ANTHOCYANINS, TOTAL PHENOLICS AND ANTIOXIDANT CAPACITIES OF COMMERCIAL RED GRAPE JUICES, BLACK CURRANT AND SOUR CHERRY NECTARS

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Introduction

Secondary plant metabolites, like polyphenols, are supposed to have protective effects against several chronic diseases. They play an important role in the human diet, because they are common constituents of almost every plant-derived food of daily use such as fruits, vegetables, cereals, coffee, tea and wine [1]. Anthocyanins are one important subclass of the widespread family of flavonoids, which these health promoting properties are attributed. The red or blue pigments are mainly found in fruits, like berries, and their corresponding red-coloured fruit juices [2].

Though, the consumption of anthocyanins in the German population is very low. It is estimated that an average of only 2.7 mg per person daily are ingested whereas in the U. S. the daily intake is between 180 and 200 mg [3, 4]. In addition the bioavailability of anthocyanins in humans is supposed to be very low, so there should be an interest to increase the anthocyanin concentration in food products during processing [5].

Commercially available juices and nectars, like red grape juice, black currant and sour cherry nectar are usually stored for a while, until the product reaches the consumer. As a result the concentration of monomeric anthocyanins can be rather low. There is not much analytical information on anthocyanin concentrations of commercial red juices or nectars, so this paper reports on anthocyanin contents of commercial red grape juice, black currant and sour cherry nectars available in the German retail sale.

MATERIALS AND METHODS

In March 2011 twenty-five commercial red grape juices, seventeen black currant nectars and eighteen sour cherry nectars were analysed at the Geisenheim Research Center. Anthocyanin concentration (sum HPLC), total phenol content (Folin-Ciocalteu), antioxidant capacity (TEAC values) and °Brix were determined. The analytical methods are described elsewhere [6, 7].

RESULTS

The soluble solids in grape juice ranged from 16.4 to 18.7 °Brix, in black currant nectars from 13.2 to 16.6 °Brix and in sour cherry nectars from 12.4 to 16.9 °Brix. Mean °Brix was the highest in grape juice (17.1 °Brix ± 0.6 SD) compared to black currant and sour cherry nectar with 14.4 ± 1.1 SD and 14.8 °Brix ± 1 SD (Tab. 1).

TAB. 1: SOLUBLE SOLIDS (°BRIX) OF 25 GRAPE JUICES, 16 BLACK CURRANT NECTARS AND 18 SOUR CHERRY NECTARS

°Brix	Grape juice	Black currant nectar	Sour cherry nectar
n	25	16	18
Median	17,0	14,0	14,9
Mean	17,1	14,4	14,8
Standard deviation	0,6	1,1	1,0
Min	16,4	13,2	12,4
Max	18,7	16,6	16,9

RED GRAPE JUICES

The grape juice anthocyanins are mainly composed of the 3-O-monoglucosides of malvidin, peonidin, cyanidin, petunidin and delphinidin (Fig. 1) whereas pelargonidins are not present in grapes. The anthocyanin composition in 24 of 25 grape juices was almost identical. The major anthocyanin was malvidin-3-glucoside.

In 24 grape juices we detected also a number of acylated anthocyanins (delphinidin-3-(6"-O-acetyl)glucoside, petunidin-3-(6"-O-acetyl)glucoside, malvidin-3-(6"-O-acetyl)glucoside). In one grape juice (no. 6) no acylated anthocyanins were found, but three different diglucosides: petunidin-3,5-diglucoside, peonidin-3,5-diglucoside and malvidin-3,5-diglucoside, which is due to the grape variety the juice was made of [8] (Fig. 2, data not shown of grape juice no. 6). The mean anthocyanin concentration in grape juices was 35.4 mg/L (± 35 mg/L SD). The highest content was 197.5 mg/L in sample no. 6. The anthocyanin concentration as sum HPLC in grape juices was calculated as malvidin-3-glucoside.

