Determination of Polyphenolic Profiles of Basque Cider Apple Varieties Using Accelerated Solvent Extraction

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Polyphenols in the peel and pulp of 15 Basque cider apple varieties were determined by accelerated solvent extraction followed by reversed phase high-performance liquid chromatography with diode array detection. It was observed that the polyphenolic composition in apple peel depended on varieties, whereas the main classes of phenolic compounds in apple pulp were flavan-3-ols and hydroxycinnamic acids in all cases, representing both together between 86 and 95% of total polyphenols assayed.

Keywords: *Cider apple; natural products; polyphenols; flavonoids; phenolic acids; accelerated solvent extraction; HPLC*

INTRODUCTION

Polyphenols have great importance in the nutritional, organoleptic, and commercial properties of agricultural foodstuffs through their contributions to their sensory properties such as color (*1*, *2*) and flavor (*3*, *4*). Thus, apple polyphenols play an important role in cider quality. The presence of procyanidins and their degrees of polymerization influence cider taste because astringency increases with the degree of polymerization, whereas bitterness follows the opposite tendency (*5*). Some phenolic compounds involved in oxidation reactions are responsible for cider color, and others, such as hydroxycinnamic acids, are precursors of volatile compounds that contribute to the aroma of cider (*6*).

Moreover, polyphenols are implicated in the formation of hazes and sediments in fruit juices and alcoholic beverages and in browning processes in fruits and vegetables. Haze formation is the result of the interaction between polyphenols and proteins by hydrophobic forces and hydrogen bondings (*7*, *8*). The amount of haze formed depends on the levels of polyphenols and proteins and their ratio, as well as their nature. In this sense, dimers of (+)-catechin (CAT) and (−)-epicatechin (EPI) have been found to be involved in this process in apple juice (*9*). Enzymatic browning processes are due to the enzymatic oxidation of phenolic compounds into quinones, which polymerize into brown products and can further react with other phenolic compounds and subsequently undergo enzymatic oxidation or couple oxidation with *o*-quinones (*10*, *11*). These browning processes are influenced not only by phenolic composition but also by polyphenol oxidase (PPO) activity, ascorbic acid content, and acidity (*12*).

Another important aspect of phenolic compounds is their benefits for health. Polyphenols seem to protect against cardiovascular diseases and have certain potential anticarcinogenic properties due to their antioxidant activity and their function as free radical scavengers (*13*−*20*).

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Knowledge of the precise composition of apple varieties may contribute to a better understanding of their role in the quality and diversity of apple products such as apple juice and cider. In this sense, characterization studies based on the polyphenolic profile have been carried out with dessert apples (*21*−*26*) and cider apple cultivars from Asturias (Spain) (*27*), France (*28*, *29*), and the United Kingdom (*26*), but there are no data about the polyphenolic content of original cider apple varieties from the Basque country (Spain).

In apple fruit, five major classes of phenolic compounds are present: flavan-3-ols [monomeric flavan-3-ols (catechins) and polymeric flavan-3-ols (procyanidins)], dihydrochalcones (phloretin glycosides), flavonols (quercetin glycosides), hydroxycinnamic acid derivatives, and, in the skin of red varieties, anthocyanins (cyanidin glycosides) (*30*). The methodology used to analyze polyphenols in apples generally includes extractions with solvents, such as methanol, ethanol, acetone, or mixtures of these with water (*28*, *29*, *31*), cleanup and further fractionation by liquid−liquid extraction (LLE) (*32*−*34*), usually with ethyl acetate, and column chromatography (CC) (*22*, *23*, *35*) or solid phase extraction (SPE) (*36*). Finally, after the extract is concentrated, polyphenols are separated by reversed phase high-performance liquid chromatography (RP-HPLC) coupled with ultraviolet (UV)−visible detection.

Accelerated solvent extraction (ASE) is a new sample preparation technique that allows the volume of extraction solvent required to be reduced, the analysis time to be shortened, and the handling necessary to produce more precise results to be decreased. In a recent work, ASE conditions have been optimized for the extraction of polyphenols from apple peel and pulp, achieving good recoveries and repeatabilities (*37*). Using this extraction method for polyphenols, the purpose of this work is to determine the polyphenolic profiles of Basque cider apple varieties at the maturity stage when they were collected to be processed for cider-making. These data may contribute to our understanding of their influence in microbiological aspects and flavor quality of cider. Therefore, further studies of the polyphenolic content

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on Basque cider cultivars would be useful, for example, along maturation, so that those cultivars with the most technological interest can be selected.

