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Effects of cultivar, yield, berry weight, temperature and ripening stage on bioactive compounds of black currants

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Summary

Total anthocyanin, total phenols, ascorbic acid, antioxidant capacity (TEAC) were quantified in fruit juice of 23 black currants cultivars grown at Geisenheim (lat. 49.6°), Germany, in the years 2003-2005. In addition, yield and 100-berry weight were recorded. There were remarkable variations in the phytochemical contents within the cultivars: 2.1-fold for TEAC and total phenols, 3.4-fold for total anthocyanins and even 4.4-fold for ascorbic acid. These differences are genetically determined but also significantly modified by plant physiology and environmental factors. The analysed compounds were negatively correlated with yield and 100 berry weight (TEAC: r = -0.34 and -0.43; total phenols: r = -0.34 and -0.48) while anthocyanins showed a significant negative relationship only with the 100-berry weight (r = -0.38). In contrast, TEAC, total phenol and total anthocyanin were significantly positive correlated with the temperature during the fruit developing phase, expressed as degree days (TEAC: r = 0.36, total phenols: r = 0.33 and total anthocyanins: r = 0.51, respectively). The ascorbic acid content was neither influenced by the plant parameters yield and 100-berry weight nor by degree days. There were also remarkable yearly changes of the analysed parameters within individual cultivars caused by low yield in 2004.

Introduction

In the last decade researchers got an increased awareness on health beneficial effects of a plant-based diet in the prevention of various diseases associated with oxidative stress and some degenerative diseases. Recent studies summarized the current knowledge on antioxidants and their possible beneficial role on human health from epidemiological, *in vitro* and in *vivo* studies in general (among others Beattie et al., 2005; Manach et al., 2005; Williamson and Manach, 2005; Duthie, 2007; McGhie and Walton, 2007) and of black currants in particular (Lister et al., 2002; Karjalainen et al., 2009). Black currants (*Ribes nigrum* L.) are rich in antioxidants when compared to other fruit species (Deighton et al., 2002; Halverson et al., 2002). The major components are the ascorbic acid, a micronutrient which is essential for human health, and polyphenols with anthocyanins as the most abundant (Kähkönen et al., 2001). Within the anthocyanins, cyanidin-3-rutinoside and delphinidin-3-rutinoside predominate, followed by cyanidin-3-glucoside and delphinidin-3-glucoside. The flavonol glycosides of quercetin, myricetin and kaempferol are only present in much lower concentrations (Herrmann, 2001).

Black currants are mainly grown for industrial use like making juices and nectars, jams and other products and only small amounts are consumed freshly. Being native to central and northern Europe and northern Asia (Brennan, 1996), main production area is still in this part of the world.

For many fruit species it has been demonstrated, that there is a great influence of cultivar on antioxidant capacity and phenolic content, whilst for black currant only limited information is available, especially for a series of cultivars grown under the same growing condition. The study of Moyer et al. (2002) reported on the phytochemical content of 32 black currant cultivars grown in Oregon. Likewise, Giné Bordonaba and Terry (2008) analysed 17 cultivars or selections only from the Scotch black currant breeding program. In both studies results are based on fruit matrix. Only a limit number of cultivars were evaluated in most other studies. Therefore, a comparison of the health beneficial components in black currants between cultivars is difficult due different growing conditions and varying analytic methods.

Furthermore, there is limited knowledge on how environmental factors such as temperature and physiological characteristics like fruit size and yield may affect health functional compounds of black currants. A better knowledge of these factors would rise up the possibility to cultivate fruits with high levels of bioactive compounds beneficial for human health.

The aim of our study was therefore to analyse antioxidative capacity (TEAC), phenolics, anthocyanins and ascorbic acid in the juice of 23 black currant cultivars grown under the same conditions and to link the analytic data with environmental and plant parameters.

Material and methods

Plants

23 varieties of black currants (listed in Tab. 2) were grown in an orchard at Geisenheim Research Station (lat. 49.6°N) in rows with a plant distance of 1.50 x 3.00 m. Fertilization, weed control, pest and disease management were done to the common standard of the region. Irrigation was provided when necessary and soil moisture tension exceeded -350 hPa.

Fruit harvest and processing

In 2003 to 2005, fruits from 5 to 8 plants per cultivar were picked when the majority of the berries per plant were sound, fully ripe and regarded as commercially ripe. Overripe fruits were avoided by the natural fruit drop of dull black fruits. All fruits per plot were weighted. Weight of 100 berries was determined as a surrogate for fruit size. After harvest, all fruits per variety were stored in plastic bags at -20 °C. For juice processing (after 2-3 month of storage), up to 5.0 kg fruits per cultivar were crushed in a disc-mill and heated to 50 °C. Mash enzyme was added for a better degradation of pectin. After standing for 2 h, the mash was pressed (HP-2H, Hafico, Fischer Maschinenfabrik, Neuss), filled into 330 mL brown glass bottles and heated to 90 °C for pasteurising.

