

Analytical composition of fruit juices from different sour cherry cultivars

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Summary

Sour cherry juices were produced from 5 different cultivars with classic processing technology. Regarding primary juice parameters, high results for dry mass (13.7-18.8 °Brix), sugar-free extract (57.5-80 g/L), total acidity (15.8-23.7 g/L), sorbitol (12.1-21.6 g/L) and minerals were found. Secondary plant metabolites were present in high amounts as well. In the sum, 651-1693 mg/L of polyphenols were found by means of HPLC/PDA. Neochlorogenic acid, 3-coumaroyl-quinic acid, chlorogenic acid, and epicatechin were the predominant polyphenols. The quercetin glycosides ranged from 31-109 mg/L. Anthocyanins were identified as cyanidin-3-(2^G-glucosylrutinoside), cyanidin-3-(2^G-xylosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside. A significant decline of the anthocyanin concentrations could be observed during a 6 months storage, which reduced the red colour of the juices drastically. The high polyphenol concentrations were responsible for the high antioxidative capacities of the juices.

Introduction

Among the domestic fruit species, sour cherries (*Prunus cerasus*) belong to the most popular fruits in professional fruit growing as well as in household or hobby horticulture. The popularity is made up by the fruity flavour, the refreshing acidity (*cerasus* = acid) and an intense colour. Apart from the fresh market, the whole fruits are utilized for cannery purposes. In the fruit juice industry, they are processed to purees and juices. Purees are used in the pastries, confectionary or dairy industry; juices or concentrates serve as source for fruit nectars and fruit beverages. Minor applications are the manufacturing of spirits and fruit wines (WILL et al., 2004). Sour cherries contain high amounts of secondary plant substances (GAO and MAZZA, 1995; GOIFFUN et al., 1999) featuring a high antioxidative capacity. For the fruit juice industry, sour cherries represent a valuable raw material. Colour and colour stability of cherry juices are important quality factors for production and marketing purposes. The present paper describes the analytical composition of 5 sour cherry juices deriving from unmixed cultivars with emphasis on secondary plant metabolites.

Material and methods

Plant material

Sour cherries cv. *Schattenmorelle* (S1, mostly planted cultivar in Germany and widely spreaded in all European growing areas), cv. *Gerema* (G2, similar to cv. *Schattenmorelle* with better suitability for mechanical harvest and a better robustness, good fruit quality for cannery purpose, one of the most new planted sour cherry cultivar in Germany), cv. *Ungarische Traubige* (U3, sufficient productivity only under optimal climate conditions, one of the main cultivars in Eastern

Hungary), cv. *Cigány 7* (C4, suitable for mechanical harvest, mostly for juice and processing purpose, low spreading out of Hungary), and cv. *Stevnsbaer Birgitte* (SB5, Danish selection, most important cultivar in Denmark, moderate productivity, mostly for juice and processing purpose, known for high juice quality) were cultivated from the department of Fruit Growing, DLR Rheinpfalz (Oppenheim).

Fruit growing data

The sour cherry trees were sited around the city of Ingelheim, a fruit and wine growing area in Rhineland-Palatinate in the southwest of Germany. The geographical position was 50° N 8° E at an altitude of 220 m. The soil type was a sandy loam (pH 7.1), which was not irrigated. The trees were planted in an experimental orchard especially for evaluations of mechanical harvest. Planting distances were 5x4 m. Each cultivar was planted with 9 trees in 3 replications. The region shows typical conditions for cherry growing with moderate climate (long-term average 10.2 °C; 2003 11.2 °C), and only little rain (long-term average 537 mm; 2003 388 mm). In 2003 there was an aride, hot summer with a sunshine periode of 1627 hours. Especially in temperature and precipitation, the weather conditions differed from the long-term averages.

Harvest, transport and fruit processing

Cherries were hand-picked (July 7th/8th 2003) into 15 kg plastic bins. The time between harvesting and processing took about 3 hours. At delivery, the fruits were mature, not injured, at outdoor temperature (24 °C), without any mold and in a proper, ready-to-eat shape. Lots of 250 kg were crushed in a stainless steel roller mill (Amos, Germany). The gap width was adjusted to 0.5 cm resulting in a limited number of destroyed pits (below 5 %). The resulting cherry mash was heated to 85 °C in a tube heat exchanger and hot-extracted in a horizontal press (Bucher HP-L, Switzerland). The raw juice was separated (Westfalia, Germany), pasteurised and finally hot filled into 0.75 L glass bottles.

