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Polyphenol profiles of apple juices

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Focusing on 17 constituents, the polyphenol profiles of juices freshly made from various dessert ($n = 4$) and cider apple cultivars ($n = 7$) as well as commercially available apple juices ($n = 24$) were investigated using high-performance liquid chromatography-photodiode array detection (HPLC-DAD) and (HPLC)-electrospray ionization-tandem mass spectrometry (ESI_{neg}-MS/MS) analyses. Significant differences in the total polyphenol content as well as the profiles of the apple cultivars under study were observed. For dessert apples the total polyphenol content ranged from 154 to 178 mg/L, whereas for 'old' German cider apple cultivars 261 – 970 mg/L were determined. Boskoop showed the highest (970 mg/L) and Granny Smith the lowest (154 mg/L) polyphenol content of the freshly prepared samples under study. Hydroxycinnamic acids, with chlorogenic acid as dominating constituent, ranged from 57 to 68 mg/L as well as from 134 – 593 mg/L in juices made from dessert apples and that from cider apples, respectively. Dessert apple juices showed lower contents of dihydrochalcones (10 – 35 mg/L) and flavan-3-ols (50 – 95 mg/L) compared to that of cider apples (34 – 171 mg/L and 70 – 393 mg/L, respectively). Quercetin and its derivatives were found from 0.4 – 4 mg/L and 0.4 – 27 mg/L in juices made from dessert apples and that of cider apples, respectively. Compared with freshly made juices, lower contents of polyphenols were determined in the commercial samples under study. Amounts ranging from 110 – 459 mg/L, dominated by chlorogenic acid with concentrations from 53 – 217 mg/L, were determined. Information about cultivar-typical apple polyphenol content and profile is important for bioactivity studies and, consequently, essential for the development of consumer-relevant products with particular nutritional functionalities.

Keywords: Antioxidants / Apple / Electrospray / Flavonoids / High-performance liquid chromatography / HPLC / Juice / MS/MS / Polyphenols / Tandem mass spectrometry

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1 Introduction

The main polyphenol sources in the Western diet are fruits and vegetables such as apples, berries, and onions. Also beverages like red wine, coffee, green and black tea as well as cocoa contribute to the total daily polyphenol intake [1]. Polyphenols show beneficial effects on degenerative diseases such as arteriosclerosis [2, 3] and play an important role as antioxidants as well and are therefore regarded to exhibit protective effects against cardiovascular diseases and cancer [4 – 7].

Apples, *Malus domestica* (Rosaceae), may contain up to 2 g of polyphenols per kilogram wet weight [1]. Apple polyphenols have shown high antioxidative capacity *in vitro* [8 – 11] and it has been reported that the consumption of apple juice increased the antioxidative status of blood [12]. In addition, apple polyphenols are known to influence carbohydrate absorption [13]. Furthermore, they have been shown to inhibit the proliferation of cancer cells [14] and to exhibit anti-arteriosclerotical activity by reducing low-density lipoprotein (LDL) oxidation [15]. Dose-dependent anti-proliferative activity on colon as well as liver cancer cells has been observed using extracts from fresh apples [16]. Just recently, it has been demonstrated that the polyphenol composition of apple juice possesses promising growth-inhibitory properties, affecting proliferation-associated signalling cascades in colon tumor cells [17]. In apples, several classes of phenolic antioxidants, such as cinnamates, flavan-3-ols, flavonols, procyanidins, dihydrochalcones, and anthocyanins have been described [18 – 20]. Major apple phenolics are chlorogenic acid (5-caffeoylquinic acid), quercetin glycosides, procyanidins, and dihydrochalcones, such as phloridzin (phloretin-2′-*O*-glucoside) and phloretin-2′-*O*-xyloglucoside [18, 21].

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Abbreviation: DAD, photodiode array detection

Approximately 69 million tons of apples are estimated to be produced in 2005 [22]. The commercially most important cultivars worldwide are Red Delicious, Golden Delicious, Granny Smith, and Fuji. Apples are mostly used for the production of apple juice, minor amounts are consumed as fresh fruits (dessert apples) [22, 23]. At present, selective information is available about the polyphenol content of dessert and cider apples [10, 21, 24, 25]. Some publications focus on the polyphenols in apple peel or pomace [18, 20]. Other research groups have investigated cider apples from Spain and France [18, 26]. In particular, information on the polyphenol content of 'old' German cider apples and commercially available clear and cloudy apple juices is rather scarce [27].

In this paper, the polyphenol profile of various apple cultivars (dessert as well as cider apples) and different commercially available apple juices was determined using high-performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD). Identification of each constituent was performed using HPLC coupled *via* electrospray to a triple-stage quadrupole mass spectrometer (HPLC-ESI-MS/MS) for structural elucidation.

