Mitteilungen Klosterneuburg 60 (2010): 287-296

Impact of variety and vintage on the polyphenol composition of apple juices

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Monovarietal apple juices from domestic table and cider apple varieties were produced in the years 2005 to 2008. Technological modifications were cloudy and clear direct juices, additionally one pomace extraction juice was manufactured. Besides standard analytical juice data the main objective was the impact of variety and vintage on the polyphenol concentrations analysed by means of RP-HPLC/UV detection. Screening of apple varieties revealed large differences in polyphenol amounts depending on variety and vintage. In cloudy apple juices, which always contained higher polyphenol amounts than clear juice, the concentrations of monomeric polyphenols ranged from 100 (cv. 'Maunzen', 2008) to 1400 mg/l (cv. 'Bittenfelder', 2008). Hydroxycinnamates with the major component chlorogenic acid represented the highest concentrations in the juices followed by the dihydrochalcones, the flavan-3-ols and the flavonols. Juice clarification decreased the total amounts of polyphenols (Folin assay) by 22 to 31 %, whereas the monomeric polyphenols determined by means of HPLC were hardly affected. Clarification removed predominantly particle-bound and high-molecular polyphenols. Enrichment of polyphenols, especially of flavonols, was achieved by producing pomace liquefaction juices. Combined treatment with cellulase and pectinase released flavonols located in the apple skin. Total polyphenols were doubled by this kind of pomace enzymation.
Keywords: apple, apple juice, polyphenols, HPLC

Einfluss von Sorte und Jahrgang auf die Polyphenolzusammensetzung von Apfelsäften. *In den Jahren 2005 bis 2008 wurden sortenreine, klare und trübe Apfelsäfte aus einheimischen Tafel- und Mostobstsorten hergestellt. Zusätzlich wurde ein Extraktionssaft aus Apfeltrester hergestellt. Neben den analytischen Standardparametern wurde der Einfluss der Sorte und des Jahrgangs auf die mittels RP-HPLC/UV-Detektion bestimmte Polyphenolzusammensetzung untersucht. Insgesamt wurde neben dem Sorteneinfluss eine starke Jahrgangsabhängigkeit festgestellt. Die trüben Apfelsäfte zeigten stets höhere Polyphenolgehalte als die klaren, wobei die Konzentrationen zwischen 100 mg/l (cv. 'Maunzen', 2008) und 1400 mg/l (cv. 'Bittenfelder', 2008) lagen. Unter den einzelnen Polyphenolen waren die Hydroxyzimtsäurederivate dominierend, gefolgt von Dihydrochalkonen, Flavan-3-olen und den Flavonolen. Während der Saftklärung wurden die Gesamtphenolgehalte um 22 bis 31 % reduziert, die mittels HPLC bestimmbaren monomeren Polyphenole blieben unverändert. Die Saftklärung reduzierte eher partikelgebundene und hochmolekulare Polyphenole. Durch die Tresterenzymierung mit Cellulasen und Pektinasen konnten die Gesamtpolyphenolgehalte verdoppelt werden.*
Schlagwörter: Apfel, Apfelsaft, Polyphenole, HPLC

L'influence de la variété et de l'année de récolte sur la composition en polyphénols des jus de pommes. *Au cours des années 2005 à 2008, des jus de pommes clairs et troubles ont été fabriqués à partir de variétés autrichiennes pures de fruits de table et de fruits à cidre. En complément, on a produit un jus d'extraction à partir de marcs de pommes. Outre les paramètres analytiques standards, l'influence de la variété et de l'année sur la composition en polyphénols, déterminée à l'aide de la détection RP-HPLC/UV, a été étudiée. Au total, on a constaté, outre l'influence de la variété, une forte dépendance de l'année. Les jus de pommes troubles présentaient toujours des teneurs en polyphénols plus élevés que les jus clairs, les concentrations se situant entre 100 mg/l (cv. 'Maunzen', 2008) et 1400 mg/l (cv. 'Bitten-*

felder', 2008). Parmi les polyphénols, les dérivés de l'acide hydrocinnamique dominaient, suivis par des dihydrochalcones, des flavane-3-ols et les flavonols. Au cours de la clarification des jus, les teneurs totales en phénols ont été réduites de 22 à 31 %, les polyphénols monomères déterminables par HPLC sont restés inchangés. La clarification des jus a réduit plutôt les polyphénols liés à des particules et ceux à haute densité moléculaire. Les teneurs totales en phénols ont pu être doublées par voie d'enzymatisation des marcs aux cellulases et aux pectinases.

Mots clés : pomme, jus de pommes, polyphénols, HPLC

Several epidemiological studies have shown protective effects of a fruit- and vegetable-rich diet concerning cardiovascular diseases and cancer (BLOCK et al., 1992). Bioactive secondary plant substances e.g. polyphenols are known to cause these effects. This substance class shows various health-promoting effects, but does not belong to the nutrients (WATZL and LEITZMANN, 1999). Besides fruits and vegetables, the corresponding juices are good sources for polyphenol intake. In Germany fruit juices are very popular with a per-capita-consumption of nearly 38 litres per year. The most popular juice is apple juice with a consumption of 11.4 litres and 8.5 litres per capita in the years 2007 and 2009, respectively (German Fruit Juice Association, VdF). Apple juice has become a recommended food stuff within the 'Five-a-Day-Campaign' of the German society for nutrition (DGE) (BITSCH et al., 2000). The average consumption is supposed to be higher, because another drink with increasing popularity is apple juice blended with sparkling water, the so-called „apple spritzer".

