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The Effect of Cultivar and cracking on Fruit quality in Black Chokeberry (Aronia melanocarpa) and Hybrids between Chokeberry and Rowan (Sorbus)

Einfluss von Sorte und Platzneigung der Früchte auf die Qualität von Aronia melanocarpa und Hybriden zwischen Aronia und Sorbus

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Summary

Eight genotypes of true black chokeberry (*Aronia melanocarpa*) (TBC) and four *Aronia* × *Sorbus* hybrid (ASH) cultivars were characterised for various fruit quality parameters in 1995 and 1996. Mean berry weight, anthocyanin (Acy) content, soluble solids content (SSC), Acy composition and content of brown compounds (browning index, BI) were studied. In 1996, fruit cracking was prevalent in the material. Cracked fruits had a significantly lower Acy content than intact fruits, whereas BI was unaffected. In general, the TBC genotypes were very similar for most characters except for one genotype belonging to var. *elata*, which had a significantly higher content of anthocyanins in both years. By contrast, the ASH cultivars differed not only from the TBC genotypes but also among each other. All ASH cultivars except 'Titan' had a lower mean berry weight than the TBC genotypes, and all of them except 'Appleberry' had a lower anthocyanin content than the TBC genotypes. 'Appleberry' also had, however, a much higher content of brown compounds, which affects fruit quality negatively. Both 'Appleberry' and 'Burka' were lower in SSC than all other genotypes in 1996. The ratio of the larger TLC-spot to the sum of both spots was the same for 'Appleberry' as for the TBC genotypes, which indicates that they all have the same Acy composition. In contrast, the other ASH cultivars had a larger proportion of the large spot, thus indicating a relatively higher content of cyanidin-3-galactoside compared to cyanidin-3-arabinoside.

Zusammenfassung:

1995 und 1996 wurden Selektionen von acht Genotypen der echten schwarzen Apfelbeere (*Aronia melanocarpa*) (TBC) und vier Aronia × *Sorbus*-Hybriden (ASH) auf verschiedene Parameter der Fruchtqualität charakterisiert. Hierzu gehörten das mittlere Beerengewicht, Anthocyane (Acy), Gehalt an löslichen Feststoffen (SSC), Anthocyan-Zusammensetzung und der Gehalt brauner Verbindungen (Bräunungsindex, BI). 1996 platzten viele Früchte. Diese hatten einen signifikant niedrigeren Gehalt an Anthocyan als intakte Früchte, während der BI nicht beeinträchtigt war. Im allgemeinen waren sich die TBC-Genotypen in den meisten Eigenschaften sehr ähnlich, nur einer der var. *elata* hatte in beiden Jahren einen signifikant höheren Gehalt an Anthocyan. Im Gegensatz dazu unterschieden sich die ASH-Sorten nicht nur von den TBC-Genotypen, sondern auch untereinander. Alle ASH-Sorten, außer 'Titan', hatten ein geringeres mittleres Beerengewicht und außer 'Appleberry' einen niedrigeren Anthocyangehalt als die TBC-Genotypen. 'Appleberry' hatte aber auch einen viel höheren Gehalt an braunen Verbindungen, die die Fruchtqualität beeinträchtigen. 'Appleberry' und 'Burka' hatten 1996 geringere Anteile löslicher Feststoffe als alle anderen Genotypen. 'Appleberry' und die TBC-Genotypen hatten die gleiche Anthocyan-Zusammensetzung, während die anderen ASH-Sorten einen relativ höheren Gehalt an Cyanidin-3-Galaktosid im Vergleich zu Cyanidin-5-Arabinosid zeigten.

Introduction

Large scale cultivation of black chokeberry (*Aronia melanocarpa* [Michx.] Ell., *Rosaceae*) started in the Soviet Union in the late 1940s, and had reached 17.800 hectares in 1984. The fruits are mainly used for juice and wine production, sometimes mixed with apple juice (KASK 1987). In 1986, a project was initiated at Balsgård with the aim to evaluate the potential for black chokeberry production in Sweden. Black chokeberry is easy to cultivate due to its shrubby growth habit and the berries can be harvested by machine (GÄTKE and WILKE 1991).