2 Materials and methods

2.1 Materials

2.1.1 Chemicals

All chemicals and solvents were of analytical grade. Solvents were redistilled before use. Acetonitrile (Lichrosolv®) was from Merck (Darmstadt, Germany); formic acid was purchased from Fluka (Deisenhofen, Germany). Chlorogenic acid (5-caffeoylquinic acid) and caffeic acid were from Roth (Karlsruhe, Germany). Phloretin (2′,4′,6′,4-tetrahydroxydihydrochalcone), quercetin-3-*O*-glucoside (isoquercitrin), quercetin-3-*O*-galactoside (hyperoside) as well as (+)-catechin and (−)-epicatechin were products from Roth (Karlsruhe, Germany). Phloridzin (2′-*O*-glucosyl-4′,6′,4-trihydroxydihydrochalcone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), quercetin-3-*O*-rutinoside (rutin), and quercetin-3-*O*-rhamnoside (quercitrin) were obtained from Sigma (Steinheim, Germany). Cyanidin-3-*O*-galactoside was purchased from Extrasynthese (Lyon, France). 4-*p*-Coumaroylquinic acid, quercetin-3-*O*-xyloside (reynoutrine), quercetin-3-*O*-arabinoside (avicularin), and phloretin-2′-*O*-xyloglucoside as well as procyanidin B_1 and procyanidin B_2 were kindly provided from Prof. Dr. H. Becker (Saarbrücken, Germany) and Prof. Dr. P. Winterhalter (Braunschweig, Germany), respectively. The internal standard 3,4,5-trihydroxycinnamic acid was purchased from Aldrich (Steinheim, Germany).

2.1.2 Apples

Different dessert apple cultivars (n = 4; Red Delicious, Golden Delicious, Granny Smith, and Fuji) were purchased from local supermarkets and fruit distributors. German cider apples (n = 7; Kaiser Alexander, Kaiser Wilhelm, Bittenfelder, Winterrambur, Bohnapfel, Brettacher, and Boskoop) were harvested in October 2004 and kindly provided by the Kreisberatungsstelle für Garten- und Obstbau, Biberach, Germany. Commercially available clear (n = 3, A–C) as well as cloudy apple juices (n = 21, D–X) were purchased at local grocery stores.

2.2 Sample preparation

Fresh apple fruits (800 g) were washed with distilled water, quartered, and blended for 30 s in a Waring blender. Aliquots of the slurry were squeezed to juice using a Hafico press (Schwanke, Neuss, Germany). The resolved juice was immediately filtered through a millipore membrane (0.45 µm; Roth), the standard was added, and the sample was analyzed by HPLC-DAD and HPLC-MS/MS. Commercial juices were filtered using a millipore membrane (0.45 µm; *cf.* above), the standard was added, and the sample submitted to HPLC-DAD and HPLC-MS/MS analysis.

2.3 HPLC-DAD analysis

The HPLC system consisted of a Hewlett-Packard 1100 HPLC gradient pump and a Hewlett-Packard 1100 photodiode array detector (Waldbronn, Germany), equipped with a Wisp 710b autosampler (Waters, Eschborn, Germany). Data acquisition and evaluation were performed with Hewlett-Packard ChemStation© software. A Hypersil™ Gold C_{18} column, 100 × 4.6 mm, with 3 µm particle size, (Thermo, Runcorn, UK) was used. The mobile phase consisted of aqueous 0.1% v/v formic acid (A) and acetonitrile (B). The gradient applied was 1–99% B in 40 min at a flow rate of 1 mL/min, and 20 µL injection volumes were used. The peaks were identified by comparison of retention time and UV spectra (200–600 nm) with that of authentic reference substances. Dihydrochalcones, catechins, and procyanidins were determined at 280 nm, hydroxycinnamic acid derivatives at 320 nm, and flavon-3-ols at 360 nm. Cyanidin-3-*O*-galactoside was detected at 520 nm.

2.4 HPLC-ESI-MS/MS analysis

HPLC-ESI-MS/MS was performed with a TSQ 7000 tandem mass spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany) and an Applied Biosystems 140b pump (BAI, Bensheim, Germany). Data acquisition and evaluation were conducted on DEC 5000/

33 (Digital Equipment, Unterföhring, Germany) using Finnigan MAT ICIS 8.1 software. HPLC chromatographic separations were carried out on a Hypersil™ Gold C_{18} column, 100 × 2.0 mm, with 3 µm particle size (Thermo, Runcorn, UK). The mobile phase consisted of aqueous 0.1% v/v formic acid (A) and acetonitrile (B). The gradient applied was 5–99% B in 40 min at a flow rate of 0.2 mL/min, and 5 µL injection volume. The analysis was performed in the negative ionization mode. Capillary voltage was set to 3.2 kV, and the temperature of the heated capillary was 200°C. Nitrogen served both as sheath (70 psi) and auxiliary gas (10 units). The mass spectrometer was operated in the full-scan mode, *m/z* 120–700, with a total scan duration of 1.0 s. MS/MS experiments were performed at a collision energy of 20–40 eV, with argon (2.0 mTorr) serving as collision gas. The obtained molecular ion peaks and product mass spectra were compared with that of references.

