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Chemical study of various Blueberry varieties introduced in Georgia

Meri Khakhutaishvili[1], Indira Djaparidze[2], Maia Vanidze[3] and Aleko Kalandia[4]

[1]Ph D students, [2]Senior Researcher, [3]Association Professor; Chemical Department Manager, [4]Professor
Batumi Shota Rustaveli State University, Georgia, 6010.
[1]Email: khakhutaishvilim@gmail.com | [2]Email: indira.djafaridze@gmail.com | [3]Email: vanidzemaia@gmail.com
[4]Email: aleko.kalandia@bsu.edu.ge

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ABSTRACT

Georgia is a small country, so it is desirable to grow profitable plants here that can easily adapt to environmental conditions. Such a plant is a blueberry. We have analyzed and studied the technical, biochemical and chemical indicators of 16 varieties of blueberries imported into western Georgia. The influence of environmental conditions and storage conditions on the organoleptic, technical, physico-chemical composition and antioxidant activity has been determined. Separation-identification and quantitative analysis were conducted using UPLC-MS (Waters Acquity QDa detector), HPLC (Waters Brceze 1525, UV-Vis 2489 detectors), pH-meters (Mettler Toledo). Refractrometer -Misco, Spectrometer –Cuvette Changer (Mettler Toledo UV5A), C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg). Antioxidant activity was defined by the DPPH method. Dry substances, titrated acidity and active pH of fruits, as well as qualitative, quantitative composition and dependence on the variety were determined. General phenols (1000 mg/100 g on average), anti-cyanins (400 mg/100 g on average) and their antioxidant dependence on storage conditions and duration were examined. The maximum preservation of antioxidants in fruits was maintained at -25°C under storage conditions.

Keywords: Blueberry, antioxidant, common phenols.

INTRODUCTION

Blueberry (Vaccinium spp.) belongs to the Ericaceae family, which includes about 450 varieties. Together with other berries (cranberries, lingonberries) the cultivation of blueberry began in the early XX century, when Frederick V. Coville selectively developed the northern highbush blueberry (Vaccinium corymbosum L.) (Mainland and Frederic, 2012). At the next stage, other blueberry varieties were brought in the United States and Canada (Westwood 1993, Prodorutti *et al.*, 2007) However,

the northern highbush blueberry is much more common in the US as well as in other countries around the world (Ehlenfeldt *et al.*, 2006).

The popularity of blueberry has increased over the last decade. In 1990 the blueberry culture was spread only in 10 countries (Statistics Division, Food and Agriculture Organization of the United Nations 2015), whereas in commercial terms, it was already common in 27 countries in 2011 (Evans and Ballen, 2014). However, according to FAO (Statistics Division, Food and Agriculture Organization of the United Nations, 2015) data, the US produces more than half of the world's blueberry production (more than 200,000 tons) (United States Department of Agriculture Economic Research Service 2015). Canada (average of 93,000 tons) is on the second place after the US, and the third place belongs to Poland (10,600 tons). Blueberry is grown in almost all member states of the EU (68 000 tons) and in some European countries (28 000 tons) (Brazelton, 2012; Connor, *et al.*, 2002).

According to the statement of the USDA, more than 50 varieties of blueberry are used for production. They differ in many agronomic properties. Among them the most important ones are: a harvest time, a fruit size, a productivity, a frost and a disease resistance, a chemical composition and a nutritional value.

The nutritional value of a raw fruit is determined by the content of carbohydrates (9.7%), proteins (0.6%) and fat (0,4%), according to which the average energy value of the 100 g of raw blueberry fetus is 192 KJ. The blueberry fruit is a good source of fiber compounds; it represents 3% -3.5% of the total mass of fruit. Many studies have shown that blueberries contain substances necessary for maintaining health: they have anti-inflammatory properties and contain a significant number of polyphenols that have cardiovascular defective effect (Routray and Orsat, 2011; Zafra-Stone *et al.*, 2007; Diaconeasa *et al.*, 2015); the content of antioxidant compounds in blueberries reduces the risk of coronary diseases, prevents cholesterol oxidation, thus reducing the risk of atherosclerosis (Ramassamy, 2006).