MATERIALS AND METHODS

Reagents and Standards. Polyphenol standards were supplied as follows: (+)-catechin, (−)-epicatechin, rutin (RUT), phloridzin (PHL-G), chlorogenic acid (CHLOR), and *p*-coumaric acid (p-COU) by Sigma-Aldrich Chemie (Steinheim, Germany); hyperoside (HYP), isoquercitrin (ISOQ), avicularin (AVI), quercitrin (QUER), and ideain chloride by Extrasynthèse (Genay, France). Phloretin-2′-xyloglucoside (PHL-XG) and procyanidin B2 (PB2) were kindly provided by Dr. F. A. Tomás-Barberán and Dr. C. Santos-Buelga, respectively. 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 and fuming hydrochloric acid 30% from Merck (Darmstdt, Germany) and ascorbic acid from Panreac (Barcelona, Spain) were of analytical quality. Diatomaceous earth was from Sigma-Aldrich Chemie (Steinheim, Germany). Nitrogen of 99.995% purity was from Carburos Metálicos (Barcelona, Spain). Stock standard solutions of (+)-catechin, (−)-epicatechin, rutin, phloridzin, chlorogenic acid, and *p*-coumaric acid at a concentration of 1 mg mL^{-1} and hyperoside, isoquercitrin, quercitrin, and ideain (IDE) at 0.6 mg mL^{-1} were prepared in methanol and stored at 4 °C in the dark. All solvents used were previously filtered through 0.45 μm nylon membranes (Lida, Kenosha, WI) and degassed by ultrasonication.

Samples. Basque cider apples (15 varieties) were harvested in an experimental orchard of a local governmental institution (Diputación Foral de Guipúzcoa, Hondarribia, Basque Country, Spain) in the second week of October 1999, at the maturity stage to be used for cider-making. About 4 kg of apple fruits of each variety was sampled from different parts of four or five apple trees. Apples were carefully peeled, and both peel and pulp were separately frozen, chopped, and homogenized with a solution of 0.1 g mL^{-1} ascorbic acid (20 mL of ascorbic acid solution for each 100 g of peel or pulp) in a conventional food processor and lyophilized. The freeze-dried material was stored at room temperature in a desiccator until analysis.

Accelerated Solvent Extraction. ASEs were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA) system. Aliquots of freeze-dried apple peel (0.5 g) or pulp (3 g) were mixed with diatomaceous earth in a proportion (1:1) and placed in an 11 or 22 mL stainless steel extraction cell, respectively. The use of a dispersion agent, such as diatomaceous earth, is recommended to reduce the solvent volume used for the extraction (*38*). At the bottom of the extraction cell were placed a stainless steel frit and a cellulose filter (Dionex Corp.) to avoid the collection of suspended particles in the collection vial. The extraction cell was arranged in the sample carousel and was extracted under the extraction conditions previously optimized (*37*).

The cell containing the sample was prefilled with the extraction solvent, pressurized, and then heated (preheating period = 5 min). The sample was extracted by two extraction cycles with methanol at 40 °C and 1000 psi during 5 min. Then, the cell was rinsed with fresh extraction solvent (60% of the extraction cell volume) and purged with a flow of nitrogen (150 psi during 90 s). The extract was collected into 60 mL glass vials. The solvent used was previously degassed to avoid the oxidation of the analytes under the operating conditions (*38*).

The extracts were stored at −20 °C in darkness until analysis. Then, each one was filtered through a 0.45 μm nylon membrane (Lida) and transferred to a 50 mL volumetric flask, which was brought up to volume with methanol. An aliquot of 3 mL of extract was diluted to 10 mL with an aqueous solution of 0.1% hydrochloric acid and filtered through a 0.45 μm PTFE filter (Waters, Milford, MA) prior to injection into the HPLC system.

HPLC Analysis. Chromatographic analyses were performed on a Hewlett-Packard series 1100 system, equipped with a vacuum degasser, a quaternary pump, an autosampler, 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 A (acetic acid/water, 10:90, v/v) and B (methanol). The elution conditions applied were as follows: 0−10 min, 0% B isocratic; 10−40 min, linear gradient of 0−15% 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. The system operated at room temperature. Flavan-3-ols and dihydrochalcones were monitored and quantified at 280 nm, hydroxycinnamic acids at 320 nm, quercetin glycosides at 370 nm, and cyanidin glycosides at 530 nm.

