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Flavonoid and Hydroxycinnamate Profiles of English Apple Ciders

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Seventeen phenolic compounds in 23 English apple ciders were identified and quantified by HPLC-PDA-MS^2. The total phenolic content of the ciders varied greatly ranging from 44 to 1559 mg/L. Four groups of compounds were identified, flavan-3-ols, hydroxycinnamates, flavonols, and dihydrochalcones. Hydroxycinnamates were the predominant group of phenolics in the majority of the ciders. Procyanidins were analyzed by HPLC after thiolysis, and total procyanidin content ranged from 8 to 722 mg/L and an average degree of polymerization of 2.5–3.5. This investigation of a wide range of ciders has shown a substantial variation in the profile and quantity of the phenolics. The analysis of single variety ciders highlighted the importance of using an apple cultivar with a high phenolic content to produce a phenolic-rich cider. Adaptations to the cider-making process could be used to increase the phenolic content with potential health benefits.

KEYWORDS: Cider; procyanidins; flavonoids; hydroxycinnamates; HPLC-PDA-MS^2

INTRODUCTION

Phenolic compounds are a class of plant secondary metabolites; compounds that are not essential for the survival of plants but are produced to enhance the plant's ability to withstand factors such as disease (*1*). Principally due to their antioxidant properties, there is much interest in the potential health benefits of phenolic compounds in our diets (*2*). Oxidative damage, caused by free radicals, has been associated with the onset of a number of chronic diseases including cancer and heart disease (*3, 4*). Phenolic compounds act as antioxidants and are able to quench free radicals, and it has been suggested that by acting as antioxidants dietary phenolics may provide protection against these diseases (*5, 6*). However, it is becoming apparent that at least some of the protective effects of dietary phenolics may be mediated by mechanisms other than a lowering of free radicals in vivo (*7, 8*).

Apples are a good source of phenolic compounds and contain five different subgroups: hydroxycinnamates, flavan-3-ols, flavonols, anthocyanins, and dihydrochalcones (*9, 10*). The levels of these compounds are dependent upon many factors including the apple cultivar, climate, and growth conditions (*9, 11–13*). Several papers have shown apple juice to be rich in phenolics and to have a high antioxidant capacity (*14–16*). A recent study highlighted the effects of both processing and cultivar on the phenolic content of apple juice. This work showed that treatment with enzymes and centrifugation both decrease the amount of phenolics present in the final product (*17*).

Since apples contain a wide variety of phenolics, with some varieties containing high levels, many studies have looked at the potential health benefits of different apple products (*18–21*). Apple juice extracts have been shown to reduce ex vivo oxidative cell damage in human colon cell lines (*20*). Feeding apple powders to obese rats lowers plasma and low-density lipoprotein (LDL) cholesterol (*18*). An in vitro study using human LDL observed that commercial apple juices inhibited LDL oxidation by 9–34%. This was also compared to whole peel, flesh, and whole Red Delicious apples which inhibited the oxidation of LDL by 38, 21, and 34%, respectively (*19*). In addition, a study using $HepG_2$ liver cancer cells demonstrated that freeze-dried apple peels have a strong antiproliferative effect (*21*).

Cider apples contain higher levels of phenolics than dessert apples (*9, 13, 15*), which gives them their characteristic bitter and astringent flavor (*22*). Cider apples are not generally eaten as fruit but traditionally have been processed to make alcoholic apple ciders typically ranging from 1.2 to 8.5% alcohol by volume. This beverage is now growing in popularity, and in the U.K. it is estimated that 13% of adults drink cider at least once a month (http://www.cideruk.com). Except for an absence of anthocyanins, ciders have been shown to have a similar phenolic profile to that of apples (*23–25*). Ciders produced in England are usually made from more than one variety of apple and are known as blended ciders. The basic method used to produce English ciders involves juice extraction, fermentation, and clarification. Some manufacturers make cider using an apple juice concentrate instead of fresh juice. Clarification can be achieved through the use of fining agents, although membrane filtration is becoming more popular. Since feeding studies have shown that phenolic compounds from both apple juice and cider are absorbed by humans (*24, 26*),

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Table 1. Details and Alcohol Content of the 23 English Ciders Investigated[a]

cider	ABV (%)	type of cider
1	7.4	B, J
2	5.2	B, J
3	7.4	S, J
4	7.4	S, J
5	3.5	S, D, J
6	8.2	B, J
7	7.0	B, J
8	5.5	B, J
9	7.0	B, J
10	6.0	B, C
11	5.3	B, C
12	1.0	B, J
13	8.2	B, J
14	7.3	B, J
15	7.3	B, J
16	7.3	B, J
17	5.3	B, C
18	5.3	B, C
19	5.5	B, C
20	6.0	B, C
21	6.8	B, J
22	7.4	S, D, J
23	5.2	S, D, J

[a] ABV, alcohol by volume; B, blended cider; S, single variety cider; D, dessert apples; C, concentrate; J, juice.

it is of interest to ask whether the phenolics in cider can make a significant contribution to the overall dietary intake of phenolic compounds.

With the gathering evidence that the phenolics in apple products may impart health benefits, this paper addresses whether the high levels of phenolics in the cider apples (*9*) is reflected in the ciders themselves by the analysis of phenolics in a wide range of commercial English ciders.

MATERIALS AND METHODS

Ciders. Table 1 provides details of the 23 commercial ciders from England analyzed in this study. All the ciders were bottled unless stated otherwise. The 23 ciders were Aspall Organic, Aspall Draught, Aspall Dry, Thatchers Criston Orchard, Thatchers Oak Matured, Thatchers Tremletts, Thatchers Redstreak, Thatchers Coxs, Thatchers Katy, Thatchers Spartan, Westons Oak Conditioned Special Vintage, Westons Stowford Press LA, Westons 1880 Anniversary Cider, Westons Vintage Cider, Westons Old Rosie Cloudy Scrumpy, Westons Organic Draught Cider, HP Bulmer Scrumpy Jack, HP Strongbow, Gaymers Olde English (can), Gaymers Olde English, Gaymers Blackthorn (can) Gaymers Blackthorn, Gaymers Blackthorn, and Gaymers Stewley Special Release Cider.

Materials. Quercetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, 5-*O*-caffeoylquinic acid, phloretin, procyanidin B2, and (−)-epicatechin were purchased from Sigma-Aldrich (Poole, U.K.). Quercetin-3-*O*-galactoside, (+)-catechin, phloretin-2′-*O*-glucoside, caffeic acid, and *p*-coumaric acid were obtained from AASC Ltd. (Southampton, U.K.). Methanol and acetonitrile were purchased from Rathburn Chemicals (Walkerburn, Peebleshire, U.K.). Hydrochloric acid was obtained from BDH Laboratory Supplies (Poole, U.K.). Formic acid was purchased from Fisher Scientific (Loughborough, U.K.). Benzyl mercaptan was obtained from Lancaster Synthesis (Morecombe, U.K.).

