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Physical properties, anthocyanins and antioxidant activity of blackcurrant berries of different maturities

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Abstract

Four blackcurrant berry cultivars, Joniniai, Kriviai, Ben Lomond and Ben Tirran, were investigated at different phases of maturity at the Lithuanian Institute of Horticulture and the Institute of Botany. The 'Joniniai' and 'Ben Lomond' had the greatest mass upon reaching technical maturity: 1.54 g and 1.27 g, respectively. When the berries were overripe, their mass increased from 3.7% ('Kriviai') up to 15.6% ('Joniniai'). During technical maturity, 'Joniniai' and 'Kriviai' berries possessed the firmest skin: 96.8 and 97.4 N cm^{-2}, respectively. The amount of anthocyanins in acidified methanol extracts was determined spectrophotometrically and calculated as Cy-3-Glu. The antioxidative (free radical scavenging) activity in fresh anthocyanin samples was determined using a DPPH model system assay. Anthocyanin antioxidative activity was compared with well-known antioxidants: 2,6-di-*tert*-butyl-4-methylphenol (BHT), ascorbic acid and anthocyanidin Cy-3-Chl. Based on a comparison of the amounts of anthocyanins accumulated in the various blackcurrant cultivars in their technical maturity phase and the antioxidative activity of their anthocyanin specimens, it was determined that 'Kriviai' may be considered a perspective candidate for use in agro-biotechnology.

Key words: Anthocyanins, antioxidative activity, berry weight, cultivar, skin firmness.

Introduction

Interest in the use of biomaterials as sources of natural antioxidants is increasing [1-3]. For this reason, investigations regarding fruit-bearing plants that accumulate high amounts of anthocyanins, which are plant polyphenols and thus members of a major anti-oxidant group as well as natural constituents of coloured fruits, are attracting much attention [4-6].

Due to their chemical structure, anthocyanins are considered powerful natural antioxidants. The antioxidative capacity, which is defined as the capacity to inhibit or delay the oxidation of other molecules, anthocyanins and their aglycones (anthocyanidins) and their free radical scavenging activity have been revealed [7-9]. Significant interest has been shown in the role of anthocyanins in plant-based foods; they are critical for maintaining optimum health as well as beneficial in the prevention of various diseases and have also been used in pharmaceutical products (phytochemicals) and as natural colorants [10].

Blackcurrant (*Ribes nigrum* L.) berries are considered rich sources of anthocyanins in Lithuania [11, 12] and other countries [1, 7, 13]. Their antimicrobial and antifilamentory properties have been demonstrated using various test systems [10, 12, 14]. Anthocyanin concentration in berries is mostly determined by the genotype. A high concentration of pigments (350-450 mg 100 g^{-1}) has been found in the berries of cultivars bred in Scandinavian countries [15]. According to our investigations, Lithuanian berry cultivars accumulate from 274.9 to 499.1 mg 100 g^{-1} of anthocyanins [5].

At the Lithuanian Institute of Horticulture, the selective breeding of blackcurrant cultivars and the evaluation of the quality of berries and their products have long been carried out. Most often, blackcurrants nutritional quality is indicated by ascorbic acid content. The agroclimatic conditions in Lithuania, Latvia, Poland and Byelorussia are favourable for the accumulation of a greater amount of vitamin C. Blackcurrant cultivars bred and grown in these countries exceed Scandinavian cultivars in the amount of ascorbic acid [5, 16, 17].

Different agroclimatic conditions influence berry quality and ripening duration [18]. The berry quality (firmness and weight) determines the blackcurrant storability, transportability and trade appearance. The results of berry biochemical composition reflect cultivars' specific quality and their intended purpose. Yet to be elucidated, however, are the dynamics of anthocyanin accumulation in various blackcurrant cultivars in relation to their country of origin, various earliness and ripening phases as well as the relationship between the amount of anthocyanins present in separate cultivars and their antioxidative capacity. An examination of the potential use of anthocyanins for the production of natural food colorants must also be accomplished.