RESULTS AND DISCUSSION

Identification and Quantitation of Phenolic Compounds. A typical RP-HPLC chromatogram of an ASE extract from freeze-dried peel from a cider apple variety is shown in Figure 1. The identification of those compounds for which we had standards [(+)-catechin, (−)-epicatechin, rutin, phloridzin, chlorogenic acid, hyperoside, isoquercitrin, avicularin, quercitrin, ideain chloride, phloretin-2′-xyloglucoside, and procyanidin B2] was carried out by comparison of their retention time and their UV−visible spectra with those obtained by injecting standards in the same conditions. Some other chromatographic peaks were assigned to a particular polyphenol class according to their UV−visible spectra and bibliographic sources. Peaks 4 and 16 exhibited spectral characteristics identical to those of flavan-3-ols and *p*-coumaric acid, respectively, so they were identified as an unknown procyanidin and an unknown *p*-coumaric derivative and were quantified as (+)-catechin and *p*-coumaric acid, respectively (*39*). Peak 11 was constituted by two peaks partially overlapped of two quercetin glycosides, being the former, unknown, and the latter, rutin. These overlapped peaks were quantified together as rutin. UV−visible spectra of peaks 5 and 6 showed that they corresponded to two unknown phloretin derivatives, so they were quantified as phloridzin. Avicularin, phloretin-2′-xyloglucoside, and procyanidin B2 were identified by comparison with standards but were quantified as rutin, phloridzin, and (+)-catechin, respectively, because the available amounts of solid standards of the former were not enough to perform an accurate quantitation.

Polyphenolic Profiles of Basque Cider Apple Varieties. The different apple varieties were harvested from the same orchard, so climatic conditions were the same for all of them. Therefore, the variations in the polyphenol contents have to be attributed more to the variety or their maturity stage than to other factors. This idea is also supported by McRae et al., who performed correspondence analysis studies with different apple varieties from different rootstocks, cultural practices, and growing conditions, concluding that the quantitative polyphenolic profiles characteristic of apple cultivars are determined principally by the breeding line rather than by growing conditions (*40*).

Quantitative data of polyphenols of low molecular weight present in Basque cider apple varieties are shown in Tables 1 and 2. It was observed that the polyphenolic composition in apple peel depended on the cultivar. In Bost Kantoi (BK), Geza Miña (GM), Moko (MK), Manttoni 111 (MN111), Manttoni EM7 (MNEM7), Mozoloa (MZ), and Txalaka (TX) varieties, flavan-3-ols