Chokeberries, *Aronia*, are native to the eastern North America. HARDIN (1973) distinguished two species: *A. arbutifolia* L. and *A. melanocarpa* (Michx.) Ell. only on fruit colour. KRÜSSMANN (1986a), however, distinguished the species on presence/absence of hairs on the leaves and inflorescences, with *A. melanocarpa* being glabrous. Furthermore, interspecific hybridisation within the genus *Aronia* occurs within the distribution range and produces offspring of intermediate appearance (HARDIN 1973). Hybridisation between *Aronia* and the closely related genus *Sorbus* (rowans and whitebeams) has occurred spontaneously (KRÜSSMANN 1986b) as well as through directed crosses. Cultivars from such directed crosses have been released in Russia (MITCHURIN 1950) and in Canada (HUTTLESTONE 1988). In Russia, black chokeberry orchards are mainly based on seed propagated plants. Due to limited genetic

Table 1. List of the genotypes with country of origin and propagation method used for this trial.
Liste der verwendeten Genotypen mit Ursprungsland und Vermehrungsmethode.

Genotype/cultivar	Origin	Propagation method
True black chokeberry (TBC) genotypes		
'Aron'	Denmark	cutting
'Nero'	Czechoslovakia	cutting
'Viking'	Finland	cutting
var. *elata* Rehd.	Rögle plant nursery, Sweden	cutting
BAr 2 12 30*	selected at Balsgård, Sweden	cutting
BAr 2 13 09*	selected at Balsgård, Sweden	cutting
BAr 2 13 19*	selected at Balsgård, Sweden	cutting
BAr C 11 28**	selected at Balsgård, Sweden	cutting
Aronia X *Sorbus* hybrid (ASH) cultivars		
'Appleberry'	Canada ('Stewart' × *Aronia melanocarpa*)	cutting
'Burka'	Soviet Union (*Sorbus aucuparia* × [*Sorbus aria* × *Aronia arbutifolia*])	in vitro
'Stewart'	Canada (*Sorbus americana* × *Aronia melanocarpa*)	cutting
'Titan'	Soviet Union (*Sorbus aucuparia* × *Aronia melanocarpa*)	in vitro

* derived from seeds from the Soviet Union
** (var. *elata* o.p.) X 'Viking'

variation, seedling populations are sufficiently homogeneous for the establishment of large scale berry orchards (KASK 1987). Restricted genetic variation in cultivated plant material has also been reported by SKVORTSOV and MAITULINA (1982) and JEPPSSON (1999a).

The berry crop of black chokeberry can be used in the processing food industry as a raw material for the production of natural food colourants (PLOCHARSKI and ZBROSSZCZYK 1989, KAACK 1992, MAZZA and MINIATI 1993, STRIGL et al. 1995). The berries contain 490 mg anthocyanins/100g fresh weight and are easily processed without preheating or depectinisation, giving a highly coloured juice with 1000 mg anthocyanins/l (PLOCHARSKI and ZBROSSZCZYK 1989). The anthocyanins (Acy) consist mainly of cyanidin-3-galactoside and cyanidin-3-arabinoside, which are reported to constitute 64 and 29% (OSZMIANSKI and SAPIS 1988) or 69 and 28% of the total content, respectively (STRIGL et al. 1995). Other berry sources commonly mentioned for food colourant production are, besides grape skin, elderberry, cranberry, and black currant (FRANCIS 1989).

Both Acy content and colour quality of juice extracts are fundamental in the evaluation of black chokeberry germplasm for berry production; a high content of anthocyanins and a low content of unfavourable brown compounds are desired. Even the composition of the anthocyanins is important in order to develop a final product with a standardised and defined colour quality. The different anthocyanins are also reported to differ slightly in stability with cyanidin-3-galactoside being more stable than cyanidin-3-arabinoside, at least in cranberry (STARR and FRANCIS 1968). Furthermore, content of soluble solids should be low since it sets the limit for gain in volume decrease when producing juice concentrates.

Fruit cracking decreases fruit quality in cherries (SEKSE 1995) and this physiological disorder has also been observed in black chokeberry. The present investigation was undertaken in order to phytochemically characterise a number of genotypes of black chokeberry and of hybrids between *Aronia* and *Sorbus*, and to study the effect of fruit cracking on fruit quality.