HPLC-PDA-MS^2 Analysis. Ciders were degassed under vacuum, centrifuged to remove any sediment, and stored at −30 °C prior to analysis on a Surveyor gradient HPLC system comprised of an HPLC pump, photodiode array (PDA) detector scanning from 250 to 700 nm, and an autosampler cooled to 4 °C (Thermo Electro, San José, CA). Separations were carried out using a Phenomenex (Macclesfield, U.K.) 4 μm Synergy RP-Max column (250 × 4.6 mm i.d.) at 40 °C, eluted with a 50 min gradient of 3–35% acetonitrile in 1% formic acid at a flow rate of 1 mL/min. After the mixture passed through the flow cell of the PDA detector, the column eluate was split and 20% was directed to a Thermo Electron LCQ Duo tandem mass spectrometer with an electrospray interface operating in negative ionization mode. Analysis was carried out using full scan, data dependent MS^2 scanning from *m/z* 100 to 1000. Capillary temperature was 375 °C, sheath gas and auxiliary gas were 80 and 60 units, respectively, and the source voltage was 2 kV. For detection of flavan-3-ols, a fluorescence detector (Jasco Corporation, Tokyo), with an excitation wavelength at 280 nm and emission wavelength at 310 nm, was also used.

All phenolics were identified based on their retention time and analysis of their absorbance and mass spectra. Quantitative estimates are based on calibrations generated by the PDA detector using the compound under study as a standard when available; see Materials. When this was not possible, a closely related derivative was used instead. Standard curves of reference curves ranged from 10 to 500 ng.

Procyanidin Analysis by HPLC after Thiolysis. Thiolytic degradation was carried out according to the method by Alonso-Salces et al. (*23*). Five hundred microliter aliquots of cider were freeze-dried and then reacted with 400 μL of benzyl mercaptan (5% in methanol, v/v) and 200 μL of acidified methanol (3.3% HCl, v/v) at 40 °C for 30 min, vortexed every 10 min. The reaction mixture was immediately cooled in an ice bath for 5 min. Samples were then filtered and stored at −80 °C prior to analysis by HPLC, as described above but using a gradient of 1% aqueous formic acid (A) in acetonitrile (B) programmed as follows: 0 min 3% B, 5 min 9% B, 15 min 16% B, 50 min 55% B, 55 min 55% B. The flow rate was 1 mL min^{-1}, and a fluorometric detector was used. The mean degree of polymerization was calculated as described by Gu et al. (*27*).

RESULTS

Qualitative Analysis. As an initial step, unthiolyzed samples were analyzed by HPLC-PDA-MS^2. Typical HPLC traces are illustrated in **Figures 1**, **2**, and **3** and HPLC and mass spectral data obtained are summarized in **Table 2**.Three flavan-3-ols were detected, (+)-catechin, (−)-epicatechin, and procyanidin B2. Peak 1 (R_t = 16.3 min, λ_{max} = 270 nm) was identified as (+)-catechin on the basis of cochromatography with a standard and the fragmentation of the negatively charged molecular ion ($[M - H]^-$) at *m/z* 289, which yielded a MS^2 ion at *m/z* 245. Peak 5, Procyanidin B2 (R_t =18.8 min, λ_{max} = 275 nm), had a $[M - H]^-$ at *m/z* 577 and MS^2 fragments at *m/z* 425, 407, and 289. Cochromatography with an authentic standard further confirmed the identity of this compound. Peak 7 (R_t = 20.1 min, λ_{max} = 275 nm) was identified as (−)-epicatechin on the basis of cochromatography with a standard and a $[M - H]^-$ at *m/z* 289 which ionized to produce a MS^2 fragment at *m/z* 245.

Seven hydroxycinnamates were detected, three isomers of *p*-coumaroylquinic acid, two isomers of caffeoylquinic acid, caffeic acid, and *p*-coumaric acid. Identification of these compounds was aided by the structural interpretation of chlorogenic acid MS^3 fragmentation patterns of Clifford et al. (*28*). Peak 2 (R_t = 16.5 min, λ_{max} = 305 nm) which had a $[M - H]^-$ at *m/z* 337 and MS^2 at *m/z* 163 was identified as 3-*O*-*p*-coumaroylquinic acid. Peak 8 (R_t = 21.2 min, λ_{max} = 310) was identified as 4-*O*-*p*-coumaroylquinic acid having a $[M - H]^-$ at *m/z* 337 and MS^2 ion at *m/z* 173. Peak 9 (R_t = 22.7 min, λ_{max} = 305 nm) had the mass spectral fragmentation pattern characteristic of 5-*O*-*p*-coumaroylquinic acid; a $[M - H]^-$ at *m/z* 337 which on MS^2 gave rise to an ion at *m/z* 191. Peak 3 (R_t =17.7 min, λ_{max} = 320 nm) was identified as 4-*O*-caffeoylquinic acid on the basis of a $[M - H]^-$ at *m/z* 353 that yielded a MS^2 ion at *m/z* 173. Peak 4 (R_t =18.3 min, λ_{max} = 320) cochromatographed with 5-*O*-caffeoylquinic acid and had a characteristic mass spectrum of this chlorogenic acid with a *m/z* 353 $[M - H]^2$ which on MS^2 produced a major fragment ion at *m* – *z* 191. Peak 6 (R_t = 19.5 min, λ_{max} = 315 nm) was identified as caffeic acid on the basis of cochromatography and