The aims of the present study were to evaluate berry quality and to investigate the accumulation of anthocyanins and antimicrobial activity in blackcurrants during ripening.

Materials and Methods

Four different blackcurrant cultivars were selected as test subjects: a) two local cultivars: Joniniai (early-ripening) and Kriviai (early/

moderate-ripening) and b) two introduced cultivars: Ben Lomond and Ben Tirran (both late-ripening). The berries ripeness was evaluated according to a scale determined by the authors: pink (beginning of ripening), dark brown (50% ripe), black (technical maturity) and overripe.

Ascorbic acid content was established by titration with 2,6-dichlorphenolindophenol sodium salt solution using chloroform for intensively coloured extracts [19]. Berry skin firmness was established with an IDP-500 penetrometer, probe diameter 1 mm.

For anthocyanin analyses, pink (beginning of ripening), dark brown (50% ripe) and black (technical maturity) berries were used. They were immediately frozen and stored at -80°C. Berry powders were extracted by aqueous methanol, acidified with HCl to 0.1 N (at a ratio of 1:20 g/ml) and stored for 16 h at 4°C in the dark. During the extraction procedure, the material was shaken twice over 30 min. The anthocyanin extract was separated from berry refuse material by filtration through membrane filters (0.45-µm pore diameter, Millipore) [9]. Washing of the material was continued until the extraction solvent became colourless [9]. The extracts were concentrated by vacuum at <40°C and stored at -18°C until use.

The anthocyanin concentration in the extracts was determined spectrophotometrically using a pH differential method [9]. The absorbance of anthocyanin was measured at λ 520 and 700 nm and determined as cyanidine-3-glucoside (Cy-3-Glu) equivalent.

The antioxidative activity of anthocyanin specimens derived from different berries was analysed with a photometric method [4, 8] according to the scavenging of free radicals in 2,2-diphenyl-1-picrylhydrazyl (DPPH) model systems. The reaction mixture contained $5x10^{-6}$ M DPPH and 10-100 mM anthocyanins. The absorbance of the mixture was fixed at 515 nm.

Meteorological conditions have a strong influence on berry biochemical composition and yield quality. According to data from the Lithuanian hydrometeorological station, the highest air temperatures were 24-27°C during the June 2007 berry ripening period (1.5-2.8°C higher than the multi-annual average). During June 2007, there were 298 sunny hours in Middle Lithuania (more than 33 hours above average). The greatest amount of precipitation fell on the last days of the month. These conditions were favourable for the growth of both early-ripening and late-ripening cultivars. The air temperature in July was similar to the multi-annual average. The hottest day was July 17th; the air temperature reached 30-34°C. Rainy weather prevailed; precipitation reached 1.5-2.5 of rate. There were 20-50 fewer hours of sunshine than the long-term average.

The air temperature in June 2008 was similar to that of June 2007. Higher temperatures were recorded on the first ten days of the month (23.3-26.6°C). Sunshine lasted for 355 hours in June 2008 and less precipitation fell. A quicker average ripening of the late-ripening blackcurrant cultivars was recorded. The air temperature in July 2008 was close to that of July 2007 and to the long-term annual average. The hottest days were July 12th and July 27th, when temperatures reached 28.2°C. The month was sunny, and the humidity was average.

Results and Discussion

According to the data obtained, the average blackcurrant berry weight depended on ripening time and the properties of each cultivar. With the exception of 'Kriviai' , berry weight increased during the ripening process until technical maturity was achieved (Fig. 1). The blackcurrant cultivars Joniniai and Ben Lomond produced the heaviest berries (1.54 and 1.27 g average weight per berry, respectively). From the beginning of ripening, their weight increased 41% and 15%, respectively. It was observed that when the berries were overripe, their weight decreased. Smaller weight losses were observed in 'Kriviai' (3.7%) and 'Ben Lomond' (4.7%); the greatest loss occurred in 'Joniniai' (15.6%). The weight of overweight 'Ben Tirran' berries decreased 6.5%.