Material and methods

The trial was performed at Balsgård – Department of Horticultural Plant Breeding (56° 07' N, 14° 10'E). Eight cultivars/selections (here referred to as genotypes) from true black chokeberry (TBC) and four cultivars derived from crosses between *Aronia* and *Sorbus* (ASH) were studied (Table 1). Plants of even size, propagated by cuttings, were used except for 'Burka' and 'Titan' which were obtained from in vitro propagation due to rooting problems when using standard cutting propagation methods. The trial was established in the spring of 1995. The experimental design was a randomised block design with four blocks where each block consisted of a single plant of each cultivar.

Berries were harvested when visually estimated to be fully ripe (Sep 4 in 1995 and Sep 13 in 1996, except 'Stewart' which in 1995 was harvested on Sep 18). In 1995 'Titan' did not set any fruits, whereas 'Burka' set fruit only in one block. Consequently, these two cultivars were excluded from the analysis in 1995. Berry weight was estimated as the mean fresh weight of fifty berries. Berry samples were stored at –20 °C until fruit quality analysis.

Juice was produced from thawed berries with a garlic press for measuring the content of anthocyanins, brown compounds and soluble solids. The anthocyanin content was measured by the pH differential method (FULEKI and FRANCIS 1968, WROLSTAD 1976) and expressed as cyanidin-3-galactoside using the molar absorbance of $\varepsilon = 30200$ and molecular weight of 445. The content of brown compounds was measured according to WROLSTAD (1976) and expressed as browning index (BI). Absorbances were recorded with a Shimadzu UV-2101 PC spectrophotometer. The content of soluble solids (SSC) was recorded with a digital refractometer (Atago, PR-100) and expressed as °Brix.

The Acy composition was investigated by thin layer chromatography (TLC) according to NILSSON and TRAJKOVSKI (1977) with modifications. Pigments were extracted in methanol (p.a.) with 1% (v/v) concentrated HCl (p.a. Merck). One-dimensional (TLC) separation on 20 × 20 cm 0.1 mm cellulose plates (Merck) was performed using 1-butanol (Kebo), acetic acid (Merck), and distilled water (90:15:30). The two spots obtained, here assumed to correspond to cyanidin-3-galactoside and cyanidin-3-arabinoside (OSZMIANSKI and SAPIS 1988), were quantified in a CS-scanner (Shimadzu CS 9001PC, supported with software v. 3.01). The relative content of cyanidin-3-galactoside to total Acy content was estimated as the area of the largest spot divided by the sum of the areas of both spots.

In 1996, flower development was quantified every second day as the ratio of open flowers to total number of flowers per cluster. Flowering date was defined as the day when 50% of the flowers were open. In the same year, colour development of the berries was recorded during ripening. On August 9, 16, 23 and 29, berry colouration was scored for each plant from 0 to 5 (0: no black fruits, 1: 1–25% black fruits, 2: 26–50% black fruits, 3: 51–75% black fruits, 4: 76–99% black fruits and 5: 100% black fruits). For each genotype the mean was calculated and these values were plotted versus date. These plots were then used to estimate the mean date of score 3 for each genotype by interpolation.

For each character and each individual plant, the differences for data obtained in 1995 and in 1996, respectively, were calculated and treated as a new character. ANOVA (SuperANOVA v. 1.11) was used (1) to test for differences between genotypes for each year separately, (2) to test whether there were significant differences between years, and (3) to test whether the differences between years varied among the genotypes. In one year (1996), fruit cracking was prevalent and for each plant, samples of intact and cracked fruits were analys-ed for Acy content and BI. The two categories were then compared by using a t-test (matched pairs, SYSTAT, v. 5.2.1).

Table 2. Flowering date and ripening date in 1996.
Blühtermin und Reifedatum 1996.

Cultivar/selection	Flowering date in June	Date in August for 50–75% black berries
'Titan'	3	13
'Burka'	3	27
'Viking'	4	19
var. *elata*	4	19
BArC 11 28	4	20
'Aron'	4	21
'Nero'	4	21
BAr 2 12 30	4	22
BAr 2 13 19	4	22
BAr 2 13 09	4	23
'Appleberry'	5	13
'Stewart'	7	23

Results

All genotypes flowered in the first week of June in 1996. Flowering date was the same for all TBC genotypes, whereas the ASH cultivars varied with 'Titan' and 'Burka' being early, and 'Appleberry' and 'Stewart' being late (Table 2). Date of ripening varied more, with 'Appleberry' and 'Titan' being early, 'Burka' being late and the remainder being intermediate (Table 2). The TBC genotypes were again quite similar and ripened within four days (19–23 Aug). There was no correlation between flowering date and ripening date. 'Appleberry' was the second latest flowering genotype but the earliest ripening.

