data of the polyphenolic profiles of these varieties were used to develop classification rules in the two categories, bitter and nonbitter, by Alonso-Salces et al. (*19*).

Table 4. Mean and Standard Deviation of the Total Polyphenol Content (Folin–Ciocalteu Method) (Grams of Tannic Acid per Liter ($n = 3$) of Basque Cider Apple Juices in the 2000 and 2001 Seasons and pH and Total Acidity (Grams of H2SO4 per Liter) in Both Seasons Altogether

	2000 and 2001 seasons						total polyphenols			
	pH		total acidity				2000 season		2001 season	
variety[a]	mean	SD	mean	SD	technological group[b]		mean	SD	mean	SD
GM	4.44	0.05	1.00	0.07	sweet	bitter			1.70	0.08
MX10	4.45	0.01	1.31	0.03	sweet	bitter			4.3	0.1
MX3	3.97	0.05	2.5	0.1	sweet	bitter			2.50	0.06
MZ	4.7	0.2	0.88	0.06	sweet	bitter	1.61	0.05	1.43	0.03
PK	4.4	0.1	1.2	0.3	sweet	bitter	1.4	0.1	1.55	0.08
PL	4.50	0.09	1.32	0.01	sweet	nonbitter	1.24	0.08	1.29	0.06
PT	4.3	0.3	1.0	0.1	sweet	bitter	1.64	0.05	1.29	0.06
UG	4.4	0.2	1.2	0.5	sweet	bitter	1.21	0.04	1.50	0.07
UGS	4.34	0.01	0.64	0.04	sweet	bitter			3.7	0.1
AG	3.62	0.06	3.97	0.02	semiacid	nonbitter	0.99	0.06	0.75	0.05
BK	3.54	0.04	4.3	1.2	semiacid	nonbitter	0.80	0.09	0.52	0.03
GZ	3.4	0.1	4.1	0.6	semiacid	nonbitter	1.09	0.04	0.80	0.07
IB	3.91	0.09	4.0	0.6	semiacid	nonbitter	0.82	0.02	1.0	0.1
MN111	3.7	0.1	3.6	0.5	semiacid	nonbitter	1.00	0.04		
MNEM7	3.8	0.2	4.0	0.7	semiacid	nonbitter	1.30	0.03	1.01	0.07
TT	3.62	0.02	4.2	0.3	semiacid	nonbitter	1.4	0.1		
UR	3.6	0.1	3.7	0.7	semiacid	nonbitter	1.53	0.05	0.83	0.03
URZ	3.62	0.03	4.4	0.5	semiacid	nonbitter	1.5	0.2	1.1	0.1
UT	3.6	0.1	4.22	0.05	semiacid	nonbitter			0.70	0.03
ER	3.54	0.01	5.8	0.2	acid	nonbitter			0.76	0.04
GG	3.34	0.05	4.9	0.3	acid	nonbitter			2.14	0.08
GK	3.54	0.09	4.8	0.8	acid	nonbitter	1.25	0.09	0.96	0.03
MK	3.22	0.07	9.0	0.5	acid	bitter			3.1	0.2
MX11	3.42	0.04	7.3	0.5	acid	nonbitter			1.23	0.06
MX2	3.23	0.04	8.3	0.1	acid	bitter			3.5	0.2
MX4	3.11	0.02	9.3	0.7	acid	nonbitter			1.25	0.09
TX	3.4	0.1	5.3	1.7	acid	nonbitter	0.9	0.1	0.80	0.05
UH	3.37	0.02	5.2	1.6	acid	nonbitter	0.95	0.06	0.79	0.03
UM	3.18	0.03	8.7	0.6	acid	nonbitter			1.0	0.1
LR	3.80[c]		1.98[c]		sweet	bitter	13.60[c]			
MX1	3.07[c]		7.34[c]		acid	bitter	3.63[c]			

[a] Abbreviations: See **Table 1**. [b] Technological classification of apple varieties according to their total acidity and their individual polyphenol composition (pattern recognition techniques). [c] Personal communication from Dr. G. del Campo, Departamento de Química Aplicada, Universidad del País Vasco, San Sebastián, Spain.

In the present work, the bitterness of other cultivars that were not clearly classified, or their apple juices not available, is predicted with the classification systems obtained by those authors.

Prediction of Apple Bitterness by Pattern Recognition Analysis. Classification models of apple varieties as bitter or nonbitter obtained from their pulp, peel, or juice polyphenolic profiles by pattern recognition techniques were used with the aim of predicting the bitterness of other apple cultivars for which this information was not known or was confusing. In a first approach, PCA was performed with the complete data set (bitter, nonbitter, and unknown samples) of each apple material; thus, the unknown samples were classified as bitter or nonbitter depending on the region where they were located in the space defined by the two first principal components. Predictions made by PCA and the models afforded by KNN, SIMCA, PLS, and MLF-ANN in apple pulp, peel, and juice are summarized in **Table 5**.

Results predicted in pulp by the different classification techniques were concordant for most varieties (**Table 5**). Predictions for GK, MX1, and URZ of the 2000 season are not conclusive, because these cultivars have intermediate polyphenolic compositions close to the limit between both classes or in the overlap region. Predictions made in peel were not as good as in pulp. Thus, it was observed that a higher number of samples were not classified or the results were not conclusive (NC) because they were different depending on the batch analyzed or they were located in overlap regions (O) [varieties GK (2000 and 2001), MX1 (2000 and 2001), UGS (2000), MX2 (2001), and MX4 (2001)] (**Table 5**). On the other hand, predictions made in peel for some varieties differed from the results obtained in pulp. In this sense, MX2 (2000) and PT (2000) classified as bitter in pulp were predicted as nonbitter in peel, whereas GG (2001) and MX11 (2001), nonbitter in pulp, were classified as bitter in peel. An explanation of this observation could be the fact that apple peel composition depends to a great extent on climatology (*6*) and sun exposure of the fruit, existing differences according to the position of the fruit in the tree and even, in the same fruit, between sun-exposed parts and shaded parts (*25*). Considering the peel and pulp distribution of polyphenols in the different apple cultivars studied, it was confirmed that the synthesis and accumulation of phenolic compounds are specific to each kind of apple tissue (peel, pulp), which had been previously reported (*25*, *26*). Taking into account the objective of this study, and as a result of the observations made, pulp predictions were considered as more reliable than those in peel, because they were more homogeneous and did not depend so much on external factors. However, it is important to note that for 80% of the samples, bitterness predictions are concordant in pulp and peel, which allows the conclusion that apple tissues present characteristic compositions that permit them to be distinguished from a technological point of view.