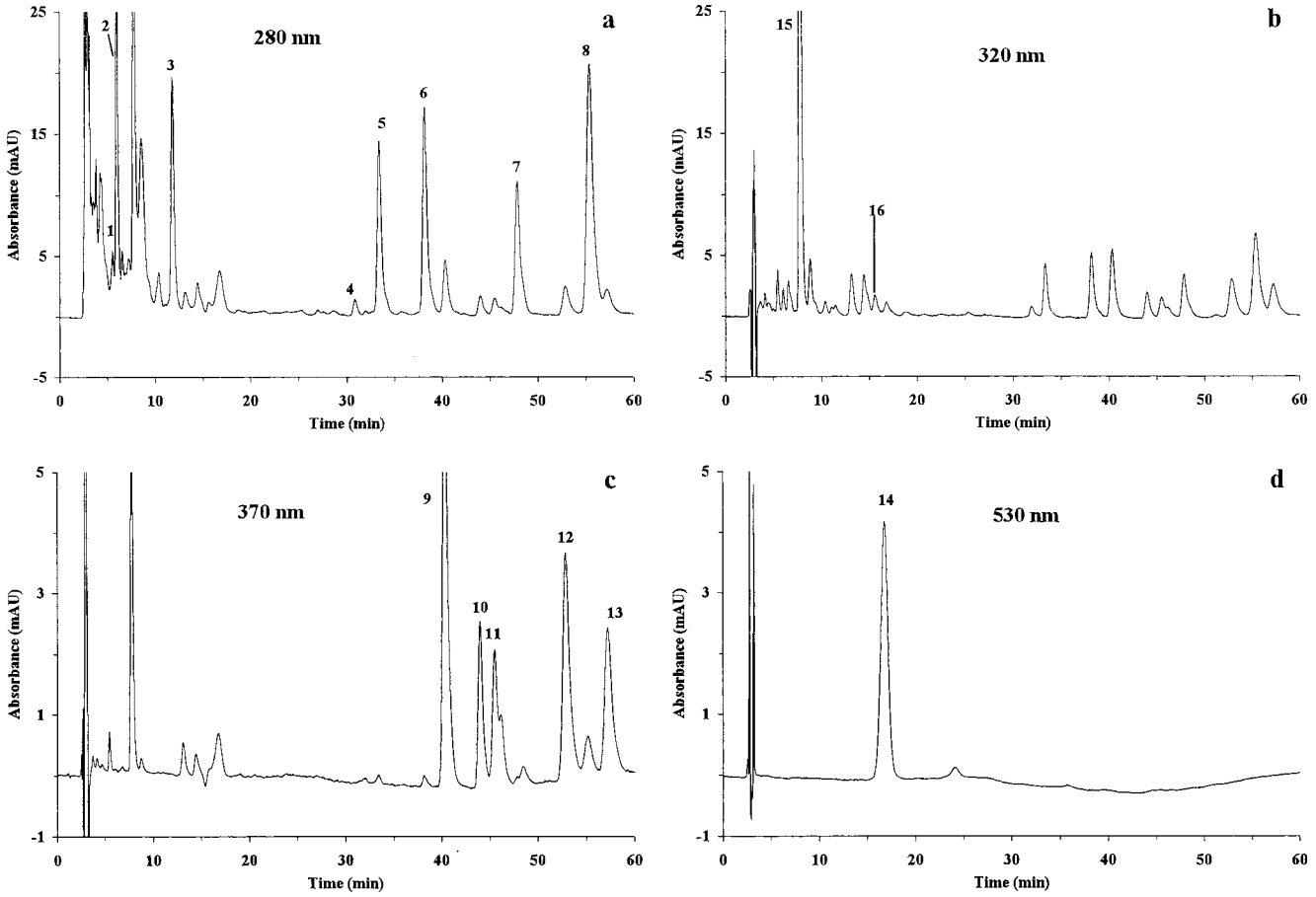

Figure 1. Chromatograms of an ASE extract of freeze-dried apple peel at (a) 280, (b) 320, (c) 370, and (d) 530 nm. Peaks: 1, (+)-catechin; 2, procyanidin B2; 3, (−)-epicatechin; 4, unknown procyanidin; 5, unknown dihydrochalcone 1; 6, unknown dihydrochalcone 2; 7, phloretin-2′-xyloglucoside; 8, phloridzin; 9, hyperoside; 10, isoquercitrin; 11, unknown quercetin glycosides plus rutin; 12, avicularin; 13, quercitrin; 14, ideain; 15, chlorogenic acid; 16, unknown *p*-coumaric acid derivative.

Table 1. Concentration of Individual Polyphenols (Micrograms per Gram of Freeze-Dried Weight) in the Peel of Cider Apple Varieties[a]