In both years, all of the TBC genotypes, as well as the ASH cultivar 'Titan', did not differ significantly in mean berry weight, whereas the other three genotypes of hybrid origin had a significantly lower mean berry weight (Table 3).

In 1995, var. *elata* had the highest Acy content of the TBC genotypes, but the differences were small and var. *elata* differed significantly only from the lowest (BAr 2 13 09). However, in 1996 var. *elata* had an Acy content that was significantly higher than that of all TBC genotypes except 'Nero'. No significant differ-

Table 3. Mean values for berry weight, Acy content, SSC, Acy composition and BI in 1995 and 1996, compared with Duncan Multiple Range Test for differences among genotypes. In each column, entries with the same letters are not different at 5% level of significance.
Mittelwertsvergleiche mit dem Multiple Range Test von Duncan für Beerengewicht, Anthocyan (Acy) – und lösliche Feststoffgehalte (SSC), Acy-Zusammensetzung und Bräunungsindex (BI). (Werte mit gleichen Buchstaben in den Spalten sind bei 5% nicht signifikant verschieden.)

Cultivar/ selection	Berry weight (g)		Acy content (mg/l)		SSC (°Brix)		Acy composition (% of largest spot)		BI (1)	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
'Aron'	66.9 a	93.8 a	1040 bc	1530 b	17.7 a	17.3 a	65.3 bc	65.0 d	0.79 b	0.80 b
'Nero'	68.9 a	91.0 a	1020 bc	1590 ab	17.8 a	16.2 ab	64.4 bc	64.2 d	0.54 b	0.74 bc
'Viking'	70.0 a	95.5 a	1150 bc	1490 b	18.3 a	15.7 ab	65.1 bc	65.3 d	0.64 b	0.65 bc
var. *elata*	69.3 a	98.1 a	1400 ab	1970 a	17.8 a	16.4 ab	63.6 c	65.0 d	0.56 b	0.81 b
BAr 2 12 30	61.3 a	95.9 a	1040 bc	1450 b	17.7 a	16.0 ab	66.1 abc	65.2 d	0.51 b	0.60 bc
BAr 2 13 09	81.5 a	98.0 a	880 c	1290 b	16.8 a	15.2 bc	66.9 ab	64.9 d	0.71 b	0.61 bc
BAr 2 13 19	65.8 a	95.7 a	1010 bc	1500 b	16.8 a	16.4 ab	65.4 bc	64.5 d	0.47 b	0.76 b
BAr C 11 28	67.7 a	91.1 a	1020 bc	1460 b	16.5 a	16.1 ab	64.9 bc	63.5 d	0.88 b	0.58 bc
'Stewart'	37.3 b	40.2 b	820 c	720 c	15.8 a	16.4 ab	68.4 a	67.9 c	0.43 b	0.50 c
'Appleberry'	31.4 b	47.5 b	1670 a	1440 b	17.6 a	13.6 cd	66.5 ab	65.5 d	2.25 a	1.45 a
'Burka'	–	59.6 b	–	170 d	–	12.4 d	–	78.6 b	–	0.70 bc
'Titan'	–	99.2 a	–	120 d	–	16.2 ab	–	88.4 a	–	0.57 bc

Table 4. Differences between 1995 and 1996 in grand mean values over all plants tested by use of ANOVA.
Mittelwertsvergleiche der Jahre 1995 und 1996 mit ANOVA für alle geprüften Pflanzen.

Trait	Berry weight (g)	Acy content (mg/l)	SSC (°Brix)	Acy composition (% of largest spot)	BI (1)
Mean 1995	63.1	1090	17.2	65.9	0.80
Mean 1996	85.9	1400	15.8	65.2	0.75
	p<0.001	p<0.001	p<0.001	p=0.01	n.s.