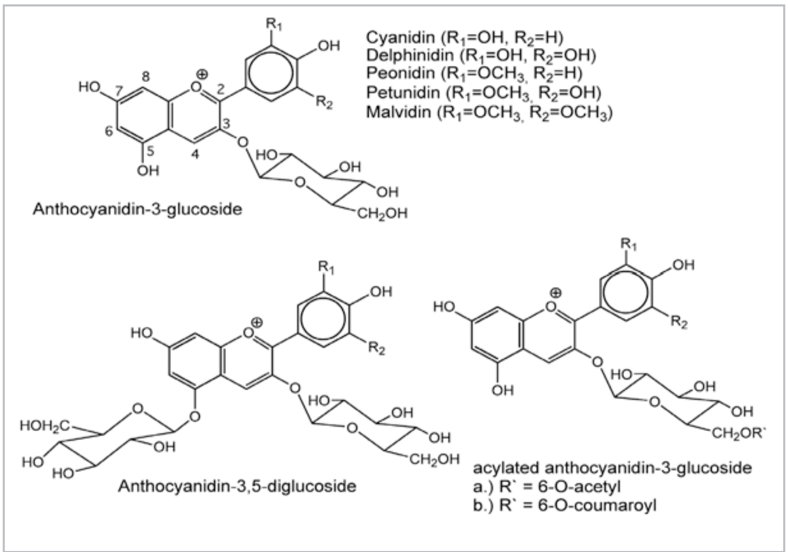

Fig. 1: Chemical structures of the major anthocyanins in red grapes

TAB. 2: PARAMETERS FOR ANTHOCYANINS, TOTAL PHENOL CONTENT AND TEAC VALUES IN 25 RED GRAPE JUICES

	Anthocyanins [mg/L]	Total phenolics [mg/L]	TEAC [mmol Trolox/L]
Median	26,3	978	8,4
Mean	35,4	1060	9,8
Standard deviation	35	378	4
Min	14,1	566	4,8
Max	197,5	2593	26,0

TAB. 3: PARAMETERS FOR ANTHOCYANINS, TOTAL PHENOL CONTENT AND TEAC VALUES IN 17 BLACK CURRANT NECTARS

	Anthocyanins [mg/L]	Total phenolics [mg/L]	TEAC [mmol Trolox/L]
Median	97	1588	16,8
Mean	120	1568	17,2
Standard deviation	97	316	3,2
Min	31	1053	11,8
Max	450	2313	23,7