Ripening stage

For evaluating the influence of ripeness on fruit quality parameters, fruits of three cultivars were harvested at three different ripening stages from the same bushes: semi-ripe (one fourth of the berry surface still red-green), ripe (all berries black and fully ripe) and slightly overripe (some berries are shrivelling or nearly dropping down). For each sampling date, 1.0 to 1.5 kg were harvested per variety in 2005 and 2006. After harvest, fruits were treated as described before.

Analytical methods

Total phenols were analyzed with the Folin-Ciocalteu method (SINGLETON and ROSSI, 1965) modified by RITTER (1994). Results are expressed as (+)-catechin equivalents. The antioxidative capacity (TEAC) was determined according to RE et al. (1999). Data are given as Trolox equivalent in mmol/L. The anthocyanins were analyzed after membrane filtration with HPLC (Dionex, P680 pump, autosampler ASI-100, PDA-100 photodiode array detector) on a Lichrospher 100 RP 18 column (250 x 3 mm, 5 µm) with 4 mm precolumn. Flow rate 0.5 mL/min, 25°C, 20µL injection volume. Gradient elution was water/acetonitile/ phosphoric acid (94/4/2 v/v and 48/50/2 v/v), respectively. The anthocyanins were calculated as cyanidin-3-glucoside (Extrasynthèse, France). Ascorbic acid was analyzed potentiometrically according to IFU method No. 17 (Anonym, 1964).

Weather and calculation of degree days

The weather conditions at ripening time were quite different in the three years examined. Especially, average temperature between May and July was lower in 2004 in comparison to the other two years (Tab. 1). For analysing the influence of temperature in different years on the content of bioactive compounds in the fruit, temperature was transformed into thermal units according to LANTIN (1986). In short: a scheme gives two values for every daily minimum and maximum temperature, which have to be summarized to the daily thermal unit. An example for a given day: the minimum temperature of 7° C becomes a value of 1.3 and the maximum temperature of 18° C is transformed to 1.8. Both values result in a thermal unit of 3.1. A calculation of degree days was made for the time between start of fruit colouring until harvest.

Tab. 1: Temperature during May to August in 2003, 2004 and 2005

		average temperature per decade (° C)			
		2002	2003	2004	2005
May	first decade	-	16.1	11.5	12.6
	second decade	-	13.1	15.2	12.1
	third decade	-	18.3	13.3	18.5
June	first decade	-	22.2	18.2	15.3
	second decade	-	21.2	16.3	18.4
	third decade	-	21.6	17.7	23.5
July	first decade	18.2	18.4	16.8	18.1
	second decade	18.4	23.0	18.4	22.3
	third decade	19.8	20.6	20.4	-
August	first decade	18.2	27.9	23.9	-
	second decade	20.9	23.4	19.6	-
	third decade	19.3	18.6	16.6	-

Time from beginning of fruit colouring until harvest of all cultivars
2003: 27 may to 8 July
2004: 21 May to 21 July
2005: 24 May to 14 July

Statistical Analyses

All statistics were performed using SPPS for Windows, version 17.0. Analyses of variance were done with cultivars and ripening stage as fixed and years as random effects. Duncan-test was applied to verify statistical differences for cultivars and ripening stage at $P \leq 0.05$. Phenotypic correlations were estimated between TEAC, total phenolics, total anthocyanins and ascorbic acid to yield, berry weight and degree days for the whole data set and individual cultivars, respectively. Data of TEAC, total phenols, total anthocyanins, ascorbic acid, yield, berry weight and degree days were subjected to variance component analyses. Missing values (3 for total anthocyanins and 7 for berry weight) were replaced by the calculated average of the individual cultivar for PCA calculation.

Results and discussion

Influence of cultivar

Mean antioxidative capacity, total phenolics, total anthocyanins and ascorbic acid for all cultivars harvested in 2003 to 2005 are shown in Tab. 2. Cultivars are listed with increasing values of mean TEAC value. The ranking in mean total polyphenols among the cultivars were similar to those of TEAC, whereas greater rank changes occurred for total anthocyanins and ascorbic acid. For each parameter, changes were great between the cultivars with the highest and those with the lowest average content: 2.1-fold for TEAC value and total phenolics, 3.4-fold for total anthocyanins and even 4.4-fold for ascorbic acid. The influence of cultivars was significant for all traits reflecting the described differences between cultivars.