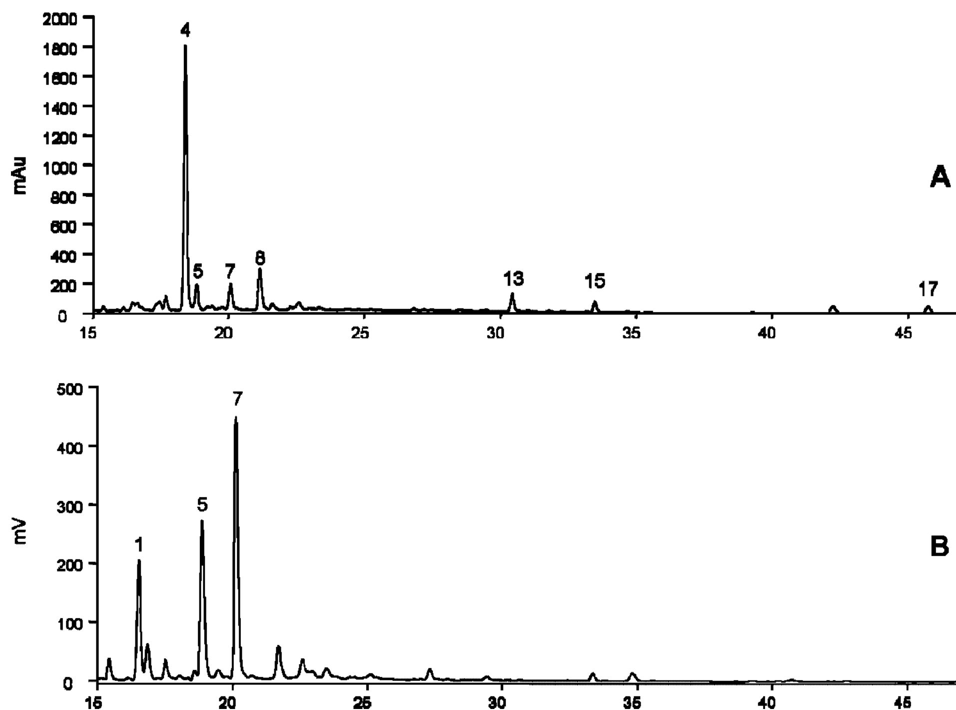

Figure 1. HPLC analysis of cider 14 with detection at (**A**) 280 nm and (**B**) fluorescence at excitation 280 nm and emission 310 nm: (1) (+)-catechin; (4) 5-*O*-caffeoylquinic acid, (5) procyanidin B2, (7) (−)-epicatechin, (8) 4-*O*-*p*-coumaroylquinic acid, (13) phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside, (15) phloridzin, (17) phloretin. For peak numbers see **Table 2**.

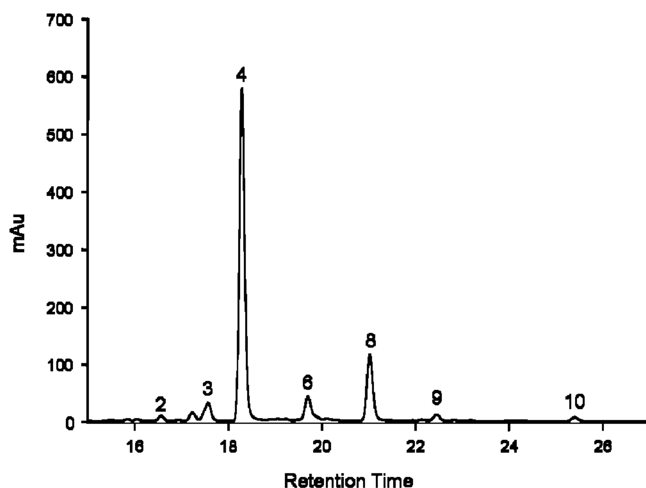

Figure 2. HPLC analysis of cider 1 with detection at 320 nm: (2) 3-*O*-*p*-coumaroylquinic acid, (3) 4-*O*-caffeoylquinic acid, (4) 5-*O*-caffeoylquinic acid, (6) caffeic acid, (8) 4-*O*-*p*-coumaroylquinic acid, (9) 5-*O*-*p*-coumaroylquinic acid, (10) *p*-coumaric acid. For peak numbers see **Table 2**.

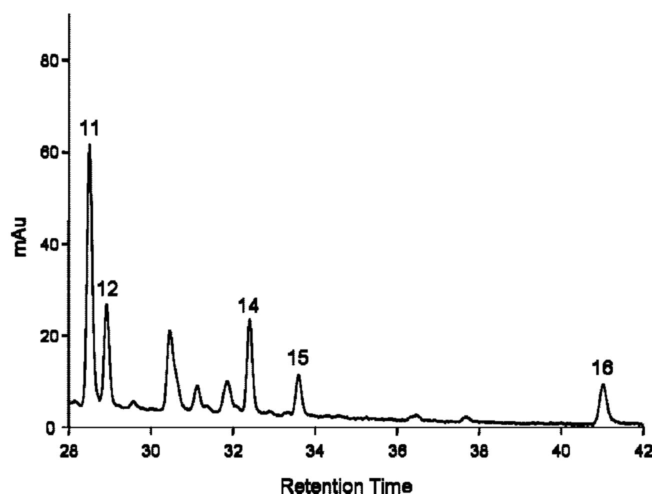

Figure 3. HPLC analysis of cider 14 with detection at 365 nm: (11) quercetin-3-*O*-galactoside, (12) quercetin-3-*O*-glucoside, (14) quercetin-3-*O*-rhamnoside, (15) phloridzin, (16) quercetin. For peak numbers see **Table 2**.

a $[M-H]^-$ at *m/z* 179 that yielded a MS^2 ion at *m/z* 135. Peak 10 (R_t = 25.6 min, λ_{max} = 300) was identified as *p*-coumaric acid. It had a $[M-H]^-$ at *m/z* 163 which fragmented to produce an MS^2 ion at *m/z* 119 and, in addition, cochromatographed with an authentic standard.

Four flavonols were detected, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, and quercetin. Peaks 11 and 12 (R_t = 29.0 and 29.5 min) had λ_{max} values of 355 and 350 nm, respectively. Both had an $[M-H]^-$ at *m/z* 463 and MS^2 fragmentation yielded a quercetin ion at *m/z* 301. The loss of 162 amu indicates the cleavage of a hexose group. This fragmentation pattern and cochromatography with reference compounds demonstrate that peak 11 is quercetin-3-*O*-galactoside and peak 12 is quercetin-3-*O*-glucoside. Peak 14 (R_t = 32.9 min, λ_{max} = 345 nm) which produced a $[M-H]^-$ at *m/z* 447 and a MS^2 fragment at *m/z* 301 was identified as quercetin-3-*O*-rhamnoside. The loss of 146 amu equates to the loss of rhamnosyl group. This identification was confirmed by cochromatography. Peak 16 (R_t = 41.1 min, λ_{max} = 370 nm) cochromatographed with quercetin and this identification was confirmed by the mass spectral data which revealed a $[M-H]^-$ at *m/z* 301 and MS^2 fragments at *m/z* 179 and 151.