	BK	GM	MK	MN111	MNEM7	MZ	TX	GK	UG	UR	ER	UM	PT	UH	UT
flavan-3-ols	*38*	*34*	*37*	*40*	*61*	*38*	*39*	*35*	*44*	*39*	*26*	*21*	*28*	*29*	*30*
CAT	123	781	160	224	311	933	67	226	594	186	143	131	191	133	225
PB2[b]	1526	2933	1423	877	2489	2700	1377	1938	1574	1802	1185	1096	1786	1462	2225
EPI	967	3995	1664	979	2960	3850	885	2612	4138	1750	792	751	1599	926	1872
unkP[b]	100	534	328	116	377	671	111	230	253	185	74	104	169	117	164
phloretin glycosides	*17*	*24*	*21*	*21*	*7*	*18*	*13*	*47*	*43*	*31*	*57*	*57*	*20*	*28*	*31*
DCH-1[c]	239	605	504	182	86	374	118	709	336	559	931	625	150	554	150
DCH-2[c]	564	1730	507	489	112	980	268	2197	1356	751	1559	1670	208	915	2190
PHL-XG[c]	61	642	372	51	115	480	53	585	561	544	419	664	572	227	89
PHL-G	351	2906	622	445	353	1991	351	3142	4153	1327	1939	2668	1796	777	2176
quercetin glycosides	*36*	*31*	*33*	*29*	*31*	*35*	*42*	*10*	*9*	*15*	*12*	*6*	*42*	*34*	*36*
HYP	762	2270	1373	465	1030	2240	862	384	249	453	398	31	1353	748	1918
ISOQ	299	702	335	232	371	705	255	101	135	160	80	61	907	397	734
QG+RUT[d]	435	1252	523	311	503	1293	536	234	245	264	142	137	1105	657	1028
AVI[d]	689	2386	780	439	784	2126	610	437	604	387	266	255	1599	750	1224
QUER	369	1105	191	142	428	1152	351	317	155	272	93	73	602	533	487
cyanidin glycoside	*nd*	*0.1*	*4*	*0.1*	*0.1*	*0.1*	*nd*	*1*	*2*	*2*	*nd*	*nd*	*0.1*	*0.01*	*nd*
IDE	nd	32	397	7	14	23	nd	210	256	228	nd	nd	11	0.5	nd
hydroxycinnamic acids	*9*	*11*	*4*	*10*	*1*	*8*	*6*	*6*	*2*	*12*	*5*	*16*	*10*	*9*	*4*
CHLOR	641	2413	347	418	77	1570	268	711	84	1141	338	1429	1055	601	435
p-COU[e]	nd	269	83	157	59	164	120	119	192	105	88	198	246	174	135

[a] Percentages of each class of phenolic compounds in relation to the total polyphenols measured are shown in italics. ′nd, not detected. [b] Quantified as (+)-catechin. [c] Quantified as phloridzin. [d] Quantified as rutin. [e] Quantified as *p*-coumaric acid.

and quercetin glycosides were the predominant classes, both present in similar proportions that varied from 30 to 40% of the total polyphenols assayed, except for MNEM7 cultivar, which had ∼61% of flavan-3-ols. In Goikoetxea (GK), Ugarte (UG), and Urdin (UR), dihydrochalcones and flavan-3-ols were the main groups accounting each one from 32 to 47% of the total phenolic compounds determined. Errezila (ER) and Udare Marroi (UM) had ∼57% dihydrochalcones and 26 and 21% flavan-3-ols, respectively. For Patzuloa (PT), Urtebi Haundia (UH), and Urtebi Txiki (UT), the highest percentages corresponded to quercetin glycosides

Table 2. Concentration of Polyphenols (Micrograms per Gram of Freeze-Dried Weight) in the Pulp of Cider Apple Varieties[a]

	BK	GM	MK	MN111	MNEM7	MZ	TX	GK	UG	UR	ER	UM	PT	UH	UT
flavan-3-ols	*52*	*36*	*58*	*39*	*69*	*43*	*70*	*33*	*52*	*41*	*46*	*25*	*29*	*30*	*67*
CAT	197	217	87	76	291	265	31	80	862	411	173	86	346	46	116
PB2[b]	1028	1536	3640	418	628	1731	1430	436	599	1134	918	549	842	534	2607
EPI	710	1348	2400	353	684	1641	860	472	1195	1130	567	391	726	364	1977
unkP[b]	71	53	276	35	58	131	101	47	51	86	60	39	54	40	202
phloretin glycosides	*5*	*4*	*7*	*6*	*4*	*4*	*5*	*14*	*13*	*7*	*13*	*6*	*6*	*11*	*7*
DCH-1[c]	7	76	135	3	2	66	6	14	132	22	34	15	86	19	7
DCH-2[c]	5	40	31	5	4	29	8	4	198	nd	8	8	25	12	27
PHL-XG[c]	58	146	429	59	29	161	70	281	114	338	307	142	158	201	127
PHL-G	112	118	159	59	60	119	83	127	256	126	131	96	113	131	356
quercetin glycosides	*0.6*	*0.2*	*0.4*	*nd*	*0.8*	*0.4*	*0.6*	*0.1*	*0.3*	*0.2*	*nd*	*0.6*	*0.7*	*1*	*0.1*
HYP	nd	nd	2	nd	nd	nd	1	nd	nd	0.7	nd	nd	nd	nd	nd
ISOQ	2	0.9	5	nd	nd	3	2	nd	0.6	nd	nd	2	6	4	1
QG+RUT[d]	5	6	11	nd	nd	10	5	nd	5	0.2	nd	7	7	7	4
AVI[d]	3	nd	6	nd	3	7	3	nd	7	0.5	nd	7	6	4	4
QUER	11	6	22	nd	17	16	10	4	2	14	nd	11	30	22	2
cyanidin glycoside	*nd*	*nd*	*0.006*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
IDE	nd	nd	0.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
hydroxycinnamic acids	*42*	*60*	*34*	*55*	*26*	*52*	*25*	*53*	*34*	*52*	*42*	*68*	*64*	*58*	*26*
CHLOR	1587	5016	3576	1134	595	4178	784	1619	920	3469	1468	2789	3885	1807	1785
p-COU[e]	26	308	189	115	38	311	72	39	843	72	99	65	387	70	86