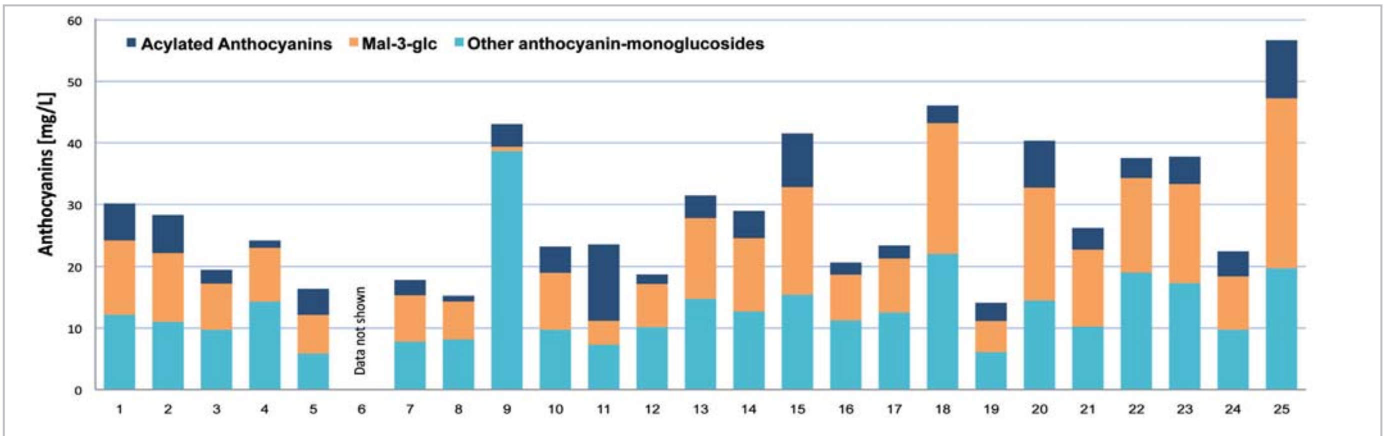

Fig. 2: Anthocyanin composition and content [mg/L as malvidin-3-glucoside] of different red grape juice (n=25); Acylated Anthocyanins = delphinidin-3-(6"-O-acetyl)glucoside, petunidin-3-(6"-O-acetyl)glucoside, malvidin-3-(6"-O-acetyl)glucoside, other anthocyanin-monoglucosides = delphinidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside

Total phenolics (Folin) in grape juices ranged from 566 mg/L to 2593 mg/L (mean 1060 ± 378 SD) and TEAC values from 4.8 to 26 mmol Trolox/L (mean 9.8 ± 4 SD). Grape juice no. 167 had also the highest content of total phenolics and TEAC value (Tab. 2).

BLACK CURRANT NECTARS

Black currant juices possess four major anthocyanins: delphinidin-3-rutinoside, cyanidin-3-rutinoside delphinidin-3-glucoside, and cyanidin-3-glucoside in a ratio of about 50:30:10:1 (Fig. 3). Acylated forms are also present as minor peaks. In 17 black currant nectars the concentrations can be rather low, due to dilution and ageing. We detected four different anthocyanins (Fig. 4): delphinidin-3-rutinoside as the major anthocyanin, delphinidin-3-glucoside, cyanidin-3-rutinoside and petunidin-3-(6"-O-coumaroyl)glucoside [9].

We detected also cyanidin-3-glucoside in black currant nectars, but the specific peak was overlaid by the major anthocyanin peak of delphinidin-3-rutinoside. The anthocyanin concentration ranged from 31 mg/L (no. 31) to 450 mg/L (no. 39) (mean 120 ± 97 SD). The anthocyanin concentration as sum HPLC in black currant nectars was calculated as cyanidin-3-glucoside (Tab. 3).

Mean of total phenolics was 1568 mg/L (± 316 SD) and of TEAC values 17.2 mmol Trolox/L (± 3.2 SD). The highest total phenolic content and TEAC value was detected in black currant nectar no. 39 (2313 mg/L, 23.7 mmol Trolox/L).

SOUR CHERRY NECTARS

The pigments in sour cherries are mainly composed of cyanidin derivatives (Fig. 5).