Three dihydrochalcones were detected, phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside, phloridzin, and phloretin. Peak 13 (R_t =31.0 min, λ_{max} = 285nm), with a $[M-H]^-$ at *m/z* 567 and a MS^2 fragment *m/z* 273, is phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside. Peak 15 (R_t = 34.0 min, λ_{max} = 285 nm) produced a $[M-H]^-$ at *m/z* 435 and a MS^2 ion at *m/z* 273. The loss of 163 amu indicates the loss of a hexose moiety. This is the

Table 2. Retention Time, λ_{max}, and MS^2 Fragmentation Data of the Major Phenolics in Ciders[a]

peak	R_t (min)	λ max (nm)	compound	[M – H]$^-$ (*m/z*)	MS^2 (*m/z*)
1	16.4	270	(+)-catechin	289	245
2	16.8	305	3-*O*-*p*-coumaroylquinic acid	337	163
3	17.7	320	4-*O*-caffeoylquinic acid	353	173
4	18.3	320	5-*O*-caffeoylquinic acid	353	191 (QA; [M – H]$^-$-caffeoyl)
5	18.8	275	procyanidin B2	577	425, 407, 289
6	19.5	315	caffeic acid	179	135
7	20.1	275	(−)-epicatechin	289	245
8	21.2	310	4-*O*-*p*-coumaroylquinic acid	337	173 (QA; [M – H]$^-$-coumaroyl)
9	22.7	305	5-*O*-*p*-coumaroylquinic acid	337	191
10	25.6	300	*p*-coumaric acid	163	119
11	29.0	355	quercetin-3-*O*-galactoside	463	301 (Q; [M – H]$^-$-Gal)
12	29.5	350	quercetin-3-*O*-glucoside	463	301 (Q; [M – H]$^-$-Glc)
13	31.0	285	phloretin-2′-*O*-(2′′-*O*-xylosylglucoside)	567	273 (Ph; [M – H]$^-$-Xyl-Glc)
14	32.9	345	quercetin-3-*O*-rhamnoside	447	301 (Q; [M – H]$^-$-Rham)
15	34.0	285	phloridzin	435	273 (Ph; [M – H]$^-$-Glc)
16	41.1	370	quercetin	301	179, 151
17	46.3	285	phloretin	273	167
18	37.1	285	(−)-epicatechin benzylthioether [b]	411	287

[a] Peak numbers and retention times refer to HPLC traces in **Figures 1**, **2**, **3**, and **4**. R_t, retention time; λ_{max}, maximum wavelength (nm); [M – H]$^-$, negatively charged molecular ion; QA, quinic acid; Q, quercetin; Ph, phloretin; Gal, galactosyl; Glc, glucosyl; Xyl, xylosyl; Rham, rhamnosyl. [b] Detected using 3–55% gradient.

Table 3. Flavonoid Content of Ciders (data expressed as mg/L ± standard error (n = 3))[a]

cider	(+)-Cat	B2	(−)-Epicat	total Flavan-3-ols	hyperoside	isoquercitrin	quercitrin	quercetin	total flavonols	Phl-2′-Xg	Phl-2′-Glc	phloretin	total DHC
1	38 ± 0	73 ± 1	97 ± 2	207 ± 2	10.1 ± 0.1	4.3 ± 0.1	8.9 ± 0.1	2.5 ± 0.1	25.9 ± 0.4	18 ± 0	71 ± 1	4.1 ± 0.1	93 ± 2
2	11 ± 0	8 ± 0	9 ± 0	29 ± 0	1.1 ± 0.0	0.6 ± 0.0	1.4 ± 0.0	0.4 ± 0.0	3.5 ± 0.1	5 ± 0	6 ± 0	n.d.	11 ± 0
3	32 ± 0	65 ± 1	87 ± 3	184 ± 2	5.5 ± 0.1	1.3 ± 0.0	4.9 ± 0.1	2.1 ± 0.1	13.9 ± 0.3	19 ± 0	27 ± 1	0.4 ± 0.0	47 ± 1
4	35 ± 1	82 ± 1	106 ± 1	224 ± 2.3	12.5 ± 0.8	4.9 ± 0.2	4.7 ± 0.2	4.2 ± 0.2	26.3 ± 0.9	31 ± 3	53 ± 4	0.7 ± 0.0	85 ± 6
5	6 ± 0	2 ± 0	4 ± 0	12 ± 0	3.3 ± 0.1	0.7 ± 0.0	1.3 ± 0.0	0.3 ± 0.0	5.6 ± 0.1	3 ± 0	6 ± 0	0.3 ± 0.0	9 ± 0
6	25 ± 0	45 ± 0	44 ± 1	114 ± 1	2.2 ± 0.0	n.d.	3.0 ± 0.1	1.0 ± 0.0	6.3 ± 0.1	13 ± 1	27 ± 1	1.1 ± 0.0	42 ± 2
7	32 ± 0	29 ± 1	23 ± 0	84 ± 2	2.9 ± 0.1	0.7 ± 0.0	2.2 ± 0.1	1.4 ± 0.1	7.2 ± 0.2	18 ± 0	32 ± 1	0.4 ± 0.0	51 ± 1
8	14 ± 0	26 ± 1	14 ± 0	54 ± 1	1.7 ± 0.0	0.4 ± 0.0	1.5 ± 0.0	1.1 ± 0.0	4.8 ± 0.1	5 ± 0	16 ± 0	n.d.	22 ± 0
9	12 ± 0	35 ± 0	22 ± 0	70 ± 1	1.0 ± 0.0	n.d.	n.d.	n.d.	1.0 ± 0	7 ± 0	12 ± 0	0.5 ± 0.0	19 ± 0
10	13 ± 0	17 ± 1	26 ± 1	56 ± 2	2.8 ± 0.1	1.3 ± 0.1	0.5 ± 0.0	1.9 ± 0.1	6.6 ± 0.3	7 ± 0	29 ± 1	n.d.	36 ± 1
11	10 ± 0	5 ± 0	8 ± 0	23 ± 0	1.4 ± 0.1	0.6 ± 0.0	1.4 ± 0.1	0.7 ± 0.0	4.1 ± 0.1	5 ± 0	12 ± 0	n.d.	16 ± 0
12	4 ± 0	2 ± 0	1 ± 0	7 ± 0	0.6 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	0.3 ± 0.0	2.3 ± 0.1	2 ± 0	2 ± 0	n.d.	4 ± 0
13	13 ± 0	38 ± 1	42 ± 1	94 ± 1	2.3 ± 0.1	0.8 ± 0.0	n.d.	0.9 ± 0.0	4.0 ± 0.1	10 ± 0	27 ± 0	1.2 ± 0.0	39 ± 0
14	16 ± 0	26 ± 1	31 ± 1	74 ± 1	1.2 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	1.6 ± 0.0	4.6 ± 0.1	10 ± 0	6 ± 0	4.2 ± 0.1	20 ± 0
15	17 ± 0	21 ± 0	27 ± 0	65 ± 1	1.5 ± 0.0	0.7 ± 0.0	1.6 ± 0.0	3.3 ± 0.1	7.0 ± 0.1	11 ± 0	10 ± 0	3.3 ± 0.1	24 ± 1
16	10 ± 0	9 ± 0	10 ± 0	28 ± 0	1.3 ± 0.0	0.6 ± 0.0	0.9 ± 0.0	2.9 ± 0.1	5.7 ± 0.1	7 ± 0	15 ± 0	0.7 ± 0.0	22 ± 0
17	8 ± 0	5 ± 0	7 ± 0	19 ± 0	0.7 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	2.3 ± 0.1	3 ± 0	6 ± 0	n.d.	9 ± 0
18	8 ± 0	5 ± 0	7 ± 0	20 ± 0	1.2 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	3.2 ± 0.1	2 ± 0	8 ± 0	n.d.	9 ± 0
19	17 ± 0	10 ± 0	15 ± 0	42 ± 0	0.6 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	1.8 ± 0.0	4 ± 0	11 ± 0	n.d.	15 ± 0
20	10 ± 0	11 ± 0	16 ± 0	37 ± 0	0.8 ± 0.0	0.6 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	3.6 ± 0.1	4 ± 0	14 ± 0	n.d.	18 ± 0
21	20 ± 0	25 ± 0	32 ± 0	76 ± 1	1.9 ± 0.0	1.4 ± 0.0	3.0 ± 0.1	2.7 ± 0.1	9.0 ± 0.2	13 ± 0	26 ± 0	n.d.	39 ± 0
22	20 ± 0	14 ± 0	16 ± 0	50 ± 0	1.6 ± 0.0	2.0 ± 0.0	2.5 ± 0.0	1.7 ± 0.0	7.9 ± 0.1	9 ± 0	14 ± 0	0.9 ± 0.0	24 ± 0
23	10 ± 0	9 ± 0	13 ± 0	31 ± 0	1.1 ± 0.0	0.7 ± 0.0	1.4 ± 0.0	n.d.	3.2 ± 0.1	3 ± 0	11 ± 0	1.3 ± 0.0	15 ± 0