[a] Percentages of each class of phenolic compounds in relation to the total polyphenols measured are shown in italics. nd, not detected. [b] Quantified as (+)-catechin. [c] Quantified as phloridzin. [d] Quantified as rutin. [e] Quantified as *p*-coumaric acid.

(34−42%), closely followed by flavan-3-ols and dihydrochalcones.

In contrast, the predominant classes of phenolic compounds in apple pulp in all cultivars were flavan-3-ols and hydroxycinnamic acids, representing both together between 86 and 95% of total polyphenols assayed. Flavan-3-ols have been mentioned in the literature to be the first polyphenol class in apple pulp (*29*, *30*). However, we have observed higher contents of hydroxycinnamic acids in some varieties (GK, GM, MN111, MZ, PT, UH, UM, and UR). This may be because the estimation of procyanidins by direct HPLC is not feasible as polymeric structures do not give well-resolved peaks (*30*). Thus, only oligomeric procyanidins have been quantified by our direct HPLC analyses and, therefore, procyanidins did not appear to be the main polyphenols in our data. The importance of procyanidins is due to their implication in cider taste, being responsible for its astringency or bitterness (*5*), and in cider quality, because they contribute to the formation of haze and precipitate during storage (*7*, *8*).

In apple peel, (−)-epicatechin was the major flavan-3-ol in GK, GM, MNEM7, MZ, and UG, in concentrations that varied from 2.6 to 3.8 mg/g of freeze-dried peel. Procyanidin B2 was present in all varieties between 0.9 and 1.9 mg/g except in GM, MNEM7, and UT, which showed higher concentrations (2.2−2.9 mg/g). In apple pulp, (−)-epicatechin and procyanidin B2 are also the main flavan-3-ols, but the latter presented higher contents in all varieties with the exception of UG. The varieties MK and UT exhibited the highest concentrations with values of 3.6 and 2.6 mg/g of freeze-dried pulp of procyanidin B2 and 2.4 and 2.0 mg/g of (−)-epicatechin, respectively. (+)-Catechin and the unknown procyanidin appeared in lower amounts than the other two flavan-3-ols assayed [procyanidin B2 and (−)-epicatechin] and exhibited similar amounts (0.07−1 mg/g of freeze-dried peel and 0.03−0.4 mg/g of freeze-dried pulp), although (+)-catechin concentration was generally slightly higher.

(−)-Epicatechin and (+)-catechin are the only monomeric flavan-3-ol constituents in both apple peel and pulp, the former always being present in considerably higher concentrations as already mentioned in the literature (*21*, *29*, *41*). The (−)-epicatechin/(+)-catechin ratio in pulp depended on the variety, and bitter varieties such as MK, UT, GM, and MZ presented higher ratios in comparison to the other cultivars, as was also observed by Sanoner et al. (*29*). Monomeric flavan-3-ols are some of the preferential substrates of PPO. Furthermore, catechins are involved in enzymatic browning processes that produce a yellow/amber color desirable for cider quality (*10*). Lower contents in flavan-3-ols should be considered an advantage in terms of the stability of cider with respect to haze, the varieties MN111, UH, GK, UM, and MNEM7 being the most suitable. However, this class of constituents is also responsible for the taste of cider and the ability of polyphenols to control microbiological spoilage. In this sense, it should be pointed out that the use of cider apple varieties with very low contents of polyphenols could lead to several faults by lactic acid bacteria, such as acidification, mannitol taint, and instability (*27*). Thus, the use of bitter varieties such as MK, GM, MZ, and UT would help to avoid these problems in cider.

