Anthocyanins Content in Blueberries (*Vaccinium corymbosum* L.) in Relation to Freezing Duration

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Abstract: The changes of total anthocyanins (TA) content in fruit of blueberries in relation to freezing duration are the aim of this study. The samples of 21 chosen cultivars obtained from the research breed station were stored in polyethylene bags in freezing box at temperature –18°C. In ethanolic blueberry extracts TA contents were by spectrophotometric method during the time of 6 months at period of 3 months determined. Significant differences of TA content among the observed cultivars were confirmed. The value interval was 615.8–3206.3 mg/kg. The average TA content in 10 very early, resp. early cultivars was 1655.8 mg/kg, in 5 medium early cultivars 1515.6 mg/kg, in 6 medium late, resp. late cultivars 1431.2 mg/kg. During 6 months of blueberries store in the freezing box the average decrease 16.7% of TA content was observed. The average decrease of TA content in very early, resp. early cultivars was 19.6%, in medium early cultivars 16.5% and in medium late, resp. late cultivars 14%.

Keywords: cultivars; blueberries; anthocyanin; freezing

INTRODUCTION

The positive influence of phytochemicals present in fruit and vegetable is accounted for their regular consumption. Small fruits as raspberries, blackberries, blueberries, blackcurrants are rich in phytochemicals such as anthocyanins which are glycosidic-linked flavonoids responsible for their red, violet, purple and blue colours. Berries are edible superstars that may protect against heart disease, cancers and ageing (Beattie *et al.* 2005). In many works a positive correlation between the intake of fruits and vegetables and prevention of diseases like atheroscelerosis, cancer, diabetes, arthritis and also ageing is documented (Kaur & Kapoor 2001). Bioactive extracts of blueberries are rich in anthocyanins and proathocyanins with a significant antioxidant activity (Smith *et al.* 2000) which is influenced by various factors (temperature, pH, oxidation) and is about 3-fold higher than either strawberries or raspberries (Kalt *et al.* 1999). Total phenolics and total anthocyanin concentrations in wild crop are higher than in cultivated blueberries (Giovanelli & Buratti 2009). The changes of total anthocyanins (TA) content in fruit of blueberries in relation to freezing duration are the aim of this study.

MATERIAL AND METHODS

The samples of 10 very early, resp. early (VE, E), 5 medium early (ME) and 6 medium late, resp. late (ML, L) blueberry cultivars were obtained from the research breed station in Kriva on Orava. Locality of blueberry cultivation is characterised by altitude 700 m, average yearly temperature 6°C and precipitation 800 till 900 mm. From manually collected blueberries 100 g samples were weighted and stored in PE bags in freezing box at temperature – 18°C. From blueberries samples 50 g were homogenised and extracted by 100 ml 80% ethanol during 12 hours. In obtained extracts total anthocyanins contents by modified method of Lapornik *et al.*

(2005) were spectrophotometrically (Shimadzu UV/VIS – 1240) determined. Into two tubes 1 cm^3 of extract was pipetted and 1 cm^3 0.01% HCl in 80% ethanol was added. Then 10 cm^3 14% HCl into the first tube and 10 cm^3 McIlvain agens (pH 3.5) into another tube were added. Absorbance was measured at 520 nm against blank sample. The obtained results were statistically evaluated by ANOVA single factor analyse (α = 0.01).

RESULTS AND DISCUSSION

Significant differences of total anthocyanin content among the observed cultivars in interval 615.8 mg/kg (Puru) – 3206.3 mg/kg (Northland) were confirmed (Table 1).

The statistically significant differences in TA content among blueberry cultivars were confirmed also by ANOVA single factor analyse (α = 0.01). In VE, E cultivars *P*-value = 4.12E-38, in ME cultivars *P*-value = 2.53E-15 and in ML, L cultivars *P*-value = 5.99E-20.

The average TA content in 10 VE, E cultivars was 1942.3 mg/kg, in 5 ME cultivars 1515.5 mg/kg, in 6 ML, L cultivars 1431.1 mg/kg. During first 3 months of blueberries store in the freezing box the average decrease (12.6%) of TA content was observed. At the some time significant differences among the observed cultivars were confirmed. The average decrease value of TA content in VE, E cultivars was 16.6%, in ME cultivars 7.5% and in ML, L cultivars 4.3%. The average decrease value of TA content after 6 months of blueberry storage at – 18°C in relationship to the first measurement in VE, E cultivars was 19.5%, in ME cultivars 16.5% and in ML, L cultivars 14%.

Our results correspond with results of Gao and Mazza (2006). They determined simple and acylated anthocyanins and other phenolics in 10 cultivars and hybrids of lowbush (*Vaccinium corymbosum*) and one cultivar of highbush (*V. angustifolium*) blueberries. TA content in blueberries ranged from 110 to 260 mg/100 g of fresh berries. Similar results (12.7–197.34 mg/100 g f.m.) are presented also by Sellapan *et al.* (2002).

Table 1. Total anthocyanins content (mg/kg) in blueberry samples during time storage at temperature –18°C

Group of cultivars	Cultivar	I. (0 months)	II. (3 months)	III. (6 months)
Very early, resp. early (VE, E)	Goldtraube 23	1971.57	1679.83	1033.04
	Earlyblue	1860.88	1202.64	1660.78
	Sunrise	2013.92	1461.46	1531.16
	Puru	615.78	566.7	575.08
	Duke	2152.18	1809.73	1934.3
	Nui	1724.56	1797.3	1642.68
	Reka	900.47	1023.54	1140.05
	Spartan	2073.89	1476.69	1596.44
	Goldtraube	2903.9	2399.34	2324.3
	Northland	3206.29	2144	2177.54
Medium late, resp. late (ML, L)	Pemberton	2145.2	1599.79	1419.8
	Blueray	1335.1	1281.48	1052.16
	Bluejay	1398.48	1217.55	1485.86
	Patriot	1882.17	1745.82	1547.9
	Bluecrop	816.75	917.96	821.66
Medium late, resp. late (ML, L)	Nelson	1852.74	1810.47	1579.1
	Berkley	1581.8	1416.17	1087.3
	Brigitte	1116.26	1090.4	1030.18
	Persey	1603.21	1382.46	1264.44
	Herbert	1183.13	1028.37	1211.2
	Darrow	1249.01	1454.01	1212.54

Cho *et al*. (2005) determined in blueberries higher TA contents (1435.2–8227.3 mg/kg). Changes of antioxidant activity of blueberries in relation to their TA, polyphenols and flavonols content and their cultivar dependence are confirmed also by Velioglu *et al*. (1998). Our results are in agreement also with data published by Häkkinen *et al*. (1999). They confirmed, that concentrations of phytochemicals in berries are influenced by many factors including environmental conditions, degree of ripeness, cultivar, cultivation site, processing and storage of the fruit. After Scibisz and Mitek (2007) the long-term frozen storage of blueberries did not induce significant changes in TA content. It is most probable that the anthocyanin in frozen fruits becomes more easily extractable. An increase in TA content in raspberry during freezing has been reported by Ancos *et al*. (2000). Gonzàlez *et al*. (2003) documented anthocyanins losses in frozen blackberry stored for 12 months at –24°C. Other investigations have demonstrated dramatic losses of anthocyanins during frozen storage. Chaovanalikit and Wrolstad (2004) reported 88% anthocyanins degradation in sweet cherries after 6 months of storage at –23°C. Our results confirmed 16.7% average decrease of TA content after 6 months of blueberrries storage at –18°C.

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