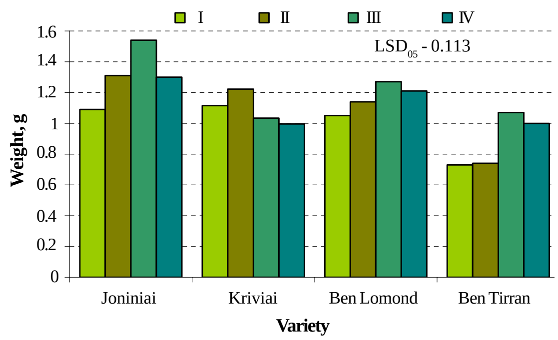

Figure 1. Change in various blackcurrant berry weights during ripening (I - beginning of ripening; II - 50% ripe berries; III - technical maturity; IV - overripe berries).

The unripe berries were green, with an unpleasant taste caused by organic acids and fermented substances. Since they contained few soluble protopectins and starch, they were firm.

During the ripening process, berry skin firmness changed. This index depended on the cultivar and the berry ripeness. The firmest skin in all cultivars belonged to berries that were just beginning to ripen. The blackcurrant berry skin firmness ranged from 218.7 N cm^{-2} ('Kriviai') to 182.5 N cm^{-2} ('Ben Lomond') (Fig. 2).

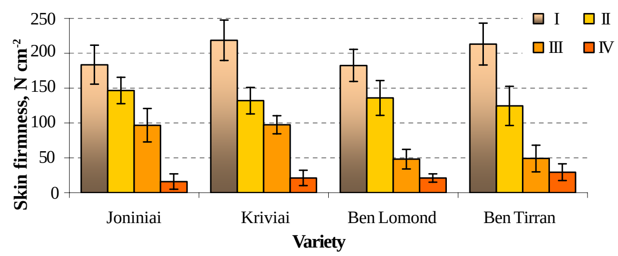

Figure 2. Change of various blackcurrant berry skin firmness during ripening (I - beginning of ripening; II -50% ripe berries; III - technical maturity; IV -overripe berries).

At the second berry ripening stage, when morphological and biochemical changes took place, pectin concentration decreased (when overripe, by as much as 51-57%) and the berries softened[20].

Significant skin softening was observed in blackcurrant berries of technical maturity. The skin firmness of berries from 'Ben Tirran' and 'Pilėnai' decreased 77.0-73.7% and in 'Kriviai' and 'Gagatai' the decrease was 55.4-47.3%. 'Joniniai' and 'Kriviai' possessed the firmest berries. When the berries from all cultivars were overripe, their skin softened; this was seen most significantly in 'Joniniai' (6.0 times) and 'Krivių' (4.6 times) (Fig. 2).

Investigations of the biochemical composition of blackcurrants of various ripeness have been carried out in Lithuania and other countries. It has been observed that the dynamics of the amounts of accumulated substances in berries depends more on the biological properties of cultivars than on growth conditions [5, 21, 22]. In the present study, the dynamics of ascorbic acid presence appeared to depend on the cultivars' physiological properties and berry ripeness. Greater amounts of ascorbic acid were observed in berries at the beginning of ripening in all investigated cultivars (Fig. 3). Significantly greater amounts of ascorbic acid were established in pink 'Joniniai' (221.0 mg/100 g) and 'Ben Tirran' (193.5 mg/100 g). During ripening, a decrease in ascorbic acid amounts was observed in berries from all cultivars. When blackcurrants reached technical maturity, ascorbic acid concentration decreased in all cultivars: in 'Kriviai' by 17.6%, in 'Ben Lomond' by 19.8%, in 'Joninai' by 23.2% and by 29.6% in 'Ben Tirran'. Vitamin concentration decreased strongly in 'Kriviai' and 'Ben Tirran' (by 21% and 29.1%, respectively). Ascorbic acid content in 'Ben Lomond' and 'Joniniai' decreased from 6.3% to 12.8% (Fig. 3). Ascorbic acid changes confirmed earlier results obtained by these and other investigators. In general, greater amounts of vitamin C are present at the beginning of berry ripening[5] (Fig. 3).