Related to bitterness predictions in apple juices, all techniques attained the same results for each variety, except PLS for PT (2000) (**Table 5**). For some cultivars, predictions in juice are different from their classifications in pulp: MZ (2000 and 2001), PT (2000 and 2001), and GM (2000) were considered as bitter

Table 5. Bitterness Predictions in Apple Pulp, Peel, and Juice Made by Pattern Recognition Techniques[a]

		pulp					peel					juice				
season	variety[b]	PCA	KNN	SIMCA	PLS	MLF-ANN	PCA	KNN	SIMCA	PLS	MLF-ANN	PCA	KNN	SIMCA	PLS	MLF-ANN
2000	GK	O	NB	B	NC	NB	B	NB	B	B	NB	NB	NB	NB	NB	NB
	GZ											NB	NB	NB	NB	NB
	LR	B	B	NC	B	B	B	NC	NC	B	B					
	MX1	B	NB	O	B	O	B	B	NC	NB	NB					
	MX2	B	B	B	B	B	NB	NB	NC	NB	NB					
	MZ											NB	NB	NB	NB	NB
	PL						NB	NB	NB	NB	NB					
	PT						NB	NB	O	NB	NB	NB	NB	NC	B	NB
	TT	NB	NB	O	NB	NB	O	NB	NC	NB	NB	NB	NB	NB	NB	NB
	UGS	B	B	B	B	B	B	NB	B	NB	NB					
	UM	NB	NB	O	NB	NB	NB	NB	NC	NB	NB					
	UR	NB	NB	O	NB	NB	O	NB	NC	NB	NB	NB	NB	NB	NB	NB
	URZ	O	NB	NC	B	NC	O	NB	NC	NB	NB	NB	NB	NB	NB	NB
2001	GG	NB	NB	O	NB	NB	O	NB	B	NB	NB					
	GK						B	NB	NC	B	NC					
	GM											NB	NB	NC	NB	NB
	MN111	NB	NB	O	NB	NB	NB	B	NB	NB	NB					
	MNEM7						NB	NB	NB	NB	NB					
	MX1	B	B	B	B	B	B	NC	NC	NC	NC					
	MX11						B	NC	B	B	NC					
	MZ											NB	NB	NC	NB	NB
	PL	NB	NB	O	NB	NB						NB	NB	NB	NB	NB
	PT											NB	NB	NC	NB	NB
	TT	NB	NB	O	NB	NB										
	UM	NB	NB	O	NB	NB										

[a] NB, nonbitter; B, bitter; O, overlapped region; NC, not classified or not conclusive results. [b] Abbreviations: See **Table 1**.

in pulp, instead of nonbitter according to predictions in juice. On the contrary, GG, which was supposed to be bitter in juice, is classified as nonbitter by pulp models. Taking into account the total acidity of these varieties, MZ, PT, and GM are nonacid varieties (sweet), whereas GG is acid. This aspect can influence the polyphenols extraction and their oxidation during juice making. In this sense, a higher acidity and, therefore, a lower pH increase polyphenol solubility and decrease polyphenol oxidase activity (*27*), minimizing the loss of polyphenols by oxidation. These considerations explain why MZ, PT, and GM varieties, with relatively high pH values (**Table 4**), are classified as nonbitter having lost part of their native polyphenols by oxidation. In contrast, GG variety, which presents a relatively low pH, presents polyphenol contents in juice higher than other varieties with a higher potential native pulp content but that are more easily oxidized. Therefore, GG is classified as nonbitter with regard to other varieties when potential concentrations in pulp are considered. As a result of these observations, and the fact that juice elaboration procedures influence its polyphenolic composition, bitterness classification made with pulp data was considered to be more accurate.

Taking into account the results obtained in the three apple materials (pulp, peel, and juice) in both harvests (2000 and 2001), the classification of the different varieties according to their bitterness is concluded, being presented in **Table 4**. For those varieties having classifications that did not coincide in the three materials, the results achieved with pulp data were considered to be the most appropriate because of the reasons explained above. The models achieved for performing bitterness classification (*19*) allow technological characterization of varieties for which this kind of information was not known; for instance, the LR variety was classified as bitter in both peel and pulp, and for the MX1 variety, even though no conclusive result was obtained in peel, predictions carried out in pulp classified it as bitter. On the other hand, the technological group of certain varieties located close to the limit of both categories (total polyphenol content versus total acidity plot) was confirmed. Thus, MZ, PK, UG, and PT were classified as bitter and TT was classified as nonbitter. Furthermore, the GG variety, which was considered as bitter in the traditional juice classification, was finally defined as nonbitter. The rest of the cider apple cultivars presented concordant results with those obtained by the traditional technological classification.

Polyphenolic Profiles and Technological Properties of Apple Cultivars. Mean concentrations of polyphenols of each cider apple variety studied at maturity in the 2000 and 2001 seasons are summarized in **Tables 1**−**3**. Total polyphenols (determined by HPLC) were distributed in a wide concentration range depending on the cultivar. Apple pulp and peel contents vary 13-fold, considering all cultivars, and 6- and 5-fold, respectively, excluding the LR variety. In apple juice, the variation factor is 8-fold (LR juice was not available). LR presents the richest composition in total polyphenols: in pulp, 13.6 g/kg of apple and in peel, 5.6 g/kg of apple. These contents are more than double those of MX10 (6.0 and 2.2 g/kg of apple in pulp and peel, respectively). In apple juice, the higher total polyphenol concentration is shown by MX10 (5.4 g/L of juice). At the opposite end of the concentration range, the BK variety has the poorest content of total polyphenols in the three apple materials: in pulp, 1.0 g/kg of apple; in peel, 0.4 g/kg of apple; and in juice, 0.7 g/L. These polyphenol concentrations in peel and pulp are comparable with the results obtained by other authors in cider apple cultivars (*3*, *26*). Nevertheless, juice polyphenolic contents of the studied varieties are higher than those found in other cider apple juices (*11*). This fact is likely due to the juice-making procedure used by those authors, which did not use any antioxidant agent for avoiding the loss of polyphenols by oxidation as far as possible. Moreover, they quantified only low molecular weight polyphenols and did not consider polymeric procyanidins. The results presented in this

paper disclose that the polyphenol concentration in cider apple cultivars is essentially higher than that in dessert apples, as has been said before in the literature (*10*).

In apple pulp, the flavan-3-ols are the major polyphenol class (63−94% of total polyphenols), followed by the hydroxycinnamic acids (4−33% of total polyphenols), dihydrochalcones (0.4−6.1%), and flavonols (<0.4%). In peel, although the flavan-3-ols also constitute the most important polyphenol class, the proportions depend on the apple cultivar. The flavonols (6−13% of total polyphenols) are the next predominant class in most varieties. However, in the case of ER, GK, MX1, MX10, PK, and UG, the dihydrochalcones (7−19%) are the second class in concentration; in MX2 and MX3, the hydroxycinnamic acids are the second class (5−11%); in LR, UM, and URZ, dihydrochalcones and hydroxycinnamic acids are present in similar percentages (6−11%); and in MX11, PL, TT, UGS, and UR, these three polyphenol classes are found at comparable rates (2−10%). Anthocyanins compose <1.1% of total polyphenols in the peel.

In apple juice, for most varieties, the preponderant polyphenols are the flavan-3-ols (55−60% of total polyphenols), followed by hydroxycinnamic acids (30−40%). GG, IB, MK, MNEM7, MX10, MX2, TX, UGS, UH, and UT cultivars show sharp differences among classes, and the flavan-3-ols represent between 66 and 88% of total polyphenols, whereas hydroxycinnamic acids represent between 8 and 28%. In other cases, for instance, in MX11, MX3, PT, and UM, hydroxycinnamic acids are in slightly higher percentages than flavan-3-ols (40−50%). Dihydrochalcones and flavonols in juice represent 2−13% and <0.8% of total polyphenols, respectively. Hence, these observations reveal that juice composition depends significantly on the variety.