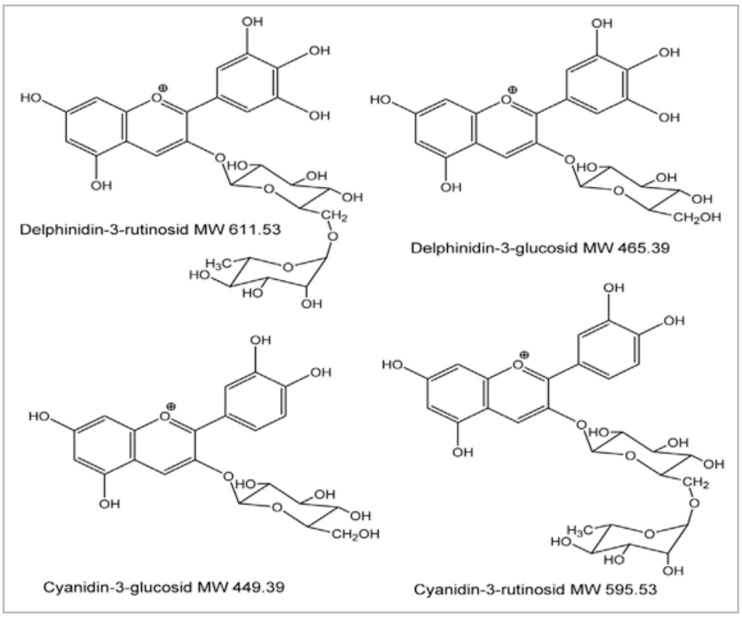

Fig. 3 Chemical structures of the major anthocyanins in black currants

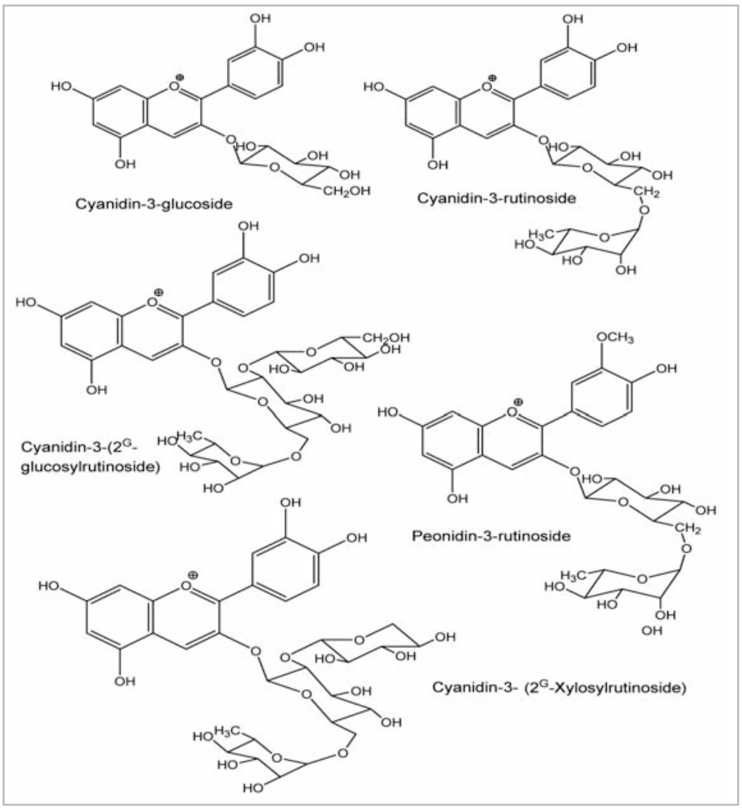

Fig. 5 Chemical structures of the major anthocyanins in sour cherries

TAB. 4: PARAMETERS FOR ANTHOCYANINS, TOTAL PHENOL CONTENT AND TEAC VALUES IN 18 SOUR CHERRY NECTARS

	Anthocyanins [mg/L]	Total phenolics [mg/L]	TEAC [mmol Trolox/L]
Median	46	1132	10,3
Mean	52	1220	11,2
Standard deviation	35	333	4,0
Min	12	800	6,3
Max	158	1940	17,3