Table 5. Changes in Acy content between years, compared with Duncan Multiple Range Test. Entries with the same letters are not different at 5% level of significance.
Multiple Range Test von Duncan zur Prüfung der Änderung der Anthocyangehalte zwischen den Jahren (Werte mit gleichen Buchstaben unterscheiden sich nicht auf dem 5% Niveau).

Cultivar/selection	Difference in Acy content between 1995 and 1996 (mg/l)
'Aron'	490 a
'Nero'	660 a
'Viking'	360 a
var. *elata*	570 a
BAr 2 12 30	400 a
BAr 2 13 09	410 a
BAr 2 13 19	490 a
BAr C 11 28	430 a
'Stewart'	–255 b
'Appleberry'	–230 b

ences were found among the remainder of the TBC genotypes. The ASH cultivars generally had a lower Acy content than the TBC genotypes except for 'Appleberry' which had the highest Acy content of all genotypes in 1995. However, in 1996, its Acy content was on the same level as the majority of the TBC genotypes. 'Appleberry' had the significantly highest browning index in both years. Among the other genotypes, only 'Stewart' differed significantly in one year (1996) by having a lower BI.

In the analysis of Acy composition, var. *elata* had, in 1995, a lower ratio when the largest TLC spot was compared to both spots, thus indicating a relatively lower content of the galactoside when compared to 'Stewart', 'Appleberry' and BAr 2 13 09. In 1996, however, all of the TBC genotypes and 'Appleberry' had the same ratio. The other three ASH genotypes had a significantly higher ratio.

No variation in SSC was found in 1995, while in 1996 'Burka' and 'Appleberry' had significantly lower values than the other ASH cultivars. Among the TBC genotypes, 'Aron' had a significantly higher SSC than BAr 2 13 09 in 1996.

There were significant differences between years for mean berry weight, Acy content, and Acy composition, but not for SSC or BI (Table 4). In 1995, mean berry weight was lower, SSC was higher and Acy content was lower. The Acy composition varied significantly between the years with the large TLC-spot being proportionally larger in 1995 than in 1996, thus indicating a relatively higher content of the galactoside in 1995. When differences among the genotypes for direction and magnitude of year response were tested, only Acy content varied with a decrease from 1995 to 1996 for 'Appleberry' and 'Stewart' and an increase for the others (Table 5).

Acy content differed significantly between intact and cracked berries, with 1700 mg/l and 1300 mg/l respectively (t-test, p<0.001). The difference in BI was, however not significant (0.76 and 0.68 respectively).

Discussion

Berry weight

Black chokeberry cultivars are generally large-fruited. Hybridisation with *Sorbus*, however, seems to have had a negative effect on berry size except in one case: 'Titan'. Still, a decrease in berry size is not necessarily detrimental in a berry crop intended for industrial processing, provided that the berries can be machine harvested (GÄTKE and WILKE 1991).

Anthocyanin content and browning index

Increased content of anthocyanins is a major aim in black chokeberry breeding. Acy content was 880–1400 mg/l in 1995 and 1290–1970 mg/l in 1996 for the TBC genotypes (Table 3), which is comparable with 1000 mg/l, reported by PLOCHARSKI and ZBROSZCZYK (1989). The genotype var. *elata* differed from the others by having a higher Acy content. This cannot be explained by differences in date of ripening since all TBC genotypes ripened simultaneously. Hybridisation between highly coloured black chokeberry with less coloured rowans results in a decrease in Acy content in the berries which is contrary to the breeding aims. When such a hybrid is backcrossed to black chokeberry, it is, however, possible to select highly pigmented genotypes from the progeny [cf. 'Appleberry' was selected from the progeny of ('Stewart' X black chokeberry) (HUTTLESTONE 1988)].

Browning of fruit products is detrimental to the appearance since it results in an undesirable colour tinge. The high rate of anthocyanins to brown compounds, at least in comparison with elderberry, makes black chokeberry a high quality raw material for the production of natural food colourants (PLOCHARSKI et al. 1989). Browning is partly caused by enzymatic degradation of phenolic compounds by enzymes. Potential quality losses due to browning should be taken into account by fruit breeders even if routine testing for enzyme activities in the seedlings is not recommended. Evaluation should instead be based on observations of the symptoms, i.e. fruit colour changes (SISTRUNK and MOORE 1983). Consequently, browning is one of the target traits to investigate when dealing with raw material for production of natural food colourants. Among the genotypes investigated, all had

a similar BI except 'Appleberry', which had a two- to threefold higher BI. The higher content of anthocyanins in 'Appleberry' did not compensate for the quality loss due to browning.