The total content of polyphenols in blueberry varies between 48 to 304 mg/100 g (up to 0.3%) (Ehlenfeldt and Prior, 2001; Moyer *et al.*, 2002,) and is strongly dependent on the variety (Taruscio *et al.*, 2004), environmental conditions and ripening stage (Zadernowski *et al.*, 2005; Castrejón *et al.*, 2008). Polyphenols in blueberries are represented by flavonoids, procyani-des (monomers and oligomeric forms) (Gu *et al.*, 2002), flavonoids (köpferol, quercetin and myricetin) (Taruscio *et al.*, 2004), phenol-carbic acid (hydroxyl chloride acid), and stylens derivatives (Wu and Kang, 2012; Howard and Hagar 2007). In the process of ripening, the synthesis of anthocyanins occurs due to the transformation of individual phenolic compounds, and at the end of the ripening the content of anthocyanins reaches its maximum (Kalt *et al.*, 2003), what significantly determines the biological activity of the blueberry fetus.

The amount of anthocyanins in blue blueberry fruits is 25-495 mg/100g, depending on the size of the fruit, the maturity stage, the climatic conditions and the time of harvest (Mazza and Miniati, 1993) Among the berries, the blueberry fruit is characterized by different types of anthocyanins (Gao and Mazza, 1994), such as malvidine, delphinidine, petunidine, cyanidine and peonidine. They occur in the form of glycoside in the fetus, where the aglycone is represented by glucose, galactose and arabinose. According to some researches, malvidine and delphinidine are the main components of blueberry fetus and its content is 75% of total anthocyanins (Scibisz and Mitek,2007) while other studies show that the percentage of delphinidine is 27% - 40%, malvidine - 22% -33%, petunidine - 19% -26%, cyanidine - 6% -14% and peonidine - 1% - 5% (Cho *et al.*, 2007).

Blueberries are summer berries and they are gathered from the end of May to August. The harvest of berries on a bush is about 8 and 12 kg. After harvesting, regardless of the gathering method, blueberries are immediately degraded and placed in a cold place to reduce fruit respiration and dehydration (Crisosto, *et al.*, 1998).

Most of the fruit is intended for consumption in raw form. However, the nutritional (original, sweet-sour taste) and functional (strong antioxidant) value of blueberry increases its popularity, and it becomes attractive for the processing industry. More number of blueberries are sold in a processed form (Brazelton, 2015; Crisosto, *et al.*, 1998).

Although some varieties of blueberries grow wild in Georgia, blueberries are a relatively new, high-yielding

agricultural crop, and its growing becomes increasingly popular. According to the research, this industry has a great growth potential, because after reducing the tea production, thousands of hectares of land (soil pH 4,5 - 5,5), intended for tea plantation, were used for planting blueberries.

Blueberry seedlings were first brought in 2006 from the United States and planted for a sample in Imereti, where the first harvest was gathered in 2009. Today, about 20 varieties of blue blueberries are introduced in Georgia. They are well adapted to local climatic conditions and, therefore, industrial plantations are being built. The number of crops gathered from a bush is 4.0 to 10.0 kg (harvesting occurs occasionally with a few days intervals). Although the process of commercialization of blueberries has begun in Georgia recently, the export indicator is growing (according to CBI statistical data it was 150,000 USD in 2014, and 215,000 USD - in 2015).

Along with the growth of production volume, it is necessary to develop modern technologies for storage-processing of berries, ensuring the storage of the fetus with fewer losses and minimal changes in chemical composition.

The aim of the research was to study the chemical composition of the different varieties of blueberry (organic acids, carbohydrates, phenolic compounds, pectic substances, their antioxidant activity) in the process of ripening of fruit and in the long-term storage of the fetus on the territory of western Georgia.