These results agree with the bitterness classification of apple cultivars according to their polyphenolic profiles: varieties with high polyphenol contents are classified as bitter and those that present low contents as nonbitter. Bitter apple cultivars present higher contents of flavan-3-ols and/or dihydrochalcones than nonbitter cultivars in their pulps, peels, and juices. Apple varieties that constitute the nonbitter class present polyphenol concentrations lower and polyphenolic profiles more homogeneous than the bitter varieties. Thus, inside the diversity of the bitter class exist three subgroups of cultivars with characteristic compositions and differences from the other bitter varieties: one composed by PK and UG cultivars and the other two by the varieties LR and MX10, respectively.

Flavan-3-ols are the major polyphenol class in cider apple pulp and peel (8.4−10.8 and 3.4−4.3 g/kg of apple, respectively). In apple juices, it is also the predominant class for most varieties (0.4−4.7 g/L of juice). (−)-Epicatechin (EC) and (+)-catechin (CAT) are the only monomers of flavan-3-ols detected in apples and constitute 3−20, 2−15, and 4−23% of total polyphenols in pulp, peel, and juice, respectively; MX10, UG, and PK present the highest rates. (−)-Epicatechin is always in larger concentration than (+)-catechin (*3*): in pulp, 41−770 mg/kg of apple; in peel, 12−302 mg/kg of apple; and in juice, 39−822 mg/L. Varieties with greater contents in (−)-epicatechin are the bitter ones. PK, UG, and MX10 present the relatively highest concentrations of (+)-catechin in all apple materials (pulp, peel, and juice). The (−)-epicatechin/(+)-catechin ratio varies according to the variety between 1 and 13 in pulp, between 4 and 24 in peel, and between 1 and 21 in juice. MK and GG present values considerably larger: 191 (pulp), 62 (peel), and 69 (juice) in MK and 72 (pulp) and 59 (juice) in GG.

Procyanidins (PC) represent between 56 and 83%, between 55 and 85%, and between 31 and 70% of the total polyphenols in pulp, peel, and juice, respectively, being the major class in pulp and peel of all varieties and in most juices. Total procyanidin concentrations estimated in pulp (0.8−4.4 g/kg of apple) and in peel (0.3−1.7 g/kg of apple) are comparable to those found in French cider apple cultivars (*3*, *26*). The LR variety, having contents of 10.4 g/kg of apple in pulp and 4.1 g/kg of apple in peel, constitutes a particular case. However, total procyanidin contents in juices (0.3−3.5 g/L of juice) are greater than those found in juices of cider apple cultivars from Asturias (Spain) (*11*), France (*28*), and the United Kingdom (*6*). This could be related to the juice extraction procedure, the analytical sample preparation, and/or the method used for procyanidin determination. By means of a direct analysis by HPLC, only some oligomeric procyanidins can be determined, whereas polymeric forms do not provide well-resolved chromatographic peaks and cannot be quantified. In this work, the analytical determination of procyanidins consisted of performing a thiolysis reaction prior to HPLC analysis, which allows estimation of the total concentration of procyanidins and their DPn. Thus, the information obtained by direct HPLC analysis of the crude extract is complemented (*14*). Procyanidin B2 (PB2) is the major procyanidin in apple, showing contents similar to (−)-epicatechin and representing <21% of total procyanidins (*10*). Bitter varieties (LR, MX10, MX2, MK, MX1, UGS, and MZ) generally contain the largest quantities of procyanidin dimers, corroborating the work of Lea and Arnold (*9*), who showed that procyanidins with polymerization degrees between 2 and 5 were particularly implicated in bitterness. Apart from PB2, another unknown procyanidin (CAT-2) was quantified in the crude extract, being present in lower concentration than PB2. Bitter varieties (LR, MX10, MK, MX2, UGS, GM, MZ, MX1, and MX3) contain higher concentrations of procyanidins than nonbitter varieties (*6*). The bitter varieties UG, PK, and PT present relatively low contents in pulp and juice with regard to the other bitter cultivars, whereas the nonbitter variety GG shows concentrated levels in pulp similar to bitter varieties such as MZ and GM. Besides, procyanidin thiolysis followed by HPLC analysis allows the identification of their constitutive units, distinguishing between terminal and extension units (*14*). Apple procyanidins are constituted fundamentally by (−)-epicatechin units (>84%) and a small proportion of (+)-catechin (**Table 6**) (*7*, *10*, *26*). Extension units are always of (−)-epicatechin, being also predominant in terminal units. (+)-Catechin rates as terminal units depend on the variety considered (certain cultivars present relatively high percentages, for instance, UG and PK). It should be pointed out that an epimerization reaction can take place under the reaction conditions of thiolysis; therefore, the percentages of (−)-catechin terminal unit would be slightly overestimated (∼3.5%) (*14*). These apple varieties also present a larger concentration of free (+)-catechin monomer.

Structural differences regarding procyanidin constitutive units can influence their spatial configuration and, thus, their properties (*29*). All varieties present average degrees of polymerization in peel (4.6−7.5) higher than in pulp (3.7−5.7) (*26*) except for the LR variety, the DPn in pulp of which was notably larger (8.3). DPn detected in juices (2.7−4.6) of the different varieties are smaller than in their corresponding pulps. This is due to procyanidin's solubility, which decreases when its molecular weight increases, whereas its ability to interact with proteins (*30*) and polysaccharides (*31*) of cell walls increases with its molecular weight, interfering in its extraction during juice making. Hence, procyanidin properties depend to a great extent

Table 6. Total Procyanidin Contents, Percentages of Constitutive Units, and Average Degree of Polymerization of Procyanidins in Cider Apple Cultivars (2000 and 2001 Seasons)[a]