It is favourable to have a high ratio of Acy content to SSC to facilitate the processing of concentrates. All the TBC genotypes and 'Stewart' were similar in SSC. In 1996, 'Appleberry' and 'Burka' had significantly lower values, suggesting that hybridisation with *Sorbus* may have lead to a decrease in SSC.

Anthocyanin composition

Acy composition in grapes (BAKKER and TIMBERLAKE 1985) and black chokeberry (JEPPSSON 1999b) is reported to be cultivar specific and relatively stable in different environments. The TBC genotypes were very similar in Acy composition, at least in 1996, which reflects their general homogeneity. In 1995 however, var. *elata* differed from BAr 2 13 09 by having proportionally less cyanidin-3-galactoside. This difference together with the significant superiority in Acy content of var. *elata* proves its distinction from the other TBC genotypes. 'Appleberry' had approximately the same Acy composition as the TBC genotypes, whereas the other ASH cultivars varied considerably, suggesting that the use of such hybrids in a breeding program affects the Acy composition and may thereby also affect the colour of the berry juice. The relative content of the galactoside was higher in 1995 but the difference was probably not big enough to contribute to any visible change in colour.

Differences between years

The direction and level of differences in fruit quality parameters between 1995 and 1996 are very consistent with data for the same two years from another black chokeberry trial (JEPPSSON 1999b). Even if the berries in both years were picked when estimated to be fully ripe, differences between years may be partly due to differences in ripening stage at harvest. The lower SSC in 1996 can be explained by a dilution effect since the berry weight was higher in 1996; a higher water uptake in that year may have lead to a lower SSC to fresh weight. This is also supported by the fact that fruit cracking, probably caused by an increased water uptake, was observed in 1996 but not in 1995. In contrast, the Acy content was higher in 1996, clearly showing that SSC and Acy content are determined by different factors.

Fruit cracking

Fruit cracking in cherries is caused by high water uptake resulting in increased turgor pressure acting on the fruit skin from the inside. In addition, osmotically moved water from a wet outer fruit surface causing degradation and thereby weakening of the fruit skin is also believed to play an important role. Fruit cracking in cherries can be reduced by keeping a uniform turgor pressure, achieved by limited and constant water supply, prior to harvest (SEKSE 1995).

For fruits produced for the fresh market, cracking is detrimental because it affects the appearance negatively. For black chokeberry, an industrial berry crop, appearance can be more or less ignored, but the negative effect on fruit quality from cracking should be taken into consideration. The Acy content was significantly lower in cracked fruits. This could be caused by dilution since cracking in black chokeberries, just like in cherries, probably is brought about by a high turgor pressure due to high water uptake. The somewhat lower (not significant) BI for cracked fruits also indicates dilution. Actually, cracking could have been expected to increase browning, since exposure of fruit tissues to oxygen causes browning. In addition, the fruit skin is an effective barrier against diseases and a damaged skin will increase the risk of disease attacks. Since cracking obviously affects fruit quality in black chokeberry, precautions should be taken to prevent it, e.g. artificial irrigation, as suggested for cherry production (SEKSE 1995).

Overall differences among the genotypes

In accordance with previous investigations (SKVORTSOV and MAITULINA 1982, JEPPSSON 1999a), the present study indicates that there is very limited variation among different cultivars/selections of black chokeberry. This suggests that they are all derived from a single accession or from a few closely related accessions. Genetic variability should be further investigated in black chokeberry by means of environmentally independent methods such as molecular markers. There is also an urgent need for inclusion of new germplasm into black chokeberry breeding. Introduction of plant material from native stands may be one way (JEPPSSON 1999a) and hybridisation with *Sorbus*, as shown here, might be another.

Among the TBC genotypes, var. *elata* had the highest content of anthocyanins. Among the ASH cultivars only 'Appleberry' seems to be worth growing for pigment production. The high BI, however, disqualifies 'Appleberry' as a candidate. In conclusion, var. *elata* seems to be the best genotype for the production of anthocyanin-rich juice concentrates.

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