2.5 Quantification

Aliquots from a stock solution of chlorogenic acid **1**, caffeic acid **2**, 4-*p*-coumaroylquinic acid **3**, phloretin-2′-*O*-xyloglucoside **4**, phloridzin **5**, phloretin **6**, procyanidin B_1 **7**, procyanidin B_2 **8**, (+)-catechin **9**, (−)-epicatechin **10**, quercetin-3-*O*-glucoside **11**, quercetin-3-*O*-galactoside **12**, quercetin-3-*O*-xyloside **13**, quercetin-3-*O*-arabinoside **14**, quercetin-3-*O*-rhamnoside **15**, quercetin **16**, and quercetin-3-*O*-rutinoside **17** (100 mg/L each) in methanol were diluted; 3,4,5-trihydroxycinnamic acid as internal standard (50 mg/L) was added. Calibration curves (at the appropriate wavelengths according to the absorption maximum of the compounds) were used for quantification. Polyphenols were quantified by means of calibration curves (peak area divided by internal standard area *versus* quotient of polyphenol and 3,4,5-trihydroxycinnamic acid concentration). Linearity was given for 0.4–600 mg/L; limits of quantification for compound **1**–**14** ranged from 0.4 to 0.9 mg/L and limits of determination from 0.2 to 0.4 mg/L with a signal-to-noise ratio of 3:1 [28], respectively. All experiments were performed in triplicate. Compounds were identified by comparison of retention time, UV-spectra and MS as well as MS/MS information using reference compounds.

3 Results and discussion

3.1 Identification and quantification of polyphenols

For reliable quantification baseline separation of the apple juice constituents **1**–**17** was achieved. Initial experiments revealed that the ESI in the negative mode [25, 29] led to higher intensities than the positive mode favored by others [18, 21].

Using model solutions of reference compounds **1**–**17**, information on chromatographic and spectroscopic data was obtained (Table 1). The deprotonated molecular ion was selected for low-energy CID fragmentation experiments to produce characteristic product ion spectra. For chlorogenic acid **1** a typical product ion spectrum of the deprotonated molecular ion of *m/z* 353.0 $[M-H]^-$ was achieved. It exhibited one main fragment at *m/z* 190.9 as a result of the loss of the caffeoyl moiety and the remaining quinic acid fragment [30–32]. The product ion mass spectrum of caffeic acid **2** (molecular ion $[M-H]^-$ *m/z* 179.1)

Table 1. Spectroscopic data (retention time, UV maximum and HPLC-ESI-MS/MS fragmentation pattern) of polyphenols **1**–**17** under study

	t_R (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z* (relative abundance, %))	eV
Chlorogenic acid (**1**)	8.8	326	353.0	190.9 (100)	15
Caffeic acid (**2**)	9.5	325	179.1	134.7 (100)	20
4-*p*-Cumaroylquinic acid (**3**)	9.9	312	337.0	172.9 (100), 190.9 (25), 163.0 (10)	19
Phloretin-2′-*O*-xyloglucoside (**4**)	13.0	284	567.3	272.9 (100)	27
Phloridzin (**5**)	13.9	284	435.3	272.9 (100), 166.8 (35), 124.8 (5)	35
Phloretin (**6**)	17.6	286	273.1	166.8 (100)	35
Procyanidin B_1 (**7**)	8.0	280	577.2	424.7 (100), 407.0 (72), 288.9 (64), 450.9 (55)	30
Procyanidin B_2 (**8**)	9.4	280	577.1	425.1 (100), 407.0 (93), 288.9 (50), 451.0 (41)	30
(+)-Catechin (**9**)	8.9	278	289.0	245.0 (100), 204.9 (29), 202.7 (28)	20
(−)-Epicatechin (**10**)	9.7	278	289.0	244.8 (100), 202.7 (83), 204.9 (40)	20
Quercetin-3-*O*-glucoside (**11**)	11.7	354	463.0	300.0 (100), 301.0 (20)	35
Quercetin-3-*O*-galactoside (**12**)	11.9	355	463.2	300.0 (100), 301.0 (26)	35
Quercetin-3-*O*-xyloside (**13**)	12.1	356	433.1	300.0 (100), 301.0 (17)	26
Quercetin-3-*O*-arabinoside (**14**)	12.5	354	433.2	300.0 (100), 301.0 (22)	35
Quercetin-3-*O*-rhamnoside (**15**)	12.8	350	447.0	300.0 (100), 301.0 (30)	35
Quercetin (**16**)	15.7	372	300.9	150.9 (100), 178.6 (40)	35
Quercetin-3-*O*-rutinoside (**17**)	11.3	356	609.3	299.8 (100), 301.0 (18)	40

For details see Section 2.