	pulp					peel					juice				
variety[b]	% CAT term.	% EC term.	% EC ext	DPn	PC (mg/kg of apple)	% CAT term.	% EC term.	% EC ext	DPn	PC (mg/kg of apple)	% CAT term.	% EC term.	% EC ext	DPn	PC (mg/L)
BK	6.3	16.9	76.8	4.4	769	3.8	14.4	81.7	5.1	322	8.2	20.5	71.3	3.5	347
MX11	7.5	14.5	78.0	4.6	813	6.4	11.0	82.6	5.8	1009	12.0	19.4	68.5	3.2	613
UH	3.6	15.5	81.0	5.4	814	2.2	11.2	86.6	7.5	604	4.7	21.6	73.7	3.9	613
MN111	6.2	17.6	76.2	4.3	839	4.1	11.8	84.0	5.9	368	6.6	20.9	72.5	3.7	543
UM	6.0	15.0	79.0	4.9	913	3.4	10.4	86.2	7.0	650	6.9	21.6	71.6	3.6	532
UR	10.5	17.4	72.2	3.7	960	3.6	15.0	81.4	5.0	511	13.5	18.3	68.2	3.2	579
PL	11.3	14.0	74.7	4.0	999	4.9	12.5	82.6	5.6	896	14.4	18.5	67.2	3.1	601
GK	4.4	20.4	75.2	4.1	1023	4.5	14.4	81.1	4.9	466	5.5	25.4	69.1	3.3	649
UT	5.0	19.3	75.7	4.2	1109	3.5	14.0	82.5	5.8	477	6.6	28.0	65.4	2.9	509
ER	5.3	14.6	80.1	5.1	1121	4.5	10.8	84.6	6.6	688	8.3	22.6	69.1	3.3	475
GZ	5.6	13.4	81.0	5.4	1132	3.1	12.6	84.2	6.0	551	9.0	18.9	72.1	3.6	600
AG	3.7	16.7	79.6	5.0	1138	2.8	13.1	84.1	5.9	599	5.3	23.8	70.9	3.5	487
PT	7.1	14.7	78.2	4.7	1174	5.2	12.7	82.1	5.3	588	11.2	18.9	69.9	3.4	674
URZ	8.3	15.7	75.9	4.2	1289	2.9	13.2	83.8	6.0	923	11.8	22.2	66.0	3.0	570
UG	12.4	11.5	76.0	4.2	1352	6.6	13.1	80.3	4.7	428	16.3	18.0	65.7	3.0	628
PK	12.1	12.3	75.5	4.2	1370	6.8	12.9	80.2	4.7	450	15.8	17.0	67.2	3.1	719
TX	2.8	15.8	81.4	5.5	1385	2.2	11.1	86.7	7.2	586	4.0	22.6	73.4	3.8	676
MX4	8.5	16.5	75.0	4.1	1428	4.0	17.6	78.4	4.7	694	13.0	24.3	62.7	2.7	786
MNEM7	6.6	15.7	77.7	4.6	1447	3.0	14.2	82.8	5.7	870	10.4	21.7	67.9	3.2	749
TT	5.3	12.6	82.1	5.7	1494	3.5	11.2	85.3	6.5	923	6.9	19.7	73.4	3.8	896
IB	3.9	15.4	80.6	5.3	1495	2.3	13.3	84.4	6.1	658	7.8	24.2	68.0	3.2	652
MX3	7.6	14.7	77.6	4.5	1513	5.0	17.0	78.0	4.6	1106	12.1	20.8	67.1	3.0	788
GG	2.4	17.7	80.0	5.1	1817	4.2	13.2	82.6	5.8	623	3.2	21.8	74.9	4.0	1258
GM	5.8	14.3	79.9	5.0	1828	6.1	12.6	81.3	5.4	877	7.1	22.9	70.0	3.3	894
MX1	6.2	17.3	76.5	4.3	1957	3.6	14.9	81.5	5.2	1092					
MZ	5.0	14.1	80.9	5.3	2000	4.4	12.2	83.3	5.7	1072	5.8	21.4	72.8	3.7	778
UGS	5.0	13.8	81.2	5.6	2941	3.6	12.4	84.0	5.9	1401	7.4	14.2	78.4	4.6	2601
MK	2.0	19.5	78.5	4.7	3041	2.2	17.2	80.5	5.2	820	2.2	20.9	76.9	4.4	2517
MX2	4.1	19.6	76.3	4.3	3088	2.4	16.3	81.3	5.0	1318	5.5	20.2	74.3	3.9	2841
MX10	9.9	14.5	75.7	4.2	4448	6.5	12.1	81.3	5.4	1676	10.7	13.9	75.4	4.1	3511
LR	1.7	10.4	87.8	8.3	10388	2.6	12.1	85.3	5.9	4058					

[a,b] Abbreviations: See **Table 1**; term., terminal unit; ext, extension unit; DPn, average degree of polymerization of procyanidins; PC, total procyanidins.

on their average degree of polymerization. The implication of procyanidins and their degrees of polymerization (DP) in the sensory properties of bitterness and astringency was studied by Lea and Arnold (*9*). According to these authors, oligomeric procyanidins with DP between 2 and 5 contribute mainly to bitterness, whereas more polymerized procyanidins (DP = 6−10) are involved in astringency. The results obtained with Basque cider apple cultivars agree with these observations. Thus, bitter (PK, UG, MX2, MX1, MX10, PT, MX3) or sweet (PL) varieties present DPn lower than acid (ER, GG, TX, UH, MX11) or semiacid (TT, GZ, IB, AG) varieties. On the other hand, the contribution of the LR variety to astringency is notable (DPn in pulp is 8.3), which was confirmed once it was tasted.

In relation to cider, the interaction susceptibility of procyanidins with other compounds in the medium could influence different aspects, such as the inhibition of fermentation microflora development, the inhibition of the enzymes implicated in polyphenol oxidation or in the clarification process, the formation of more or less stable complexes during storage that could play an important role in the colloidal stability of ciders (*32*), and the interaction with certain aldehydes generated in some faults developed in cider by the action of microorganisms (bitterness) (*33*). In a first approach, low contents in procyanidins would be considered advantageous in terms of cider stability with regard to the formation of precipitates and cloudiness, the most suitable varieties being BK, UH, MN111, UR, MX11, and UM. However, these classes of polyphenols are responsible for cider flavor and contribute to control the microbiological spoilage of cider. In this sense, it should be noted that the use of cider apple varieties with low phenolic contents could lead to some faults caused by lactic acid bacteria, such as acidification, mannitol taint, and ropiness (*11*). Therefore, the use of bitter varieties such as LR, MX10, MK, MX2, UGS, GM, MZ, MX1, and MX3 would help to avoid this kind of trouble in cider.

Hydroxycinnamic acids contents depend on apple variety, presenting concentrations of 153−820 mg/kg of apple in pulp, 10−181 mg/kg of apple in peel, and 226−1320 mg/L of juice. The LR variety shows especially high contents: 2531 and 631 mg/kg of apple in pulp and peel, respectively. Bitter varieties generally contain higher concentrations of hydroxycinnamic acids than nonbitter ones. However, the bitter cultivars MX10, PK, and UG present relatively low quantities, whereas the nonbitter varieties URZ, MX4, UR, MX11, and UM contain intermediate concentrations, which are comparable to those of some bitter varieties (MK). The concentrations obtained in apple juice are higher than those found in juice of cider apple cultivars from Asturias (Spain) (*11*) and France (*28*), probably owing to the juice extraction procedure used and/or sample preparation, as has been said above. However, those contents in juice are comparable to those in juices made with apple cultivars from the United Kingdom (*6*). It was also observed that the concentrations of phenolic acids in cider apples are considerably greater than those in dessert apples (*10*).

