CHANGES IN BIOLOGICALLY ACTIVE CONSTITUENTS DURING RIPENING IN BLACK CURRANTS

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A B S T R A C T

The aim of the research was to investigate dynamics of changes in ascorbic acid, anthocyanins and other chemical compounds in different black currant cultivars during the ripening process. Nine black currant (*Ribes nigrum* L.) cultivars cultivated in Lithuania were investigated. Ascorbic acid content was highest in green and reddish berries. Ascorbic acid content was lowest in overripe berries, which contained 14.7 to 29.5% less ascorbic acid than black berries. Ascorbic acid content was particularly high in black berries of 'Vakariai' and 'Joniniai'. Anthocyanins content was highest in overripe berries of 'Ben Alder', 'Vakariai' and 'Ben Tirran'. In black berries, cyanidin-3-rutinoside was the most abundant anthocyanin present and made up 44.1% of the total. Delphinidin-3-rutinoside accounted for 33.7%, cyanidin-3-glucoside for 8.7%, and delphinidin-3-glucoside for 13.4%. The cultivars with the highest soluble solids content were 'Vakariai' and 'Ben More'. Titratable acidity was highest in berries at the beginning of the ripening process. The decrease in titratable acidity in overripe berries ranged from 3.2 to 30.6%, depending on cultivar.

Key words: anthocyanins, ascorbic acid, black currants, cultivar, soluble solids, titratable acidity.

INTRODUCTION

Black currants (*Ribes nigrum* L.) are perennial bush plants of the gooseberry family (*Grossulariaceae*). Their berries are rich in vitamin C (Hummer and Barney, 2002).

While the berries grown and ripen, many morphological and biochemical changes take place, which depend on the physiological state of the plant, agrotechnical techniques and weather conditions. Warmer weather increases soluble solids content (Maksimenko, 1997). Environmental conditions have a larger impact on acidity than on sugar content (Ogolcova and Kniazev, 1997; Brennan, 1996). In berries which ripen at different times, soluble solids content increased 18-30 days before the normal harvest time (Severin et al., 1997). When the weather is warm and dry, less acid accumulates in the berries. Nevertheless, black currants are sour even when fully ripe. Citric acid is the main acid in black currants.

The dynamics of ascorbic acid content over the course of the year are not well understood. In one study, ascorbic acid content highest in green, unripe berries (Maksimenko, 1999). In another study, ascorbic acid content highest in brown berries (Bickauskiene, 1981). Ascorbic acid content and its dynamics depend on the cultivar (Viola et al., 2000).

Anthocyanins content also depends on the developmental stage of the berries (Maksimenko, 1997; Clifford, 2000).

Black currants are a very popular garden berry plant in Lithuania. The Lithuanian Institute of Horticulture has an active black currant selection program. The composition and characteristics of currant berry pigments are studied in Lithuania. Further study is needed to elucidate the dynamics of bioactive compounds during the ripening process and its dependence on the genetic make-up of the cultivar.

The aim of this study was to investigate the chemical properties of different black currant cultivars and to determine which varieties contain high amounts of ascorbic acid, anthocyanins and other bioactive compounds.

MATERIAL AND METHODS

Biochemical studies on black currant berries were carried out in the Biochemistry and Technology Laboratory in 2001 and 2002. Anthocyanins were qualitatively analyzed at the Food Institute of the Kaunas University of Technology. Black currants for analyses were collected at the breeding plantation of the Lithuanian Institute of Horticulture. The cultivars studied included local and foreign varieties which are grown in Lithuania, including:

- ‘Joniniai’: a very early local cultivar;
- ‘Almiai’: an early local cultivar;
- ‘Vakariai’: a very late local cultivar;

- 'Öjebyn', 'Titania' and 'Minaj Shmyriov': moderately late introduced cultivars; and
- 'Ben Alder', 'Ben More' and 'Ben Tirran': late introduced cultivars.

Chemical compounds were measured at different stages of maturity:

- green: 4 to 6 days before the ripening process begins;
- reddish: the beginning of the ripening process;
- dark brown: half ripe;
- black: fully ripe; and
- deep black: overripe.