MATERIALS AND METHODS

The object of research: The object of research was the variety of raw (Mist, O'Neal, Elizabeth, Blueray, Berkeley, Earlyblue, Sunrise, Toro, Duke, Legacy, Chandler, Spartan, Reka, Brigittarey, Bluegold, Bluecrop), shockingly frozen (-45^{0}C) and stored in a freezer (-25^{0}C).

Preparation of the sample for the test: Blueberry fruits were first cooled (heat retention) for 1 - 2 hours at less than 10^{0}C, then sorted and stored in different conditions, depending on the duration of storage. The samples were extracted by alcohol (anthocyanins were supplemented with 1% formic acid at 45^{0}C) for the chromatographic analysis. After extraction the samples were filtered, replenished with eluent and centrifuged. Before injecting the analyzed samples were filtered into 0,45-μm membrane filters.

Research Methods: Determination of dry substance in juice with a refractometer - Digital Refractometer # PA202 (Palm Abbe) MISCO;

Determination of pH and titrated acidity with a potentimeter (Mettler Toledo), by AOAC method;

Qualitative and quantitative determination of carbohydrate - HPLC (Waters (USA), 525 pump, 2414 Refractive Index Deteqtor and Carbohydrate column, the motion phase was 75% acetonitrile (SIGMA-ALDRICH).

Total monomeric anthocyanins were determined by using pH differentiated method - AOAC International Official Method 2005.02.

Antioxidant activity has been determined with respect to stable radical -DDPPH (2,2-Diphenyl-1-picrylhydrazil) by a free radical 50% inhibition method. DPPH – (Aldrich), LOT#STBD4147V (producvt of Germany).

Folin-Ciocalteu reagent was used for **determination of total phenols.**

RESULTS AND CONCLUSIONS

According to the variety, blueberries are distinguished by a sour and sourish taste (Bluecrop, Sunrise, Mist), a sweetish taste (Patriot, Toro), others have a sourish-sweetish one. The color of all the varieties is mainly blue with different shades. The aroma is peculiar to all of them. The size, volume and roundness of a fruit depends on its variety. The largest fruits belong to Chandler (3,8 g) and Berkeley (2,63 g), therefore, the fetal volume is greater. Duke have the smallest fruits (1,04 g) (Table 1).

Blueberry is distinguished by a high content of juice (70-92%). There is a difference between the varieties: the most juicy sort is Mist (92%), while the less juicy variety is Berkeley (70%). Dry substances in the juice slightly vary depending on the variety (about 10% brix); however, Reka, Sunrise common acidity, sugar-acid index, current acidity pH and Chandler lag behind (8.0; 9.0 respectively), while it is slightly more (11.0%)

in Torso. Fruits also differ in acidity content. Bluecrop (0,89%) and Sunrise (0,71%) are in the lead in this regard. The lowest indicator of these features refers to Brigettarey, Toro, Berkeley and Mist (0,38; 0,39; 0,40; 0,41% respectively). Approximately the same regularity was observed when determining the actual acidity (ranges from 3,4 to 4,12). It is logical that the dry substance and acidity ratio of fruit taste characteristics is quite high in most cases (more than 20). As for the most acidic Bluecrop, this characteristic is naturally low (11, 2) (Table 2).

The main components of the fetus juice dry substance are carbohydrates. HPLC study of carbohydrates (Fig. 1) has showed that the main components are fructose and glucose. Their ratio varies from 0,86 to 1,13 (Table 3). The sucrose trace can be observed in some varieties of blueberry.