Sampling and controlled storage

Pasteurised juices were stored in 0.75 L glass bottles for 6 months at 20.0 °C in a temperature-controlled incubator. Samples were taken monthly and frozen at -20 °C. The control samples of the storage experiments were taken directly after pasteurisation and frozen.

Analytical methods

IFU-methods (International Fruit Juice Union) were used for the routine parameters. Brix and density were measured by means of digital refractometry and densitometry. Total titratable acid, ascorbic acid, and pH were determined potentiometrically. Sugars were analyzed with enzymatic kits; organic acids were determined by means

of HPLC/UV. Nitrate and phosphate were assayed photometrically, sulphate and ash gravimetrically. Cations were analyzed by AAS, trace elements by ICP-OES. The TEAC assay on antioxidative capacity was performed according to RE et al. (1999), and total phenolics were assayed with the Folin-Ciocalteu-method (SINGLETON and ROSSI, 1965). Sugar alcohols were determined by HPAEC/PAD on a Dionex Bio-LC. Samples were 1:10 diluted, filtered and analysed on a 250/4 mm MA-1 column guarded with 50/4 MA-1 precolumn (Dionex, Idstein, Germany). The eluent was 612 mmol/L NaOH. Quantitation was carried out using peak areas from external calibration. Colloid concentrations were determined by size-exclusion chromatography on a Pharmacia FPLC-system (ZIMMER et al., 1992). Colourless polyphenols were separated on an HP 1090 HPLC/PDA system equipped with a 250/4 Phenomenex aqua column (Aschaffenburg, Germany) protected by a corresponding 50/4 guard column (SCHIEBER et al., 2001). Quantification was carried out using peak

areas from external calibrations with commercially available reference substances. Standards of neochlorogenic and 4-coumaroylquinic acid were isolated by preparative RP-chromatography of *Sorbus domestica* and apple extracts, respectively. 3- and 5-coumaroylquinic acid were calculated with the calibration curve based on 4-coumaroylquinic acid. The red colour was determined photometrically at 520 nm in 2 mm pathway cuvettes after 1:1 dilution of the juices with distilled water.

HPLC-PDA/electrospray ionization (ESI)-MS of anthocyanins

Mass detection and MSⁿ experiments were performed on a ThermoFinnigan Surveyor HPLC system (MS pump, PDA, AS) coupled to a ThermoFinnigan LCQ Advantage Max mass spectrometer (Dreieich, Germany) equipped with an ESI source and an ion trap mass analyzer.

Tab. 1: Basic analytical parameters of the sour cherry juices: S1, cv. *Schattenmorelle*; G2, cv. *Gerema*; U3, cv. *Ungarische Traubige*; C4, cv. *Cigány 7*; and SB5 cv. *Stevnsbaer Birgitte*.

	unit	S1	G2	U3	C4	SB5
relative density	20/20	1.0546	1.0607	1.0681	1.0761	1.0756
brix	°	13.71	15.41	16.89	18.62	18.81
conductivity	µS/cm	3860	4170	4080	4660	4490
total extract	g/L	141.9	157.8	177.2	198.2	196.9
sugar free extract	g/L	57.5	65.1	62.4	79.1	80.0
glucose	g/L	48.4	53.3	63.8	67.0	62.0
fructose	g/L	38.9	44.4	52.2	53.7	53.0
pH		3.07	3.32	3.30	3.19	3.15
total acid (expressed as malic acid, pH 8.1)	g/L	21.2	15.8	16.3	23.7	23.7
acetic acid	g/L	0.05	0.05	0.06	0.05	0.06
ascorbic acid	mg/L	116	83	50	142	56
lactic acid	mg/L	11	16	15	16	21
malic acid	g/L	23.5	18.8	20.2	27.2	26.6
citric acid	g/L	<0.1	<0.1	<0.1	<0.1	<0.1
shikimic acid	mg/L	11	16	15	16	21
fumaric acid	mg/L	<0.2	<0.2	<0.2	<0.2	<0.2
sulphate	mg/L	36	10	87	72	73
phosphate	mg/L	478	541	640	718	804
nitrate	mg/L	2	5	3	2	3
colloids	mg/L	1862	1859	925	2743	2358
ash	g/L	3.98	4.70	5.23	5.79	6.43
inositol	mg/L	106	189	70	38	65
erythritol	mg/L	5	5	6	3	3
xylitol	mg/L	8	8	12	10	11
sorbitol	g/L	12.1	17.6	12.3	21.6	19.5
potassium	mg/L	2229	2575	2625	3291	3345
calcium	mg/L	160	228	244	192	224
magnesium	mg/L	107	143	147	128	122
sodium	mg/L	1	3	4	2	4
total phenolics	mg/L	3054	3702	1707	2577	5498
TEAC	mmol/L Trolox	32.6	27.2	16.9	20.8	44.4