Biochemical composition of berries was determined by standard methods. The following analyses were carried out each year:

- soluble solids content (SSC) by refractometer;
- titratable acidity expressed as citric acid by titration with 0.1 N NaOH;
- ascorbic acid content by titration with 2,6-dichloro-phenolindophenol, using chloroform for intensely collared extracts;
- inverted sugar and sucrose by the Bertrand method (Ermakov, 1987).

Anthocyanins were extracted from 5 g of berries with 95% (v/v) food grade ethanol acidified with 0.1N HCl. The berries were ground with quartz sand and the extraction was continued with 20 ml portions of solvent until the sample became colourless. The extract was diluted with acidified ethanol. Absorption at 544 nm was measured using a Genesys-10 spectrophotometer (Thermo Spectronic, Rochester, USA). Concentration of anthocyanins was determined from the calibration curve.

Anthocyanin profiles were obtained by fractioning the ethanol extracts using HPLC with a reversed phase C_{18} LiChrospher®100 RP 18e column (5μm, 125 x 4 mm) a LiChrospher®100 RP 18 guard column (5μm) (Merck, Darmstadt, Germany). The eluents were:

A: 4% H_3PO_4 in water, and

B: 100% HPLC grade acetonitrile (Merck, Darmstadt, Germany).

Chromatographic conditions were as follows: 3% B in A at the time of injection (20 μl), 45 min – 25% B in A, 46 min – 30% B in A, 47 min – initial conditions. Flow rate was 1 ml/min. 20 µl was injected. Samples were filtered through a 0.45 µm cellulose syringe filter before analysis. Absorbance at 520 nm was measured with a Merck Hitachi L-7400 LaChrom UV detection system (Merck KGaA, Darmstadt, Germany).

Data were elaborated using analysis of variance (ANOVA), followed by mean separation at $P \leq 0.05$. Dependence among separate parameters was established using correlation and regression methods.

RESULTS

During the two years of the study, ascorbic acid content was high in green and reddish berries, especially in 'Joniniai' (402-435 mg 100 g^{-1}) (Fig. 1). During ripening, ascorbic acid content suddenly dropped in black berries: by 52.2% in 'Titania', 48.9% in 'Öjebyn' and 47.8% in 'Almiai'. This drop was less sudden with late ripening cultivars. In black berries of 'Ben More', 'Vakariai', 'Ben Tirran' and 'Ben Alder', ascorbic acid content dropped by 27.3 to 33.5%. In all cultivars, ascorbic acid content was lowest in overripe berries, which contained 14.7 to 29.5% less ascorbic acid than black berries. Ascorbic acid content was particularly high in black berries of 'Vakariai' and 'Joniniai'.

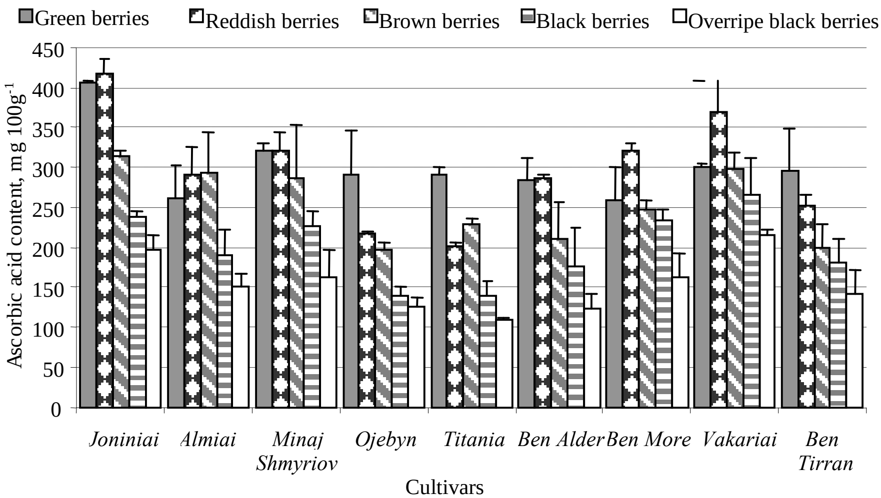

Figure 1. Ascorbic acid content of black currants during ripening [mg 100 g^{-1}]

