

VARIATION IN THE CONTENT OF TOTAL PHENOLICS, ANTHOCYANINS AND ANTI-MICROBIAL EFFECTS IN TWO FRACTIONS OF BLUEBERRIES DIFFERENT CULTIVARS

Kristina LOŽIENĖ^{1*}, Juozas LABOKAS¹, Algimantas PAŠKEVIČIUS¹, Loreta LEVINSKAITĖ¹, Petras Rimantas VENSKUTONIS², Jurgita ŠVEDIENĖ¹, Giedrė ABRUTIENĖ¹

¹Nature Research Centre, Institute of Botany, Žaliųjų Ežerų Str. 49, LT-08406 Vilnius, Lithuania

²Kaunas University of Technology, Department of Food Science and Technology, Radvilėnų Rd 19, LT-50254 Kaunas, Lithuania

*Corresponding author. E-mail: kristina.loziene@gmail.com

Abstract

Ložienė K., Labokas J., Paškevičius A., Levinskaitė L., Venskutonis P.R., Švedienė J., Abrutienė G., 2016: Variation in the content of total phenolics, anthocyanins and antimicrobial effects in two fractions of blueberries different cultivars [Fenolinių junginių ir antocianinų kiekio bei antimikrobinio poveikio skirtumai dviejose šilauogių veislių uogų frakcijose]. – Bot. Lith., 22(1): 78–86.

Two fractions (pomace and juice) of blueberries of seven commercial cultivars growing in the same plantation were analysed for the content of total phenolics (TPC), total anthocyanins (TAC) and effects on food pathogens over different harvesting years. TPC in pomace did not differ significantly between years only in ‘Gretha’ and ‘Northblue’ cultivars; they accumulated stable content of phenolics. The variation in TAC in pomace across seasons was 2–5 times higher than that in TPC. The variations in TPC and TAC in juice across harvesting years were lower than in pomace, but both TPC and TAC in this fraction also differed significantly ($p < 0.05$) between years in all studied cultivars. The activity of juice and pomace of cultivars against food pathogens was mostly stable and did not show significant differences between harvesting years; the significant correlations were not established between antimicrobial activity of both berries fractions and TPC and TAC in all investigated cultivars. Bacteria were the most susceptible to blueberry juice with *Micrococcus* spp., *Bacillus macerans* and *Escherichia coli* being the most sensitive species. The evaluation of antifungal effect shows that only *P. chrysogenum* was sensitive to the juice of blueberries, while *Aspergillus niger*, *Cladosporium sphaerospermum* and *Penicillium chrysogenum* showed resistance to the tested blueberries juice and pomace extracts.

Keywords: antimicrobial effects, blueberries, food pathogens, juice, pomace, total anthocyanins, total phenolics.

INTRODUCTION

It is well-known that fruits are of vital importance in human’s everyday diet contributing to the nutritional value of food and health-protective effects. The small berry fruits are increasingly often referred to as natural functional foods; they are a particularly rich source of natural antioxidants, mainly represented by phenolic compounds, which are the essential qualitative index in small fruits (HOWARD et al., 2003; KONDAKOVA et al., 2009). Among small

fruits, blueberries have exceptionally high content of phenolic compounds. Although they are native species of North America, highbush blueberries (*Vaccinium corymbosum* L.) and lowbush blueberries (*Vaccinium angustifolium* Aiton) are extensively cultivated and commercially produced over a wide geographic range. About 30 hectares of blueberry plantations are established in Lithuania, the country of the Baltic region. Also there are three field collections of blueberries in Lithuania, where the studies on acclimatization and possibilities of cultivation

of different cultivars are carried out (STACKEVIČIENĖ et al., 1997; STACKEVIČIENĖ, 1998; BUDRIŪNIENĖ et al., 2002; ČESONIENĖ et al., 2010).

Blueberries have become popular not only with small consumers, but are usable in drinks, yogurts and as ingredients in sweet manufacture (CHANDAN & SHAHANI, 1993; ROBICHAUD, 2006). As blueberries are commercial and marketable berries, the demand in quantity of their production is being satisfied by growers and traders. However, very important is the other side of production – the quality of berries. Although the quality of production is treated as the marketable appearance or “outside quality” of berries frequently, the “inside quality” of berries, i. e. the content of biologically active compounds is also very important. The amounts of phenolic compounds in blueberry cultivars are affected genetically; however, the environmental conditions of the same field can differently influence on biosynthesis and accumulation of phenolics in different cultivars (CONNOR et al., 2002; SKUPIEŃ, 2006; DRAGOVIĆ-UZELAC et al., 2010). Therefore, the stability of “inside quality” of berries across harvesting years depends on the ability of cultivars to produce stable amounts of phenolics independently of annual variation of meteorological conditions.