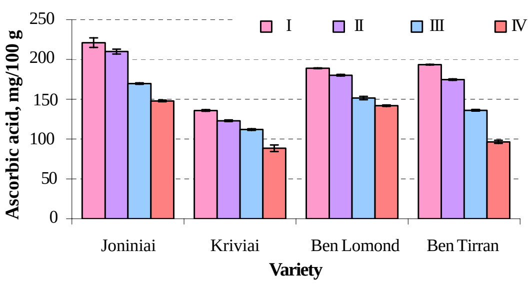

Figure 3. Change dynamic of ascorbic acid in blackcurrants during ripening.

At the beginning of ripening (pink phase) there were no significant differences between the accumulated anthocyanin concentrations in berries of four different blackcurrant cultivars examined. At the beginning of the ripening process, the concentration of anthocyanins in both local cultivars (the early-ripening 'Joniniai' and early/moderate-ripening 'Kriviai') as well as in the introduced, late-ripening cultivar Ben Lomond was on average, 9-10 mg/L. However, in 'Ben Tirran' the anthocyanin concentration reached 13-14 mg/L. That necessitated an accumulation of about 38 mg major amount of anthocyanins in 100 g fresh weight, than in 'Joniani', 'Kriviai' and 'Ben Lomond' (Fig. 4). This result was seen for both years (2007 and 2008).

During the next two ripening phases, the anthocyanin concentration in all blackcurrant berries increased, though not identically. According to results from both years tested, the amount of anthocyanins in all tested cultivars increased most significantly during the shift from the pink to the dark brown (50% ripe) phase (Fig. 4). During this period, the most significant amount of anthocyanins accumulated in the berries, and the differences in accumulation between cultivars appeared. The amount of anthocyanins in the local, early-ripening cultivar Joniniai increased about 2.5 times; in the early/moderate-ripening cultivar Kriviai it increased by more than 3.0 times. In the late-ripening 'Ben Lomond' and 'Ben Tirran', it increased by no less than 5-7 times. During the next ripening phase, the shift from the dark brown (50% ripe) to the black (technical maturity) phase, the amount of anthocyanins

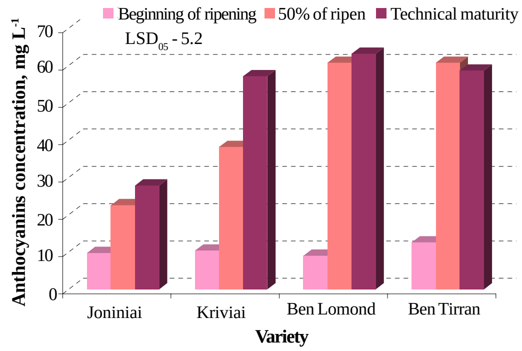

Figure 4. Dynamics of anthocyanin accumulation in berries of different blackcurrant cultivars.

(though synthesis or better accumulation) increased, although not significantly. The most intensive anthocyanin accumulation occurred in berries of 'Kriviai': an 1.5- times increase from 38 to 57 mg L^{-1}.

According to our results, the greatest amounts of anthocyanin were accumulated in the berries of the late-ripening 'Ben Lomond' and 'Ben Tirran'. In the year 2008, anthocyanin content averaged 548 mg/100 g fresh weigh in 'Ben Lomond' and 626 mg/100 g fresh weight in 'Ben Tirran'. The amount of anthocyanin accumulated in the berries of the local early/moderate-ripening 'Kriviai' seems insignificant. In 2007 and 2008, the amount of anthocyanins in these berries at their technical maturity phase averaged about 541 mg per 100 g of fresh weight. According to results from both years, the smallest amount of anthocyanins accumulated in 'Joniniai' berries: from 314 to 380 mg per 100 g of fresh weight only. On the basis of our results, the amount of anthocyanin accumulated in the berries of the tested blackcurrant cultivars at technical maturity phase may ranged: 'Joniniai', 'Kriviai', 'Ben Lomond' and 'Ben Tirran'. However, it must be noted that the amount of accumulated anthocyanins in berries from the local cultivars 'Joniniai' and particularly 'Kriviai' fluctuated less than in the introduced cultivars over both years. This is not surprising, however, because the local cultivars have been selected in accordance with our climate conditions [6].