In Germany approximately 500 million litres of pipfruit juices per year were produced in the years 1992 to 2005 which is equivalent to the processing of about 670,000 tons of raw material. On average the raw material was 50 % cider apples, 35 % table apples and 15 % unspecified imported material (ELLINGER, 2007). Apples and apple juices are rich in polyphenols, mainly hydroxycinnamates, flavonoids (flavanols, flavonols) and characteristically dihydrochalcones, which occur essentially in apples. The amounts of these polyphenols depend on variety (THIELEN et al., 2006; KELLER et al., 2001; SCHMITZ-EIBERGER et al., 2003) and processing modifications (DIETRICH et al., 2003; WILL et al., 2007). During processing apples to apple juices potential losses of 50 to 90 % of the polyphenols have to be considered. In respect to health claims of fruit juices, this is counterproductive. The majority of clear apple juices available at food retailers contain only minor amounts of polyphenols. They are made from table apples or are extensively stabilised by polyphenol removal techniques to prevent potential haze formations or astringency. The popular apple spritzers often originate from low-cost Asian apple juice concentrates with low contents of polyphenol.

In contrast to that natural cloudy apple juices manufactured from cider apples contain distinctly higher polyphenol amounts. For that reason, they are in the focus of nutritional research. Within the BMBF (German Ministry of Education and Research) research project „Role of Food Ingredients in the Development of Intestinal Diseases and Potentiality of their Prevention by Nutrition", polyphenol amounts and composition of technological modifications of apple juices should be investigated. Previous animal studies concerning colon cancer revealed significantly better effects of cloudy apple juices in comparison to the corresponding clear apple juices (BARTH et al., 2007). The aim of this study was the characterisation of monovarietal cloudy apple juices made from cider apple varieties. Pure cider apple juices are sometimes hardly drinkable because of strong astringency and high amounts of total titratable acid. Therefore, monovarietal cloudy cider apple juices were blended with juices from table apple varieties resulting in polyphenol-rich cloudy apple juices with more moderate amounts of titratable acids. These juices were used for cell culture, animal and human studies carried out in the above mentioned project. Furthermore, pomace liquefaction experiments using pectinases and cellulases were performed to increase polyphenol yields.

Materials and methods

Juice samples

All monovarietal juices were produced from different cider and table apple varieties from the harvests 2005 to 2008. Table apples were provided from the department of fruit growing of the Research Center Geisenheim, the variety 'Topaz' was processed in the year 2005 and 2006, additionally a mixture of different table apples was available. The mixture of table apples consisted predominantly of the varieties 'Jonagold' and 'Elstar'. Cider apples were purchased at regional German fruit growers: cv. 'Bittenfelder' (2005/2006/2007/2008), 'Boertlinger Weinapfel' (2005/2008), 'Bohnapfel'

(2005/2006), 'Brettacher' (2006/2007), 'Erbacher Hofer' (2006), 'Gehrers Rambour' (2006/2008), 'Haux' (2005), 'Luxemburger Rambour' (2006), 'Maunzen' (2006/2008), 'Seestermueher' (2005), 'Winesap' (2006/2007/2008) and 'Weisser Trierer' (2006).

Chemicals

All chemicals used were of analytical grade. Methanol and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany). (+)-Catechin, phloridzin, chlorogenic acid, caffeic acid and coumaric acid were purchased from Sigma-Aldrich (Munich, Germany). Quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-rhamnoside, quercetin-3-arabinoside, procyanidin B1, procyanidin B2 and procyanidin C1 were purchased from Extrasynthèse (Genay Cedex, France). 4-coumaroyl-quinic acid and phloretin-2-xyloglucoside were isolated as described by Will et al. (2006).

Processing of apple juices

Cloudy apple juices (A-juices): The process followed the standard procedure of the fruit juice industry for direct cloudy juice. After washing and selection, apples were crushed with a BTM macerator (Seepex, Bottrop, Germany) and the mash was pressed with a horizontal press (HP-L 200, Bucher-Guyer, Switzerland) or dejuiced in a decanter (Flottweg Z3, Vilsbiburg, Germany). 200 mg/l ascorbic acid were added to prevent oxidation of the juices, followed by centrifugation of the juice (separator SAMR 3036, Westfalia, Germany). The juices were short-time heated (87 °C, 35 s; Sigmatherm, Schmidt-Bretten, Germany) and stored in sterile stainless steel

Table 1: Chemical parameters of monovarietal apple juices of the vintages 2005 to 2008