The data on blueberries effect against different microorganism groups are quite scarce except for bacteria species of *Salmonella*, *Escherichia* and *Staphylococcus* (PUUPPONEN-PIMĪA et al., 2005; BISWAS et al., 2012). It has been demonstrated that *Listeria monocytogenes* is more sensitive to the effect of blueberry extracts than *Salmonella enteritidis* (SHEN et al., 2014). Mostly, the antibacterial effects of blueberries are being studied by using water and ethanolic extracts (CHATTERJEE et al., 2004; SILVA et al., 2013; PERVIN et al., 2013). Because blueberries are the raw material in different branches of food industry (in drinks, yogurts, and sweet manufacture) (CHANDAN & SHAHANI, 1993; ROBICHAUD, 2006; CINBAS & YAZICI, 2008), the two blueberries fractions – pomace and juice – are often used separately. Therefore, the studies on antimicrobial properties of the two fractions of berries are reasonable.

The main objective of this study was to evaluate variation in the content of total phenolics (TPC), total anthocyanins (TAC) as well as antimicrobial effects against food pathogens of two fractions (pomace and

juice) of berries of some more popular cultivars of blueberries in Lithuania.

MATERIALS AND METHODS

Plant material and meteorological data. The blueberries were cultivated in the field collection of the Nature Research Centre (Vilnius, Lithuania, N 54°46' and E 25°17') under the same environmental and growing conditions in rows along east-west direction at the distances of 1.5 m within and 2 m between rows. Seven of the more popular cultivars in Lithuania were selected for the study: six cultivars of *Vaccinium corymbosum* L. ('Northblue', 'Northland', 'Gretha', 'Dixi', 'Gila', and 'Bluecrop') and one of *V. angustifolium* Ait. ('Putte'). The ripe berries of these cultivars (from three bushes of each cultivar separately) were sampled at the full-mature stage and analysed annually over the three consecutive harvesting years period. The meteorological data (temperature (C°), precipitation (mm), photosynthetically active solar radiation (PAR) (Mj/m²) and sunshine duration (h)) were obtained from the meteorological bulletins (2010–2012) of the closest meteorological station of Lithuanian Hydrometeorological Service under the Ministry of Environment. The TPC and TAC increase in blueberries during ripening period and top out maximum at the stage of full ripening (CONNOR et al., 2002). In Lithuania, the picking of blueberries generally takes place from late July to the end of August; the cultivar 'Dixi' is characterized as the late cultivar (STACKEVIČIENĖ et al., 1997; ČESONIENĖ et al., 2010). Therefore, the meteorological conditions of July and August in 2010–2012 are presented in Table 1.

Preparation of juice and extracts of pomace. The defrosted berries were homogenized in a blender Braun Mix and centrifuged using centrifuge Sigma 2–16PK at 9.000 rpm for 10 min. The obtained juice was used for further tests. The berries pomace (centrifugation residue) was dried at 40°C and triturated using ultra-centrifuge mill Retsch ZM200 at 10.000 rpm with a separator of 1 mm in diameter. The consecutive extraction was carried out with acetone/methanol (1:1 v/v) (Sigma-Aldrich, United Kingdom) into an accelerated solvent extractor ASE 350 (Dionex, Sunnyvale, CA, USA) by using the following extraction parameters: pressure 10.3 MPa, tem-

Table 1. The average monthly temperature (T), rainfall (R), sunshine duration (Sd) and photosynthetically active solar radiation (PAR) in July–August 2010–2012

Meteorological factor	July					August				
	2010	2011	2012	Average ± SD	CV (%)	2010	2011	2012	Average ± SD	CV (%)
T (°C)	21.8	19.6	19.5	20.3 ± 1.3	6	19.8	17.3	16.5	17.9 ± 1.7	10
R (mm)	208	155	81	148 ± 64	43	117	101	83	100 ± 17	17
PAR (Mj/m ²)	330	285	258	291 ± 36	12	263	250	220	244 ± 22	9
Sd (h)	300	220	280	267 ± 42	16	215	250	205	223 ± 24	11

SD – standard deviation, CV – coefficient of variation.

perature 60°C at heating time 5 min, static time 3 × 5 min, and purging with nitrogen for 120 s. The extracts were centrifuged at 12.000 rpm for 10 min and concentrated in a rotary vacuum evaporator Büchi (Flavil, Switzerland) at a temperature not higher than 45°C. Dry extracts were stored in a refrigerator.

Determination of the content of total phenolics (TPC) and anthocyanins (TAC). Soluble TPC in juice and in the dried pomace extracts of blueberries were determined by Folin-Ciocalteu colorimetric method using gallic acid as a standard (SINGLETON et al., 1999). The absorption was measured on a spectrophotometer Biochrom Libra S32PC (Biochrom Ltd, England) at 765 nm. Results were expressed as mg/g and mg/L wet weight (gallic acid equivalents) in berries pomace and juice, respectively. Folin-Ciocalteu reagent was purchased from Sigma-Aldrich, and gallic acid from MERCK KGaA (Germany).