The whole system was controlled by Xcalibur software. For anthocyanins, the mass spectrometer was operated in the positive mode with the following conditions: source voltage 4.5 kV; capillary voltage 32 V; capillary temperature 275 °C; collision energy 30 (MS²) and 33 % (MS³). Chromatographic separation was achieved on a 125 x 2 mm i.d., 5 µm RP-Reprosil-Pur 120 ODS-3 column (Maisch, Germany) protected with a guard column of the same material in a cartridge holder. Injection volume was 5 µL, elution conditions were: 200 µL/min flow rate at 25 °C; solvent A was water/trifluoroacetic acid (100:1, v/v); solvent B, acetonitrile/water/TFA (50:50:1, v/v/v); 1 min isocratic conditions with 10 % B, linear gradient from 10 to 25 % B in 24 min, followed by washing with 100 % B and re-equilibrating the column. During the first 7 min of the run the effluent was drained into the waste before switching the divert valve to the ion source to avoid contamination of the MS with sugars, organic acids, and minerals from the juices. Quantitation was carried out using peak areas (520 nm) from external calibration via the reference substance cyanidin-3-glucoside.

Results and discussion

Basic juice parameters

The pasteurised samples were analyzed for routine parameters. There were large dry mass differences in the different varieties (Tab. 1). The results were ranged from 13.71 (S1) to 18.81 °Brix (SB5). Due to a dry vegetation period in Germany, the results for total (142-198 g/L) and sugar-free extract (57.5-80 g/L) were rather high. Among the sugar alcohols, sorbitol contributed with 12.1 - 21.6 g/L to the sugar-free extract. Inositol was present in minor amounts, erythritol and xylitol were only present in trace amounts. The total acid concentrations (15.8-23.7 g/L) were nearly exclusively made up from malic acid (18.8-27.2 g/L); and the natural ascorbic acid concentrations were between 50 and 142 mg/L. Due to the optimal harvest conditions and the fast processing, acetic and lactic acid as spoiling indicators could be kept very low. The juices also contained 925-2743 mg/L of colloids, mainly composed of pectic fragments. The high concentrations of ash and minerals such as potassium (2229-3345 mg/L), calcium (160-244 mg/L) and magnesium (107-147 mg/L) underline the positive nutritional properties of sour cherry juices. Boron, copper, iron, manganese and zinc were the most important trace elements (Tab. 2). The high polyphenol concentrations (1707-5498 mg/L, Folin) were responsible for the high antioxidative capacities. Expressed in TEAC-values, we found 16.9-44.4 mmol/L Trolox equivalents. Regarding the juice ingredients monitored by the sugar-free-extract, cv. *Stevnsbaer Birgitte* was on the top of the 5 cultivars.

Tab. 2: Trace elements in sour cherry juices (mg/L)

	S1	G2	U3	C4	SB5
Al	0.4	2.5	0.6	0.5	0.3
Ba	0.1	0.2	0.1	0.3	0.3
B	4.2	8.0	6.8	7.4	7.7
Cu	1.1	1.1	0.5	1.2	0.9
Fe	3.0	3.4	2.2	2.8	2.6
Mn	1.2	1.4	1.4	1.5	1.4
Ni	0.1	0.2	0.1	0.1	0.1
Zn	1.0	0.9	0.6	0.7	0.6

Colourless polyphenolic compounds

Polyphenols listed in Tab. 3 were identified properly and quantitated on the basis of external calibrations. In the sum, 651-1693 mg/L of polyphenols were found by means of HPLC/PDA. As demonstrated in Tab. 3, the composition was very inconsistent throughout the 5 varieties. Neochlorogenic acid (125-995 mg/L), 3-coumaroylquinic acid (91-554 mg/L), chlorogenic acid (81-183 mg/L), and epicatechin (17-368 mg/L) were the predominant polyphenols. Neochlorogenic acid was also found in various other fruits. It is the main polyphenol of plums and sloes, and it also appears in the fruits of the service tree (*Sorbus domestica*, WILL et al., 2000; ÖLSCHLÄGER et al., 2004), quince, black currant and aronia. Neochlorogenic acid and 3-coumaroylquinic acid had already been described earlier as main polyphenols of sweet cherries (MÖLLER and HERRMANN, 1983; GAO and MAZZA, 1995). Sour cherries are well-known for their quercetin contents (HERRMANN, 1996). The fraction of quercetin glycosides was present from 31-109 mg/L. Referring to antioxidative capacity, total phenolics and the colourless polyphenols analysed monthly, no significant alterations could be detected over 180 days storage at 20 °C.