5-Caffeoylquinic acid (CQA) is the most abundant hydroxycinnamic acid in all varieties and apple materials, except for UG and PK peel, where 4-*p*-coumaroylquinic acid (PCQ) is more concentrated. Bitter varieties present higher concentrations, except for MX10, PK, and UG, that present relatively low contents. Another two species that present UV−visible spectra

Table 7. Mean Concentrations (Milligrams per Kilogram of Fresh Apple Pulp) of Polyphenols Present in Apple Pulp (2000 and 2001 Seasons)[a]

	flavan-3-ols					hydroxycinnamic acids				dihydrochalcones				flavonols			
variety[b]	CAT	EC	PB2	CAT-2	PC	CA	CAA-1	CMA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QCI
AG	18.0	116.5	136.9	14.5	1836.3	275.8	31.9	1.8	28.1	3.5	4.5	15.0	17.7	nd	0.3	0.6	0.8
BK	23.3	81.1	93.4	8.9	1031.6	206.2	10.6	0.6	7.3	3.3	4.4	8.7	13.5	nd	0.5	0.8	1.6
ER	17.6	100.7	94.1	10.3	1556.6	275.8	24.9	nd	31.1	9.1	4.8	63.2	25.8	nd	nd	nd	0.6
GG	2.5	184.7	221.6	22.5	2512.7	370.1	14.8	nd	6.2	2.1	2.0	39.0	5.9	nd	0.3	0.4	2.5
GK	23.9	159.4	169.2	16.4	1424.0	480.5	18.3	1.1	11.9	14.0	6.2	76.5	37.6	nd	0.6	1.3	2.3
GM	27.5	202.0	180.1	17.7	2376.5	745.9	45.3	nd	53.3	3.4	3.5	38.8	16.9	nd	0.7	1.0	1.4
GZ	28.8	84.9	92.0	9.0	1713.5	359.0	40.7	1.7	35.1	5.9	4.4	14.9	10.5	0.6	0.8	0.8	1.7
IB	29.8	127.4	145.4	16.2	2179.4	158.4	35.6	2.6	35.9	5.1	4.7	33.6	20.9	nd	0.5	0.8	2.9
LR	49.2	626.7	875.5	76.3	17198.2	4005.8	76.4	2.8	105.3	nd	16.0	78.2	263.0	1.5	5.3	10.0	7.9
MK	1.6	336.4	478.8	40.3	4419.4	637.5	29.2	nd	24.4	2.8	4.2	77.3	19.7	nd	1.2	2.3	5.9
MN111	24.1	87.3	81.1	9.3	1155.9	231.4	31.7	0.8	26.4	2.5	3.2	10.7	9.6	nd	nd	0.4	0.6
MNEM7	59.0	227.2	225.3	19.5	2139.7	231.4	15.1	nd	1.4	1.4	3.8	11.9	21.3	nd	0.3	0.9	4.6
MX1	63.8	318.7	326.2	31.2	3039.6	570.7	51.5	0.7	38.0	12.2	5.4	76.8	34.9	nd	0.7	1.7	6.8
MX10	580.4	1148.1	769.3	64.9	6570.3	349.4	26.4	nd	22.8	11.3	8.9	101.1	40.7	nd	1.2	1.1	8.4
MX11	39.0	97.1	87.8	9.2	1502.8	854.7	32.6	nd	15.3	3.6	3.2	49.6	30.1	nd	0.7	1.0	3.1
MX2	53.4	615.6	686.6	55.0	4533.2	925.5	15.6	nd	6.3	0.9	nd	13.3	9.6	nd	0.3	0.7	3.2
MX3	94.0	372.4	261.2	22.6	2505.4	1110.4	80.2	nd	99.2	8.3	7.3	78.2	55.8	nd	0.7	2.0	3.3
MX4	98.0	235.8	245.0	23.2	2096.6	728.6	31.0	nd	20.5	1.4	2.5	18.0	14.8	0.7	1.0	1.2	5.9
MZ	45.2	314.4	277.2	23.6	2773.3	1038.6	52.7	1.4	70.0	5.2	4.9	44.2	24.9	nd	1.2	1.9	2.1
PK	147.8	259.9	149.4	15.5	1920.1	231.7	89.3	6.1	117.8	19.7	30.1	59.4	70.6	nd	0.6	1.7	1.3
PL	92.3	125.8	95.4	9.1	1493.4	575.6	9.5	0.4	7.6	3.9	4.2	11.7	10.7	nd	0.8	0.6	1.5
PT	69.3	130.2	114.8	10.9	1566.3	771.2	63.7	1.1	87.8	8.2	5.3	50.2	22.5	nd	1.1	1.4	5.8
TT	38.0	108.1	106.2	11.0	2277.3	411.6	38.4	1.5	21.9	2.6	4.0	13.3	16.4	nd	0.6	1.3	5.5
TX	9.5	104.3	134.9	12.7	1866.9	264.3	18.4	1.2	23.6	3.3	2.7	21.5	15.7	nd	0.5	1.2	2.5
UG	156.0	280.0	161.9	14.4	1856.2	249.9	75.4	2.2	135.4	13.7	21.9	62.9	69.7	nd	0.4	1.4	0.8
UGS	65.6	513.6	362.1	32.2	4853.9	651.3	31.8	nd	28.0	2.9	2.4	26.5	17.1	nd	3.4	1.4	6.0
UH	7.7	67.1	76.2	7.2	1104.0	254.1	17.0	1.1	16.2	3.6	2.2	24.7	15.9	nd	0.4	0.9	2.5
UM	12.2	56.8	56.7	7.1	1266.9	593.6	27.2	nd	16.6	2.4	2.8	22.2	18.3	0.6	0.5	1.7	2.5
UR	70.1	187.1	153.3	14.8	1346.3	632.0	20.8	nd	13.8	10.0	3.4	41.3	15.0	nd	nd	1.1	2.7
URZ	89.5	241.4	213.0	19.6	1922.8	848.2	29.0	0.3	20.0	10.6	3.7	51.0	17.8	nd	nd	1.2	2.6
UT	18.5	142.2	147.0	13.8	1463.0	256.2	18.3	nd	11.2	7.0	3.3	39.6	13.7	nd	0.3	nd	1.7

[a,b] Abbreviations: See **Tables 1** and **6**.

of caffeic acid, CAA-1 and CAA-2, are determined. The former is found in quantities similar to those of 4-*p*-coumaroylquinic acid in pulp and peel and in less concentrations in apple juice. The latter was detected in pulp at trace levels in some varieties and in peel and juice in relatively low concentrations. Also determined was a species with the UV−visible spectrum of *p*-coumaric acid, CMA-2. These hydroxycinnamic derivatives are likely to be different isomers of 5-caffeoylquinic acid and 4-*p*-coumaroylquinic acid, the presence of which has been described previously in the literature (*8*). UG, PK, PT, MX3, and LR show the highest contents in CAA-1 and 4-*p*-coumaroylquinic acid.

The 5-caffeoylquinic acid/4-*p*-coumaroylquinic acid ratio varies widely according to apple variety: between 2 and 159 in pulp, between 0.6 and 147 in peel, and between 0.9 and 101 in juice. This ratio is important when fruits are processed into juices and ciders, because CQA is considered to be a preferential natural substrate of polyphenol oxidase (PPO), whereas 4-*p*-coumaroylquinic acid seems to be a competitive inhibitor of the cresolase activity of the enzyme. Thus, as has been demonstrated for *p*-coumaric acid, 4-*p*-coumaroylquinic acid could be hydroxylated by the cresolase activity of that enzyme (*27*). Therefore, relative concentrations of these compounds could influence the oxidation processes and color development during cidermaking. Moreover, the enzymatic oxidation product of CQA (their *o*-quinones) can co-oxidize other substances, such as flavan-3-ols, by means of coupled mechanisms, generating colored products. Thus, the browning degree depends not only on the CQA contents but also on the flavan-3-ols/hydroxycinnamic acids ratio (*8*). Hence, those varieties with balanced compositions of flavan-3-ols and hydroxycinnamic acids, low CQA contents, and a small 5-caffeoylquinic acid/4-*p*-coumaroylquinic acid ratio would be the most appropriate to make apple juices in order to minimize the enzymatic browning and control the stability of the final product. In this sense, the varieties LR, MX2, URZ, MX4, UR, MK, MX11, UM, UGS, PL, and GK would be the least suitable.