The TAC in pure juice and in the dried extracts of berries pomace was measured by pH differential method (LEE et al., 2005). Absorbance was measured in a Biochrom Libra S32PC spectrophotometer at 510 nm and 700 nm in buffers at pH 1.0 and 4.5.

Investigation of the antimicrobial effects. All microorganisms used in this study were isolated from foods: bacteria *Enterococcus faecalis* 2EL, *Listeria monocytogenes* 10LR, *Micrococcus* spp. 1414, *Bacillus macerans* 22LB, *Salmonella* spp. 17L, *Staphylococcus aureus* 5LL, *Escherichia coli* 18EL, *Pseudomonas aeruginosa* 24RL; yeasts *Candida parapsilosis* BICP1, *Debaryomyces hansenii* BID4T, *Geotrichum fermentans* BIG1, *Yarrowia lipolytica* BIY6.1, *Kluyveromyces marxianus* BIK7.1T, *Pichia fermentans* BIP.12, *Rhodotorula mucilaginosa* BIRH6, *Saccharomyces cerevisiae* BIS1.5T, *Torulasporea delbrueckii* BIT1, *Zygosaccharomyces bailii* BIZ.1T; fungi *Aspergillus niger* 11AL, *Cladosporium sphaerospermum* 3-1L, *Penicillium chrysog-*

num 18-1L. All microorganisms were obtained from the collection of the Laboratory of Biodeterioration Research of the Nature Research Centre (Lithuania).

The activity of blueberries juice and extracts of pomace were screened for antimicrobial activity for three years annually following the disc diffusion method (GULLUCE et al., 2007). The microorganisms were grown on Nutrient Agar (bacteria) (Liofilchem, Italy) and on Sabouraud dextrose agar (yeasts and fungi) (BioMérieux, France). Inoculum was obtained from overnight bacterial strains on Nutrient agar slants at 28°C, yeasts were cultured for three days, fungi – seven days. The suspensions of bacteria and yeast cells were equal to 0.5 McFarland turbidity standards. The concentrations of fungal spores in the suspension were determined using a hemacytometer, which was adjusted to 1.0 × 10⁶ cfu/mL. The resulting suspension of microorganisms was mixed for 15 s with a vortex. For the disc diffusion assay 100 µl of each suspension of microorganisms (bacteria and yeasts) was uniformly spread on a Sabouraud Agar in a Petri dish (90 mm). The suspensions of spores of micromycetes (one by one ml) were poured in Petri dishes with Sabouraud Agar at 45°C. After absorption of inoculate by agar, the sterile paper disc (6 mm in diameter) was placed on the surface of each Petri dish. The discs were impregnated separately using 10 µL of the pure juice and extracts of pomace. The inoculated Petri dishes were incubated at 37°C for 48 h with bacteria, 28°C for 72 h with yeasts and for five days with fungi. Antibacterial and antifungal activity was determined by the measurement of the zone of inhibition (mm) around the discs after the period of incubation. The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone (KALEMBA & KUNICKA, 2003; JAGESSAR et al., 2008). All tests were performed in triplicate for all strains. After the incubation period, the diameters of the inhibition zones were measured in mm.

Statistical analysis. Statistical data processing (the calculation of means, standard deviations (SD), coefficients of variation (CV), Spearman's rank correlation coefficients (r), reliabilities (p), and the analysis of variance (ANOVA)) was carried out using the STATISTICA® 7 and MS Excel software.

RESULTS AND DISCUSSION

Variation in the content of total phenolics and anthocyanins in two fractions of berries. The total phenolics highly varied in berries pomace of blueberry cultivars (Table 2). The highest variation in TPC in berries of pomace fraction between harvesting years was established in 'Dixi'. Also the average of TPC in berries pomace of this genotype was the lowest. The temperature, light intensity and light duration are those meteorological factors, which directly or indirectly influence on ripening of small fruits (JAAKOLA et al., 2004; KRÜGER et al., 2011). The full-maturity stage of 'Dixi' occurred in August, when the averages of temperature, PAR and sunshine duration were lower compared to July almost annually (Table 1). Therefore, the late ripening can result in low TPC in pomace of 'Dixi'. The cultivar 'Northland' was distinguished by the highest TPC in berries pomace, as its total phenolics exceeded the other cul-

tivars by 15–37%. However, there was a fairly high variation in TPC between harvesting years in berries pomace of 'Northland' compared to most of the studied cultivars (Table 3).

The cultivars 'Northblue', 'Gila', and 'Gretha' particularly, distinguished by the lowermost variation in TPC in pomace of blueberries through the study period (Table 2). The analysis of variance (ANOVA) showed that TPC in berries pomace of 'Gretha' and 'Northblue' did not differ significantly between harvesting years ($F = 3.3$, $p < 0.1$ and $F = 4.9$, $p < 0.06$, respectively) (Table 4, Fig. 1A). The implication is that the variation in meteorological conditions did not significantly influence on accumulation of TPC in pomace of 'Gretha' and 'Northblue' berries. Meanwhile, TPC in pomace of 'Gila' significantly differed in 2010–2012 (Fig. 1A).