showed the loss of the carboxylic acid group (−44 u; *m/z* 134.7). In agreement with the literature the product ion spectrum of 4-*p*-coumaroylquinic acid **3** (*m/z* 337.0) gave the same fragment at *m/z* 190.9 for the quinic acid besides a main fragment of *m/z* 172.9 derived from the coumaric acid by the loss of the quinic acid moiety (at 19 eV) [27]. Phloretin-2′-*O*-xyloglucoside **4** (*m/z* 567.3) and phloridzin **5** (*m/z* 435.3) revealed an almost similar fragmentation pattern, dominated by the fragment of *m/z* 272.9 for the aglycon phloretin by loss of the sugar moiety. The product ion spectrum of **4** showed the protonated molecular ion of *m/z* 567.3 and the fragment ion of *m/z* 272.9 due to the loss of 132 u (xyloyl moiety) and 162 u corresponding to the loss of the glucosyl moiety [18, 27]. The deprotonated molecular ion of *m/z* 273.1 for phloretin **6** revealed the characteristic product ion of *m/z* 166.8 by the loss of 107 u. Procyanidin B_1 **7** and procyanidin B_2 **8** gave the same molecular ion of *m/z* 577. Product ion spectra showed a characteristic ion at *m/z* 288.9 by the loss of 288 u equivalent to the loss of a (+)-catechin **9** or (−)-epicatechin **10** (288 u) moiety [18]. As structurally identical isomers, **9** and **10** gave similar spectroscopic data [32]. Due to their chromatographic separation, however, **9** and **10** could be quantified easily. The product ion spectra of **11**–**15** gave as most abundant product ion *m/z* 300.0 by the homolytic cleavage to the remaining quercetin fragment $[M-H]^-$ after the loss of the sugar moieties, whereas *via* heterolytic cleavage the product ion of *m/z* 301.0 occurred [33]. Because of the identical molecular ions of quercetin-3-*O*-glycoside **11** and quercetin-3-*O*-galactoside **12** (each with *m/z* 463) as well as quercetin-3-*O*-xyloside **13** and quercetin-3-*O*-arabinoside **14** (each with *m/z* 433) these glycosides were elucidated by their retention times. The product ion spectrum of quercetin **16** showed losses of 150 u and 122 u, respectively [34–36].

Quantification was performed using the internal standard method using 3,4,5-trihydroxycinnamic acid. Compounds were quantified by means of calibration curves at the appropriate wavelength at maximum UV absorption [18]. Limits of quantification ranged from 0.4 to 0.9 mg/L, limits of determination from 0.2 to 0.4 mg/L, respectively.

3.2 Total polyphenol content

The polyphenol content in the juices freshly made from eleven apple cultivars varied significantly as displayed in Tables 2 and 3. Amounts ranging from 154 to 178 mg/L and

Table 2. Polyphenol content (mg/L) of dessert apples under study

	Granny Smith	Golden Delicious	Red Delicious	Fuji
Chlorogenic acid (**1**)	54.0	37.6	32.7	54.1
Caffeic acid (**2**)	3.8	4.8	6.1	2.5
4-*p*-Cumaroylquinic acid (**3**)	9.1	14.4	22.1	11.1
Σ Hydroxycinnamic acids	66.9	56.8	60.9	67.7
Phloretin-2′-*O*-xyloglucoside (**4**)	25.9	7.6	2.7	7.2
Phloridzin (**5**)	9.3	4.1	7.1	8.5
Phloretin (**6**)	n.d.	n.d.	n.d.	n.d.
Σ Dihydrochalcone derivatives	35.2	11.7	9.8	15.7
Procyanidin B_1 (**7**)	2.7	5.5	3.2	4.3
Procyanidin B_2 (**8**)	29.6	38.7	36.9	42.5
(+)-Catechin (**9**)	2.5	3.8	3.2	7.0
(−)-Epicatechin (**10**)	15.1	46.6	51.4	36.6
Σ Flavan-3-ols	49.9	94.6	94.7	90.4
Quercetin-3-*O*-glucoside (**11**)	+	+	+	+
Quercetin-3-*O*-galactoside (**12**)	+	+	+	2.2
Quercetin-3-*O*-xyloside (**13**)	+	+	+	+
Quercetin-3-*O*-arabinoside (**14**)	0.5	+	+	+
Quercetin-3-*O*-rhamnoside (**15**)	1.9	1.6	+	1.4
Quercetin (**16**)	n.d.	n.d.	n.d.	n.d.
Quercetin-3-*O*-rutinoside (**17**)	n.d.	n.d.	n.d.	n.d.
Σ Flavonols	2.4	1.6	+	3.6
Total polyphenol amount	**154.4**	**164.8**	**165.4**	**178.0**

n.d., not detectable; +, < limit of quantification. Standard deviation < 1.0% of triplicate determinations
For details see Section 2.

Table 3. Polyphenol content (mg/L) of cider apples under study

	Boskoop	Bittenfelder	Brettacher	Winterrambur	Kaiser Wilhelm	Kaiser Alexander	Bohnapfel
Chlorogenic acid (**1**)	487.6	223.1	448.2	230.0	117.3	80.6	305.3
Caffeic acid (**2**)	+	3.2	4.0	7.3	4.2	3.0	5.5
4-*p*-Cumaroylquinic acid (**3**)	29.2	43.6	140.4	78.0	17.0	50.0	48.9
Σ Hydroxycinnamic acids	516.8	269.9	592.6	315.3	138.5	133.6	359.7
Phloretin-2′-*O*-xyloglucoside (**4**)	36.6	44.8	63.2	63.0	36.3	20.3	135.9
Phloridzin (**5**)	24.7	34.9	93.6	27.6	15.3	13.2	35.1
Phloretin (**6**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Dihydrochalcone derivatives	61.3	79.7	156.8	90.6	51.6	33.5	171.0
Procyanidin B_1 (**7**)	5.0	20.4	2.6	14.0	2.8	3.0	2.4
Procyanidin B_2 (**8**)	138.4	87.9	34.2	82.6	29.2	32.2	36.8
(+)-Catechin (**9**)	60.0	11.7	3.0	21.5	8.4	3.6	12.0
(−)-Epicatechin (**10**)	189.1	77.6	29.8	90.0	64.7	50.3	66.8
Σ Flavan-3-ols	392.5	197.6	69.6	208.1	105.1	89.1	118.0
Quercetin-3-*O*-glucoside (**11**)	+	1.0	+	4.0	+	1.3	1.9
Quercetin-3-*O*-galactoside (**12**)	+	6.9	2.9	8.1	2.4	1.6	7.1
Quercetin-3-*O*-xyloside (**13**)	+	5.0	4.7	4.5	3.3	+	4.1
Quercetin-3-*O*-arabinoside (**14**)	+	5.0	0.9	6.1	0.4	0.4	4.9
Quercetin-3-*O*-rhamnoside (**15**)	+	4.3	2.8	4.0	2.8	1.6	4.6
Quercetin (**16**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-*O*-rutinoside (**17**)	n.d.	0.8	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Flavonols	+	23.0	11.3	26.7	8.8	4.9	22.6
Total polyphenol amount	**970.0**	**570.2**	**829.7**	**641.3**	**304.1**	**261.2**	**671.4**