Chlorogenic acid was the most abundant hydroxycinnamic acid in all varieties. UG peel is an exception, having a higher content of the *p*-coumaric acid derivative. GM exhibited the highest concentration of chlorogenic acid in peel (2.4 mg/g of freeze-dried peel), followed by MZ, PT, UM, and UR (1.1−1.6 mg/g), and the rest of the cultivars that presented amounts under 0.71 mg/g. In pulp, chlorogenic acid was the major compound in most varieties or it was present at a similar concentration as (−)-epicatechin or procyanidin B2. This had been also observed in cider apple cultivars from Asturias (Spain) (*27*, *42*) and in dessert apples (*12*, *21*, *23*, *43*), when polymerized procyanidins were not determined. GM showed the highest content of chlorogenic acid in pulp, 5.0 mg/g of freeze-dried pulp, followed by MK, MZ, PT, UM, and UR with values that varied from 2.8 to 4.2 mg/g, and the rest of cultivars under 1.8 mg/g. The amounts of chlorogenic acid in pulp are between 2- and 11-fold higher than in peel depending on the variety. A *p*-coumaric acid derivative was a minor component with concentration values under 0.27 mg/g of freeze-dried peel and 0.84 mg/g of freeze-dried pulp.

The chlorogenic acid/*p*-coumaric acid derivative ratio is very dependent on the cultivar, varying from 0.4 to 11 in peel and from 1 to 61 in pulp. This ratio may be important when apple fruits are processed into juices and ciders, because chlorogenic acid is considered to be the preferential natural substrate of PPO, whereas a *p*-coumaric acid derivative is supposed to be a competitive inhibitor of the catecholase activity of this enzyme, as has been previously shown for its aglycon, *p*-coumaric acid (*44*). Therefore, the relative concentrations of these compounds may influence the oxidation process and color development during cider-making. Furthermore, the oxidation product of chlorogenic acid by PPO (its *o*-quinone) can co-oxidize other substances, such as flavan-3-ols, by a coupled mechanism and produce highly colored pigments; therefore, the degree of browning depends not only on the chlorogenic acid content but also on the flavan-3-ols/hydroxycinnamic acids ratio (*12*). Therefore, those cider varieties with low concentrations of chlorogenic acid would be better for making apple juice in order to minimize enzymatic browning and to control the stability of the final product. This fact led us to think that varieties such as GM, MZ, PT, MK, UR, and UM are less suitable for making apple juice. Moreover, hydroxycinnamic acids can contribute to cider flavor. They are precursors of the volatile phenols, which may positively contribute to cider flavor when they are present at low concentrations (*45*) but that can produce undesirable off-flavors when they surpass the acceptability threshold. Ethylphenol and ethylcatechol may be products from the metabolism of *p*-coumaroylquinic acid and chlorogenic acid, respectively, produced during the fermentation process (*46*). Chlorogenic acid also contributes to the astringency of beverages (*47*).

Phloridzin was the main compound among dihydrochalcones in the peel of GK, GM, MNEM7, MZ, PT, UG, UM, and UR, being the major polyphenol in the peel of UG variety (4.2 mg/g of freeze-dried peel). The other cultivars presented similar concentrations of phloridzin and the unknown dihydrochalcone 2, varying from 0.3 to 2.2 mg/g of freeze-dried peel. Phloretin-xyloglucoside and the unknown dihydrochalcone 1 contents were <0.93 mg/g of freeze-dried peel in all varieties. Dihydrochalcones were minor components in pulp, being present in concentrations <0.43 mg/g of freeze-dried pulp. Phloridzin is the major dihydrochalcone in the pulp of BK, MNEM7, UG, and UT, and phloretin-xyloglucoside is the major dihydrochalcone in GK, MK, MZ, PT, UH, UM, and UR. It was observed that phloridzin and the unknown dihydrochalcones 1 and 2 were present in considerably greater amounts in peel than in pulp, whereas phloretin-2′-xyloglucoside appeared in similar amounts in both matrices. Dihydrochalcones are believed to play an important role in apple juice and cider quality. In this sense, when phloridzin and epicatechin coexist in apple juice, they are involved in the formation of orange oxidation products, which account for about half of the juice color (*4*, *48*). Furthermore, phloridzin and some of its oxidation derived products may contribute to the potential antioxidant activity of apple products (*50*).