Anthocyanins are the most important phenolic compounds of blueberries and the TAC values increase during ripening of the berries (KALT et al., 2001). The current study showed that TAC in blueberries pomace amounted to 11–15% of TPC, and that TPC and TAC significantly positively correlated ($r = 0.67$, $p < 0.05$). The variation of TAC in pomace across three harvesting years was notably (2–5 times) higher than that of TPC (Table 2). Though pathway of biosynthesis of anthocyanins is encoded genetically, it is

Table 2. Variation of total phenolics and total anthocyanins in blueberries pomace of different cultivars in 2010–2012

Cultivar	Total phenolics (mg/g)		Total anthocyanins (mg/g)	
	Average \pm SD	CV (%)	Average \pm SD	CV (%)
'Bluecrop'	34.96 \pm 5.11	15	5.68 \pm 3.79	67
'Northland'	41.07 \pm 7.26	18	4.67 \pm 1.50	32
'Northblue'	26.96 \pm 2.72	10	3.64 \pm 1.75	48
'Putte'	30.63 \pm 5.72	19	3.82 \pm 1.61	42
'Gila'	32.35 \pm 3.36	10	4.85 \pm 2.35	49
'Gretha'	34.51 \pm 3.09	9	5.05 \pm 2.03	40
'Dixi'	25.68 \pm 5.90	23	3.09 \pm 1.25	41

Table 3. Variation of total phenolics and total anthocyanins in blueberries juice of different cultivars in 2010–2012

Cultivar	Total phenolics (mg/L)		Total anthocyanins (mg/L)	
	Average \pm SD	CV (%)	Average \pm SD	CV (%)
'Bluecrop'	553.45 \pm 54.28	10	63.74 \pm 15.06	24
'Northland'	607.41 \pm 23.75	4	78.92 \pm 14.19	18
'Northblue'	562.35 \pm 36.38	7	74.49 \pm 24.17	32
'Putte'	561.82 \pm 41.50	7	67.26 \pm 33.05	49
'Gila'	615.59 \pm 30.46	5	60.91 \pm 40.89	67
'Gretha'	626.18 \pm 7.53	1	83.36 \pm 7.76	9
'Dixi'	605.80 \pm 19.27	3	112.62 \pm 37.24	33

Table 4. One-way analysis of variance (ANOVA) of total phenolics and anthocyanins in the cultivars of blueberries between harvesting years

Cultivar		Fruit pomace		Fruit juice	
		Total phenolics	Total anthocyanins	Total phenolics	Total anthocyanins
‘Bluecrop’	F	8.6	2209.6	239.5	365.8
	P	0.02*	< 0.001*	< 0.001*	< 0.001*
‘Northland’	F	28.9	576.9	6.6	21.2
	P	< 0.001*	< 0.001*	0.03*	0.002*
‘Northblue’	F	4.9	227.5	11.7	832.8
	P	0.06	< 0.001*	0.009*	< 0.001*
‘Putte’	F	15.0	322.3	61.2	6053.6
	P	0.005*	< 0.001*	< 0.001*	< 0.001*
‘Gila’	F	12.8	392.1	253.6	543.2
	P	0.007*	< 0.001*	< 0.001*	< 0.001*
‘Gretha’	F	3.3	416.8	7.2	593.5
	P	0.1	< 0.001*	0.03*	< 0.001*
‘Dixi’	F	14.8	155.9	7.5	13784.5
	P	0.005*	< 0.001*	0.02*	< 0.001*

*Significant differences between harvesting years (selected significance level $p < 0.05$). F – Fisher’s criterion.

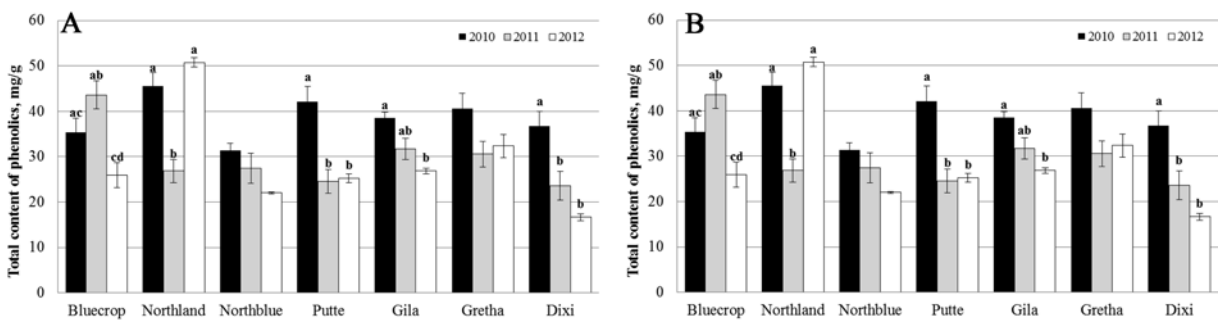


Fig. 1. Comparison of total phenolics (A) and total anthocyanins (B) in pomace of different blueberries cultivars between harvesting years. Different letters above columns indicate significant differences between harvesting years within a cultivar at $p < 0.05$. Means with the same letter and without letter do not differ significantly between harvesting years within cultivar