Dihydrochalcones. Phloretin and hydroxyphloretin glycosides are the dihydrochalcones detected in apple (*35*). Their concentrations depend on the variety: 16−128 mg/kg of apple in pulp, 19−168 mg/kg of apple in peel, and 32−182 mg/L in juice. The LR variety stands out because of its particularly high contents: 216 mg/kg of apple and 502 mg/kg of apple in peel and pulp, respectively. Altogether, as was observed in previous works, bitter varieties present higher concentrations (*3*). In this sense, MX2 and UGS varieties constitute two exceptions. On the other hand, the nonbitter varieties GK and ER show relatively high contents. In the three apple materials studied, four dihydrochalcones were found: phloridzin (phloretin 2′-*O*-glucoside) (PLG), phloretin 2′-*O*-xyloglucoside (PLXG), and two glycosides of hydroxyphloretin, PLD-1 and PLD-2 (*35*), the most abundant being the first two. In pulp and juice, the preponderance of one over the other depends on the variety; however, in peel, phloridzin is present in concentrations higher than or similar to that of phloretin 2′-*O*-xyloglucoside in all varieties. Hydroxyphloretin glycosides are in minor concentration, being found in the peel and pulp of all varieties, except for PLD-1 in LR pulp and PLD-2 in MX2 pulp. They are also detected in most apple juices.

Flavonols. Quercetin glycosides are the flavonols that are essentially in apple. Six isorhamnetin glycosides have been also detected by Alonso-Salces et al. (*35*) in apple peel in some of the cultivars studied, among which isorhamnetin 3-*O*-glucoside and isorhamnetin 3-*O*-rutinoside were identified. In the literature,

Table 8. Mean Concentrations (Milligrams per Kilogram of Fresh Apple Peel) of Polyphenols Present in Apple Peel (2000 and 2001 Seasons)[a]

	flavan-3-ols					hydroxycinnamic acids					dihydrochalcones				flavonols							anthocyanins				
variety[b]	CAT	EC	PB2	CAT-2	PC	CA	CAA-1	CMA-2	CAA-2	PCQ	PLD-1	PLD-2	PLXG	PLG	HYP	IQC	QG-1	QG-2	QG-3	AVI	QCI	IDE	CG-1	CG-2	CG-3	CG-4
AG	15.3	186.8	207.6	27.7	4789.3	120.6	26.0	5.8	5.2	13.2	15.0	31.3	76.3	123.6	249.7	44.5	118.1	17.3	nd	128.0	83.7	0.9	nd	nd	nd	nd
BK	25.6	133.0	152.2	18.9	3045.6	128.1	7.7	1.6	2.1	3.0	11.7	24.9	49.6	86.7	173.9	57.2	90.7	8.2	nd	111.9	59.0	nd	nd	nd	nd	nd
ER	28.2	155.7	153.7	18.6	5590.2	143.5	27.2	2.5	nd	8.2	107.9	172.8	170.0	449.2	165.8	28.6	52.7	6.4	nd	87.9	43.0	0.2	nd	nd	nd	nd
GG	31.5	310.4	304.3	30.8	5237.9	190.7	17.1	nd	8.4	2.3	20.6	30.9	159.4	141.3	262.7	67.4	122.5	30.2	17.8	166.2	66.2	22.3	1.1	0.5	0.9	0.7
GK	65.3	503.0	413.2	47.2	4784.7	367.5	40.9	3.1	9.1	10.7	56.1	217.0	215.4	1069.0	428.2	71.8	117.3	13.4	nd	188.7	131.7	65.9	3.4	nd	0.6	0.3
GM	74.9	806.9	523.2	56.5	7834.2	514.5	65.5	5.9	nd	42.7	58.3	108.8	187.2	543.5	494.6	110.6	210.1	17.7	3.4	424.5	228.4	0.3	nd	nd	nd	nd
GZ	16.8	136.7	183.7	22.3	4548.3	125.9	51.3	5.2	6.3	16.9	25.7	61.2	68.0	152.8	277.7	103.9	125.3	28.8	20.7	109.5	59.1	4.1	0.1	nd	nd	nd
IB	18.2	268.2	318.4	42.9	6498.9	39.8	39.5	8.4	2.7	19.9	36.5	45.5	147.4	200.2	313.0	96.5	182.1	40.4	25.0	191.9	136.8	7.5	nd	nd	nd	nd
LR	172.2	1179.5	1520.7	153.1	28571.8	3575.0	101.0	8.6	11.3	106.0	67.8	338.7	337.2	2278.0	361.2	123.9	200.0	14.1	nd	401.8	101.0	152.1	5.1	nd	3.8	3.4
MK	5.8	344.0	469.8	44.7	6405.7	332.7	31.4	2.3	nd	11.1	23.4	27.9	181.2	159.2	378.8	67.2	111.4	44.4	27.6	156.1	47.4	48.9	2.1	nd	2.0	1.8
MN111	20.4	114.5	103.0	16.7	3906.0	124.5	42.8	5.1	5.5	11.6	16.3	68.4	40.5	99.4	184.1	62.9	86.4	22.8	22.3	106.0	36.1	1.1	nd	nd	nd	nd
MNEM7	40.6	482.5	583.7	61.8	8276.9	64.4	10.7	nd	13.5	nd	12.3	20.5	57.6	117.3	175.0	60.5	100.0	9.6	1.1	160.7	91.5	nd	nd	nd	nd	nd
MX1	68.9	785.5	791.4	78.6	10071.1	133.7	82.2	6.0	29.0	30.8	105.5	233.1	278.5	664.9	96.6	40.0	69.9	6.2	nd	128.2	72.9	nd	nd	nd	nd	nd
MX10	321.6	2461.6	899.4	111.4	12992.5	39.2	21.8	4.3	14.9	23.3	87.5	111.3	500.3	667.7	139.3	113.5	122.3	5.1	1.0	144.0	78.5	6.9	nd	0.7	nd	nd
MX11	55.8	197.1	213.5	26.3	6043.6	860.1	46.7	2.4	nd	18.2	24.8	65.6	191.0	639.1	285.2	83.0	123.0	10.8	nd	179.9	83.9	6.2	0.3	nd	nd	nd
MX2	35.7	847.6	1076.1	96.3	12423.6	649.6	21.4	nd	6.9	4.4	16.3	25.0	74.3	98.3	96.6	41.3	44.7	4.1	nd	108.1	86.9	1.8	nd	nd	nd	nd
MX3	87.7	1106.4	755.3	89.3	7898.8	968.0	161.0	14.5	13.6	125.3	31.2	80.0	172.0	579.1	216.5	55.7	125.6	8.9	nd	189.8	154.4	16.9	0.4	nd	nd	nd
MX4	26.1	502.5	496.1	48.2	5179.0	351.4	41.9	3.1	25.7	15.9	7.8	3.6	94.2	47.9	325.0	192.5	124.1	12.3	0.6	143.3	185.6	9.3	0.2	nd	nd	nd
MZ	78.6	810.5	660.5	75.5	12185.4	677.3	65.9	7.8	3.0	63.1	44.1	109.7	237.3	782.3	637.3	173.2	271.1	24.6	4.5	511.1	295.4	1.1	nd	nd	nd	nd
PK	154.9	643.1	276.8	42.5	4071.8	57.8	52.1	22.0	3.5	82.1	44.7	134.6	193.4	947.5	235.9	63.7	111.5	8.0	nd	210.3	57.6	73.4	2.6	nd	0.8	0.4
PL	92.4	605.6	459.8	48.8	7581.4	310.8	12.4	nd	3.4	3.4	17.3	22.1	93.2	114.1	120.5	64.5	94.8	6.5	nd	144.8	34.7	3.1	nd	nd	nd	nd
PT	73.1	384.1	387.3	43.5	5796.4	333.8	53.7	5.9	2.4	48.1	35.3	60.0	213.1	390.0	286.4	156.2	159.8	13.0	2.6	232.9	91.2	0.9	0.4	nd	nd	nd
TT	37.6	279.7	297.7	35.6	7599.4	256.8	50.3	4.8	19.4	20.8	19.4	36.9	78.6	117.5	153.9	50.9	96.9	7.1	nd	111.1	48.2	32.3	1.5	nd	0.3	nd
TX	7.1	130.9	226.0	27.0	6007.1	126.0	29.5	3.9	7.4	10.7	25.1	40.9	71.3	159.0	319.8	77.2	137.1	12.6	1.2	122.1	84.5	0.4	nd	nd	nd	nd
UG	143.0	667.1	280.4	41.7	4079.5	53.4	59.8	19.4	3.7	80.4	38.4	100.8	180.3	967.4	136.5	46.3	89.5	5.3	nd	158.2	46.9	40.9	1.6	nd	0.5	0.3
UGS	74.8	1035.0	665.4	69.0	11590.8	371.1	36.1	4.5	4.8	22.7	20.5	20.4	103.2	101.3	105.1	56.3	116.6	18.2	8.1	191.8	89.3	nd	nd	nd	nd	nd
UH	9.3	147.1	232.9	28.7	6492.8	170.6	42.9	4.0	9.3	14.4	30.7	54.5	108.4	243.7	318.4	94.7	152.8	11.2	0.5	156.2	106.5	0.5	nd	nd	nd	nd
UM	19.9	120.8	168.5	30.9	7159.8	553.8	62.7	2.5	7.2	21.4	67.7	244.6	80.0	446.3	30.2	19.9	48.1	3.2	nd	98.7	26.5	0.8	nd	nd	nd	nd
UR	41.3	305.3	370.0	46.5	5178.8	414.9	31.8	nd	17.5	9.3	40.3	52.3	147.6	314.8	210.5	52.6	89.1	8.6	1.6	103.6	84.7	27.3	0.9	nd	nd	nd
URZ	49.3	417.8	496.8	50.2	8017.1	629.6	48.4	2.4	27.9	18.9	48.8	67.7	157.0	237.2	47.8	15.5	30.1	2.6	nd	51.4	35.0	6.2	0.3	nd	nd	nd
UT	20.1	334.2	294.9	32.1	4546.9	124.0	31.7	4.0	7.6	9.7	47.7	112.0	121.4	336.6	286.9	77.7	109.0	8.3	nd	130.2	105.1	0.5	nd	nd	nd	nd