[a] (+)-cat, (+)-catechin; B2, procyanidin B2; (−)-Epicat, (−)-epicatechin; hyperoside, quercetin-3-galactoside; isoquercitrin, quercetin-3-glucoside; quercitrin, quercetin-3-rhamnoside; Phl-2′-Xg, phloretin-2′-*O*-(2′′-*O*-xylosyl)glucoside; Phl-2′-Glc, phloretin-2′-*O*-glucoside; DHC, dihydrochalcones; n.d., not detected.

fragmentation pattern phloretin-2′-*O*-glucoside (aka phloridzin) and cochromatography confirmed this identification. Peak 17 (R_t = 46.3 min, λ_{max} = 285 nm) cochromatographed with and had the same mass spectrum ([M – H]$^-$ at *m/z* 273 and MS^2 ion at *m/z* 167) as phloretin.

(+)-Catechin and (−)-epicatechin were also detected in the thiolyzed cider samples. One additional compound was detected in these samples, peak 18 (R_t = 37.1 min) which had a [M – H]$^-$ at *m/z* 411 and a MS^2 fragment at *m/z* 287, a well-known mass spectrum characteristic of the presence of (−)-epicatechin benzylthioether (*13*). Previous studies have shown that the extension units of procyanidins in apples are composed of (−)-epicatechin which when subjected to thiolysis and cleaved releases (−)-epicatechin benzylthioether (*29*).

Quantitative Analysis. The 17 phenolics identified by HPLC-PDA-MS^2 (**Table 2**) were quantified using PDA (**Figures 1A**, **2**, and **3**) and fluorescence detection (**Figure 1B**). Of the three flavan-3-ols detected in the unthiolyzed samples, (−)-epicatechin was the most abundant with concentrations ranging from 1 mg/L in cider 12 to 106 mg/L in cider 4. Procyanidin B2 was again found in the highest concentrations in cider 4 (82 mg/L) with the lowest concentration in ciders 5 and 12 (2 mg/L). (+)-Catechin was found in lower concentrations than the other two flavan-3-ols with concentrations ranging from 4 mg/L in cider 12 to 38 mg/L cider 1 (**Table 3**). The three flavan-3-ols comprised between 17% of the total phenolics in cider 12 and 41% in cider 23.

The hydroxycinnamates were the major compounds in unthiolyzed samples contributing up to 71% of the total phenolics in cider 11 and 27% in cider 5. The major hydroxy-cinnamate was 5-*O*-caffeoylquinic acid with concentrations ranging from 4 mg/L in cider 5 to 437 mg/L in cider 4. 4-*O*-

Table 4. Hydroxycinnamate Content of Ciders (data expressed as mg/L ± standard error ($n = 3$))[a]

cider	3pCoQA	4-CQA	5-CQA	caffeic acid	4-*p*CoQA	5pCoQA	pCoA	total
1	2.4 ± 0.1	21 ± 0	300 ± 5	16 ± 0	32 ± 1	3.2 ± 0.1	2.7 ± 0.0	378 ± 5
2	0.1 ± 0.0	1 ± 0	23 ± 0	4 ± 0	1 ± 0	0.2 ± 0.0	0.7 ± 0.0	31 ± 0
3	2.5 ± 0.0	10 ± 0	179 ± 1	7 ± 0	19 ± 0	1.7 ± 0.0	n.d.	218 ± 1
4	8.0 ± 0.5	47 ± 3	437 ± 4	n.d.	81 ± 1	10.9 ± 0.8	n.d.	584 ± 4
5	0.1 ± 0.0	2 ± 0	4 ± 0	2 ± 0	2 ± 0	n.d.	0.7 ± 0.0	10 ± 0
6	4.2 ± 0.2	16 ± 1	165 ± 1	14 ± 0	33 ± 1	1.9 ± 0.1	n.d.	235 ± 2
7	1.2 ± 0.0	13 ± 0	142 ± 3	24 ± 0	35 ± 1	3.3 ± 0.1	1.0 ± 0.0	220 ± 5
8	1.0 ± 0.0	10 ± 0	103 ± 2	n.d.	26 ± 1	1.4 ± 0.0	n.d.	141 ± 3
9	1.1 ± 0.0	10 ± 0	95 ± 1	n.d.	29 ± 0	1.1 ± 0.0	n.d.	136 ± 2
10	3.5 ± 0.1	9 ± 0	166 ± 1	1 ± 0	35 ± 1	4.0 ± 0.1	n.d.	218 ± 2
11	1.0 ± 0.0	3 ± 0	89 ± 2	1 ± 0	13 ± 0	1.9 ± 0.0	n.d.	110 ± 2
12	0.1 ± 0.0	1 ± 0	21 ± 0	3 ± 0	2 ± 0	0.1 ± 0.0	0.6 ± 0.0	28 ± 1
13	4.0 ± 0.0	19 ± 0	81 ± 1	21 ± 0	49 ± 1	1.9 ± 0.0	0.7 ± 0.0	176 ± 2
14	1.4 ± 0.1	8 ± 0	139 ± 2	n.d.	11 ± 0	1.5 ± 0.0	n.d.	161 ± 2
15	1.8 ± 0.0	8 ± 0	98 ± 1	6 ± 0	14 ± 0	1.7 ± 0.0	n.d.	130 ± 2
16	2.9 ± 0.0	10 ± 0	62 ± 0.3	3 ± 0	29 ± 0	1.0 ± 0.0	n.d.	109 ± 0
17	0.1 ± 0.0	1 ± 0	34 ± 0	3 ± 0	5 ± 0	0.2 ± 0.0	0.2 ± 0.0	44 ± 0
18	0.2 ± 0.0	2 ± 0	34 ± 0	2 ± 0	5 ± 0	0.2 ± 0.0	n.d.	43 ± 0
19	0.8 ± 0.0	5 ± 0	71 ± 0	2 ± 0	18 ± 0	1.2 ± 0.0	n.d.	98 ± 1
20	2.4 ± 0.0	5 ± 0	95 ± 1	2 ± 0	15 ± 0	1.2 ± 0.0	n.d.	121 ± 1
21	5.6 ± 0.3	17 ± 0	105 ± 1	n.d.	26 ± 0	1.2 ± 0.0	n.d.	155 ± 2
22	n.d.	1 ± 0	38 ± 0	10 ± 0	1 ± 0	0.2 ± 0.0	0.7 ± 0.0	50 ± 0
23	n.d.	n.d.	12 ± 0	11 ± 0	2 ± 0	n.d.	0.4 ± 0.0	26 ± 0