influenced by different environmental factors also (temperature, rainfall, ultraviolet light) (KRÜGER et al., 2011; CASTELLARIN et al., 2007; GUO et al., 2008). July and August of 2012 were distinguished by lower temperatures, PAR and rainfall (Table 1). All studied cultivars did not cope with such unfavourable meteorological conditions: TAC in berries pomace was the lowest that year (Fig. 1B). The highest and the lowest TAC variations in berries pomace were established in ‘Bluecrop’ and ‘Northland’, respectively (Table 2). As distinct from TPC the variation in TAC in berries pomace of ‘Gretha’ and ‘Northblue’ was not the lowest compared to other cultivars, and the ANOVA showed that TAC in berries pomace significantly ($p < 0.05$) differed in different years in all studied genotypes (Table 4).

The current study showed that TAC amounted to

10–13% of TPC in blueberries juice fraction (except for ‘Dixi’, which reached an average of 19%), and that TPC and TAC in juice significantly positively correlated ($r = 0.72$, $p < 0.05$). In juice, like in berries pomace, the variation in TAC across harvesting years was 2 to 13 times higher than variation of TPC (Table 3). Differently from berries pomace, the highest TPC and TAC in juice were observed in 2012 (Fig. 2). The year 2012 distinguished by low rainfall in July and August: in July it was lower by 61% and 48% than in 2010 and 2011, respectively, and in August – by 29% and 18%, respectively (Table 1). It has been reported that dry weight content in blueberries ranges from 14.13% to 32.9% and can depend on meteorological conditions of harvesting season (SKUPIEŃ, 2006). Therefore, markedly lower rainfall in 2012 might be a reason for the decrease

of water in berries. As a result, significantly higher TPC and TAC in juice of blueberries cultivars were established that year.

The highest average of TPC in blueberries juice was established in ‘Gretha’: its total phenolics exceeded the other cultivars by 2–12% (Table 3). The TAC in juice of this cultivar was also high compared to that of the most of other genotypes. Also TAC and particularly TPC in juice of ‘Gretha’ berries varied very slightly across harvesting years. However, although the variations in TPC and TAC in blueberries juice were lower than in pomace, the ANOVA showed that both TPC and TAC in juice differed significantly ($p < 0.05$) between years not only in ‘Gretha’, but also in all studied cultivars (Table 4).

Antimicrobial activity of two fractions of blueberries. The effects of blueberries juice and extracts of pomace on microorganisms isolated from food were different; the controls (50% acetone and 50% methanol) did not inhibit tested bacteria. The activity of juice and pomace extracts of blueberries cultivars

against food pathogens with several exceptions was quite stable and did not show any significant differences between harvesting years (Table 5, 6). Only the activity of ‘Bluecrop’ juice against most bacteria differed significantly between years.

Blueberries juice had the most prominent effect on bacteria, and *Micrococcus* sp., *Bacillus macerans* and *Escherichia coli* were the most susceptible. The zones of antibacterial inhibition ranged from 7.4 to 9.8 mm (Table 4). The lowest effect of the juice was observed on *Enterococcus faecalis* and *Pseudomonas aeruginosa* (6.2–7.4 mm). It was impossible to single out the most antimicrobially effective cultivar: different bacteria were affected slightly more effectively by juice from different cultivars. Blueberry juice had almost no effect on microscopic fungi, except for *P. chrysogenum*, which was slightly inhibited by ‘Dixi’ juice. That could be related to the highest TAC in the juice of that cultivar (Table 3). No effects were observed of blueberry juice from any cultivar on the studied yeasts.

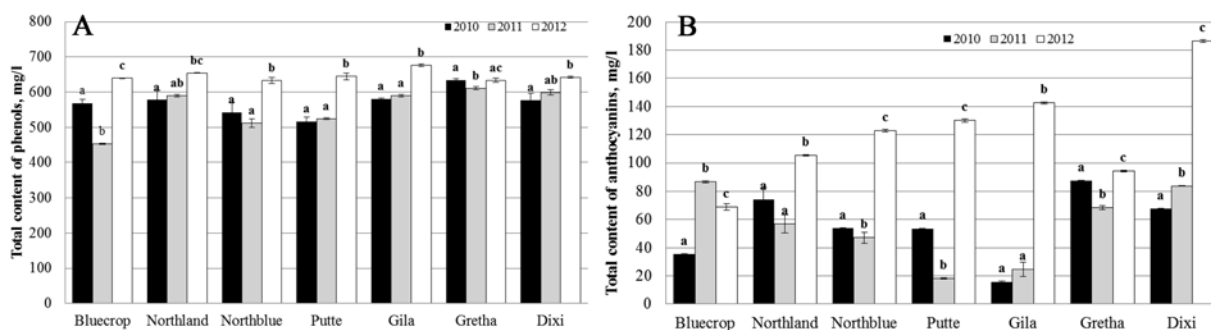


Fig. 2. Comparison of total phenolics (A) and total anthocyanins (B) in blueberries juice of different cultivars between harvesting years. Different letters above columns indicate significant differences between harvesting years within a cultivar at $p < 0.05$. Means with the same letter and without letter do not differ significantly between harvesting years within cultivar