The changes in anthocyanins content during the ripening process are presented in Figure 2. There was an increase in anthocyanins when the berries turned dark brown. At this stage, anthocyanins content increased by a factor of 4.0 to 4.6 times its level in reddish berries. Anthocyanins content was higher in 2002, when the weather was warm and dry, than in 2001. Anthocyanins content was highest in overripe berries, which contained 5.8 to 17.2% more anthocyanins than black berries in all cultivars. Anthocyanins content was highest in late ripening cultivars. Anthocyanins content was highest in overripe berries of 'Ben Alder' (520.1 mg 100 g^{-1}), 'Vakariai' (514.7 mg 100 g^{-1}) and 'Ben Tirran' (508.8 mg 100 g^{-1}) and lowest in very early berries of 'Joniniai'.

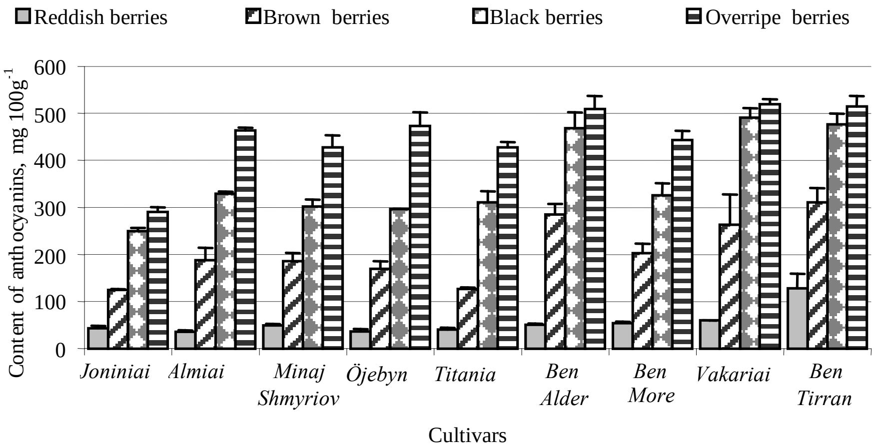

Figure 2. Anthocyanins content of black currants during ripening [mg 100 g^{-1}]

In 'Ben Alder', 'Minaj Shmyriov', 'Joniniai', 'Almiai', 'Vakariai', four anthocyanins were identified:

- Cyd-3-rut (cyanidin-3-rutinoside);
- Cyd-3-glu (cyanidin-3-glucoside);
- Dpd-3-rut (delphinidin-3-rutinoside); and
- Dpd-3-glu (delphinidin-3-glucoside) (Fig. 3).

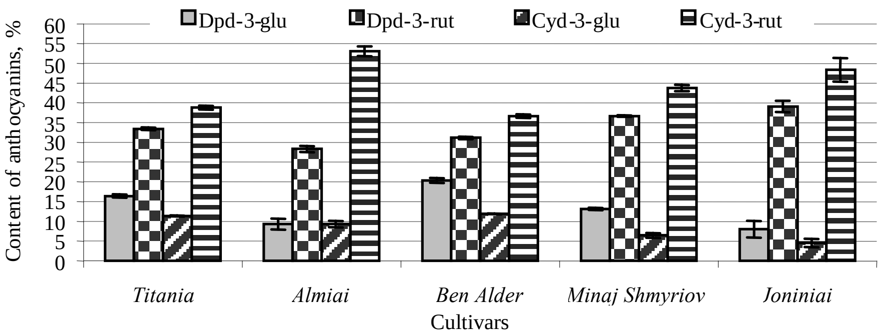

Figure 3. Anthocyanin composition [%] in black currant berries

In black berries, cyd-3-rut was the most abundant anthocyanin present, especially in 'Almiai' (53.08%) and 'Joniniai' (48.33%). Dpd-3-glu was the most abundant glucoside in 'Almiai' and 'Joniniai'. In the native Lithuanian cultivars, anthocyanins were distributed as follows: 44.14% cyd-3-rut, 33.74% dpd-3-rut, 11.24% dpd-3-glu, and 8.40% cyd-3-glu.