The accumulated data shows the high index of free radical scavenging activity [2, 10]. This biological effectiveness has been detected in anthocyanin specimens derived from all tested blackcurrants at different phases of their ripening. At the beginning of the ripening (pink) phase, the free radical scavenging activity of anthocyanin samples (10-13 µM concentration) in a 6.5x 10^{-5} M DPPH test system ranged from 64% in 'Ben Tirran' to 76% in 'Joniniai'. In such systems, the DPPH solution scavenged the free protons and shifted the colour of solution.

During the black (technical maturity) phase, the highest antioxidative activity was observed in 'Joniniai', despite the fact that anthocyanin concentration in these berries is about 1-2 times less than that in the berries of other tested cultivars (Fig. 4, Table 1).

In antioxidative activity of anthocyanins the local cultivars Joniniai and Kriviai outperformed the introduced Ben Lomond and Ben Tirran cultivars. Comparisons were made between the antioxidative activity of anthocyanin specimens derived from all tested cultivars with the antioxidative activity of analogous concentrations of well-known antioxidants, which included ascorbic acid, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and anthocyanidin (cyanidin-3-chloride). These comparisons revealed that blackcurrant anthocyanin outperforms ascorbic acid and BHT and Cy-3-Glu (oxidation rates about 35% and 52%, respectively). Our results (Table 1) therefore support the proposition [4] that there is no direct correlation between anthocyanin concentration and antioxidative activity.

Based on the amount of anthocyanins accumulated in various blackcurrant cultivars in their technical maturity phase and the antioxidative activity of their anthocyanin specimens, it is supposed that the local, early/moderate-ripening cultivar Kriviai may be considered for prospective use in agro-biotechnology and in native colorant preparations.

Table 1. Comparison of the antioxidative activity of anthocyanin specimens derived from different blackcurrant cultivars.

Variety	Characteristic	Anthocyanin concentration, μM	Antioxidative activity, %
'Joniniai'	early	8-36	76-82
'Kriviai'	early/moderate	10-57	69-79
'Ben Lomond'	late	10-63	64-78
'Ben Tirran'	late	13-72	64-72

Conclusions

1) Berries from 'Joniniai' and 'Ben Lomond' blackcurrants were distinguished for the heaviest berry weight during technical maturity (1.54 and 1.27 g, respectively). When berries were overripe, their weight increased from 3.7% ('Kriviai') to 15.6% ('Joniniai'). During technical maturity, 'Joniniai' (96.8 N cm^{-2}) and 'Kriviai' (97.4 N cm^{-2}) berries had the firmest skin.

2) Greater amounts of ascorbic acid were established in all berries at the beginning of ripening. Overripe berries were the least vitaminous. 'Joniniai' berries of various maturities contained most ascorbic acid (221-148 mg/100 g^1).

3) 'Joniniai', 'Ben Lomond' and 'Ben Tirran' accumulated anthocyanins most intensively between stages I and II of ripening. The greatest anthocyanin concentrations accumulated in the berries of the late-ripening 'Ben Tirran' and 'Ben Lomond'.

4) The blackcurrant cultivars Joniniai and Kriviai were distinguished for the greatest relative antioxidative activity according to the joining of free radicals.

5) The antioxidative activity of anthocyanin preparations extracted from the investigated blackcurrant cultivars was equal to that of synthetic antioxidants of analogical concentrations.

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