Table 5. Antimicrobial effects of extracts (600 $\mu\text{g}/\text{mL}$) of berries pomace of blueberry cultivars

Bacterium species	Cultivars						
	Blue crop	Dixi	Gretha	Gila	Putte	Northland	Northblue
<i>Enterococcus faecalis</i>	0.0	0.0	6.4 ± 0.4	0.0	0.0	0.0	0.0
<i>Micrococcus</i> spp.	6.7 ± 0.6	$7.6 \pm 1.4^*$	0.0	0.0	0.0	0.0	0.0
<i>Bacillus macerans</i>	0.0	0.0	0.0	6.8 ± 0.7	$6.7 \pm 0.9^*$	0.0	0.0
<i>Escherichia coli</i>	0.0	6.9 ± 1.3	6.9 ± 1.3	0.0	6.7 ± 0.6	$7.4 \pm 1.3^*$	6.3 ± 0.3
<i>Pseudomonas aeruginosa</i>	0.0	6.7 ± 0.9	0.0	0.0	0.0	0.0	0.0

*Significant differences between harvesting years (selected significance level $p < 0.05$). Values are mean \pm standard deviation measured for samples in 2010–2012, and differences between harvesting years were compared by one-way analysis of variance (ANOVA). Listed are only those microorganisms, which responded to extracts of berries pomace. Lysis zones (mm) including disk (6 mm).

Table 6. Antimicrobial effects of berries juice of blueberry cultivars

Microorganism species	Cultivars						
	Bluecrop	Dixi	Gretha	Gila	Putte	Northland	Northblue
Bacteria							
<i>Enterococcus faecalis</i>	6.8 ± 1.3*	7.2 ± 1.0	6.9 ± 1.0	6.6 ± 0.7	6.7 ± 0.1	6.6 ± 0.2	7.0 ± 0.9
<i>Listeria monocytogenes</i>	7.3 ± 2.3*	8.4 ± 3.1	8.0 ± 2.0	7.4 ± 1.2	8.3 ± 1.0	6.9 ± 1.5	6.8 ± 1.1
<i>Micrococcus</i> spp.	7.7 ± 1.0*	9.0 ± 0.6	8.9 ± 1.9	7.3 ± 1.8	8.1 ± 0.7	7.5 ± 1.0	9.8 ± 2.7*
<i>Bacillus macerans</i>	8.2 ± 3.3*	9.7 ± 3.3	7.4 ± 1.5	8.8 ± 2.5*	9.7 ± 3.4	8.3 ± 2.0	7.6 ± 1.9
<i>Salmonella</i> spp.	6.7 ± 0.3	6.7 ± 0.6	6.7 ± 0.7	6.8 ± 0.2	6.4 ± 0.8	7.8 ± 2.3	6.9 ± 0.8
<i>Staphylococcus aureus</i>	6.6 ± 1.0	7.3 ± 1.2*	8.4 ± 2.5	8.9 ± 3.3	7.9 ± 1.8	7.4 ± 1.5	6.8 ± 0.8
<i>Escherichia coli</i>	8.4 ± 3.9*	8.6 ± 2.5	8.8 ± 2.3	7.4 ± 2.0	8.9 ± 2.5	8.0 ± 1.8	8.1 ± 1.9
<i>Pseudomonas aeruginosa</i>	7.4 ± 2.5*	6.6 ± 0.7	6.4 ± 0.5	6.4 ± 0.4	6.2 ± 0.2	6.3 ± 0.6	6.3 ± 0.3
Fungi							
<i>Penicillium chrysogenum</i>	0.0	6.5 ± 0.4	0.0	0.0	0.0	0.0	0.0

*Significant differences between harvesting years (selected significance level $p < 0.05$). Values are mean ± standard deviation measured for samples in 2010–2012, and differences between harvesting years were compared by one-way analysis of variance (ANOVA). Listed are only those microorganisms, which responded to berries juice. Lysis zones (mm) including disk (6 mm).

The extracts of berries pomace showed fairly weak antibacterial effects and had no effect on other microorganisms (Table 6). The most prominent effect was established with ‘Dixi’ pomace on *Micrococcus* spp. and ‘Northland’ on *E. coli*. In other cases the antibacterial effect was lower or absent at all.