Parameters	Density (20/20)	Soluble solids (°Bx)	Glucose (g/l)	Fructose (g/l)	Sucrose (g/l)	Sugar-free extract (g/l)	Sorbitol (g/l)	pH-value	Titratable acid (g/l)	Total PP* (mg/l)	TEAC (mmol/l)
Bittenfelder 2005	1.0617	14.43	21.7	55.1	48.3	31.9	8.6	3.02	12.60	1191	9,0
Bittenfelder 2006	1.0674	16.09	21.3	61.3	55.2	37.6	15.8	3.07	14.46	2051	15,2
Bittenfelder 2007	1.0570	13.81	21.0	57.1	31.1	39.0	8.7	2.92	11.08	1281	9,0
Bittenfelder 2008	1.0802	18.81	24.4	67.8	58.0	58.7	23.6	3.07	16.80	2967	25,2
Boertlinger Weinapfel 2005	1.0632	15.09	20.2	67.8	48.3	28.0	8.4	3.23	8.80	1298	11,0
Boertlinger Weinapfel 2008	1.047	11.46	16.3	58.2	32.4	15.0	2.6	3.40	6.3	953	6.7
Bohnapfel 2005	1.0625	14.97	23.5	82.2	34.5	23.4	6.3	3.09	7.30	1464	12,6
Bohnapfel 2006	1.0597	14.34	15.6	78.0	37.7	23.7	9.5	3.18	8.35	1934	13,9
Brettacher 2006	1.0482	11.66	17.5	71.9	25.0	10.7	5.0	3.14	6.12	720	5,1
Brettacher 2007	1.0454	11.12	13.2	64.3	25.0	15.4	3.4	3.05	6.22	579	4,5
Erbacher Hofer 2005	1.0516	12.42	20.2	63.2	23.0	27.5	7.5	3.21	8.50	1670	13,7
Gehrers Rambour 2005	1.0421	11.13	13.6	50.3	19.7	25.8	3.3	2.85	13.70	1246	10,1
Gehrers Rambour 2008	1.0531	12.78	15.9	57.3	33.5	31.4	7.6	3.12	13.4	1978	15.8
Haux 2005	1.0470	11.54	13.2	56.5	23.0	29.3	3.3	2.87	14.80	796	7,3
Luxemburger Rambour 2006	1.0478	11.64	19.6	63.3	18.6	22.1	3.4	3.25	7.43	1464	12,8
Maunzen 2006	1.0466	11.38	16.7	68.0	11.2	25.1	3.5	3.04	9.04	2184	16,5
Maunzen 2008	1.0450	10.94	15.9	66.9	19.6	14.4	2.3	3.23	6.3	384	2.6
Seestermueher 2005	1.0452	12.26	22.0	65.0	15.5	14.8	2.0	3.16	6.20	716	5,5
Topaz 2005	1.0617	14.65	19.3	68.0	49.8	23.3	4.3	3.11	10.20	1061	8,6
Topaz 2006	1.504	12.13	16.6	62.4	40.1	11.7	3.1	3.24	6.89	530	4,2
Weisser Trierer 2006	1.0481	11.65	17.1	54.5	23.5	29.3	4.6	2.98	12.18	2983	25,5
Winesap 2006	1.0660	15.70	31.1	89.9	22.7	27.9	10.8	3.48	6.61	1572	13,1
Winesap 2007	1.0570	13.73	26.8	73.8	22.5	25.0	7,1	3.26	6.81	819	6,3
Winesap 2008	1.0664	15.57	29.8	81.8	29.2	31.9	11.4	3.40	7.2	1956	13.9

* PP = Polyphenols

Table 2: Polyphenol composition of monovarietal apple juices of the vintages 2005 to 2008 (mg/l)

	Bittenfelder 2005	Bittenfelder 2006	Bittenfelder 2007	Bittenfelder 2008	Boertlinger Weinapfel 2005	Boertlinger Weinapfel 2008	Bohnapfel 2005	Bohnapfel 2006	Brettacher 2006	Brettacher 2007	Erbacher Hofer 2005	Gehrers Rambour 2005	Gehrers Rambour 2008	Haux 2005	Luxemburger Rambour 2006	Maunzen 2006	Maunzen 2008	Seestermueher 2005	Topaz 2005	Topaz 2006	Weisser Trierer 2006	Winesap 2006	Winesap 2007	Winesap 2008
Procyanidin B1	7.4	16.6	13.5	71.0	16.6	12.0	11.0	13.4	1.1	2.5	9.0	7.7	31.9	4.5	7.6	11.2	3.6	4.2	4.6	2.1	8.9	5.6	9.9	31.2
(+)-Catechin	2.3	26.5	28.3	53.3	2.1	9.6	4.2	30.8	7.5	5.7	4.3	5.6	32.5	2.4	8.8	14.9	2.2	13.9	1.9	3.6	13.0	22.2	21.8	27.7
Procyanidin B2	12.0	66.6	85.5	169.2	17.0	16.5	32.5	59.6	6.7	10.1	23.2	18.6	130.8	5.0	22.4	125.1	n.d.	3.5	6.3	11.8	153.2	30.5	37.0	75.3
(-)-Epicatechin	23.1	60.7	60.8	121.4	38.8	12.2	64.8	87.7	15.2	12.2	38.6	42.0	102.5	13.6	29.9	120.9	6.2	8.0	17.1	18.5	92.6	45.0	42.8	57.5
Procyanidin C1	9.7	40.8	36.2	74.3	14.1	1.5	18.6	33.6	8.7	5.5	17.4	11.6	74.0	4.1	24.1	82.6	n.d.	4.3	3.7	8.4	100.2	20.5	17.6	41.2
Σ Flavan-3-ols	54.5	211.1	224.3	489.2	88.6	51.8	131.1	224.9	39.3	35.9	92.5	85.5	371.7	29.6	92.8	354.7	12.0	34.0	33.6	44.4	367.9	123.7	129.1	232.9
Phloretin-2'-xyloglucoside	51.2	107.5	59.3	99.9	47.7	25.6	189.7	268.0	104.1	94.5	238.3	85.8	34.6	122.8	34.6	36.9	24.7	15.7	18.7	17.6	114.7	156.9	47.7	86.2
Phlorizin	49.6	42.3	45.2	48.6	75.2	32.3	21.0	32.3	20.8	20.8	41.1	13.4	14.4	44.6	7.0	11.7	10.8	26.3	3.0	8.5	91.6	45.3	16.3	19.4
Σ Dihydro-chalcones	100.9	149.8	104.5	148.5	122.9	57.9	210.7	300.2	125.0	115.3	279.4	99.2	49.0	167.4	41.6	48.5	35.5	42.0	21.8	26.1	206.3	202.2	64.0	105.6
Cumaroyl-glucose*	n.d.	2.8	1.6	1.9	n.d.	2.7	n.d.	0.9	0.9	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	0.0	1.0	0.9	0.5
5-Caffeoyl-quinic acid	197.9	286.7	198.6	374.0	214.2	109.2	219.6	257.4	131.1	123.1	299.3	223.7	87.2	128.1	199.8	211.9	30.1	184.1	49.1	39.5	313.2	257.6	133.2	250.6
4-Caffeoyl-quinic acid**	24.0	28.9	29.8	41.2	29.9	28.8	7.0	10.2	11.5	11.6	33.3	11.8	7.5	3.6	13.2	3.9	3.4	26.1	0.0	0.1	4.8	10.0	7.4	18.8
Caffeic acid	4.4	1.1	1.0	1.2	10.7	n.d.	15.2	1.4	0.5	0.3	6.5	1.0	n.d.	1.0	5.6	0.4	n.d.	0.0	0.0	0.1	1.3	1.4	0.4	0.7
3-Cumaroyl-quinic acid	4.4	4.3	20.2	16.1	4.0	3.5	2.1	3.2	0.5	8.9	3.7	3.2	n.d.	2.4	n.d.	n.d.	1.2	3.4	0.0	0.3	0.0	3.6	6.8	5.3
4-Cumaroyl-quinic acid	59.6	99.0	176.2	314.9	91.2	179.3	20.1	28.2	28.4	76.2	79.4	41.5	37.1	11.0	135.1	46.4	16.7	89.0	1.5	1.2	39.4	34.1	54.7	114.9