n.d. not detectable; +, < limit of quantification. Standard deviation < 1.2% of triplicate determinations.
For details see Section 2.

from 304 to 970 mg/L were determined in dessert apple juices and cider apple juices, respectively. Lowest contents were found in Granny Smith and Golden Delicious cultivars with 154 and 165 mg/L, respectively. This finding is in good agreement with previously published data [37]. The highest total polyphenol content (970 mg/L) was measured in Boskoop apple juice.

The polyphenol profile of commercial apple juices under study is presented in Table 4; the distribution of the constituents is shown in Figs. 1 and 2. Differences in the polyphenol content of cloudy products in comparison to that of clear apple juices are obvious. Total polyphenol amounts in cloudy apple juices varied between 152 and 459 mg/L, whereas in clear apple juices ranges varying from 110 to 173 mg/L were determined.

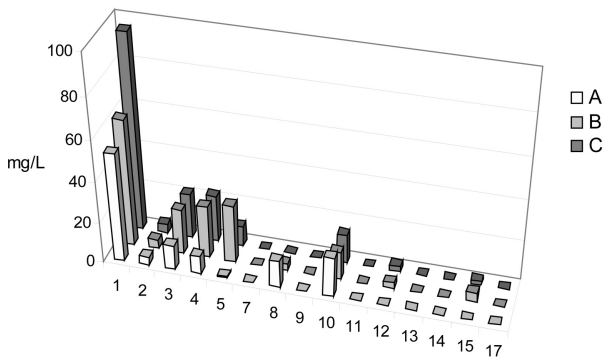

Figure 1. Polyphenol profile (mg/L) of commercial clear apple juices (n = 3) determined by HPLC-DAD analysis (for details see Section 2). Numbering corresponds to that given in Section 2.

Selective information is available in the literature about the total polyphenol content of dessert apple cultivars such as Elstar and Jonagold [25]. Among the cultivars Renetta, Morgenduft, Granny Smith, Red Delicious, Golden Delicious, Fuji, Braeburn, and Royal Gala, the first-mentioned revealed the highest polyphenol content with 213 mg/100 g fresh weight [10]. Tsao *et al.* [21] have reported amounts of polyphenols in Red Delicious, Golden Delicious, McIntosh, Empire, Ida Red, Northern Spy, Mutsu, Cortland and Fuji. Whereas the latter showed the lowest amounts with 66 mg/

Table 4. Polyphenol content (mg/L) of commercial clear ($n = 3$, A–C) and cloudy apple juices ($n = 21$, D–X)

	A	B	C	D	E	F	G	H	I	J	K	L
Chlorogenic acid (**1**)	52.9	61.8	95.6	88.2	217.4	79.4	85.3	181.5	115.9	188.2	85.0	98.2
4-*p*-Cumaroylquinic acid (**2**)	11.8	21.8	21.8	13.7	37.3	10.0	14.8	37.2	21.0	38.0	20.4	22.5
Caffeic acid (**3**)	3.9	4.4	4.4	4.1	4.3	4.4	4.3	5.4	4.9	5.7	5.7	5.2
Σ Hydroxycinnamic acids	68.6	88.0	121.8	106.0	259.0	93.8	104.4	224.1	141.8	231.9	111.1	125.9
Phloretin-2′-*O*-xyloglucoside (**4**)	8.8	25.5	23.0	20.0	57.7	32.0	22.1	45.4	48.0	54.3	35.3	30.6
Phloridzin (**5**)	0.6	28.0	9.7	7.6	18.1	12.2	10.7	23.2	9.5	32.8	14.8	18.8
Phloretin (**6**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Dihydrochalcone derivatives	9.4	53.5	32.7	27.6	75.8	44.2	32.8	68.6	57.5	87.1	50.1	49.4
Procyanidin B_1 (**7**)	+	+	+	+	+	4.3	+	4.1	5.2	3.8	14.8	+
Procyanidin B_2 (**8**)	12.9	3.2	+	10.3	21.4	19.7	19.2	19.7	17.1	27.4	18.9	31.3
(+)-Catechin (**9**)	+	+	+	+	0.4	1.8	1.9	2.8	3.2	1.7	+	0.8
(−)-Epicatechin (**10**)	19.0	13.5	14.0	35.3	97.9	86.9	31.3	48.6	45.8	59.3	43.5	31.7
Σ Flavan-3-ols	31.9	16.7	14.0	45.6	119.7	112.7	52.4	75.2	71.3	92.2	77.2	63.8
Quercetin-3-*O*-glucoside (**11**)	+	+	n.d.	+	+	+	+	1.8	1.4	2.2	+	+
Quercetin-3-*O*-galactoside (**12**)	+	2.7	2.5	+	1.2	+	2.7	2.0	2.2	6.5	1.7	3.7
Quercetin-3-*O*-xyloside (**13**)	n.d.	+	n.d.	+	+	+	+	5.0	+	5.6	+	+
Quercetin-3-*O*-arabinoside (**14**)	+	+	+	+	0.6	0.4	+	0.7	+	1.5	0.5	0.5
Quercetin-3-*O*-rhamnoside (**15**)	+	4.6	1.7	2.8	2.7	3.1	4.1	3.2	1.4	4.2	2.8	3.5
Quercetin (**16**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-*O*-rutinoside (**17**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Flavonols	+	7.3	4.2	2.8	4.5	3.5	6.8	12.7	5.0	13.5	4.5	7.7
Total polyphenol amount	**109.9**	**165.5**	**172.7**	**182.0**	**459.0**	**254.2**	**196.4**	**380.6**	**275.6**	**431.1**	**243.4**	**246.6**