[a,b] Abbreviations: See **Tables 1**, **2**, and **6**.

as far as we know, there is only one recent work in which two isorhamnetin glycosides were detected in apple, one of them being identified as isorhamnetin 3-*O*-glucoside (*38*). In addition, quercetin aglycon was found in apple peel in some of the varieties studied (*35*), which has not been earlier reported. Commonly, quercetin is glycosylated and is a minor component, essentially located in apple peel (*36*) [21−200 mg/kg of apple (**Table 2**)], but it is also present in pulp (*12*) [0.5−17 mg/kg of apple (**Table 1**)] and juices (*37*) [2−14 mg/L (**Table 3**)]. Although the highest contents are due to bitter apples, no great differences exist between both classes (bitter and nonbitter). Hyperoside (HYP) and avicularin (AVI) are the most abundant quercetin glycosides in peel, ranging from 3 to 60 mg/kg of apple and from 6 to 7 mg/kg of apple, respectively. Isoquercitrin (IQC), QG-1, and quercitrin (QCI) show concentrations of <33 mg/kg of apple. Unknown flavonols QG-2 and QG-3 are found at quantities lower than 6 mg/kg of apple. In apple pulp, QG-1, quercitrin, and isoquercitrin present similar contents (<6 mg/kg of apple), and hyperoside, avicularin, QG-2, and QG-3 have been detected at trace levels in some varieties. The same observations were made in apple juices, although hyperoside is detected in higher concentrations, comparable to that of isoquercitrin.

Anthocyanins. Cyanidin glycosides are essentially located in apple peel. Ideain (IDE) is the major anthocyanin, present in concentrations of <25 mg/kg of apple (LR variety) in red or partially red varieties. The other anthocyanins detected in apple peels (CG-1, CG-2, CG-3, and CG-4) of unknown structures show concentration levels of <1 mg/kg of apple.

DISCUSSION

A detailed analysis of the polyphenols present in different apple varieties allows them to be classified technologically and also provides information about the most interesting polyphenols owing to the particular properties that they give to apples. In this sense, knowledge of the polyphenolic profile of each apple cultivar affords information about their susceptibility to oxidation, their sensory properties (bitterness, astringency), and their possible influence on the characteristics and quality of the final product (juice, cider) when apples are processed.

It is interesting to note that in all varieties, apple contents are higher than in their corresponding juices (taking into account the pressing yield) (*28*), which is likely due to the fact that certain polyphenols, such as procyanidins, are adsorbed onto the pomace, whereas others are oxidized during apple crushing and pressing (*4*). Pearson correlation coefficients between pulp and juice variables and between peel and juice showed that all variables with major concentrations (CAT, EC, PB2, CAT-2, PC, CQA, PCQ, PLXG, and PLG) presented high and positive correlation coefficients between pulp and juice, whereas correlations between peel and juice were lower. These results seemed to indicate that the polyphenols in a juice come mainly from apple pulp, which has been noted before in the literature (*6*). On the other hand, the most modern techniques of apple crushing and pressing and juice extraction could manage to extract peel polyphenols (*41*); therefore, knowledge about its composition is also useful.