The study of antimicrobial effects of different blueberry cultivars on microorganisms showed that juice and extracts of pomace possessed a certain inhibitory effect. Bacteria showed the highest susceptibility towards juice. The juice of all cultivars had the effects against the tested bacteria, whereas fungi and yeast were more resistant. A good effect of blueberry juice on foodborne bacteria was also reported by BISWAS et al. (2012). Our study showed that the susceptibility towards blueberry juice was variable between the tested bacteria. A different sensitivity of bacteria towards methanolic blueberry extracts was also reported by SHEN et al. (2014).

The extract of blueberry pomace had a slight inhibitory effect only on some bacteria. Our study showed that fungi were the most resistant group of microorganisms: only negligible effects of the juice were noticed against *P. chrysogenum*.

The results showed that, independently of annual variation of meteorological conditions, the amounts of total phenolics were significantly stable only in pomace fraction of blueberries ‘Gretha’ and ‘Northblue’ cultivars. The blueberries juice and pomace revealed low activity against food pathogens and did not show significant differences between harvesting years.

ACKNOWLEDGEMENTS

This study was funded by the Research Council of Lithuania, Grant No. SVE-04/2011. Also we express our thanks to Dr Elicija Stackevičienė for providing the background information about blueberry field collections at the Institute of Botany of the Nature Research Centre; PhD student of Kaunas University of Technology Paulius Kraujalis for his kind technical assistance in preparing extracts.

REFERENCES

- BISWAS D., WIDEMAN N.E., O’BYRAN C.A., MUTHAIYAN A., LINGBECK J.M., CRANDAL P.G., RICKE S.C., 2012: Pasteurized blueberry (*Vaccinium corymbosum*) juice inhibits growth of bacterial pathogens in milk, but allows survival of probiotic bacteria. – *Journal of Food Safety*, 32: 204–209.
- BUDRIŪNIENĖ D., AŠMONTIENĖ V., DAUBARAS R., 2002: Sodinė šilauogė. – Kaunas.
- CASTELLARIN S.D., MATTHEWS M.A., GASPERO G.D., GAMBETTA G.A., 2007: Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. – *Planta*, 227: 101–112.
- ČESONIENĖ L., DAUBARAS R., VIŠKELIS P., 2010: Agrobiological properties and berry quality of high-bush blueberry cultivars. – *Horticulture and olericulture, scientific articles*, 29: 3–12 (in Lithuanian with English summary).

- CHANDAN R.C., SHAHANI K.M., 1993: Dairy Science and Technology Handbook: Product Manufacturing, 2. – New York.
- CHATTERJEE A., YASMIN T., BAGCHI D., STOHS S.J., 2004: Inhibition of *Helicobacter pylori* in vitro by various berries extracts, with enhanced susceptibility to clarithromycin. – Molecular and Cellular Biology, 265: 19–26.
- CINBAS A., YAZICI F., 2008: Blueberry Addition to Yoghurts, Effect of the Addition of Blueberries on Selected Physicochemical and Sensory Properties of Yoghurts. – Food Technology and Biotechnology, 46: 434–441.
- CONNOR A.M., LUBY J.J., TONG C.B.S., FINN C.E., HANCOCK J.F., 2002: Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. – Journal of the American Society for Horticultural Science, 127: 89–97.
- DRAGOVIĆ-UZELAC V., SAVIĆ Z., BRALA A., LEVAJ B., KOVAČEVIĆ D.B., BIŠKO A., 2010: Evaluation of Phenolic Content and Antioxidant Capacity of Blueberry Cultivars (*Vaccinium corymbosum* L.) Grown in the Northwest Croatia. – Food Technology and Biotechnology, 48: 214–221.
- GULLUCE M., SAHIN F., SOKMEN M., OZER H., DAFERERA D., SOKMEN A., POLISSIOU M., ADIGUZEL A., OZKAN H., 2007: Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. – Food Chemistry, 103: 1449–1456.
- GUO J., HAN W., WANG M.-H., 2008: Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: a review. – African Journal of Biotechnology, 7: 4966–4972.
- HOWARD L.R., CLARK J.R., BROWNMILLER C., 2003: Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. – Journal of the Science of Food and Agriculture, 83: 1238–1247.
- JAAKOLA L., MÄÄTTÄ-RIIHINEN K., KÄRENLAMPI S., HOHTOLA A., 2004: Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. – Planta, 218: 721–728.
- JAGESSAR R.C., MARSA A., GOMES G., 2008: Antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. – Nature and Science, 6: 24–38.
- KALEMBA D., KUNICKA A., 2003: Antibacterial and antifungal properties of essential oils. – Current Medicinal Chemistry, 10: 813–829.
- KALT W., RYAN D.A.J., DUY J.C., PRIOR R.L., EHLENFELDT M.K., KLOET S.P.V., 2001: Interspecific Variation in Anthocyanins, Phenolics, and Antioxidant Capacity among Genotypes of Highbush and Lowbush Blueberries (*Vaccinium* Section *cyanococcus* spp.). – Journal of Agriculture and Food Chemistry, 49: 4761–4776.
- KONDAKOVA V., TSVETKOV I., BATCHVAROVA R., BADJAKOV I., DZHAMBAZOVA T., SLAVOV S., 2009: Phenol compounds – qualitative index in small fruits. – Biotechnology & Biotechnological Equipment, 23: 1444–1448.
- KRÜGER E., DIETRICH H., HEY M., PATZ C.-D., 2011: Effects of cultivar, yield, berry weight, temperature and ripening stage on bioactive compounds of black currants. – Journal of Applied Botany and Food Quality, 84: 40–46.
- LEE J., DURST R.W., WROLSTAD R.E., 2005: Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. – Journal of AOAC International, 88: 1269–1278.
- PERVIN M., HASNAT M.A., LIM B.O., 2013: Antibacterial and antioxidant activities of *Vaccinium corymbosum* L. leaf extract. – Asian Pacific Journal of Tropical Disease, 3: 444–453.
- PUUPPONEN-PIMIA R., NOHYNEK L., ALAKOMI H.-L., OKSMAN-CALDENTY K.-M., 2005: Bioactive berry compounds – novel tools against human pathogens. – Applied Microbiology and Biotechnology, 67: 8–18.
- ROBICHAUD M.-J., 2006: Blue skies for blueberries. Statistics Canada. Available: <http://www.statcan.gc.ca/pub/21-004-x/21-004-x2006001-eng.pdf> (accessed 12 May 2015).
- SHEN X., SUN X., XIE Q., LIU H., ZHAO Y., PAN Y., HWANG CH.-A., WU V.C.H., 2014: Antimicrobial effect of blueberry (*Vaccinium corymbosum* L.) extracts against the growth of *Listeria monocytogenes* and *Salmonella enteritidis*. – Food Control, 35: 159–165.