	M	N	O	P	Q	R	S	T	U	V	W	X
Chlorogenic acid (**1**)	120.8	94.1	181.8	95.0	135.4	125.6	106.9	134.0	125.8	122.5	131.9	60.5
Caffeic acid (**2**)	4.2	4.6	4.8	4.0	3.9	4.0	+	5.0	4.3	4.3	4.9	3.7
4-*p*-Cumaroylquinic acid (**3**)	18.1	21.4	23.8	16.4	24.4	18.8	14.2	23.7	26.8	21.9	34.4	10.1
Σ Hydroxycinnamic acids	143.1	120.1	210.4	115.4	163.7	148.4	121.1	162.7	156.9	148.7	171.2	74.3
Phloretin-2′-*O*-xyloglucoside (**4**)	26.0	26.5	14.8	15.0	32.0	15.8	46.4	57.6	54.5	21.9	33.5	10.0
Phloridzin (**5**)	10.1	16.0	n.n.	7.7	20.0	7.2	9.0	15.2	3.4	8.7	26.5	4.0
Phloretin (**6**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Dihydrochalcone derivatives	36.1	42.5	14.8	22.7	52.0	23.0	55.4	72.8	72.1	30.6	60.0	14.0
Procyanidin B_1 (**7**)	+	2.0	+	+	+	2.7	+	2.2	20.8	5.7	+	+
Procyanidin B_2 (**8**)	15.7	26.7	20.4	22.0	19.7	26.0	10.3	20.3	40.1	33.8	18.8	25.5
(+)-Catechin (**9**)	1.9	+	3.4	2.2	3.6	0.4	+	0.4	3.4	5.9	3.6	0.5
(−)-Epicatechin (**10**)	49.0	40.5	67.9	45.5	98.5	95.3	40.9	86.3	50.4	70.2	56.8	33.0
Σ Flavan-3-ols	66.6	69.2	91.7	69.7	121.8	124.4	51.2	109.1	114.7	115.6	79.2	59.0
Quercetin-3-*O*-glucoside (**11**)	+	+	+	+	+	4.4	+	+	+	+	+	+
Quercetin-3-*O*-galactoside (**12**)	+	1.8	1.6	+	+	1.9	+	2.7	1.6	1.6	1.7	1.8
Quercetin-3-*O*-xyloside (**13**)	+	+	0.5	+	+	1.1	+	+	+	+	+	+
Quercetin-3-*O*-arabinoside (**14**)	+	0.6	3.8	0.4	+	0.4	+	+	+	+	+	+
Quercetin-3-*O*-rhamnoside (**15**)	2.9	3.0	3.8	2.7	2.3	2.5	1.5	2.2	3.0	2.2	2.5	3.1
Quercetin (**16**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-*O*-rutinoside (**17**)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Flavonols	2.9	5.4	9.2	3.1	2.3	10.3	1.5	4.9	4.6	3.8	4.2	4.9
Total polyphenol amount	**248.8**	**237.0**	**368.1**	**211.0**	**340.8**	**306.1**	**229.3**	**349.6**	**348.3**	**298.7**	**314.6**	**152.2**

n.d., not detectable; +, < limit of quantification. Standard deviation < 0.8% of triplicate determinations.
For details see Section 2.

100 g, Northern Spy and Red Delicious provided highest polyphenol contents with 934 and 534 mg/kg fresh fruit. In another study [24], polyphenol amounts of 624, 929, and 550 mg/kg fresh weight have been reported for Golden Delicious, Granny Smith and Braeburn, respectively.