The range of values found for antioxidant capacity, phenolic content and ascorbic acid in this study were similar to a previous study with the same sample preparation and juice processing method but with different cultivars (DIETRICH et al., 2000). The average value for TEAC (53.0 mmol/l) as an average of all cultivars and years exceeded widely the average of the first study (32.4 mmol/L) showing that newly bred cultivars may exhibit higher antioxidative capacity. Average phenolic (5534 mg/L) and ascorbic acid content (1307 mg/L) in this study was slightly lower than in the previous study (6622 mg/L and 1449 mg/L, respectively). Due to different analysing methods and different analysing matrices such as juice or berries, our results are not always comparable to the literature. In addition, data for anthocyanin content of the juices may be reduced by aging when the monomeric anthocyanins of fresh processed juices changed to polyphenols (EDER, 1996; IVERSEN, 1999; RUBINSKIENE et al., 2005). This might be one reason for changes in the anthocyanin content between the different years, whilst cultivar variation could not be explained because the analysis of juices from one year were always done at the same time for all cultivars.

Big variation for antioxidative capacity among cultivars was also described by DEIGHTON et al. (2002) and MOYER et al. (2002). Also, genotypic effects on content of polyphenols and anthocyanins of black currants are well known in the literature (MOYER et al., 2002; RUBINSKIENE et al., 2006; GINÉ BORDONABA and TERRY, 2008). The great differences of ascorbic acid within the cultivars examined are in good agreement with VIOLA et al. (2000) who also described a threefold variation of ascorbic acid content among 6 different cultivars.

In our study, there were also partially remarkable variations of the different traits within individual cultivars from year to year (see standard deviation). For TEAC the greatest changes were found for cvs. Kristin and Ometa and total phenols varied highly within cvs. Farleigh, Kristin, Ben Lomond and PC 96. Very big differences between the years were found for total anthocyanins, especially within cvs. Farleigh, PC 95, Foxendown and PC 96. For ascorbic acid the greatest variation were found within cvs. PC 110, Tifon, Narwe Viking, and Ben Lomond, respectively. These changes of the different parameters within individual cultivars resulted in significant differences between the years (Tab. 4). The lowest value for TEAC and total phenols as an average of all cultivars was found in 2003 while the content of anthocyanins was lowest in 2005. The content of ascorbic acid was not altered by yearly influences.

Significant differences were also present for yield per plant and berry weight between cultivars (Tab. 3). The highest average yield was recorded for cv. Tsema and the lowest for cv. Titania. Average berry weight was high for cv. Intercontinental and low for cv. Triton.

Tab. 2: Antioxidative capacity (TEAC), total phenols (Folin), total anthocyanins (sum HPLC) and ascorbic acid of 23 black currant cultivars harvested in 2003-2005 (cultivars are arranged in increasing order of TEAC-value)

	TEAC (mmol/L)	Total Phenols (mg/L)	Total Anthocyanins (mg/L)	Ascorbic Acid (mg/L)
Ben Loyal	34.1 ± 3.2 a	3726 ± 299 a	493 ± 55 a	970 ± 208 abc
Ben Connan	38.6 ± 5.9 ab	4069 ± 460 ab	720 ± 79 ab	1098 ± 303 abcde
PC 106	39.2 ± 7.3 ab	3789 ± 454 a	871 ± 228 ab	821 ± 249 ab
Bona	40.5 ± 6.8 abc	4481 ± 759 abc	1357 ± 377 bc	1027 ± 325 abcd
PC 110	43.0 ± 6.0 abcd	4223 ± 47 ab	847 ± 292 ab	1289 ± 405 bcdef
Farleigh	46.1 ± 9.0 abcde	4424 ± 1071 abc	1038 ± 550 abc	616 ± 44 a
Intercontinental	46.6 ± 9.8 abcdef	4425 ± 602 abc	1004 ± 437 abc	1117 ± 215 abcde
Polar	48.4 ± 6.6 abcdef	5012 ± 527 abcd	1452 ± 407 bc	1168 ± 198 bcdef
Triton	49.4 ± 9.9 abcdef	5171 ± 401 bcd	1188 ± 281 abc	1355 ± 286 cdef
Titania	50.6 ± 7.0 abcdefj	5305 ± 260 bcde	1100 ± 261 abc	1330 ± 284 bcdef
Andega	53.3 ± 6.2 abcdefg	4859 ± 136 abc	1077 ± 334 abc	997 ± 163 abc
Tisel	53.6 ± 4.3 bcdefg	6151 ± 586 defg	755 ± 278 ab	2718 ± 243 g
PC 95	53.7 ± 9.3 bcdefg	5587 ± 928 cdef	968 ± 579 abc	2563 ± 256 g
Kristin	54.8 ± 20.7 bcdefg	5675 ± 1078 cdefg	1149 ± 269 abc	836 ± 233 abc
Ben Tron	56.6 ± 11.2 bcdefg	6476 ± 640 efg	1243 ± 141 bc	1318 ± 170 bcdef
Foxendown	59.6 ± 1.6 cdefg	6696 ± 428 fgh	1291 ± 493 bc	1562 ± 238 ef
Tifon	61.4 ± 5.7 defg	6213 ± 544 defg	1070 ± 64 abc	1243 ± 364 bcdef
Narwe Viking	61.8 ± 11.9 defg	6711 ± 622 fgh	1442 ± 200 bc	1274 ± 438 bcdef
Tiben	62.4 ± 8.3 defg	6475 ± 960 efg	1683 ± 433 c	940 ± 285 abc
Ben Lomond	64.1 ± 6.7 defg	6287 ± 1024 defg	1455 ± 365 bc	1646 ± 362 f
Ometa	65.8 ± 21.0 efg	6904 ± 516 gh	1375 ± 353 bc	1105 ± 85 abcde
Tsema	66.1 ± 13.2 fg	6868 ± 401 fgh	1323 ± 273 bc	1534 ± 197 def
PC 96	70.0 ± 11.6 g	7765 ± 1095 h	1690 ± 715 c	1521 ± 125 def
mean	53.0	5534	1156	1307