[a] *p*-CoQA, *p*-coumaroylquinic acid; CQA, caffeoylquinic acid; *p*-CoA, *p*-coumaric acid; n.d., not detected.

Caffeoylquinic was found in highest amounts in cider 4 (47 mg/L) with levels as low as 1 mg/L occurring in several ciders (1 mg/L). 4-*O*-*p*-Coumaroylquinic acid was the most abundant isomer of the *p*-*O*-coumaroylquinic acids with concentrations ranging from 1 mg/L in ciders 2 and 22 to 81 mg/L in cider 4. The lowest concentrations of 5-*O*-*p*-coumaroylquinic acid were detected at 0.1 mg/L in cider 12, while cider 4 once again contained the highest levels at 10.9 mg/L. 3-*O*-*p*-Coumaroylquinic acid had concentrations ranging from 0.1 mg/L in several ciders to 8 mg/L in cider 4 (**Table 4**). Caffeic acid was most abundant in cider 7 (24 mg/L) and least abundant in ciders 10 and 11 (1 mg/L). Cider 17 contained the lowest levels of *p*-coumaric acid with 0.2 mg/L and the highest levels were found in cider 1 (2.7 mg/L).

The four flavonols were minor phenolics in the ciders presenting 0–15% of the total phenolics. Of the four flavonols, quercetin-3-*O*-galactoside was found in higher concentrations ranging from 0.6 mg/L in cider 19 to 12.5 mg/L in cider 4. Quercetin-3-*O*-glucoside concentrations ranged from 0.3 mg/L in cider 19 to 4.9 mg/L in cider 4. Cider 1 contained the highest levels of quercetin-3-*O*-rhamnoside with 8.9 mg/L with cider 10 containing the lowest at 0.5 mg /L. Quercetin levels ranged from 0.2 mg/L in cider 19 to 4.2 mg/L in cider 1 (**Table 3**).

The major dihydrochalcone, phloretin-2′-*O*-(2′′-*O*-xylosyl) glucoside, was found in concentrations ranging from 2 mg/L in cider 18 to 19 mg/L in cider 3. The lowest levels of phloretin-2′-*O*-glucoside were detected in cider 12 (2 mg/L) and highest concentrations in cider 1 (71 mg/L). Concentrations of the aglycone phloretin ranged from 0.3 mg/L in cider 5 to 4.2 mg/L in cider 14 (**Table 3**). The dihydrochalcones were found in varying amounts in the different ciders with their contribution to the overall levels of phenolics ranging from 8 to 25%.

Procyanidins were identified by HPLC-PDA-MS^2 (**Table 2**) and quantified using PDA (**Figure 4A**) and fluorescence detection (**Figure 4B**). All the ciders analyzed using thiolysis were shown to contain procyanidins with the highest levels in cider 4 (722 mg/L) and the lowest in cider 12 (8 mg/L) (**Table 5**).

DISCUSSION

With the knowledge that cider apples contain higher levels of phenolics than dessert apples, this study set out to determine whether the higher levels in the apples were transferred to the final product, cider. If this is achieved, consumption of moderate amounts of such a cider may, like red wine, enhance the health benefits of a fruit- and vegetable-rich diet.

The total phenolics analyzed, excluding procyanidins, ranged from 35 mg/L in cider 5 to 837 mg/L in cider 4. There could be many reasons for this wide variation including the apples used and the process employed to produce the cider. The phenolic profile of the ciders is similar to the phenolic profile of cider apples, except for two major differences. There are less flavonol glycosides present in ciders, a maximum of three compared to seven in cider apples. The second difference is the presence of free caffeic acid, *p*-coumaric acid, quercetin, and phloretin. This suggests that some conjugates are cleaved by the cider-making process releasing aglycones. Pectolytic enzymes used in the cider-making process have been shown to possess some glycosidase activity and are likely to be the explanation for this observation. (*30*). The procyanidin contents ranged from 8 mg/L in cider 12 to 722 mg/L in cider 4. The average degree of polymerization of the procyanidins ranged from 2.5 to 3.5. Analysis of cider apples has shown a wider range of average degree of polymerization (*13*), indicating that the cider-making process removes the higher molecular weight procyanidins. A recent study has shown the oligomeric procyanidins are potentially vasoactive (*7*) and increasing the procyanidin content of cider could, therefore, have health benefits. However, procyanidins can impart taste and mouthfeel properties. Procyanidins with a degree of polymerization of 2–5 impart predominately bitterness, while those with a degree of polymerization of 6–10 are astringent. Both are characteristic of traditional English ciders made from bittersweet apples (*22*).

The effect of the choice of apple can be seen in two of the ciders. Both cider 4 and cider 5 are produced by the same manufacturer using the same method, but there is a massive difference in their phenolic profiles, with cider 5 having a much