For cider apples information on polyphenols was provided for Spanish cultivars from the Basque region [18] and 12 French cider and two dessert apple cultivars [26]. In the latter study cider apple cultivars showed higher polyphenol concentrations than dessert apples. In addition, high

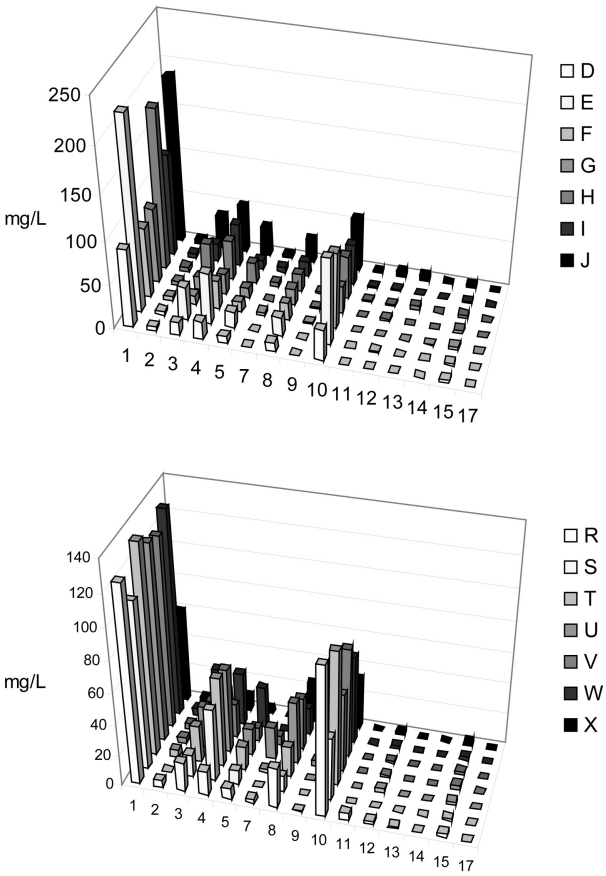

Figure 3. Polyphenol profile (mg/L) of juices freshly made from dessert apple cultivars determined by HPLC-DAD analysis (for details see Section 2). **6** and **16** were not detectable. Numbering corresponds to that given in Section 2.

amounts of polyphenols have been found in the parenchym of Red Boskoop apples (506 mg/kg) and their core (927 mg/kg) [27].

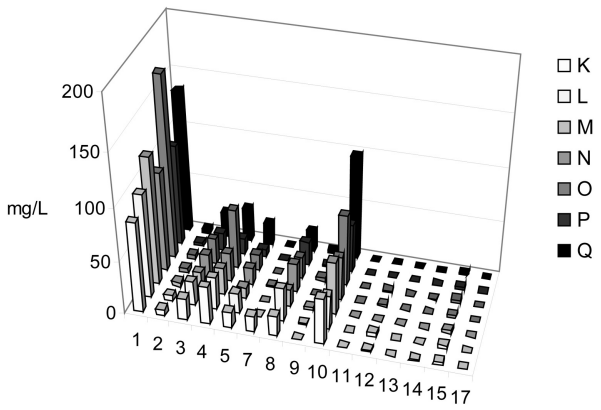

Figure 2. Polyphenol profile (mg/L) of commercial cloudy apple juices (n = 21) determined by HPLC-DAD analysis (for details see Section 2). Numbering corresponds to that given in Section 2.

Among the five major groups of polyphenols, only hydroxycinnamic acids, flavan-3-ols, flavonols, and dihydrochalcones play an important role in the polyphenol profile of apples. We have observed chlorogenic acid **1**, 4-*p*-coumaroylquinic acid **3**, procyanidin B_2 **8**, and (−)-epicatechin **10**, as major phenolics in the cultivars under study as shown in Figs. 3 and 4.

3.3 Hydroxycinnamic acids

Hydroxycinnamic acids, with chlorogenic acid **1** as dominating constituent, ranged from 57 to 68 mg/L as well as from 134 to 593 mg/L in juices made from dessert apples and that from cider apples, respectively (*cf.* Tables 2 and 3). Highest amounts for **1** were determined in Boskoop apples with 488 mg/L, whereas in the other cultivars under study amounts of 33 to 54 mg/L for dessert apples and 81 to 448 mg/L for cider apples were achieved.

The polyphenolic profile of commercial clear and cloudy apple juices was also dominated by **1** (53 – 217 mg/L). Total hydroxycinnamic acid amounts varied from 74 to 259 mg/L in cloudy apple juices, whereas in clear juices the amounts ranged from 69 to 122 mg/L. The results are in good agreement with literature data where **1** appeared as the main