The values represent means of three years ± standard deviation; p = < 0.05

The results in Tab. 4 also demonstrate the remarkable differences in yield and berry weight as an average of all cultivars in the three years examined. The highest yield was obtained in 2003 and the lowest in 2004. Berry weight was highest in 2005. The low yields in 2004 reflect the bad growing conditions during mid July until the end of August 2003, where the daily average temperature rose up to 29° C and hampered flower initiation (Tab. 1). Plants were in a very bad condition at the time and photosynthetic activity was strongly reduced which might be also a result of abiotic stress. Normally it should be expected that a reduced fruit set leads to an increased fruit size. But, in 2004, fruit size was substantially reduced for some cultivars, which might be a result of this stress, also.

Correlations of phytochemicals

Antioxidative capacity was strongly related to the phenolic content (r = 0.84) whereas only moderate correlations exist to the anthocyanin content and among total phenolic content and total anthocyanin content (Tab. 5). Moyer et al (2002) mentioned for 32 black currants a much lower correlation among antioxidative capacity measured as ORAC (Oxygen Radical Absorbance Capacity) and total phenols (r = 0.44, $P < 0.005$) and between ORAC and anthocyanins (r = 0.38, $P < 0.005$) while they observed a much higher correlation between total phenols to anthocyanins (r = 0.63, $P < 0.005$) than we did. It should be noted that the mechanism of TEAC and ORAC assay are quite different in measuring antioxidative capacity. However, anthocyanins are the most abundant polyphenols in black currants and phenolic acids and other phenolic compounds are of minor importance, both for the content of polyphenols and for the antioxidant level of black currant (Kähkönen et al., 2001). Applying HPLC-analysis Dietrich et al. (2002) found a total phenol content of 2791 mg/L (1805-4126 mg/L) in 9 black currant cultivars, which was dominated by an anthocyanin content of 2600 mg/L (1688-3846 mg/L). In contrast, Giné Bordonaba and Terry (2008) found no correlation between total phenols and anthocyanins.

Beside the fact that ascorbic acid is a powerful antioxidant, no correlation between TEAC and ascorbic acid could be found in our study (Tab. 5). Deighton et al. (2002) showed that Vitamin C is the major contributor to the antioxidant capacity of black currants. In contrast to this result, Dietrich et al., (2002) reported on a vitamin C ratio of 22 % on the TEAC value of black currants.

Correlations to yield, berry weight and degree days

Slight negative, but highly significant correlations were determined between yield or berry weight and TEAC-value or total phenolic content, respectively (Tab. 6). No correlation was found between yield and anthocyanin content, but a negative influence existed between berry size and anthocyanin content. Fig. 1 demonstrates the correlation between yield and TEAC and total phenols within the whole data set, all 5 varieties with the lowest average yield over the three years examined exhibit high TEAC and total phenol values and are mainly concentrated for each of the three years on the left side of the graph while all 5 varieties with the highest average yield had low values and are located more on the right side. However, some exceptions occur, for example for cvs. Ben Loyal, Tiben and Tsema in one of the three years when yield was extremely low for cv. Ben Loyal in 2004, or TEAC values were higher than in the other two years for cv. Tiben in 2004 and for cv. Tsema in 2005. There were also low negative but highly significant correlations between berry weight and TEAC

Tab. 3: Yield and 100-berry weight of 23 black currant cultivars harvested in 2003-2005 (cultivars are arranged in increasing order of TEAC-value)