Table 2 (continued): Polyphenol composition of monovarietal apple juices of the vintages 2005 to 2008 (mg/l)

	Bittenfelder 2005	Bittenfelder 2006	Bittenfelder 2007	Bittenfelder 2008	Boerlinger Weinapfel 2005	Boerlinger Weinapfel 2008	Bohnapfel 2005	Bohnapfel 2006
Cumaric acid	n.d.	1.9	0.8	2.8	n.d.	0.1	n.d.	1.3
Σ Hydroxy-cinnamates	290.4	424.7	428.2	752.1	349.9	323.6	264.0	302.6
Quercetin-3-galactoside	1.5	1.0	2.1	1.9	1.7	1.6	3.0	4.4
Quercetin-3-glucoside	0.6	0.6	0.7	0.6	0.7	0.5	1.1	1.8
Quercetin-3-xylosid	1.9	1.2	1.2	2.2	1.7	0.5	1.3	1.3
Quercetin-3-arabinoside	n.d	1.7	2.8	3.9	n.d.	1.8	n.d	3.5
Quercetin-3-rhamnoside	1.6	1.6	1.6	3.3	1.2	1.9	3.9	6.2
Σ Flavonols	5.5	6.1	8.3	11.9	5.2	6.3	9.3	17.2
Total	451.2	791.6	765.3	1401.7	566.6	439.6	615.2	844.9

	Brettacher 2006	Brettacher 2007	Erbacher Hofer 2005	Gehrers Rambour 2005	Gehrers Rambour 2008	Haux 2005	Luxemburger Rambour 2006	Maunzen 2006
Cumaric acid	0.8	1.9	n.d.	n.d.	n.d.	n.d.	1.1	0.8
Σ Hydroxy-cinnamates	173.7	222.6	422.2	281.2	131.8	146.1	354.8	263.3
Quercetin-3-galactoside	1.3	1.8	1.7	1.1	2.7	0.9	0.8	1.2
Quercetin-3-glucoside	1.0	0.9	0.7	1.2	0.8	0.8	0.4	0.7
Quercetin-3-xylosid	0.6	0.5	1.7	1.0	0.5	1.3	0.4	0.8
Quercetin-3-arabinoside	0.9	1.3	n.d.	n.d.	1.0	n.d.	0.6	0.9
Quercetin-3-rhamnoside	2.0	1.9	1.2	2.9	4.2	1.1	1.6	2.3
Σ Flavonols	5.9	6.5	5.2	6.3	9.2	4.0	3.7	6.0
Total	343.8	380.3	799.3	472.2	561.7	347.1	493.0	672.5

	Maunzen 2008	Seestermueher 2005	Topaz 2005	Topaz 2006	Weisser Trierer 2006	Winesap 2006	Winesap 2007	Winesap 2008
Cumaric acid	n.d.	n.d.	n.d.	0.2	1.0	2.0	1.1	1.4
Σ Hydroxy-cinnamates	51.6	302.6	50.6	41.5	359.7	309.7	204.4	392.2
Quercetin-3-galactoside	0.6	1.7	0.6	0.3	3.7	2.7	1.6	1.3
Quercetin-3-glucoside	n.d.	0.8	n.d.	n.d.	1.6	1.3	0.4	0.5
Quercetin-3-xylosid	n.d.	1.1	n.d.	n.d.	1.9	1.2	0.7	0.7
Quercetin-3-arabinoside	n.d.	n.d.	n.d.	n.d.	3.9	2.8	1.4	1.7
Quercetin-3-rhamnoside	0.5	1.1	2.3	1.2	3.6	2.4	2.2	2.0
Σ Flavonols	1.1	4.7	2.8	1.5	14.8	10.3	6.3	6.2
Total	100.2	383.3	108.8	113.5	948.6	645.9	403.8	736.9

*expressed as coumaric acid; ** expressed as 5-caffeoylquinic acid; n.d. = not detected

tanks. Apple juices (AJ05-07) were blended with monovarietal juices after analytical characterisation and sensory evaluation.