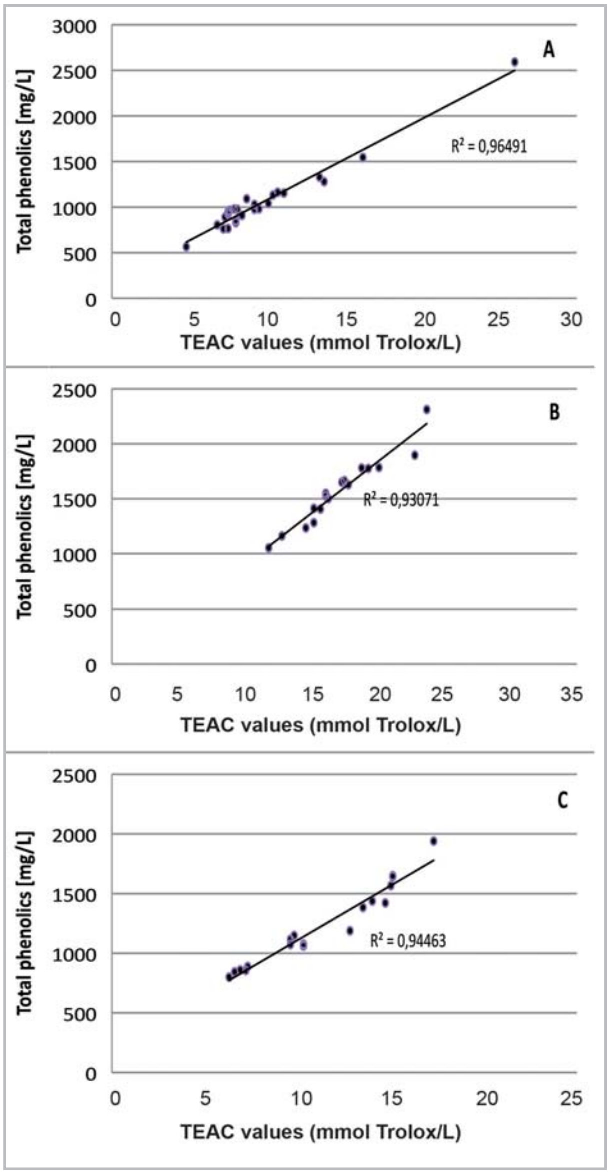

Fig. 7: Correlation between total phenolics [mg/L] and TEAC values [mmol Trolox/L] in 25 red grape juices (A), 17 black currant nectars (B) and 18 sour cherry nectars (C)

The major anthocyanin in sour cherry nectar was cyanidin-3-(2-glucosyl)rutinoside, followed by cyanidin-3-rutinoside, cyanidin-3,5-diglucoside and 5-carboxycyano-cyanidin-3-(2-glucosyl)rutinoside (Fig. 6). The last mentioned pigment is not native but is produced by processing and storage [7, 10]. Sour cherry nectars had a mean anthocyanin content of 52 mg/L (± 35 SD) and ranged from 12 mg/L (no. 55) to 158 mg/L (no. 52). The anthocyanin concentration as sum HPLC in sour cherry nectars was calculated as cyanidin-3-glucoside (Tab. 4).

Total phenolics were in a concentration range of 800 mg/L to 1940 mg/L (mean 1220 ± 333 SD). Mean TEAC value of sour cherry nectar was 11.2 mmol Trolox/L (± 4 SD) and ranged from 6.3 to 17.3 mmol Trolox/L.

DISCUSSION

The results show that the anthocyanin profiles of juices and nectars can be clearly attributed to the corresponding fruit variety, which is due to the genetic characteristics of the cultivar. In all three groups of juices and nectars the variation of anthocyanin and total phenol content between different samples was very high. The correlation between total phenolic content and TEAC values was very clear in all three groups of juices (Fig. 7). Such dependence between TEAC values and total polyphenols content is reported in many studies.

The content of antioxidants like anthocyanins and also total phenolics in fruit juice is mainly influenced by four important factors:

- selection of the fruit variety,
- processing
- fruit content of nectars
- storage conditions and ageing

To develop a fruit juice with high content of anthocyanins it is necessary to select the right cultivar. Among the analysed products, grape juice no. 6 is remarkable and was produced from another variety then the remaining juices. This has been demonstrated due to the anthocyanin composition (3,5-diglucosides). This relationship applies not only for grape products but also for juices made of black currant and sour cherry. Dietrich et al. reported a mean of 2599 mg/L anthocyanins and 6082 mg/L total phenolics in different black currant juices (100 % fruit content, 16 °Brix) [13]. Bonerz et al. detected a range of 569-858 mg/L anthocyanins and 2704-4998 mg/L total phenol content in juice from five different sour cherry cultivars (15.3 °Brix) [7]. The comparison with these results shows that the contents of bioactive compounds in commercial juices and nectars are rather low.

Oh et al. analysed different juices from Korean grape varieties and found a range of 184-1044 mg/L anthocyanins. Mullen et al. reported a range of 13-133 mg/L anthocyanins in commercial red grape juice in the UK, which is similar to the contents we detected [11].