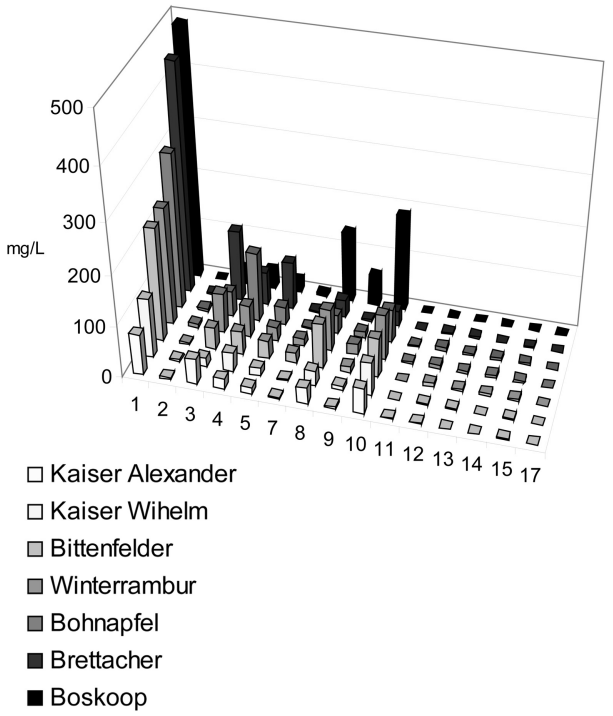

Figure 4. Polyphenol profile (mg/L) of juices freshly made from cider apple cultivars determined by HPLC-DAD analysis (for details see Section 2). Numbering corresponds to that given in Section 2.

polyphenolic substance in apple flesh [10, 18, 21, 26]. Tsao *et al.* [21] have reported main differences between the polyphenol profiles of apple peel and apple flesh. In these studies the main polyphenol in apple flesh was **1,** whereas in peel phloridzin **5**, procyanidin B_2 **8**, and (−)-epicatechin **10** dominated.

3.4 Flavan-3-ols

The flavan-3-ol dimers procyanidin B_1 **7** and B_2 **8** as well as the monomers (+)-catechin **9** and (−)-epicatechin **10** were present in the juices made from dessert and that from cider apples under study. As shown for hydroxycinnamic acids, flavan-3-ol concentrations were fairly low for dessert apples and high for cider apples (see Figs. 3 and 4). As to compound **9**, amounts ranged from 3 to 7 mg/L in dessert apples and 3 to 60 mg/L in cider apples. Higher concentrations for **10** ranging from 15 to 51 mg/L and 30 to 189 mg/L for dessert and cider apples, respectively, were measured. The dimeric **7** varied from 3 to 6 mg/L in dessert apples and 3 to 14 mg/L in cider apples. In particular, compound **8** exhibited higher amounts varying from 30 to 43 mg/L and 29 to 138 mg/L in dessert and in cider apples, respectively.

In commercial apple juices the group of flavan-3-ols ranged from 46 to 124 mg/L in cloudy and 14 to 32 mg/L in clear juices, showing highest amounts of **8** and **10**. The flavan-3-ol data provided by the literature show wide ranges of concentrations and an inhomogeneous distribution in different apple cultivars. Compounds **8** and **10** have been reported to be the most predominant flavan-3-ols in apples [21]. In general, highest concentrations of procyanidins up to 1655 µg/g occurred in apple peels.

3.5 Dihydrochalcones

The dihydrochalcones phloretin-2′-*O*-xyloglucoside **4** and phloridzin **5** were also quantified. In dessert apple juices amounts of 3 to 26 mg/L of **4** were determined, whereas higher amounts in juices from cider apples (20–136 mg/L) were measured. The highest amount of **4** was observed in 'Bohnapfel' with 136 mg/L. The quantification of **5** revealed concentrations of 4 to 9 mg/L and 13 to 94 mg/L in the juices from dessert apples and cider apples, respectively. In the commercial apple juices under study dihydrochalcone derivatives ranged from 9 to 54 mg/L and 14 to 87 mg/L for clear and cloudy apple juice, respectively. In general, **4** predominated; only in one sample (juice B) the ratio between **4** and **5** was found to be nearly equal. No phloretin **6** was found in the samples under study.

3.6 Flavonols

Main flavonols in the freshly produced juices from apples were quercetin glycosides with highest amounts determined in juices from the cider apple cultivars 'Winterrambur' and 'Bittenfelder'. The flavonol concentrations in juices produced from all apple cultivars under study ranged from the limit of detection up to 27 mg/L. In dessert apples very low concentrations of quercetin derivatives were observed, whereas in cider apples quercetin glycosides were dominated by quercetin-3-*O*-galactoside **12** (2–8 mg/L), quercetin-3-*O*-arabinoside **14** (0–6 mg/L), and quercetin-3-*O*-rhamnoside **15** (2–5 mg/L). Only in freshly made juice from 'Bittenfelder' quercetin-3-*O*-rutinoside **17** was determined, as previously described elsewhere [37]. In contrast to previously published data [18] no free quercetin **16** was observed in the apple samples under study.

In commercially available apple juices compounds **16** and **17** were not detectable. The amounts of quercetin glycosides varied significantly among each juice. In clear juices amounts from 0 to 7 mg/L were measured, whereas in cloudy apple juices concentrations spread from 2 to 14 mg/L. In freshly prepared juices from all apple cultivars as well as the commercial available juices no cyanidin-3-*O*-galactoside was identified using HPLC-DAD at 520 nm. In the literature, anthocyanin concentrations of 95–100 mg/kg, *i.e.*, mainly cyanidin-3-*O*-galactoside, have been reported [38].

In conclusion, significant differences in the polyphenol profile of the apple cultivars under study were observed. Information about cultivar-typical apple polyphenol content and profile is important for bioactivity studies and, consequently, essential for the development of consumer-relevant products with particular nutritive functionalities.

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