	Yield per plant (g)	100-berry-weight (g)
Ben Loyal	1712 ± 736 cd	84.2 ± 6.9 fg
Ben Connan	1523 ± 367 abcd	68.7 ± 0.2 cdef
PC 106	1480 ± 521 abcd	78.0 ± 7.9 efg
Bona	1684 ± 571 bcd	88.0 ± 1.5 gh
PC 110	1240 ± 488 abcd	80.9 ± 8.7 efg
Farleigh	865 ± 409 abc	69.7 ± 9.8 def
Intercontinental	1168 ± 635 abcd	101.6 ± 13.9 h
Polar	861 ± 278 abc	51.0 ± 2.1 abcd
Triton	1088 ± 282 abcd	46.5 ± 4.3 a
Titania	680 ± 198 a	64.9 ± 13.5 abcde
Andega	959 ± 387 abcd	53.6 ± 1.3 abcd
Tisel	1015 ± 588 abcd	64.0 ± 4.8 abcde
PC 95	1064 ± 810 abcd	64.0 ± 12.4 abcde
Kristin	697 ± 320 a	62.2 ± 15.2 abcde
Ben Tron	1073 ± 392 abcd	61.9 ± 9.0 abcde
Foxendown	694 ± 329 a	49.2 ± 11.1 abc
Tifon	1239 ± 390 abcd	47.4 ± 3.8 ab
Narwe Viking	719 ± 329 a	56.7 ± 4.8 abcd
Tiben	1763 ± 413 d	66.2 ± 3.3 bcdef
Ben Lomond	890 ± 100 abcd	69.0 ± 3.9 def
Ometa	1279 ± 255 abcd	52.4 ± 1.1 abcd
Tsema	1719 ± 323 cd	66.2 ± 19.8 bcdef
PC 96	821 ± 294 ab	53.1 ± 4.1 abcd
mean	1141	65.2

The values represent means of three years ± standard deviation; $p = < 0.05$

Tab. 4: Yearly means of antioxidative capacity (TEAC), total phenols (Folin), total anthocyanins (sum HPLC) and ascorbic acid, yield and 100-berry weight of 23 black currant cultivars

	2003	2004	2005
TEAC (mmol/L)	46.3 a	59.0 b	53.9 b
Total Phenols (mg/L)	5019 a	5772 b	5813 b
Total Anthocyanins (mg/L)	1172 ab	1322 b	985 a
Ascorbic Acid (mg/L)	1278 a	1429 a	1213 a
Yield per plant (g)	1533 c	818 a	1132 b
100-berry-weight (g)	62.8 ab	61.5 a	72.4 b

The values represent means of all cultivars; means followed by the same letter in a row do not differ significantly; $p = < 0.05$

Tab. 5: Correlations coefficient within TEAC-value, total phenols, total anthocyanins and ascorbic acid in black currant juice based on 23 cultivars

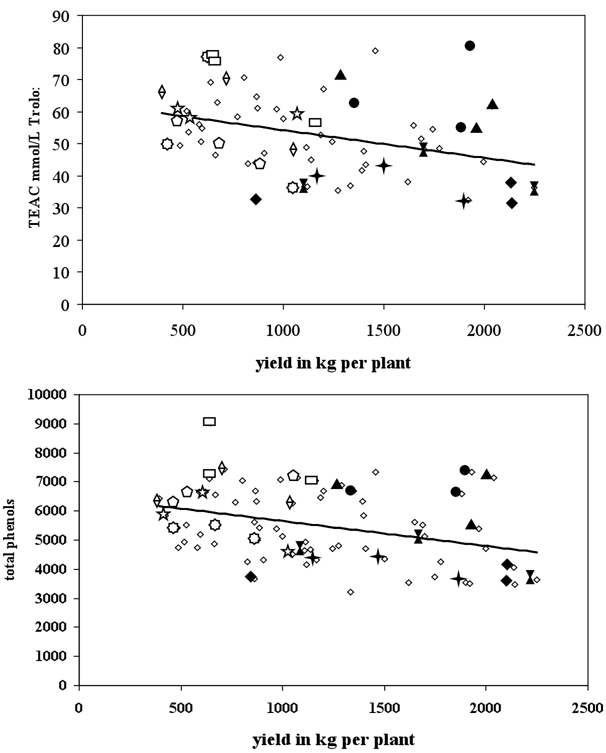
	TEAC	Total Phenols	Total Anthocyanins	Ascorbic acid
TEAC	1	0.84**	0.56*	ns
Total Phenols		1	0.52**	-
Total Anthocyanins			1	-

Correlation after Pearson, bivariat; * $p \geq 0.05$; ** $p \geq 0.01$, ns = not significant