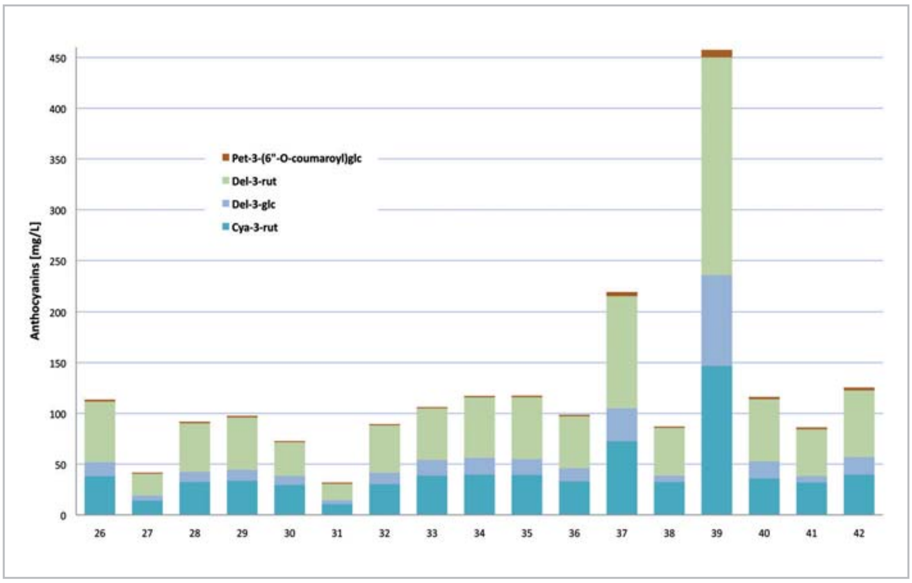

Fig. 4: Anthocyanin composition and content [mg/L as cyanidin-3-glucoside] of different black currant nectars (n=17); Pet-3-rut = petunidin-3-(6"-O-coumaroyl)glucoside, Del-3-rut = delphinidin-3-rutinoside, Del-3-glc = delphinidin-3-glucoside, Cya-3-rut = cyanidin-3-rutinoside

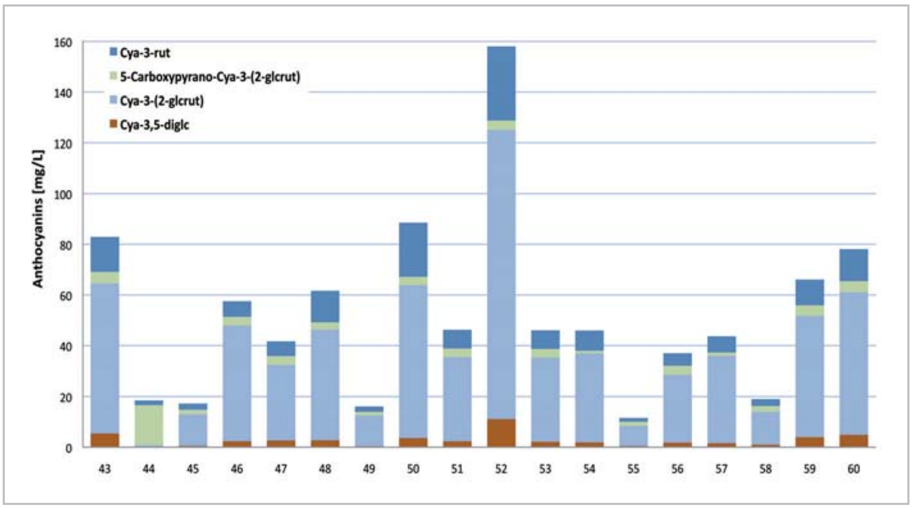

Fig. 6: Anthocyanin composition and content [mg/L as cyanidin-3-glucoside] of different sour cherry nectars (n=18); Cya-3-rut = Cyanidin-3-rutinoside, Cya-3,5-diglc = Cyanidin-3,5-diglucoside, Cya-3-(2-glcrut) = Cyanidin-3(2-glucosylrutinoside), 5-Carboxypyrano-Cya-3-(2-glcrut) = 5-Carboxypyrano-Cyanidin-3-(2-glucosylrutinoside)