Quercetin glycosides are essentially located in the peel (*24*, *30*, *49*), but they have also been detected in pulp (*21*, *26*). Hyperoside and avicularin were the major quercetin glycosides in peel; the amounts observed varied from 1.9 to 2.3 mg/g of freeze-dried peel (GM, MZ, and UT) and from 1.2 to 2.4 mg/g (GM, MZ, PT, and UT), respectively. Quercetin glycosides have been detected in pulp in most varieties, quercitrin being the most abundant.

Among cyanidin glycosides, ideain was present in the peel of red apple varieties, such as GK, MK, UG, and UR, in concentrations that varied from 0.21 to 0.40 mg/g of freeze-dried peel, and GM, MN111, MNEM7, MZ, PT, and UH, from 0.005 to 0.032 mg/g. Moreover, ideain has been also detected at trace levels in the pulp of one variety (MK), which has not been earlier reported as far as we know. Cyanidin glycosides may contribute to the potential antioxidant activity of apple fruit (*51*, *52*).

In all varieties, the amounts of flavan-3-ols, dihydrochalcones, quercetin glycosides, and cyanidin glycoside were higher in peel than in pulp, whereas hydroxycinnamic acids appeared in lower concentration in peel, facts that had been already observed (*21*, *30*). The MK variety was an exception to this observation because it showed higher flavan-3-ols and total polyphenol contents in pulp than in peel. The peel/pulp total polyphenol content ratio varied from 1.5 to 4.5 depending on the cultivar and was 0.9 for MK.

CONCLUSION

Basque cider apple varieties were characterized on the basis of their composition in low molecular weight polyphenols. ASE followed by RP-HPLC-DAD was used to determine these phenolic compounds in both apple peel and pulp. Large difference have been found between the polyphenol composition of cider and dessert apple varieties (*21*, *23*, *29*, *41*), showing that cider apple cultivars present considerably higher amounts of polyphenols in either peel and pulp. On the whole, our results are in good agreement with previous studies on cider apple varieties, showing that the apple varieties employed in the Basque country for cider-making have polyphenolic contents similar to those employed in other countries and regions. The data obtained about total polyphenols contents, (−)-epicatechin/(+)-catechin ratio, and dihydrochalcone concentrations are in accordance with the organoleptic properties known for the studied cultivars (*29*, *41*). Thus, bitter varieties such as MK, UT, GM, and MZ showed the highest polyphenol contents, (−)-epicatechin/(+)-catechin ratios, and concentration levels of dihydrochalcones. Sharp and acidulous varieties, such as MN111, MNEM7, UH, TX, ER, and BK, had the lowest total polyphenol contents. Sweet varieties (PT) and bittersweet varieties (UR, UG, UM, and GK) presented intermediate total polyphenol concentrations and high dihydrochalcone contents. Knowledge of the polyphenolic profiles of cider apple varieties, at the moment of processing for cider-making, may contribute to our understanding of their influence in microbiological aspects and flavor quality of Basque ciders. Further studies on the polyphenolic content of Basque apple cider cultivars would be useful, so that those varieties with the most technological interest can be selected.

ABBREVIATIONS USED

CAT, (+)-catechin; EPI, (−)-epicatechin; PB2, procyanidin B2; unkP, unknown procyanidin; DCH-1, unknown dihydrochalcone 1; DCH-2, unknown dihydrochalcone 2; PHL-XG, phloretin-2′-xyloglucoside; PHL-G, phloridzin; CHLOR, chlorogenic acid; p-COU, *p*-coumaric acid derivative; AVI, avicularin; QG, quercetin glycoside; RUT, rutin; HYP, hyperoside; ISOQ, isoquer-

citrin; QUER, quercitrin; IDE, ideain; PPO, polyphenol oxidase; ASE, accelerated solvent extraction; RP-HPLC, reversed phase high-pressure liquid chromatography; DAD, diode array detection; LLE, liquid−liquid extraction; CC, column chromatography; SPE, solid phase extraction; BK, Bost Kantoi; ER, Errezila; GM, Geza Miña; GK, Goikoetxea; MN111, Manttoni 111; MNEM7, Manttoni EM7; MK, Moko; MZ, Mozoloa; PT, Patzuloa; TX, Txalaka; UM, Udare Marroi; UG, Ugarte; UR, Urdin; UH, Urtebi Haundia; UT, Urtebi Txiki.

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