Tab. 3: Polyphenols in cherry juices (mg/L, n.d. = not detected)

	S1	G2	U3	C4	SB5
procyanidin B1	41	24	12	25	92
catechin	34	34	4	21	77
epicatechin	139	50	17	119	369
neochlorogenic acid	200	995	930	126	181
3-coumaroylquinic acid	459	241	91	236	555
5-coumaroylquinic acid	20	81	70	12	30
chlorogenic acid	85	183	100	82	148
quercetin-3-rut	50	24	17	14	29
quercetin-3-gal	4	3	2	n.d.	4
quercetin-3-ara	16	12	5	n.d.	7
quercetin-3-rha	38	45	18	18	35
sum HPLC	1088	1694	1265	652	1526

Anthocyanins

In the HPLC-chromatograms (Fig. 1) we found 8 peaks with UV-spectra similar to anthocyanins. Five of them, representing >90 % of the total peak area, were identified with the assistance of UV/VIS-spectra, MSⁿ and literature data (JEFFREY et al., 1999) as cyanidin-3-(2^G-glucosylrutinoside), cyanidin-3-(2^G-xylosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside. Furthermore a pyranoanthocyanin probably resulting from the reaction of cyanidin-3-(2^G-glucosylrutinoside) with pyruvic acid was detected. This anthocyanin-pyruvic acid adducts are already known from red wine (SCHWARTZ et al., 2003) and red onions (FOSSEN and ANDERSEN, 2003). They seem to be more stable than their anthocyanin precursors during wine aging (MATEUS and DE FREITAS, 2001). Compared to the PDA-UV-spectra of cyanidin-3-(2^G-glucosylrutinoside), its λ_{\max} was hypsochromically shifted from 515 to 509 nm. In aging red wines or port wines, this may contribute to the development of the orange-red colour (Mateus et al., 2003) The retention times, λ_{\max} , and MS-data are given in Tab. 4.

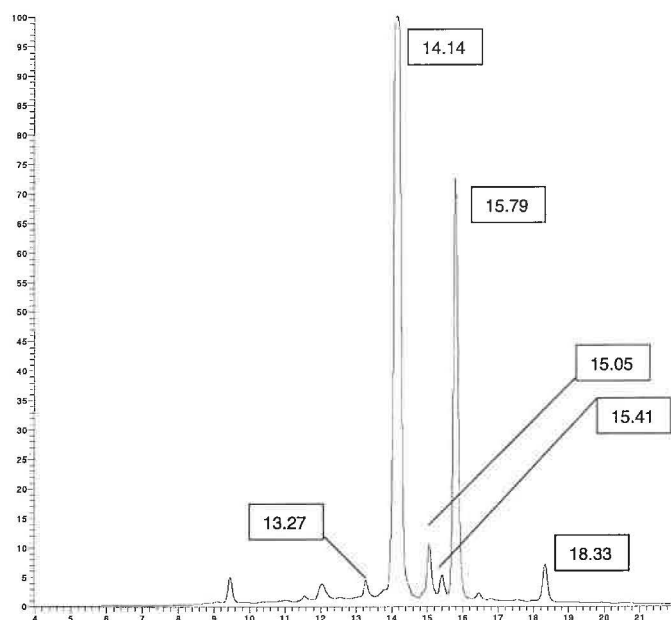


Fig. 1: RP-HPLC chromatogram of anthocyanins from sour cherry juice (cv. Gerema)

Tab. 4: Short characterisation of identified anthocyanins from sour cherry

	RT (min)	λ max (nm)	[M] ⁺ (m/z)	Fragments (m/z)
pyranoanthocyanin	13.27	509	825	355
cyanidin-3-(2 ^o -glucosylrutinoside)	14.14	515	757	611, 287
cyanidin-3-(2 ^o -xylosylrutinoside)	15.05	518	727	581, 287
cyanidin-3-glucoside	15.41	515	449	287
cyanidin-3-rutinoside	15.79	515	595	287
peonidin-3-rutinoside	18.33	517	609	301

Due to the lack of corresponding reference substances, the identified anthocyanins were quantitated via a calibration curve determined with commercially available cyanidin-3-glucoside. Response factors could not be determined. For this reason the concentration results listed in Tab. 5 might be possibly charged with an unknown mis-