The new grape cultivars Dakapo and Accent are worth mentioning, because they have anthocyanin concentrations over 1000 mg/L. Fig. 8 shows the difference between the commercially available juices and the juices from grape cultivars Dakapo and Accent in 2009, 2010 and 2011. Furthermore it shows the anthocyanin content of commercial red grape juices in 2010 (n=20). Compared to 2011 the concentration of monomeric anthocyanins was lower than in 2010 (sum HPLC 15 mg/L ± 9 SD). In commercial red grape juices, it seems to be common practice to blend red and white grape juices, so therefore the anthocyanin concentration is lower. Pour-Nikfardjam et al. [12] could show that the ratio between

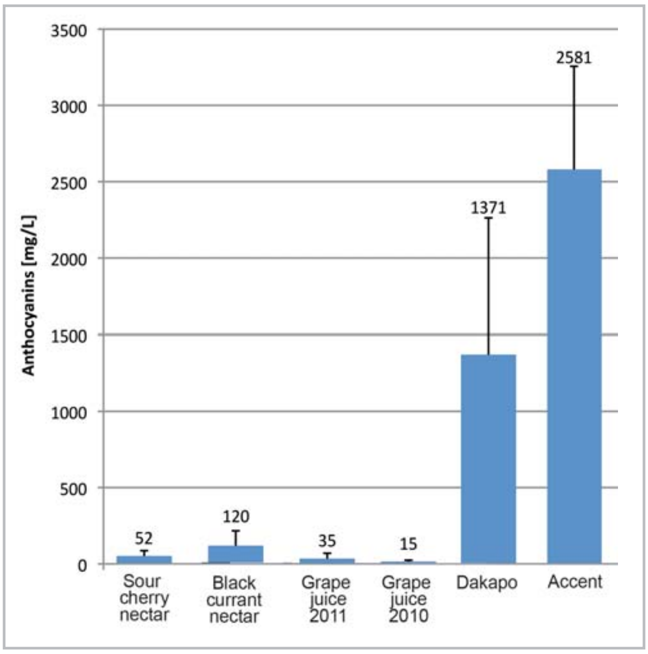

Fig. 8: Mean anthocyanin content [mg/L] in sour cherry nectars (n=18), black currant nectars (n=17), grape juice 2011 (n=25), grape juice 2010 (n=20), and in juices from grape variety Dakapo and Accent in 2009, 2010 and 2011 (both n=3)

anthocyanins and non-coloured phenolics (phenolic acids, flavonoids) can be used to differentiate red grape juices made from 100% red varieties from blends between white and red grapes.

The processing method plays also an important role for the content of anthocyanins and polyphenols in juices. For example, clarification influences the phenolic content in juice very strong, because these compounds are partially bound to cloud particles [13]. As anthocyanins are not stable at high temperatures, pasteurisation of juice plays also an important role particularly the time until they reach room temperature after pasteurisation at 85 °C. For example Kechinski et al. demonstrated that degradation of anthocyanins follows a first-order reaction kinetic, so as higher the temperature and heating time as lower the stability of anthocyanins [14].

The storage conditions influence the anthocyanin concentration in fruit juices exceedingly. Previous studies showed that only about 50 % of anthocyanins remain in the juice after 6 months storage at 20 °C [15]. This correlation applies not only for grape juices, but also for black currant [16] and sour cherry nectars [17]. Storage temperature at 4 °C or lower seems to be optimal to preserve the native antioxidants over a certain period.

CONCLUSION

In comparison to previous studies and data from literature the anthocyanin concentration, total polyphenols content and TEAC values in the commercial juices and nectars seem to be very low. Studies rarely are focused on commercial fruit juice, because anthocyanin research for example in human trials or in cell culture studies usually requires a high content in the beverage which is tested. And if we have a look on the bioavailability of anthocyanins in humans, which is supposed to be lower than 1 %, it is evident to maximise the anthocyanin content in fruit juice to have health benefits. Even if it doesn't seem to be possible for economic reasons to store juice at 4 °C for a short period in the retail sale, it is still feasible to select the right fruit cultivar and to optimise the processing method.

Especially in the case of red grape juices the contents of anthocyanins is surprisingly low. The reasons could be blends of red and white varieties, selection of the wrong varieties and ageing.

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