Table 1: Blueberry fruit size, mass, volume, taste, color, smell, roundness index according to the varieties

Blueberry fruits physical and organoleptic characterization								
Blueberry Name	**Berry size**			**Berry mass, g**	**Berry volume, ml**	**Color**	**Taste**	**Aroma**
	Bunches Height, mm	Bunches Width,mm	roundness index RI					
Berkeley	18,8±0,44	13,85±0,33	1,36±0,032	2,63±0,022	2,55±0,032	Blue	Sweet Sour	Characteristics
Bluegold	14,2±0,33	12,5±0,29	1,13±0,027	1,35±0,011	1,35±0,017	"	„	"
Bluecrop	16,37±0,38	13,9±0,33	1,18±0,028	1,68±0,014	1,65±0,021	"	Sour	"
Brigitta-rey	17,4±0,41	13,9±0,33	1,25±0,029	1,7±0,014	1,67±0,021	"	Sour	"
Duke	12,95±0,30	11,1±0,26	1,16±0,027	1,04±0,009	1,04±0,013	"	Sweet Sour	"
Elizabeth	16,9±0,40	13,3±0,31	1,27±0,030	2,13±0,018	1,96±0,025	"	„	"
Misty	16,8±0,39	12,45±0,29	1,35±0,032	1,92±0,016	1,78±0,022	"	„	"
Patriot	17,88±0,42	13,7±0,32	1,3±0,031	1,86±0,016	1,82±0,023	"	Sweet	"
Reka	13,75±0,32	13,6±0,32	1,01±0,024	1,59±0,014	1,54±0,019	"	Sweet Sour	"
Sunrise	16,44±0,39	12,9±0,30	1,27±0,030	1,44±0,012	1,42±0,018	"	Sour	"
Toro	15,85±0,37	12,1±0,28	1,3±0,031	1,45±0,012	1,44±0,018	"	Sweet	"
Chandlery	15,17±0,36	19,78±0,46	0,76±0,018	3,5±0,030	3,8±0,048	"	Sweet Sour	"

Tables -2. Blueberry fruit juice ness, Juice brix, Titr., Acidity, Activity acidity, pH, Index brix./t.a

Blueberry name	**Blueberry Juice physical and chemical characterization**					
	Juice ness %	Juice brix, (20 °C)°brix	Correct brix, (20 °C)	Titr. Acidity by citric acid (pH8.1)%	Index brix./t.a	Activity acidity, pH
Berkeley	70±1,47	10,0±0,11	10,2±0,11	0,40±0,007	25,0±0,73	3,91±0,08
Bluegold	75±1,58	10,0±0,11	10,12±0,11	0,59±0,011	16,94±0,49	3,7±0,08
Bluecrop	75±1,58	10,0±0,11	10,18±0,11	0,89±0,016	11,23±0,33	4,0±0,08
Brigitta-rey	72±1,51	10,0±0,11	10,08±0,11	0,38±0,007	26,31±0,76	4,02±0,08
Duke	70±1,47	10,0±0,11	10,1±0,11	0,40±0,007	25,0±0,73	3,62±0,08
Elizabeth	80±1,68	10,2±0,11	10,3±0,11	0,63±0,011	16,19±0,47	3,5±0,07
Misty	92±1,93	10,0±0,11	10,08±0,11	0,41±0,007	24,39±0,71	3,52±0,07
Patriot	82±1,72	10,0±0,11	10,2±0,11	0,45±0,008	22,22±0,64	3,42±0,07
Reka	78±1,64	8,0±0,09	8,1±0,09	0,48±0,009	16,67±0,48	3,58±0,08
Sunrise	84±1,76	9,0±0,10	9,12±0,10	0,71±0,013	12,67±0,37	4,12±0,09
Toro	80±1,68	11,0±0,12	11,08±0,12	0,39±0,007	28,21±0,82	3,55±0,07
Chandlery	80±1,68	9,0±0,10	9,12±0,10	0,59±0,011	15,25±0,44	3,61±0,08