Tab. 6: Correlation coefficients between TEAC value, total phenols, total anthocyanins and ascorbic acid with yield, berry weight and temperature units

	Yield (g/plant)	100-berry-weight (g)	Degree days
TEAC	-0.34**	-0.43**	0.36**
Total Phenols	-0.34**	-0.48**	0.33*
Total Anthocyanins	ns	-0.38**	0.51**
Ascorbic Acid	ns	ns	ns

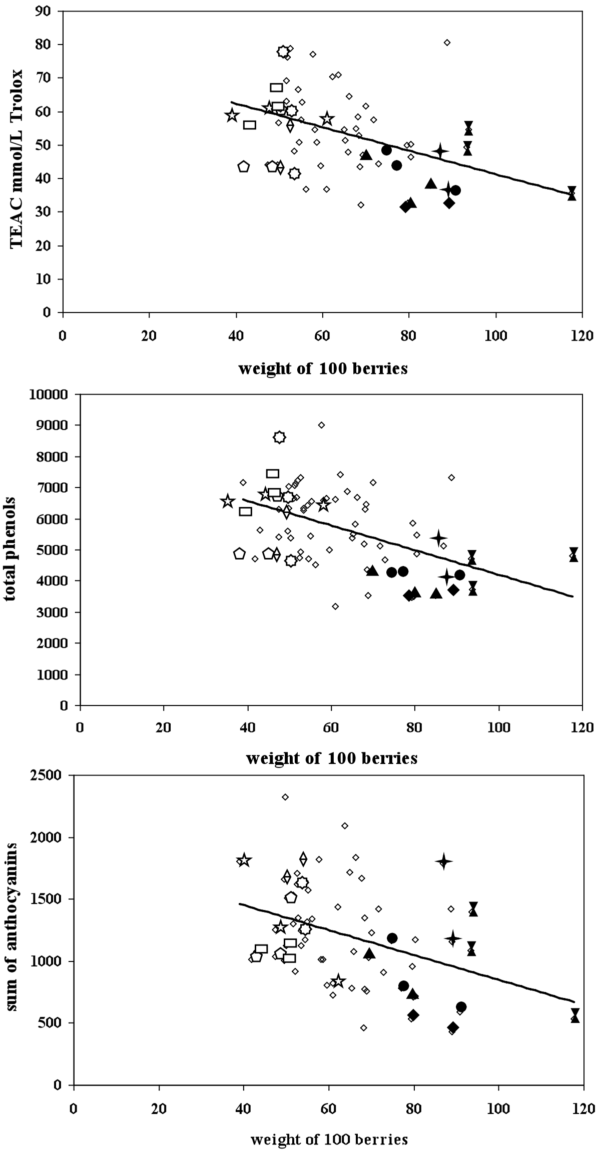
Correlation after Pearson, bivariat; * $p \geq 0.05$; ** $p \geq 0.01$
degree days: calculated temperature units from beginning of fruit colouring until harvest



highest average yield: ◆ Ben Loyal, ✚ Ben Connan, ⧗ Bona, ▲ Tiben, ● Tsema
lowest average yield: ⬡ Foxendown, ☆ Kristin, ⧫ Narve Viking, ▭ PC 96, ⬠ Titania

Fig. 1: Correlation of yield with antioxidant activity (TEAC) and total phenol content of 23 black currant cultivars.

value, total phenolic and anthocyanin content, respectively (Tab. 6). As shown in Fig. 2 for all traits, cultivars with the lowest average berry size are again more present on the left side whereas those with the highest average berry size are more concentrated on the right side of the graphs. Neither yield nor berry weight had any influence on the content of ascorbic acid.
To the best of our knowledge, there is no information available on the influence of yield on bioactive components of berry fruits. The observed negative influence of berry weight, as a surrogate of berry size, on the examined biochemical compounds are in agreement with other studies. While anthocyanins are mainly localized in the skin of black currant fruit, an increase of fruit size will decrease the surface area : volume ratio and consequently the concentrations of anthocyanins. Anthocyanins are an important fraction of total phenolics and both are substantial contributors to the antioxidant capacity.



highest average berry weight: ◆Ben Loyal, ✚Bona, ⧗Intercontinental, ▲PC 106, ● PC 110
lowest average berry weight: ☆Foxendown, ⬡Ometa, ◊Polar, ▭Tifon, ⬠Triton

Fig. 2: Correlation of 100-berry weight with antioxidant activity (TEAC), total phenol and total anthocyanin content of 23 black currant cultivars.