Tab. 5: Anthocyanin concentrations in freshly pasteurised sour cherry juices (mg/L, expressed as cya-3-glc)

	S1	G2	U3	C4	SB
cyanidin-3-(2 ^o -glucosylrutinoside)	182	207	155	185	218
cyanidin-3-(2 ^o -xylosylrutinoside)	13	17	11	18	26
cyanidin-3-glucoside	11	22	5	9	18
cyanidin-3-rutinoside	120	108	84	111	135
peonidin-3-rutinoside	7	17	8	13	13
sum HPLC	334	371	262	336	410

take. However, the proportions are quite certainly in the right perspective. Anthocyanin concentrations ranged from 262-410 mg/L in the different juices, cv. *Stevnsbaer Birgitte* again being on the top. Cyanidin-3-(2^o-glucosylrutinoside) and cyanidin-3-rutinoside were the major peaks, representing about 80 % of the total peak area. Peonidin-3-rutinoside, a major anthocyanin in fresh plums and plum juices, was only present in minor amounts. In opposite to the colourless polyphenols, the peak ratio of the anthocyanins was rather regularly in all cultivars. Referring to the order presented in Tab. 5, the ratio was on average 55/5/4/33/3. The pyranoanthocyanin is not commercially available, and because of structural differences to anthocyanins it was not quantitated via the cya-3-glc calibration. Although pyranoanthocyanins are considered as aging pigments of anthocyanins, there was no significant increase of the peak areas during storage.

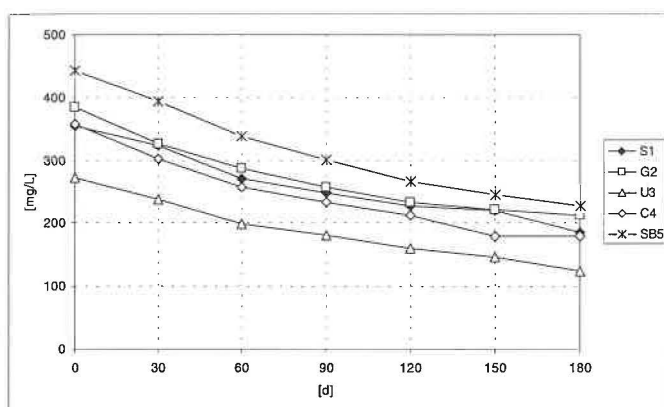


Fig. 2: Decline of anthocyanin concentrations in pasteurised sour cherry juices during 180 days of storage at 20 °C (mg/L, sum HPLC)

During storage of the pasteurised samples at 20 °C in the dark over a period of 6 months, the anthocyanins were not as stable as the polyphenols. About half of the anthocyanins got lost during storage, and a significant decline of the concentrations could be observed. Fig. 2 presents the results expressed as sum of total anthocyanins in mg/L. All individual substances were affected equally. The 50 % loss of the anthocyanins was running in line with a 30-50 % loss of colour expressed as photometric absorption at 520 nm and 10 mm pathway (Fig. 3).

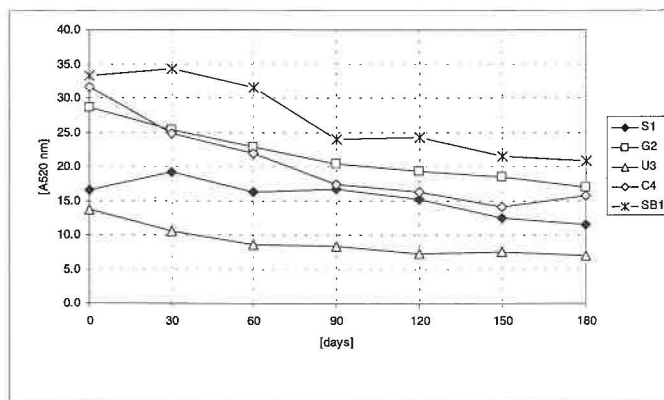


Fig. 3: Colour losses of sour cherry juices during 180 days of storage at 20 °C

In conclusion, sour cherry juices were rich in primary and secondary plant ingredients. One of the most remarkable features was the high content of polyphenols and anthocyanins, resulting in high antioxidative capacities. The 50 % decline of the anthocyanins within 180 days at 20 °C seemed to be a function of temperature. During storage at 4 or 10 °C, the losses were essentially lower (unpublished data). For industrial purposes, cold storage is advisable to keep the nutritional value near the starting point at processing.

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