Soluble solids content increased during ripening, although the dynamics differed from cultivar to cultivar. At the beginning of ripening, soluble solids content suddenly increased by 33.3% in 'Vakariai', 20.0% in 'Ben Tirran' and 18.6% in 'Titania' (Tab. 1). The cultivar with the highest soluble solids content was 'Vakariai' (16.8%). As the ripening process proceeded, soluble solid content increased at a slower pace, by 2.8% in 'Joniniai' to 12.2% in 'Titania'). Soluble solids content in overripe berries was 10% higher in 'Ben Alder', 5.6% higher in 'Titania', and only 1.1% higher in 'Vakariai' (Tab. 1).

T a b l e 1. Soluble solid (SS) and titratable acid (TA) contents of black currants during ripening

Cultivar	Berry maturity									
	green		reddish (beginning of ripening)		dark brown (50% of ripen fruits)		black (technical maturity)		black (overripe fruits)	
	SS	TA	SS	TA	SS	TA	SS	TA	SS	TA
Joniniai	12.0	4.05	13.0	3.90	14.1	3.80	14.5	2.81	15.3	2.81
Minaj Shmyriov	11.4	3.23	13.0	3.30	13.6	2.48	14.5	2.45	14.9	2.25
Almiai	10.9	3.01	11.0	3.03	12.9	2.80	14.3	2.49	14.5	2.27
Öjebyn	12.9	2.98	12.6	3.05	14.3	2.88	15.0	2.67	15.3	2.27
Titania	11.8	3.49	14.0	3.39	14.8	3.20	16.6	3.01	16.9	2.12
Ben Alder	12.8	3.08	14.5	3.28	15.0	3.13	15.9	2.86	17.6	2.68
Ben More	14.1	3.50	15.1	3.80	17.3	3.48	17.9	3.00	18.2	2.35
Vakariai	12.6	2.75	16.8	2.92	17.1	2.80	18.2	2.75	18.4	2.65
Ben Tirran	10.5	3.18	12.6	3.26	14.3	3.05	15.9	2.85	16.7	2.76
Mean of cultivars	**12.11**	**3.25**	**13.62**	**3.32**	**14.81**	**3.07**	**15.87**	**2.76**	**16.42**	**2.55**
LSD_{05}	0.72	0.23	0.25	0.10	0.22	0.18	0.27	0.33	0.35	0.15

Titratable acidity depended on the stage of berry maturity (Tab. 1). Titratable acidity increased at the beginning of the ripening process except in 'Joniniai' and 'Titania'. The increase in titratable acidity in reddish berries ranged from 0.7% in 'Almiai' to 8.6% in 'Ben More'. The cultivars with the highest titratable acidity were 'Joniniai' (3.9%) and 'Ben More' (3.8%). As the ripening process proceeded, titratable acidity decreased as soluble solids content and anthocyanins content increased. Titratable acidity was on average 7.5% lower in brown berries than in reddish berries, and 24.8% lower in 'Minaj Shmyriov'. In black berries, titratable acidity ranged from 2.45% in 'Minaj Shmyriov' to 3.01% in 'Titania'. Titratable acidity continued to decline in overripe berries. The decrease in titratable acidity was especially high in 'Titania' (29.6%), and especially low in 'Vakariai' and Ben 'Tirran' (3.2%).

DISCUSSION

The dynamics of ascorbic acid contents depended on the physiological properties of the cultivars and on the growth conditions in some cultivars. Ascorbic acid synthesis was most intense in berries which were not fully ripe. The level of ascorbinoxidase in unripe fruits is low (Rubin, 1970). Ascorbinoxidase activity presumably determines the concentration of ascorbic acid in the berries. In early and moderately early cultivars, ascorbic acid content suddenly decreased as berry color intensified, which agrees well with an earlier study (Maksimenko, 1999).