Therefore, part of the variability of the TEAC values and the anthocyanin and phenolic contents among the cultivars might be attributed to increased berry size of individual cultivars. MOYER et al. (2002) showed for different cultivars of black currants and highbush blueberries, which are similar in shape to black currants, a significant impact of berry size on anthocyanins content. CONNOR et al. (2002) reported for blueberry of inverse significant to high significant correlations between berry weight (fresh berries) and antioxidative capacity (r = -0.28), total phenolics (r = -0.44) and anthocyanin content (r = -0.41).

The observed phenomenon illustrates that, beside the cultivar influence on the accumulation of biochemical components, the genetically based variations in yield and berry weight among cultivars also account for some of the observed variation in TEAC value and total phenolic content and, in addition, berry weight also for anthocyanin content. This suggests that breeding for higher yield and greater berry size might concomitantly limit the values for antioxidative capacity, anthocyanin and phenolic content in black currant.

However, when considering the relationship between yield and TEAC value or total phenolic content for individual cultivars during the three years of the study (Tab. 7), it could be noted, that 60.8 % and 65.2 % of all cultivars showed a close negative relationship between both parameters (r = > 0.5 to 1.0) while yield affected negatively the anthocyanin content only by 40 % (r = > 0.5 to 1.0). The frequency of moderate to very high negative correlation coefficients between berry weight and TEAC-value, total phenolic or anthocyanin content raised up to 37.5 % and 43.8.0 % (r = > 0.5 to 1.0), giving variation of berry size within a cultivar a less pronounced effect on the examined traits than yield.

This demonstrates that, within a cultivar, other factors than genetics may influence TEAC value and total phenolic content as well. It is well known, that the yield potential can be modified by unfavourable conditions such as temperatures during flower initiation or nutrition and carbohydrate status of the plant. In our study, the high temperatures at flower initiation in 2003 reduced the yield potential in 2004 and thus seem to promote an accumulation of the TEAC value and the phenolic content. Similar results are reported from tree fruits: STOPAR et al. (2002) found an increased polyphenol level both in apple skin and cortex with decreasing fruit load and just recently, ANDREOTTI et al. (2010) demonstrated also for nectarine that a reduced fruit load increased the content of polyphenols in the pulp.

Positive correlations were observed between degree days and anthocyanins (r = 0.51) and to a much lesser degree to phenolics and TEAC (Tab. 6). When looking at individual cultivars, degree days also affected in 60 % of all cultivars the concentration of anthocyanin in black currant fruit (Tab. 7).

Degree days might be depending on:

a) differences in daily temperature from the beginning of fruit colouring to harvest of each cultivar; this was the case in our study, when temperature during fruit ripening was highest in 2003 and lowest in 2004 (Tab. 1);
b) variation in the visual rating of the beginning of fruit colouring; this might be one factor influencing the value of the degree day, but beginning of fruit colouring is far away (around 3-4 weeks) from harvest. However, from a physiological point of view, conditions around the potential harvest date seem to be more important.
c) decision when to harvest the individual cultivar and thus by the maturity of the berries.

As was shown by a sub trial conducted in 2005-2006, TEAC value und total phenolic content increased in tendency and anthocyanin level significantly from the semi- ripe (one fourth of the berry surface still red-green), to ripe stage (all berries black and fully ripe) whereas ascorbic acid content declined with proceeding ripening (Tab. 8). After delayed harvest, (slightly overripe berries, which were already shrivelling or nearly dropping down) TEAC values and phenolic and anthocyanin contents remained nearly unaffected when compared to ripe berries while ascorbic acid level was still decreasing. However, differences of TEAC value and total phenol and anthocyanin content between the different ripening stages were less pronounced compared to the observed variations within individual cultivars in the years 2003 to 2005. Only anthocyanin level increased substantially during fruit ripening, which is in agreement with results reported by TOLDAM-ANDERSON and HANSEN (1997) or RUBINSKIENE and VIŠKELLIS (2002). However, berries of the main trial from 2003 to 2005 were never harvested in a semi-ripe stage but after visual survey of colouring (black and fully ripe) and softening of the berries and their affinity to pre-harvest fruit drop. Thus, the data of our study may be an indication that the weather conditions, mainly temperature, during ripening time of an individual cultivar determines to a much higher degree the accumulation of these parameters than a delayed harvest can do.