Clear Apple Juices (A-juices): Cloudy apple juices were treated with pectinase (50 ml/t Fructozym P; Erbsloeh, Geisenheim, Germany) and fined with gelatine, silica sol and bentonite (80 g/t gelatine, 400 ml/t silica sol, 500 g/t bentonite; all: Erbsloeh, Geisenheim, Germany). Afterwards juices were separated (SAMR 3036, Westfalia, Germany) and cross-flow filtered (0.2 µm; Pall-Seitz, Bad Kreuznach, Germany). The juices were short-time heated and stored in sterile stainless steel tanks. All laboratory samples were hot-filled (85 °C) into 0.75 l glass bottles.

Pomace liquefaction juices (B-juices): Pomace from the juice production described above was resuspended in hot demineralised water (1:1, w/v, 95 °C). This mixture showed the optimum temperature for enzymation (50 °C). 300 g/t pectinase (MA-X-Press, Erbsloeh, Geisenheim, Germany) and 300 g/t cellulase (Distizym, Erbsloeh, Geisenheim, Germany) were added to the mixture. The enzymation time was 2 h in a jacketed stirring tank at 50 °C. After decanting (Flottweg Z3, Vilsbiburg, Germany) the juice was again depectinised with 100 ml/t Fructozym P for one hour. After separation the juice was concentrated with a 3-step rising film evaporator (Unipektin 103B, Eschenz, Switzerland) and finally hot-filled (85 °C) into 0.75 l glass bottles (MEHRLÄNDER et al., 2002).

Fruit juice analysis

IFU methods were used for the standard parameters. Density and soluble solids (°Brix) were determined by digital refractometry (Abbemat, Dr. Kernchen, Seelze, Germany) and densitometry (DMA 48, Anton Paar, Ostfildern, Germany). Total titratable acid (expressed as malic acid at pH 8.1) and

Table 3: Composition of blended apple juices AJ05, AJ06, and AJ07 (in %)

	AJ05	AJ06	AJ07
Bittenfelder	10		50
Bohnapfel	20	10	
Boertlinger Weinapfel	20		
Maunzen		45	
Seestermüher	25		
Brettacher			30
Luxemburger Rambour		10	
Topaz	25		
Mixed table apples		35	20

pH-value were measured potentiometrically. Glucose, fructose, sucrose, and sorbitol were determined enzymatically. Total phenolics were assayed with the Folin-Ciocalteu-reagent based on a (+)-catechin calibration (SINGLETON and ROSSI, 1965). The TEAC assay on antioxidant capacity was performed according to RE et al. (1999).

Polyphenol analysis (RP-HPLC/UV)

Single polyphenols were determined as described by WILL et al. (2008). Briefly, the system was a Surveyor HPLC-PDA system (ThermoFisher, Dreieich, Germany), the chromatographic separation was achieved on a 150 x 2 mm i.d., 3 µm RP-Reprosil-Pur C18-AQ column (Dr. Maisch, Ammerbuch, Germany) protected with a guard column of the same material. Flow rate was 0.2 ml/min, injection volume of the samples was 3 µl, column oven temperature was 40 °C. Solvent A was 0.2 % acetic acid, Solvent B was acetonitrile/water/acetic acid (50/49.5/0.5; v/v/v). The separation was achieved by the following gradient: 0 to 31 min from 10 to 55 % B, 31 to 37.5 min 55 % to 100 % B, washing with 100 % B (4.5 min) and 10 min re-equilibration of the column. Flavanols and dihydrochalcones were detected at 280 nm, phenol carbonic acids at 320 nm and flavonols at 360 nm. Juices were injected after centrifugation and 0.45 µm membrane filtration. Quantification was carried out using peak areas from external calibration curves. HPLC results are given as means from duplicates.

Results and discussion

Table 1 shows the analytical parameters of the cloudy apple juices produced in the years 2005 to 2008. There were large differences between the varieties as well as between harvest years. Relative densities of the juices varied from 1.0421 ('Gehrers Rambour' 2005) to 1.0802 ('Bittenfelder' 2008), the corresponding soluble solids were between 11.13 and 18.81 °Bx. The results found were within the limits of variations of the AIJN code of practise reference guide lines (www.aijn.org; November 2010). Glucose varied from 13.2 g/l ('Haux' 2005; 'Brettacher' 2007) to 31.1 g/l ('Winesap' 2006), fructose was between 50.3 g/l ('Gehrers Rambour' 2005) and 89.9 g/l ('Winesap' 2006). Sucrose concentrations differed from 11.2 g/l ('Maunzen' 2006) to 58.0 g/l ('Bittenfelder' 2008). These amounts exceeded the variation cited in the AIJN Code of Practice (5 to 30 g/l) distinctly. The sugar-free extract represents the soluble ingredients in the juice except sugars (organic acids, minerals, sorbitol, polyphenols, and polysaccharides). The juices of the varieties 'Brettacher' (10.7 g/l; 2006) and 'Topaz' (11.7 g/l; 2006) showed extremely low values, falling below the AIJN Code of Practice variation limits of (18 to 29 g/l). In contrast, the juices of the variety 'Bittenfelder' in the years 2005, 2006 and 2007 showed high amounts of sugar-free extract (31.9 g/l; 37.6 g/l; 39 g/l). Total titratable acid (pH 8.1; calculated