Polyphenol oxidase activity depends on several factors, among them the concentration of substrates (5-caffeoylquinic acid and catechins), the presence of inhibitors such as 4-*p*-coumaroylquinic acid, and the juice pH. The optimum pH for PPO activity is generally considered to be in the range from 4.5 to 5 (*39*). GM, MZ, PT, UGS, and PL present high concentrations of 5-caffeoylquinic acid, intermediate-high CQA/PCQ ratios, and relatively high pH values (4.3−4.7), close to the optimum pH for the enzyme activity. These conditions favor enzymatic oxidation by PPO of CQA and the following coupled oxidation reactions of their *o*-quinones with other polyphenols (*27*). As a result, a decrease of the juice polyphenol content takes place. This could be the reason some varieties in which these phenomena are favored, for instance, GM, MZ, and PT, are classified in juice as nonbitter although being bitter according to their potential polyphenolic contents in pulp and peel.

PK and UG varieties, which are located in the same area in the total polyphenol versus total acidity plot (**Table 4**) as GM, MZ, PT, and PL, actually present particular and different compositions in relation to these other four varieties, forming a subgroup inside the bitter class, as could be observed in CA and in PCA in the different apple materials (*19*).

PK and UG show similar polyphenolic profiles between them but different with respect to the other bitter varieties. In this sense, they are the poorest varieties in total polyphenols (together with PT) among the bitter class, presenting high concentrations of (+)-catechin, which leads them to have a high percentage of catechins and a high level of (+)-catechin terminal units in procyanidins, and large percentages of dihydrochalcones. Their procyanidin (also procyanidin B2) and CQA contents are low, but they contain the highest concentrations of 4-*p*-coumaroylquinic acid (PCQ). Therefore, they present the lowest CQA/PCQ ratios in pulp. In peel, 4-*p*-coumaroylquinic acid concentrations are greater than those of 5-caffeoylquinic acid, and in apple juice, CQA/PCQ ratios are 0.9 and 1.1 for UG and PK, respectively. Thus, at first, it could be expected that their susceptibility to oxidation would be lower than the rest of sweet−bitter varieties. However, the relatively high pH (4.4) of the juices of the varieties and the fact that (+)-catechin is also a preferential substrate of PPO are the reasons for their sensitivity to oxidation (*40*).

The LR variety shows notably higher contents than the other bitter cultivars, considering either total polyphenols or the different classes of polyphenols. However, in pulp, it presents a low concentration of (+)-catechin, the lowest percentages of this monomer in procyanidins, and the greatest average degree of polymerization of procyanidins (DPn = 8.3). All of this gives this variety certain particular organoleptic characteristics, as has been commented before. The high 5-caffeoylquinic acid content and the large CQA/PCQ ratio, together with the intermediate pH (3.8) (**Table 4**) that its juice presents, are responsible for the high browning sensitivity of this variety.

The bitter variety MX10 has considerably larger catechin contents than the rest of the cultivars. Moreover, its high procyanidin content makes it the second richest variety in polyphenols, even though it presents low concentrations of hydroxycinnamic acids. This variety's pH is relatively high (4.5), and, as for UG and PK, its oxidation susceptibility is due to its high catechin contents.

The MX3 variety presents certain similarities to PK and UG in its pulp and juice composition, as was observed in the principal component plots (*19*). In this sense, they show similar contents of procyanidins, catechins, and dihydrochalcones. Moreover, these varieties have the greatest concentrations of 4-*p*-coumaroylquinic acid. However, MX3 has high CQA contents, whereas PK and UG are poor in this compound. In juice, the three varieties present the same DPn.

The GG variety is a nonbitter variety; its pulp presents relatively high concentrations of procyanidins, comparable to bitter varieties such as MZ. Its contents in the other polyphenol classes are intermediate−low. However, in juice, the differences

with the bitter class are smaller, likely due to a lower occurrence of polyphenol oxidation in this variety or a smaller oxidation rate than in other sweet−bitter varieties, as a result of presenting a lower CQA content and pH (3.3). Therefore, GG juice was classified as bitter.

PT is a bitter variety characterized by low procyanidin contents with regard to the other cultivars of its category. However, it presents the largest hydroxycinnamic acid rates. Indeed, in juice, the content of this polyphenol class is slightly higher than flavan-3-ols.

Taking into account concentrations on fresh material (pulp or peel) weight basis (**Tables 7** and **8**), total polyphenols are more concentrated in apple peel than in pulp for all varieties, as was observed by other authors (*25*). Peel/pulp total polyphenol ratio varies in the range from 1.5 to 5.4 according to the cultivar. It is the same when individual polyphenols are considered, except for hydroxycinnamic acids and, in some varieties, for (+)-catechin, which are present in greater concentrations in pulp than in peel (*26*).

Definitively, cider should be made with a mixture of different cider apple cultivars in order to obtain an apple juice with a balanced composition in the components of technological interest, which allows an adequate fermentation process and gives the juice certain characteristics related to flavor, color, product stability, microbiological control, etc., so as to achieve a cider with quality and special organoleptic properties.

ABBREVIATIONS USED

AVI, avicularin; CQA, 5-caffeoylquinic acid; CAA-1, -2, unknown hydroxycinnamic acids with caffeic acid UV spectra; CAT, (+)-catechin; CAT-2, unknown flavan-3-ol; CG-1, -2, -3, -4, unknown anthocyanins; CMA-2, unknown hydroxycinnamic acid with *p*-coumaric acid UV spectra; DPn, average degree of polymerization of procyanidins; EC, (−)-epicatechin; HYP, hyperoside; IDE, ideain; IQC, isoquercitrin; PB2, procyanidin B2; PC, total procyanidins; PCQ, 4-*p*-coumaroylquinic acid; PLD-1, hydroxyphloretin diglycoside; PLD-2, hydroxyphloretin monoglycoside; PLG, phloridzin; PLXG, phloretin 2′-*O*-xyloglucoside; PPO, polyphenol oxidase; QCI, quercitrin; QG-1, -2, -3, unknown flavonols, CA, cluster analysis; KNN, *K*-nearest neighbors; LDA, linear discriminant analysis; MLF-ANN, multilayer feed-forward−artificial neural network; PCA, principal component analysis; PLS, partial least-squares; RMSE, root medium square error; SD, standard deviation; SIMCA, soft independent modeling of class analogy; DAD, diode array detector; HPLC, high-performance liquid chromatography; nd, not detected; t, traces; AG, Azpuru Garratza; BK, Bost Kantoi; ER, Errezila; GG, Gazigorri; GK, Goikoetxea; GM, Geza Miña; GZ, Gazilokia; IB, Ibarra; LR, Larrabetzu; MK, Moko; MN111, Manttoni 111; MNEM7, Manttoni EM7; MX1, Mendexa 1; MX10, Mendexa 10; MX11, Mendexa 11; MX2, Mendexa 3; MX3, Mendexa 2; MX4, Mendexa 4; MZ, Mozoloa; PK, Piko; PL, Palazio; PT, Patzuloa; TT, Txistu; TX, Txalaka; UG, Ugarte; UGS, Urdai Goika Santutxu; UH, Urtebi Haundia; UM, Udare Marroi; UR, Urdin; URZ, Urdin Zalla; UT, Urtebi Txiki.

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