Table 3. Quantity content of carbohydrate in blueberries

	Name	Fructose g/kg	Glucose g/kg	Fructose/Glucose	Total sugars g/kg
1	Reka	44,164±0,22	48,31±0,24	0.91±0,005	92,474±0,46
2	Spartan	49,46±0,25	51,876±0,26	0.95±0,005	101,336±0,51
3	Sunrise	48,062±0,24	55,988±0,28	0.86±0,004	104,05±0,52
4	Toro	50,43±0,25	52,456±0,26	0.96±0,005	102,886±0,51
5	Misty	43,68±0,22	48,55±0,24	0.90±0,005	92,23±0,46
6	Duke	53,652±0,27	56,6±0,28	0.95±0,005	110,252±0,55
7	Brigitta-rey	35,91±0,18	39,29±0,20	0.91±0,005	75,2±0,38
8	Bluerey	41,112±0,21	43,902±0,22	0.94±0,005	85,014±0,43
9	Legacy	42,554±0,21	42,788±0,21	0.99±0,005	85,342±0,43
10	Bluecrop	51,31±0,26	45,254±0,23	1.13±0,006	96,564±0,48
11	Bluegold	39,28±0,20	35,844±0,18	1.10±0,006	75,124±0,38
12	Chandlery	48,452±0,24	44,6±0,22	1.09±0,005	93,052±0,47
13	Onile	39,67±0,20	35,808±0,18	1.11±0,006	75,478±0,38
14	Erliblue	45,774±0,23	53,99±0,27	0.85±0,004	99,764±0,50
15	Elizabeth	43,382±0,22	40,764±0,20	1.06±0,005	84,146±0,42
16	Berkeley	49,564±0,25	45,992±0,23	1.08±0,005	95,556±0,48

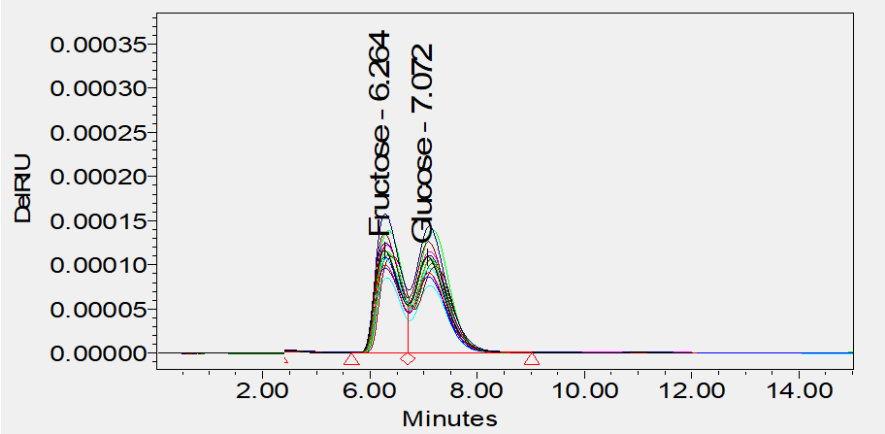

Fig. 1. Blueberry 16 varieties Carbohydrates HPLC, RI Deteqtor, column - Carbohydrate, a motion phase of 75% acetonitrile.