Table 4: Chemical parameters of the apple juice blends AJ05 to AJ07

Parameters	AJ05 cloudy	AJ05 clear	AJ06 cloudy	AJ06 clear	AJ07 cloudy	AJ07 clear
Density (20/20)	1.0566	1.0559	1.0506	1.0500	1.0535	1.0529
Soluble solids (°Bx)	13.63	13.62	12.21	12.05	13.03	12.87
Glucose (g/l)	21.2	26.7	18.9	19.6	23.9	28.6
Fructose (g/l)	67.0	76.5	69.2	68.2	65.4	70.2
Sucrose (g/l)	36.2	17.6	21.7	20.2	25.2	15.6
Sugar-free extract (g/l)	22.7	25.5	21.6	21.8	24.4	22.9
Sorbitol (g/l)	5.4	3.4	4.7	4.6	6.9	6.7
pH-value	3.13	3.28	3.13	3.13	3.12	3.09
Titratable acid (g/l)	8.13	7.58	7.44	7.36	8.21	8.07
Total polyphenols (mg/l)	1070	739	1343	1027	1070	844
TEAC (mmol/l)	7.9	5.6	9.8	8.5	8.6	7.4

Table 5: Polyphenol composition of the apple juice blends AJ05 to AJ07 (mg/l)

	AJ05 cloudy	AJ05 clear	AJ06 cloudy	AJ06 clear	AJ07 cloudy	AJ07 clear
Procyanidin B1	3.8	1.2	8.5	5.7	4.9	3.2
(+)-Catechin	5.9	2.5	6.9	8.7	6.6	6.2
Procyanidin B2	13.8	2.9	71.2	62.0	32.6	27.2
(-)-Epicatechin	14.7	5.3	36.3	57.2	18.6	20.3
Procyanidin C1	6.1	n.d.	35.8	32.3	5.5	2.4
Σ Flavan-3-ols	44.2	11.9	158.7	165.8	68.2	59.3
Phloretin-2'-xyloglucoside	65.8	25.1	40.9	40.6	57.7	58.8
Phlorizin	22.8	24.4	9.0	11.6	23.9	24.8
Σ Dihydrochalcones	88.6	49.5	49.8	52.2	81.6	83.7
Cumaroylglucose*	0.9	1.7	0.1	0.2	1.9	1.8
5-Caffeoylquinic acid	168.8	187.3	177.8	186.8	162.0	160.4
4-Caffeoylquinic acid**	14.1	26.3	5.3	4.7	23.0	22.2
Caffeic acid	n.d.	n.d.	0.2	n.d.	0.3	0.9
3-Cumaroylquinic acid	n.d.	n.d.	4.5	4.9	6.8	5.6
4-Cumaroylquinic acid	38.3	79.9	41.8	49.3	125.0	110.2
Coumaric acid	n.d.	n.d.	0.4	0.4	1.2	1.1
Σ Hydroxycinnamates	222.1	295.2	230.1	246.2	320.2	302.1
Quercetin-3-galactoside	1.2	1.3	1.3	1.5	1.0	1.2
Quercetin-3-glucoside	0.4	0.5	0.6	0.7	0.3	0.4
Quercetin-3-xyloside	n.d.	n.d.	0.3	0.5	0.4	0.5
Quercetin-3-arabinoside	n.d.	n.d.	0.4	0.8	0.7	0.7
Quercetin-3-rhamnoside	1.3	1.2	1.5	2.1	1.3	1.7
Σ Flavonols	3.0	2.9	4.1	5.6	3.7	4.5
Total	357.7	359.6	442.8	469.9	473.7	449.5

*expressed as coumaric acid; ** expressed as 5-caffeoylquinic acid; n.d. = not detected

as malic acid) varied from 6.1 g/l ('Brettacher' 2006) to an outstanding concentration of 16.8 g/l ('Bittenfelder' 2008) with a mean value of 9.3 g/l. Titratable acid concentrations distinctly above 8.0 g/l are sensory unpleasant, because the juices will become too sour and will also tend to a certain bitterness. A striking parameter is the high concentration of sorbitol in 'Bittenfelder' juice (2006: 15.8 g/l; 2008: 23.6 g/l) which exceeds the typical range of sorbitol in apple juices. It is known from literature (Dietrich et al., 2007) that sorbitol increases after water stress and dry weather conditions. This effect was found also for the year 2003 with a maximum value of 25.5 g/l for the variety 'Bittenfelder'. The sorbitol metabolism has an adaptive function under water stress. Under these conditions the range of 2 to 7 g/l for sorbitol stated within the AIJN Code of Practice cannot be used for evaluation of authenticity of cider apple juices, like 'Bittenfelder'.

Total polyphenol concentrations (Folin assay) differed between the cider apple varieties, ranging from 384 mg/l ('Maunzen' 2008) to 2983 mg/l ('Weisser Trierer' 2006). The 'Maunzen' juices also showed a strong vintage influence with an untypical low total polyphenol concentration of 384 mg/l in 2008 and a more typical high concentration in 2006 with 2184 mg/l. In the case of the latter, the raw material was from different locations. The juices of the variety 'Topaz', which is a typical table apple variety, showed a polyphenol concentration of 530 mg/l in the year 2006 and 1061 mg/l in the year 2005. For this variety the lower concentration was more typical. Apples of the variety 'Bittenfelder' were processed in four vintages, and the total polyphenol concentrations ranged from 1191 to 2967 mg/l (Table 1). Depending on natural growing conditions, e.g. the apple-typical biannual bearing, it was not always possible to obtain each variety in every year. But it became evident, that besides variety the harvest year is an important factor influencing total polyphenol concentrations of apple juices. Antioxidant capacities are predominantly affected by the polyphenols present in the juice, hence a good correlation (R^2 = 0.98) was observed between the total polyphenols and the TEAC-results.