- SILVA S., COSTA E.M., PEREIRA M.F., COSTA M.R., PINTADO M.E., 2013: Evaluation of the antimicrobial activity of aqueous extracts from dry *Vaccinium corymbosum* extracts upon food microorganism. – Food Control, 34: 645–650.
- SINGLETON V.L., ORTHOFER R., LAMUELA-RAVENTOS R.M., 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. – Methods in Enzymology, 299: 152–178.
- SKUPIEŅ K., 2006: Chemical composition of selected cultivars of highbush blueberry fruit (*Vaccinium corymbosum* L.). – Folia Horticulture, 18: 47–56.
- STACKEVIČIENĖ E., 1998: Highbush blueberry cultivars promising in Lithuania. – Forestry Studies (Tartu), 30: 180–186.
- STACKEVIČIENĖ E., BUTKUS V., BUTKIENĖ Z., 1997: Introduction and essentials of cultivation of American cranberries, highbush and lowbush blueberries and lingonberries. – Agriculture Science, 2: 58–70 (in Lithuanian).

FENOLINIŲ JUNGINIŲ IR ANTOCIANINŲ KIEKIO BEI ANTIMIKROBINIO POVEIKIO SKIRTUMAI DVIEJOSE ŠILAUOGIŲ VEISLIŲ UOGŲ FRAKCIJOSE

Kristina LOŽIENĖ, Juozas LABOKAS, Algimantas PAŠKEVIČIUS, Loreta LEVINSKAITĖ, Petras Rimantas VENSKUTONIS, Jurgita ŠVEDIENĖ, Giedrė ABRUTIENĖ

Santrauka

Buvo tirti fenolinių junginių bendro kiekio (TPC) ir antocianinų bendro kiekio (TAC) bei poveikio maisto patogeniniams mikroorganizmams pokyčiai vienodose aplinkos sąlygose augintų septynių komercinių šilauogių veislių uogų sultyse ir išspaudose. Uogų išspaudose TPC tarp derėjimo metų patikimai nesiskyrė tik 'Gretha' ir 'Northblue' veislėse. TAC įvairavimas uogų išspaudose tarp sezonų buvo 2–5 kartus didesnis nei TPC įvairavimas. TPC ir TAC įvairavimas uogų sultyse buvo silpnesnis nei išspaudose, tačiau ir TPC, ir TAC šioje uogų frakcijoje taip pat patikimai ($p < 0,05$) skyrėsi tarp skirtingų derėji-

mo metų visose tirtose veislėse. Uogų sulčių ir išspaudų aktyvumas prieš maisto patogeninius mikroorganizmus tarp derėjimo metų patikimai nesiskyrė; nei vienoje veislėje nebuvo nustatyti patikimi koreliaciniai ryšiai tarp uogų frakcijų antimikrobinio aktyvumo ir TPC bei TAC. Bakterijos buvo labiau jautrios šilauogių uogų sultims; *Micrococcus* spp., *Bacillus macerans* ir *Escherichia coli* buvo labiausiai jautrios rūšys. Mikrogybai *Aspergillus niger*, *Cladosporium sphaerospermum* ir *Penicillium chrysogenum* buvo labiausiai atsparūs tirtų šilauogių veislių uogų sulčių ir išspaudų poveikiui.