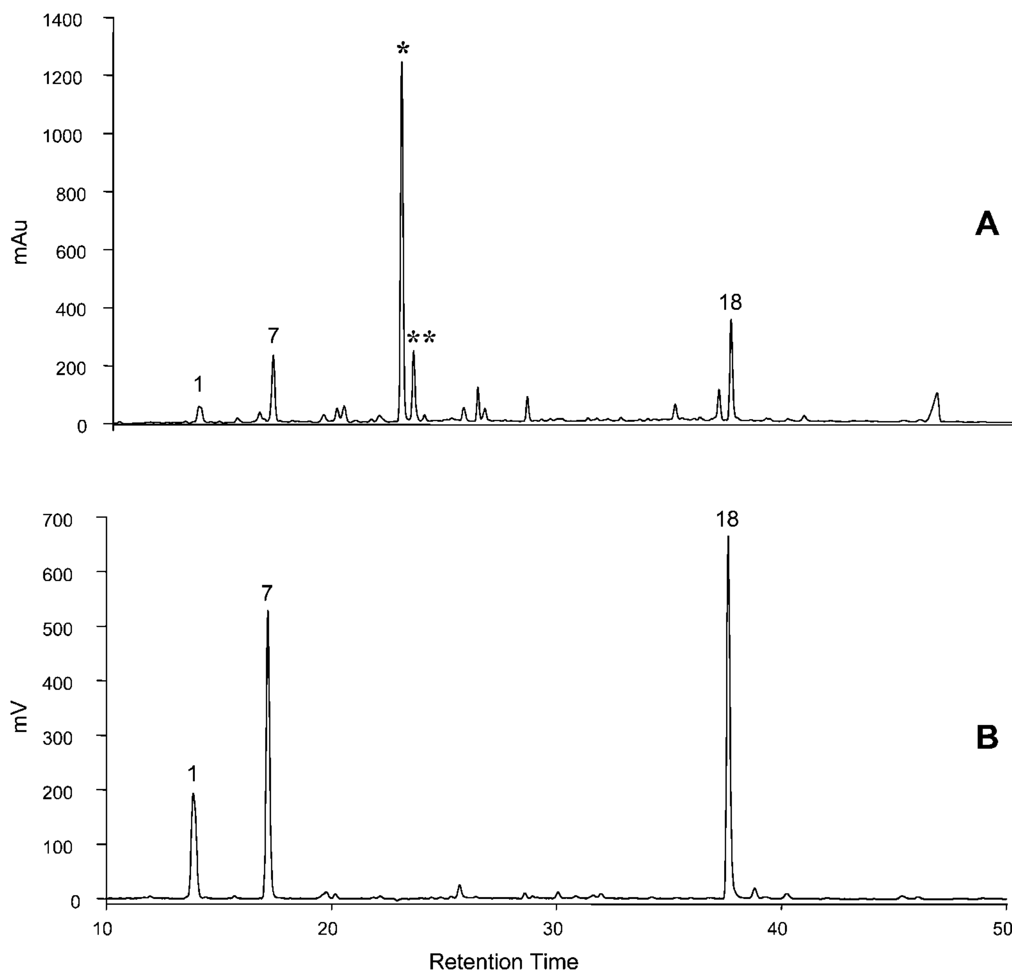

Figure 4. HPLC analysis of cider 14 with detection at (**A**) 280 nm and (**B**) fluorescence at excitation 280 nm and emission 310 nm: (1) (+)-catechin; (7) (−)-epicatechin; (18) (−)-epicatechin benzylthioether; ∗, methylated 5-*O*-caffeoylquinic acid ∗∗, methylated 4-*O*-*p*-coumaroylquinic acid. For peak numbers see **Table 2**.

lower phenolic content of 44 mg/L compared with the 1559 mg/L in cider 4. Both of these ciders are single variety ciders, cider 4 being produced from Somerset Redstreak apples and cider 5 from Cox apples. It has been shown in previous studies that cider apples have higher phenolic content than dessert varieties of apples (*9, 13*) and that the Somerset Redstreak cider apple is especially rich in phenolics (*9*). This indicates that the choice of apples can play a major part in the final phenolic content of the cider. Quercetin glycosides are found mainly in the peel of the apple and have been found in this study to be minor compounds in cider. This suggests that current methods used to produce cider do not give an efficient extraction of the phenolics in peel. Previous studies have shown that the waste product from apple juice making, pomace, contains high levels of phenolics and has a high antioxidant activity (*31, 32*), again indicating that processing adaptations could produce ciders with higher levels of phenolics.

Previous work has examined the effect processing can have on the phenolic content of cider (*22*). This study found that the main areas of loss were incomplete extraction of apple tissue, oxidation during milling and pressing, and clarification and fining. Cider-making techniques have evolved since this study was carried out, with some cider producers choosing apple juice which allows production to continue all year round. The use of concentrates does not appear to have a negative effect on the phenolic content of the cider; one example is cider 10, which

Table 5. Procyanidin Content and Composition of Ciders (data expressed as mean values ± standard error ($n = 3$))[a]

cider	total PC (mg/L)	DPn	(−)-Epicat_EU (%)	(−)-Epicat-TU (%)	(+)-Cat-TU (%)
1	452 ± 6	3.2 ± 0.0	53.6 ± 0.2	35.1 ± 0.1	11.3 ± 0.1
2	35 ± 1	2.9 ± 0.0	46.1 ± 0.5	29.1 ± 0.3	24.8 ± 0.2
3	539 ± 10	2.9 ± 0.0	47.2 ± 0.7	40.0 ± 0.7	12.8 ± 0.5
4	722 ± 12	3.3 ± 0.0	55.6 ± 0.3	35.1 ± 0.3	9.3 ± 0.5
5	9 ± 0	2.5 ± 0.0	31.9 ± 0.3	28.8 ± 0.6	39.3 ± 0.8
6	347 ± 15	3.1 ± 0.0	51.7 ± 0.5	33.1 ± 0.1	15.2 ± 0.6
7	192 ± 3	3.0 ± 0.0	50.0 ± 0.2	30.7 ± 0.2	19.3 ± 0.1
8	160 ± 3	3.5 ± 0.0	60.1 ± 0.3	25.9 ± 0.1	14.0 ± 0.2
9	240 ± 6	3.2 ± 0.1	54.9 ± 2.0	31.4 ± 1.4	13.7 ± 0.6
10	139 ± 1	3.0 ± 0.0	48.9 ± 0.6	33.4 ± 0.3	17.7 ± 0.3
11	41 ± 1	2.7 ± 0.0	39.8 ± 0.4	37.1 ± 0.5	23.1 ± 0.2
12	8 ± 0	3.4 ± 0.0	58.7 ± 0.3	31.5 ± 0.3	9.8 ± 0.4
13	243 ± 5	3.1 ± 0.0	51.9 ± 0.2	31.9 ± 0.3	16.2 ± 0.1
14	153 ± 4	3.3 ± 0.0	57.2 ± 0.4	28.1 ± 0.4	14.7 ± 0.1
15	158 ± 1	3.1 ± 0.0	52.4 ± 0.3	32.0 ± 0.2	15.6 ± 0.1
16	83 ± 1	3.0 ± 0.0	50.1 ± 0.2	31.9 ± 0.2	18.1 ± 0.3
17	33 ± 1	2.9 ± 0.0	46.4 ± 1.1	32.9 ± 0.6	20.7 ± 0.5
18	35 ± 1	2.9 ± 0.0	47.2 ± 0.5	32.4 ± 0.2	20.4 ± 0.3
19	74 ± 2	2.9 ± 0.0	47.8 ± 0.1	28.5 ± 0.2	23.7 ± 0.2
20	122 ± 3	2.9 ± 0.0	47.0 ± 0.2	35.1 ± 0.1	17.8 ± 0.1
21	206 ± 3	3.1 ± 0.0	51.3 ± 0.2	30.6 ± 0.2	18.1 ± 0.1
22	109 ± 1	2.8 ± 0.0	45.7 ± 0.1	36.8 ± 0.1	17.6 ± 0.2
23	44 ± 0	2.8 ± 0.0	42.9 ± 0.2	39.4 ± 0.1	17.7 ± 0.3

[a] total PC, total procyanidin; DPn, mean degree of polymerization; (−)-Epicat-EU, (−)-epicatechin as extension unit; (−)-Epicat-TU, (−)-epicatechin as terminal unit; (+)-Cat-TU, (+)-catechin as terminal unit; n.d., not detected.