Cyanidin-3-rutinoside content was about 9% higher in the native Lithuanian cultivars than in the introduced European cultivars. In ‘Minaj Shmyriov’, anthocyanin content and distribution was different in 2002 than it was in 2001. In comparison with earlier data (Jasutiene and Viskelis, 1998), In our study, cyanidin and delphinidin rutinosides were 20% higher than in previous studies (Jasutiene and Viskelis, 1998). Anthocyanins content and distribution in ‘Ben Alder’ agree well with earlier studies (Cacace and Mazza, 2003).

Table 2. Dependency of soluble solids and titratable acids amounts on meteorological conditions

Index		Correlation coefficient, r			
X	Y	very early cultivar	medium late cultivars	late cultivars	very late cultivars
Rainfall [mm]	soluble solids content [%]	-0.75	-0.72*	-0.74	-0.70*
	titratable acidity [%]	0.59	0.62	0.50*	0.63*
Temperature [°C]	soluble solids content [%]	0.84	0.80*	0.80*	0.81*
	titratable acidity[%]	0.49	0.58*	0.42*	0.60
Hydrothermal coefficient	soluble solids content [%]	-0.76	-0.79*	-0.80*	-0.78
	titratable acidity [%]	0.61	0.64*	0.73*	0.78*

*coefficient significant at the probability level of 5%

Respiration increases at the end of the ripening process and in overripe berries (Dolgaeva et al., 1987; Montero et al., 1996). This brings about the decomposition of organic acids in the berries. For example, titratable acidity in overripe berries was 29.6% lower in ‘Titania’ and 21.7% lower in ‘Ben More’ than in black berries. During the ripening process, weather conditions have a large effect on titratable acidity (Volodina, 1987; Banaszczyk and Pluta, 1997). Correlation analysis confirmed that titratable acidity depends on weather conditions, especially the hydrothermal coefficient (Tab. 2). The effect of the hydrothermal coefficient was greater in late or very late ripening cultivars (r = 0.78 to 0.73).

Soluble solids content also depended on weather conditions. Soluble solids content depended on the sum of active temperatures in all cultivars tested, especially in the early cultivar 'Joniniai' and the very late ripening cultivars(r = 0.84 to 0.81) (Tab. 2). Soluble solids content was negatively correlated with the hydrothermal coefficient and with the amount of precipitation during ripening in all cultivars (r = -0.76 to -0.80).

CONCLUSIONS

Ascorbic acid content was highest in unripe black currants. Ascorbic acid in overripe berries is 14.7-29.5% lower than in fully ripe black berries. Anthocyanins content and soluble solids content were highest in overripe berries. Titratable acidity was highest at the beginning of the ripening process. Titratable acidity was 21.7 to 29.6% lower in overripe berries.

In five native cultivars, by HPLC were identified four anthocyanins: cyanidin-3-rutinoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and delphinidin-3-glucoside. In black berries, cyanidin-3-rutinoside predominated and made up 44.1% of the total. Delphinidin-3-rutinoside accounted for 33.7%, cyanidin-3-glucoside for 8.7%, and delphinidin-3-glucoside for 13.4%.

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REFERENCES

Banaszczyk J., Pluta S. 1997. Quality characteristics of fruits of new black currant selections bred in Skierniewice. J. FRUIT ORNAM. PLANT RES. 5: 10-19.

Bickauskiene S. 1981. Chemical composition and technological properties of black currant berries. In: Selection and cultivar testing a black currant, Barnaul, (in Russian) vol. 1, pp. 132-134.

Brennan R. 1996. Currants and gooseberries. In: J. Janick, J. N. Motore (eds.), Fruit breeding, vol.2. Small fruit and vine crops, New York, pp. 191-295.

Cacace J.E., Mazza G. 2003. Optimization of extraction of anthocyanins from black currant with aqueous ethanol. J. FOOD SCI. 68: 240-248.

Clifford M. 2000. Review: Anthocyanins – nature, occurrence and dietary burden. J. SCI. FOOD AGRIC. 80: 1063-1072.