Tab. 7: Frequency of correlation coefficient between TEAC value, total phenols, total anthocyanins and ascorbic acid with yield, berry weight and temperature units based on single cultivar calculation

			negative correlation frequency of coefficent			%	positive correlation frequency of coefficent			%
		n	< 0.5	>0.5-0.7	>0.7-1.0	∑ >0.5 -1.0	<0.5	>0.5-0.7	>0.7-1.0	∑ >0.5-10
TEAC	yield	23	4	3	11	60.8	4	1	-	4.3
	berry weight	16	3	2	4	37.5	1	1	5	37.5
	degree days	23	2	-	3	13.0	7	3	8	47.8
Phenols	yield	23	4	3	12	65.2	3	-	1	4.3
	berry weight	16	1	3	4	43.8	2	2	4	37.5
	degree days	23	5	1	3	17.4	4	3	7	43.5
Anthocyanins	yield	20	3	4	4	40.0	3	1	5	30.0
	berry weight	13	3	-	5	38.5	4	-	1	7.7
	degree days	20	-	-	2	10.0	6	1	11	60.0

An impact of environmental pre-harvest factors such as temperature on these phytochemicals is shown by Wang and Zheng (2001) who found for strawberries a more pronounced accumulation of anthocyanins, phenolics and antioxidative capacity under warm night/day temperature compared to low day/night temperatures. In addition, at constant day temperature, the accumulation of flavonoids in strawberries was favoured by higher night temperature in comparison to low night temperature (Wang et al., 2003). Davik et al. (2006) demonstrated that antioxidative capacity of strawberries was negatively correlated with rainfall and positively to minimum temperature on the day prior to harvest.

The observed decline of ascorbic acid during maturity (Tab. 7) is in accordance to findings of Rubisnkiene et al. (2006) and Viola et al. (2000). The results of Viola et al. (2000) indicate that the genotypic ranking of ascorbic acid content is already manifested at the berry expansion phase, before the fruits start to get coloured. This might explain why no correlation was found between ascorbic acid content and degree days calculated from fruit colouring until harvest in our study. However, Lee and Kader (2000) and Atkinson et al. (2006) reported of higher ascorbic acid content with increasing intensity of radiation. Redalen (1993) showed that the ascorbic acid content of black currants was reduced by elevated temperature when grown in controlled growth chambers. Comparable results are reported by Wang and Camp (2000) for strawberries.

Tab. 8: TEAC-value, total phenols, total anthocyanins and ascorbic acid of black currant affected by ripening stage (average of the cvs. PC 73, PC 106 and Tsema in 2005 and 2006)

	TEAC (mmol/L)	Total Phenols (mg/L)	Total Anthocyanins (mg/L)	Ascorbic Acid (mg/L)
Ripening stage				
Semi-ripe	49.8	5041.5	133.2 a	1919.3 a
Ripe	59.0	5919.3	818.0 b	1471.3 ab
Slightly overripe	61.1	5638.3	891.3 b	1135.2 a
significance	ns	ns	***	*

ns = non significant, * significant at p<0.05, *** significant at p<0.001

PC-Analysis

To simplify the influencing factors and for easier interpretation, all data were subjected to principle component analyses. Three significant PCs were derived accounting for 39.5, 18.1 and 13.1 % of the total variance (70.7 %), respectively (Tab. 9). The first PC was mainly dominated by the total phenols and TEAC value and, less important, total anthocyanins and berry weight (vector loadings are 0.899, 0.876, 0.693 and -0.6635, respectively). The parameter cultivar loaded to only -0.437. This is surprising low, but underlined, that in this data set other factors than only genetic determines the accumulation of the phytochemicals. The second PC is mainly attributed to ascorbic acid and its influencing factor degree days (vector loadings -0.719 and 0.705) while PC 3 is defined by cultivar and the genetically based yield (scores -0.724 and 0.642).

Tab. 9: Principal component analyses of ratings for selected phytochemicals, yield, berry weight and degree days of black currant juices of 23 cultivars

	Principal component		
item	1	2	3
Cultivar	-0.437	0.263	-0.724
Ascorbic Acid	0.347	-0.719	0.228
Total Phenols	0.899	-0.021	-0.028
Total Anthocyanins	0.693	0.432	-0.002
TEAC	0.876	0.098	-0.098
Yield	-0.448	0.305	0.642
Berry Weight	-0.635	0.272	0.124
Degree Days	0,436	0.705	0.191
Variance explained by PC, %	39.5	18.1	13.1

Conclusion

The study shows the great genetical variation in content of anthocyanins, total phenols, and ascorbic acid, and in the level of TEAC within different cultivars, giving the possibility to enhance their level of health functional phytochemicals by breeding. Also fruit size is often regarded as an important breeding aim. However, while anthocyanins are mainly localized in the skin of black currant fruit, big fruit size might imply the risk that the bioactive compounds will be reduced when the ratio between surface area and fruit volume becomes too wide.

The study also showed that environmental factors like temperature during the fruit period and fruit load may influence the accumulation of health functional phytochemicals in black currant fruit. This underlines that investigation on the phytochemical properties of fruits and the comparison of different cultivars have to be done for more than one year to minimize influences of external factors such as weather parameters, fruit load, etc.. Finally, objective tools to determine the harvest date of the berries seem to be helpful ensuring to get fruits with high contents of bioactive compounds.

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