Single polyphenols of cloudy apple juices were determined using RP-HPLC/UV; the results are given in Table 2. Like total polyphenols determined with the Folin assay the concentrations of the single polyphenols differed in the processed juices depending on the variety and on the vintage. As expected the juice of the table apple variety 'Topaz' contained the lowest concentrations of polyphenols (109 mg/l in 2005, 114 mg/l in

Table 6: Chemical parameters of the Bittenfelder A- and B-juices

Parameters	A-juice	B-juice
Density (20/20)	1.0674	1.0358
Soluble solids (°Bx)	16.09	8.8
Glucose (g/l)	21.3	11.2
Fructose (g/l)	61.3	28.4
Sucrose (g/l)	55.2	24.6
Sugar-free extract (g/l)	37.6	28.6
Sorbitol (g/l)	15.8	7.4
pH-value	3.07	3.03
Titratable acid (g/l, pH 8.1)	14.46	7.93
Total polyphenols (mg/l)	2051	1157
TEAC (mmol/l)	15.2	11.9

2006). It is well-known that table apple juices contain lower polyphenol concentrations than cider apple juices. The low amount of HPLC-determinable polyphenols in 'Topaz' juice of the harvest year 2005 was not in line with the high total polyphenol concentration analysed with the Folin assay (1061 mg/l).

The amounts of polyphenols (analysed by HPLC) in cider apple juices ranged from 100 mg/l with the variety 'Maunzen' 2008 (untypically low) to 1401 mg/l with the variety 'Bittenfelder' 2008. The average concentration of the cider apple juices was about 600 mg/l. Generally, all cider apple juices were good sources for polyphenol uptake. Hydroxycinnamates represented the highest concentrations in all juices and varied from 41.5 mg/l ('Topaz' 2006) to 752.1 mg/l ('Bittenfelder' 2008). The major component in all cider apple juices was chlorogenic acid (from 30.1 mg/l in 'Maunzen' 2008 to 374 mg/l in 'Bittenfelder' 2008), followed by 4-coumaroyl-quinic acid (1.2 mg/l in 'Topaz' 2006, 314.9 mg/l in 'Bittenfelder' 2008). The exception was 'Boertlinger Weinapfel' 2008, in which the chlorogenic acid (5-caffeoylquinic acid) was always the main polyphenol. Other major components were the dihydrochalcones phloretin-2'-xyloglucoside and phloridzin, two characteristic polyphenols which are essentially present in apples. Total dihydrochalcones varied from 21.8 mg/l ('Topaz' 2005) to 300.2 mg/l ('Bohnapfel' 2006). The amounts of monomeric flavan-3-ols ranged from 12 mg/l ('Maunzen' 2008) to 489.2 mg/l ('Bittenfelder' 2008); flavonols varied from 1.1 mg/l ('Maunzen' 2008) to 17.2 mg/l in 'Bohnapfel l' 2006. Outstanding flavan-3-ol concentrations were also found in the juices of the varieties 'Weisser Trierer' 2006 (367.9 mg/l), 'Maunzen' 2006 (355 mg/l) and 'Gehrers Rambour' 2008 (371.7 mg/l).

Table 7: Polyphenol composition of the Bittenfelder A- and B-juices; results of the B-juice (8.80 °Bx) calculated to the original °Bx of the A-juice (16.09 °Bx)

	A-juice	B-juice
Procyanidin B1	16.6	14.7
(+)-Catechin	26.5	29.9
Procyanidin B2	66.6	54.1
(-)-Epicatechin	60.7	91.9
Procyanidin C1	40.8	47.1
Σ Flavan-3-ols	211.1	237.7
Phloretin-2'-xyloglucoside	107.5	128.6
Phlorizin	42.3	179.8
Σ Dihydrochalcones	149.8	308.4
Cumaroylglucose*	2.8	1.8
5-Caffeoylquinic acid	286.7	374.3
4-Caffeoylquinic acid**	28.9	29.1
Caffeic acid	1.1	6.2
3-Cumaroylquinic acid	4.3	4.3
4-Cumaroylquinic acid	99.0	93.6
Cumaric acid	1.9	4.4
Σ Hydroxycinnamates	424.7	513.8
Quercetin-3-galactoside	1.0	14.4
Quercetin-3-glucoside	0.6	4.0
Quercetin-3-xylosid	1.2	10.6
Quercetin-3-arabinoside	1.7	2.0
Quercetin-3-rhamnoside	1.6	8.1
Σ Flavonols	6.1	39.1
Total	791.6	1098.9

*expressed as cumaric acid; ** expressed as 5-caffeoylquinic acid

Production of blended apple juices

Most of the pure cider apple juices tasted very astringent and sour. They were not suitable to be merchandised in the retail sale. Apple juices AJ05, AJ06 and AJ07 from the vintages 2005, 2006, and 2007 were blended to single lots from monovarietal juices (2000 to 4000 litre) following the recipe listed in Table 3. The blending partner „mixed table apples“ consisted predominantly of the varieties 'Jonagold' and 'Elstar'. After blending the juices still contained high amounts of polyphenols, but in respect to astringency and acidity they were more consumer-friendly. The addition of table apple juices decreased the amounts of total titratable acid to achieve a pleasant drinkability. A concurrent decrease of polyphenols was unavoidable. Table 4 shows the standard parameters of the juice blends which were all in typical ranges corresponding to the AIJN Code of Practise. Soluble extracts were in the range of 13 °Bx (12.05 to 13.63 °Bx), total titratable acids were near the sensory acceptable upper limit of about 8 g/l. Total polyphenols varied from 739 mg/l to 1343 mg/l. Clarification of juices caused big losses of total polyphenols ranging from 21 to 31 %. These results confirm the data of Will et al. (2007) with losses of 34 to 74 % for table apple juices and of 3 to 26 % for polyphenol-rich cider apple juices. The amounts of single polyphenols (Table 5) were similar in all blended juices, the juice AJ05 contained lower concentrations in all polyphenol classes. Because of high amounts of flavan-3-ols (cloudy 159 mg/l; clear 166 mg/l) in combination with pleasant sensory properties the juice AJ06 and extracts of it turned out to be a very successful blend for in vitro and in vivo studies (Bellion et al., 2009; Soyalan et al., 2009). The juice AJ07 contained high concentrations of dihydrochalcones (about 80 mg/l); the concentration of flavonols was in typical concentration ranges in all juices.