Dolgaeva N., Vasiljeva G., Ryzkova T. 1987. Results of studies of varieties of Altay and Omsk selection black currant. In: Selection and studies of berry varieties. Michurinsk, pp. 39-44.

Ermakov A.I. (ed.). 1987. Methods of plant biochemical investigations. Leningrad, pp. 431.

Hummer K., Barney D. 2002. Crop Reports. Currants. HORT. TECHNOLOGY. 12: 377-387.

Jasutiene I, Viskelis P. 1998. Pigments of black currants and their stability. (Lithuanian with English summary). FOOD CHEM. TECHNOLOGY. 32: 12-15.

Maksimenko M. 1997. Accumulation of nutrients in black currant berries during ripening. In: Situation and Problems of Russian Horticulture, Novosibirsk, vol. 2, pp. 162-170.

Maksimenko M.G. 1999. Dynamics of vitamin C and its forms in the process of ripening and technological processing of black currants. HORTICULTURE. 12: 155-159.

Montero T., Molla E., Esteban R., Lopez-Andreu F. 1996. Quality attributes of strawberry during ripening. SCI. HORT. 65: 239-250.

Ogolcova T.P., Kniazev S.D. 1997. The use of black currant of Altay varieties in selection program of VNII selection of fruits. In: Situation and Problems of Russian Horticulture, Novosibirsk, vol. 1, pp. 235-239.

Rubin B.A. 1970. Physiology of grapes and tea. In: Physiology of Agriculture Plants, vol. 9. Moscow, pp. 277-280.

Severin V., Vorobiova N., Volgin V., Smirnich D. 1997. Cultivar testing a black currant in Novosibirsk area. In: Situation and Problems of Russian Horticulture, Novosibirsk, vol. 1, pp. 208-213.

Viola R., Brennan R., Davies H., Sommerville L. 2000. L-ascorbic acid accumulation in berries of Ribes nigrum L. J. HORT. SCI. BIOTECHNOL. 75: 409-412.

Volodina E.B. 1987. Modern assortment of black currant and source material in selection. In: Selection and Studies of Berry Varieties. Michurinsk, pp. 30-34.

ZMIANY SKŁADNIKÓW BIOLOGICZNIE CZYNNYCH W PORZECZCE CZARNEJ PODCZAS DOJRZEWANIA

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STRESZCZENIE

Celem pracy było zbadanie dynamiki zmian zawartości kwasu askorbinowego, antocyjanów i innych chemicznych składników w dojrzewających porzeczkach czarnych różnych odmian. Badaniami objęto dziewięć odmian porzeczki czarnej (*Ribes nigrum* L.) uprawianych na Litwie. Wyższą zawartość kwasu askorbinowego miały zielone i czerwonawe jagody. Wykazano, że w porównaniu do jagód o dojrzałości zbiorczej, zawartość kwasu askorbinowego w przejrzałych jagodach zmniejszała się o 14,7-29,5%. Wysoką zawartość kwasu askorbinowego miały jagody odmiany 'Vakariai' i 'Joniniai'. Najwyższą zawartość antocyjanów zaobserwowano w przejrzałych jagodach odmian: 'Ben Alder', 'Vakariai' i 'Ben Tirran'. W jagodach czarnych w stadium dojrzałości zbiorczej przeważał 3-rutynozyd cyjanidyny stanowiący średnio 44,1% ogólnej zawartości antocyjanów, 3-rutynozyd delfinidyny stanowił 33,7%, 3-glukozyd cyjanidyny – 8,7%, 3-glukozyd delfinidyny – 13,4% ogólnej zawartości antocyjanów. Najwyższą zawartość związków rozpuszczalnych oznaczono w jagodach odmian 'Vakariai' i 'Ben More'. Wyższą kwasowość miareczkową owoców czarnej porzeczki otrzymano w pierwszym okresie dojrzewania. W przejrzewających jagodach różnych odmian zawartość kwasów zmniejszała się o 3,15-30,6%.

Słowa kluczowe: antocyjany, kwas askorbinowy, czarna porzeczka, odmiany, związki rozpuszczalne, kwasowość miareczkowa