Table 6. Summary of Flavonoid and Hydroxycinnamate Profiles of Ciders (data expressed as mg/L)[a]

cider	total flavan-3-ols	total flavonols	total dihydrochalcones	total hydroxycinnamates	total procyanidins	total phenolics
4	224 (*1*)	26 (*1*)	85 (*2*)	584 (*1*)	722 (*1*)	1559 (*1*)
1	207 (*2*)	26 (*2*)	93 (*1*)	378 (*2*)	452 (*3*)	1083 (*2*)
3	184 (*3*)	14 (*3*)	47 (*4*)	218 (*5*)	539 (*2*)	938 (*3*)
6	114 (*4*)	6 (*9*)	42 (*5*)	235 (*3*)	347 (*4*)	699 (*4*)
7	84 (*6*)	7 (*6*)	51 (*3*)	220 (*4*)	192 (*8*)	526 (*5*)
13	94 (*5*)	4 (*15*)	39 (*7*)	176 (*7*)	243 (*5*)	518 (*6*)
21	76 (*7*)	9 (*4*)	39 (*6*)	155 (*9*)	206 (*7*)	460 (*7*)
10	56 (*11*)	7 (*8*)	36 (*8*)	218 (*6*)	139 (*12*)	439 (*8*)
9	70 (*9*)	1 (*23*)	19 (*14*)	136 (*11*)	240 (*6*)	431 (*9*)
14	74 (*8*)	5 (*13*)	20 (*13*)	161 (*8*)	153 (*11*)	385 (*10*)
15	65 (*10*)	7 (*7*)	24 (*9*)	130 (*12*)	158 (*10*)	364 (*11*)
8	54 (*12*)	5 (*12*)	22 (*12*)	141 (*10*)	160 (*9*)	356 (*12*)
20	37 (*15*)	4 (*16*)	18 (*15*)	121 (*13*)	122 (*13*)	292 (*13*)
16	28 (*18*)	6 (*10*)	22 (*11*)	109 (*15*)	83 (*15*)	240 (*14*)
22	50 (*13*)	8 (*5*)	24 (*10*)	50 (*17*)	109 (*14*)	227 (*15*)
19	42 (*14*)	2 (*22*)	15 (*17*)	98 (*16*)	74 (*16*)	221 (*16*)
11	23 (*19*)	4 (*14*)	16 (*16*)	110 (*14*)	41 (*18*)	189 (*17*)
23	31 (*16*)	3 (*18*)	15 (*18*)	26 (*22*)	44 (*17*)	111 (*18*)
18	20 (*20*)	3 (*19*)	9 (*22*)	43 (*19*)	35 (*19*)	106 (*19*)
17	19 (*21*)	2 (*21*)	9 (*20*)	44 (*18*)	33 (*21*)	103 (*20*)
2	29 (*17*)	4 (*17*)	11 (*19*)	31 (*20*)	35 (*20*)	100 (*21*)
12	7 (*23*)	2 (*20*)	4 (*23*)	28 (*21*)	8 (*23*)	47 (*22*)
5	12 (*22*)	6 (*11*)	9 (*21*)	10 (*23*)	9 (*22*)	44 (*23*)

[a] Figures in italics in parentheses represent the rank of each cider based on concentration for each class of compound with 1 being the highest; n.d., not detected.

is made from concentrate, and is ranked 8 for total phenolics, which is higher than some ciders made directly from juice (**Table 6**).

The phenolic content of a number of French and Basque ciders have been analyzed (*23*). The English ciders analyzed in the current study have similar phenolic content and procyanidin qualities to the French ciders. The French ciders had a degree of polymerization ranging from 1.6 to 4.7 and total procyanidin content from 0 to 1050 mg/L with a mean value 307 mg/L. In contrast, the Basque ciders had very low levels of phenolics but higher degrees of polymerization of procyanidins than both the French and English cider. The lower levels of phenolics can be attributed to the long maceration and pressing times, which allows greater oxidation by polyphenol oxidase. The high degree of polymerization is thought to be due to the lack of processes such as enzymatic clarification, centrifugation, and filtration which are utilized in the production of the French ciders and the majority of the English ciders analyzed in this study.

Ciders from the Asturian region of Spain seem to have different phenolic profiles than the English ciders analyzed in this study. Hydrocaffeic acid was a major compound, and maximum levels of 5-*O*-caffeoylquinic acid were 13 times lower than the maximum levels found in the English ciders and 24 times lower than those found in the French ciders analyzed by Alonso-Salces et al. but double the levels found in the Basque ciders (*23, 25*). Dihydrochalcone levels were also lower in the Asturian ciders than those obtained in the English ciders. All these ciders do, however, share the low levels of flavonols. Since these are found predominately in the peel, this would indicate that current methods do not extract the phenolics from the peel effectively, and processing methods may need to be adapted to increase the level of these compounds in cider.

In **Table 6** the English ciders have been ranked according to the concentration of each class of compound. Cider 4 was ranked first for all but one class of compounds, the dihydrochalcones which were highest in cider 1.

A previous study which analyzed both commercially clear and cloudy apple juices found levels ranging between 110 and 182 mg/L and 152 and 459 mg/L, respectively (*15*). Although, the majority of ciders had higher levels of phenolics than these apple juices, there were some ciders in the present study that had a lower phenolic content than apple juices. This may be attributed to the additional steps that are employed in the cider-making process. The cider with the highest levels of phenolics had almost 10 times the amount of phenolics compared to the cloudy apple juice, possibly highlighting once again the importance of the variety of apple utilized with the apple juices being produced from dessert apples which contain much lower levels of phenolic compounds than those contained in cider apples (*9, 13*).

This study has shown that ciders can provide a wide range of phenolics to supplement a healthy, well-balanced diet. It has demonstrated that ciders have varied phenolic content which may be due to the processing or choice of the variety of apple. The majority of ciders were also shown to contain higher levels of phenolics than apple juices analyzed in a previous study (*15*). By manipulating the cider-making process and choosing an apple cultivar with a high phenolic content, it may be possible to produce a cider with a higher phenolic content and increased potential health benefits when consumed in moderation.

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