Losses of single polyphenols (HPLC) caused by clarification were neglectable compared to total polyphenols measured with the Folin assay. The concentrations remained nearly unchanged. Clarification did hardly affect the composition of HPLC-determinable polyphenols, but removed in case of cloudy juices particle-bound polyphenols and probably polymeric polyphenols like higher molecular procyanidins which cannot be analysed with standard HPLC. In summary, polyphenol-rich apple juices with good sensory properties could be produced with similar polyphenol amounts, compositions, and good antioxidant capacities.

Pomace liquefaction juices from the cider apple variety 'Bittenfelder' (2006)

In the first step a cloudy apple juice (A-juice, Table 1 and 2) was produced followed by the combined enzymatic treatment of the pomace with a pectinase and a cellulase preparation (B-juice). It should be mentioned that the B-juices are no fruit juices in the legal sense because of the water extraction and the use of cellulases. The analytical parameters of these apple juices are shown in Table 6. Due to the hot water extraction the soluble solids in the B-juice were about 40 % lower than in the corresponding A-juice (8.80 vs. 16.09 °Bx). Table 7 shows the polyphenol compositions of the A- and B-juices both calculated to the original Brix of the A-juice. There were high increases in the amounts of flavonols as well as in dihydrochalcone concentrations in the B-juice. The increase in flavonols (quercetin derivatives) was about 600 % (A-juice: 6 mg/l; B-juice: 39 mg/l). The dihydrochalcone concentration was doubled, concentrations of flavan-3-ols increased as well. Flavonols are mainly located in the skin of apples (Thielen et al., 2004). Under usual conditions their limited water solubility prevents a better transfer into the A-juices. Combined pectinase and cellulase activities break down the apple cell walls and release the flavonols into the B-juice.

Summarizing the results, polyphenol compositions of apple juices depend on various parameters. Screening of different cider apples of four harvest years (2005 to 2008) showed large differences between the different cider apple varieties. Total polyphenol amounts varied in a wide range (400 to 3000 mg/l), single, HPLC-determinable polyphenols nearly in the same manner (100 to 1400 mg/l). Major polyphenols were chlorogenic acid, 4-coumaroyl-quinic acid and the two dihydrochalcones phloretin-2'-xyloglucoside and phloridzin, which occur characteristically only in apples. The amounts of these components varied from variety to variety. Cider apple juices contained higher polyphenol concentrations than table apple juices and should be preferred for juice production in respect to health-beneficial effects.

The harvest year affected the amounts of apple polyphenols considerably. Many factors influence the formation of polyphenols, e.g. insolation, abiotic stress and attacks from pathogens and viruses. For this reasons it is not possible to produce a „standardised" apple juice from year to year. But generally it was possible to produce polyphenol-rich apple juices with sensory acceptable amounts of titratable acids by careful selection and appropriate blending of raw materials. In respect to health-beneficial effects the choice of appropriate apple varieties and the vintage were the most important variables. A possibility to increase polyphenols, especially flavonols and dihydrochalcones, in juices is the production of pomace liquefaction juices. Combined pectinase and cellulase treatment breaks down the apple cell walls and releases more flavonols into the juices. The increase of flavonol concentration was about 600 %, dihydrochalcone amounts could be duplicated. The so-called B-juices show minor amounts of soluble primary ingredients and are not appropriate for direct drinking. However the juices or their corresponding concentrates may be used as additives to enrich other juices with polyphenols. In respect to legal regulations, these products would not have a juice status.

Because of the gathering pace of urbanisation cider apples are more and more disappearing from rural agriculture. Domestic fruit juice processors have to switch to table apples which are easier to grow and will deliver higher and more predictable yields. This is not only a loss of genetic resources, but also a disadvantage in respect to a healthy nutrition. All efforts to change this situation and to sustain traditional apple varieties should be supported. The juices introduced above were processed in a technical scale on industry-like equipment. In most cases the lots ranged from 500 to 2000 kg raw material. This is done because the strong impact of technology and the batch sizes on juice composition and quality are well-known. This knowledge prevents us from carrying out lab-scale trials which are not comparable with industrial or technical scales. With respect to scientifically working principles, a disadvantage of fruit processing from larger amounts of authentic crops is the lacking reproducibility. Especially the time-delayed technical scale processing of an equal apple batch will always result in analytically different juices although originating from the same raw material. The differences between technical and laboratory scale juices will become measurable not so much for the primary juice ingredients (sugars, fruit acids and minerals), but clearly for the secondary plant substances like polyphenols.

Acknowledgement

The project was funded by the German Ministry of Education and Research (BMBF #01EA0104). We would like to thank Prof. Dr. Dr. SCHRENK from the faculty of Food Chemistry and Toxicology of the University of Kaiserslautern for his support and the project coordination.

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Received September 6, 2010