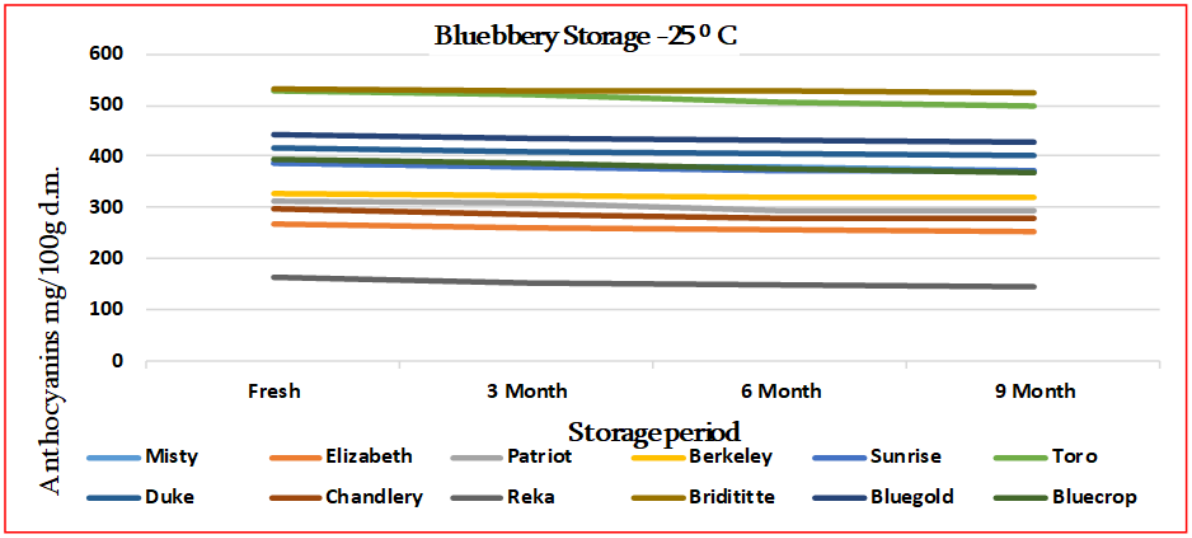

Fig. 2. Total content variability of anthocyanins during blueberry fetus storage.

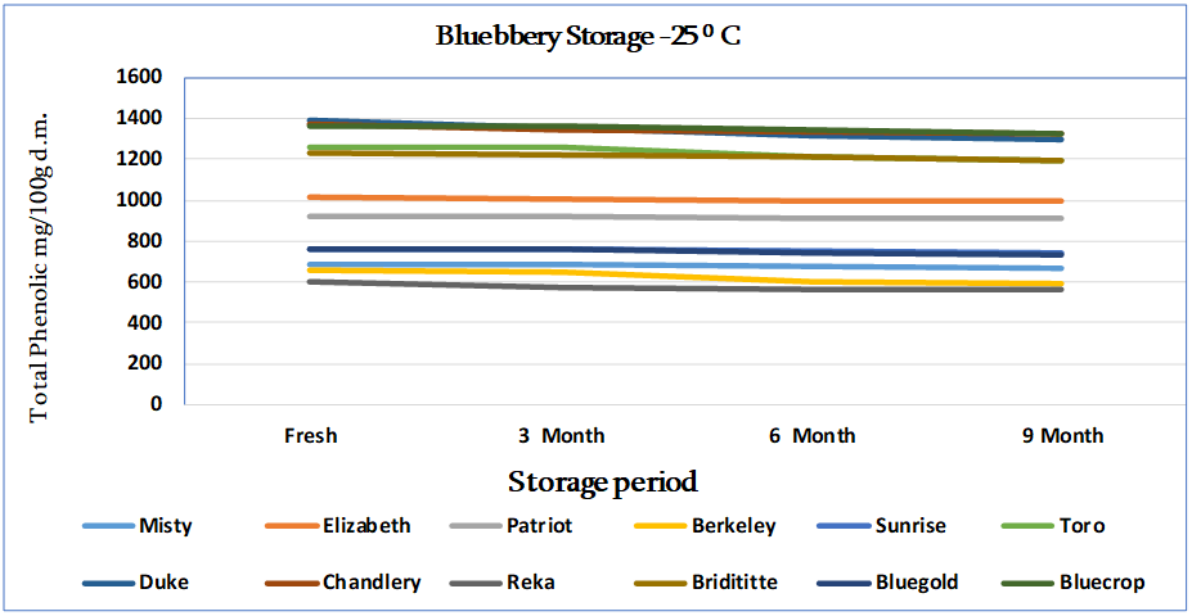

Fig. 3. Variability of the total composition of phenolic compounds during fruit storage.

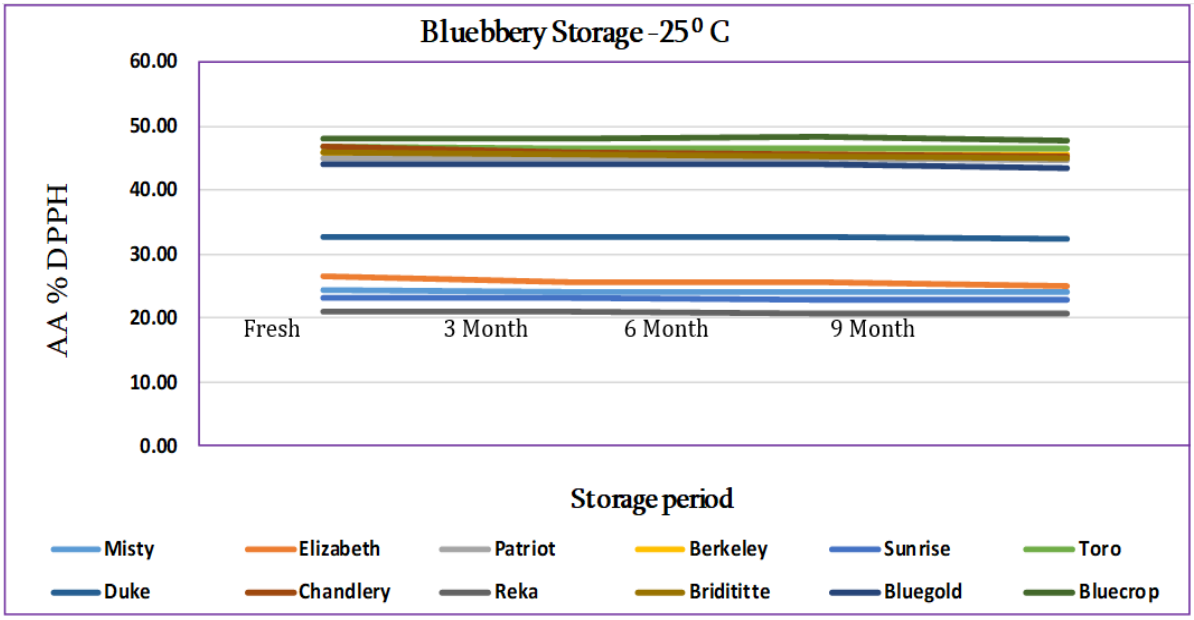

Fig. 4. Variability of antioxidant activity during the storage of blueberry fruits.

The total content of the anthocyanins is correlated with the color of the fetus (approximately 400 mg / 100g). The lowest number of blueberry fetus is in Reka (142,21 mg / 100g), while the highest number is in Brigittarey (564,37 mg / 100g). It should be noted that the shocking frost and the further storage allows to keep the antioxidant content in the fetus almost completely (Fig. 2).

After 9 months of storage the amount of anthocyanins was reduced by 5-7%. No special pattern has been observed in any of the varieties. The total content of phenolic compounds has also been maintained (Fig.3). It is also very important that the fetus keeps the antioxidant activity during 9 months of storing (Fig. 4).

CONCLUSIONS

The analysis of chemical composition of the blueberry fruit has shown that it was adapted to the soil-climatic conditions of Georgia. Among the studied varieties,

Chandler (3,87 ml) and Berkeley (2,55 ml) have the biggest fruits, while the smallest fruits are peculiar to Duke (1,04 ml). The largest amount of carbohydrates was observed in Duke (110.25 g / kg) and Sunrise (104 g / kg), while Brigittarey had the smallest one (75,1). The largest amount of anthocyanins was collected in Brigittarey (564.37 mg / 100g) and Bluegold (463 mg / 100g), while Reka was characterized by the smallest number (142,2 mg / 100 c). It is noteworthy, that after the shocking freezing, the chemical compounds and the antioxidant activity of the fetus practically were not changed.

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Conflict of interest:

The Authors declare no conflict of interest.

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