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INVESTIGATION OF THE BIOCHEMICAL COMPOSITION OF FRUITS FROM TWO GENOTYPES SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.)

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Abstract

The aim of present study is to make a comparative analysis of biochemical composition of fruits of selected genotypes and their suitability for food products.
The experiment was conducted between RIMSA-Troyan and FRDI - Plovdiv. Fruits of two perspective genotypes of sea buckthorn were included in the experiment in the collection plantation of the institute. Biochemical composition (dry matter refractometric (%), total sugars (%), sucrose (%), inverted sugar (%), organic acids (%) tannins (%), pectin (%), ascorbic acid (mg%)), antioxidant activity, total polyphenols of fresh fruits and were studied.
Data analyses show that the measured dry matter has higher values for the Siberian shed - 13.25%. The total polyphenols defined in the Siberian berries are three times more than the total Caucasian berries. Antioxidant activity was comparatively with similar values for both genotype due to the content of total polyphenols in fruits. The data were statistically distinguishable due to genotypes difference (p <0.05).

Key words: *antioxidant activity, biochemical compounds, Hippophae rhamnoides L., total polyphenols.*

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*), is a hardy thorny bush or small tree of family *Eleagnaceae.* It is commonly known as sea buckthorn, , sandthorn distributed on a sandy soil near the rivers, the dunes and the coastline in Europe, Japan, Himalayas, Altai, Tibet. (Li, 2003; Li & Schroeder, 1996). The female plants produce ripe sea buckthorn berries yellow, orange, or red , are spherical in shape, and range in size between 3 and 8 mm in diameter weighting from 0.2 g to 1 g, (Li, 2003).

The sea buckthorn berries has been used for medicinal and nutritional purposes in Russia, Europe, and Asia for many centuries. As an agricultural plant is grown in Germany, France, Finland, India and China, which is the largest agricultural producer of sea buckthorn. Many of the substances that are found in the sea buckthorn are known to have beneficial effects on health (Li and Wang, 1998). It has been well established in the literature that berries and seeds contain high amounts of natural antioxidants including ascorbic acid, tocopherols, carotenoids, flavonoids, as well as health beneficial fatty acids (Gao et al., 2000; Kallio et al., 2002; Rosch et al., 2003; Mondeshka, 2005).

The chemical and nutritional composition of sea buckthorn berries vary considerably between different subspecies, with the origin, climate, time of harvesting and method of processing. The chemical composition of *H. rhamnoides* ssp. sinensis varies greatly with growth locations (variation in latitudes and altitudes). It is also reported that sea buckthorn fruit berries and seed oil contain various kinds of bioactive substances. Clinically proven are the regenerative properties of the oil on gallbladder, duodenal and epithelial cells in skin burns. It acts favorably in colitis, gastritis and ulcers, as well as on lipid exchange in the liver. Its antioxidant properties protect the body from cardiovascular disease, hypertension, atherosclerosis and lower blood cholesterol levels (Mingyu et al., 2001; Yang, 2001; Yang, 2009).

In taste the fruits resemble the gums - sweet, slightly acidic but with a specific flavor. All food products in the diet normalize the functioning of the gastrointestinal tract Products on the market from sea buckthorn range from oil, juice, and food additives to candies, jellies, cosmetics, and shampoos. Sea-buckthorn fruit can be used to make pies, jams, lotions and liquors. The juice or pulp has other potential applications in foods or beverages. For example, in Finland, it is used as a nutritional ingredient in baby food. It provides a nutritious multi-vitamin beverage, rich in ascorbic acid and carotenes (Dharmananda, 2004; Schroeder & Yao, 1995; Mondeshka, 2005; Cenkowski et al., 2006).

MATERIALS AND METHODS

Fruit materials

The fruits of the Siberian variety have been grown and supplied by IPJZ-Troyan. Siberian is early spring - end of July - until mid-August, the bushes are up to 2.50 m high.

Siberian is early, fruits are large and oval, mature from the end of July to mid-August, the height of the bushes is up to 2.50 m.

The Caucasus has small berries, ripening from October to November, woody in appearance, with a height of 5.00 m.

Methods

- Determination of dry matter, % - BDS EN 12143-00;
- Determination of active acidity (pH) - BDS 11688-93;
- Total titrable acidity, % - BSS 6996-93;
- Active acidity (pH) - BDS 11688;
- Total sugars, % - BDS 7169-89;
- Ascorbic acid, mg% - BDS 11812-91;
- Pectin, % - BDS 16491-86;
- Tanning substances, % - Levental-Nibbaur method by (Bukharina et al., 2015).

Chemical compounds

For the analytical purposes, the following reagents were used: DPPH (2,2-diphenyl-1-picrylhydrazide) and Trolox [(±) -6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); Folin-Ciocalteau reagent (FC-reagent) (Merck, Darmstadt, Germany); gallic acid monohydrate (Fluka, Buchs, Switzerland). All other reagents and solvents analytical purity.

Preparation of samples for chemical analysis

5 g of each fruit are placed in a 50 mL volumetric flask. The contents of the flask were adjusted to ~ 2/3 of the volume with acidified methanol. After standing overnight under refrigeration conditions (10°C), the contents of the flask were added to the mark. The resulting methanol extracts were filtered through a crimped filter and analyzed.

All measurements were performed with a Helios Omega UV-Vis Spectrophotometer with VISIONlite software installed (Thermo Fisher Scientific, Madison, WI, USA) using 1 cm optical paths.

Total polyphenols (TPP)

The content of total polyphenols was determined by the Singleton and Rossi method in the following modification: In a 10 ml measuring tube, 0.1 ml of extract (base solution or fraction), ~ 7 ml of distilled water, 0.5 ml of FC reagent (diluted 1: 4 with distilled water) and 1.5 ml of a 7.5% (w / v) aqueous solution of sodium carbonate. After shaking, the tubes are poured to the mark with distilled water. After standing at rest for 2 hours at room temperature, absorbance of the reaction mixture was measured at 750 nm. An analogous blank was prepared using distilled water instead of extract. The results obtained are presented as gallic acid equivalents (GAE).

Antioxidant capacity

Radical scoring ability is determined by the method of Brand-Williams et al 2005. in the following modification: In a cuvette, 2250 μL of DPPH solution (2.4 mg DPPH in 100 mL of methanol) and 250 μL of extract (base extract or fraction) previously diluted with distilled water in a 1:3 volume ratio was dispensed sequentially prepared blank sample using methanol instead of extract. After the closed cuvettes were kept for 15 minutes in the dark at room temperature, the absorption of the reaction mixture at 515 nm was measured. The results obtained are presented as Trolox equivalents (TE).

Color measurements

Color measurements were performed instrumentally with COLORGARD SYSTEM 2000 colorimetry of BYK-GARDNER JNC., USA. The indicators are accounted for by the CIELab system. Three color coordinates are taken: L - luminosity (L = 0 - black, L = 100 - white), + a - red color, -a - green color, + b - yellow color Five measurements were performed on each sample. The color coordinates of each sample represent the arithmetic average of the measured coordinates.

Mathematical and statistical processing

The results presented are arithmetic mean values of at least three parallel definitions, with coefficients of variation less than 5%. Statistical data processing was performed with ANOVA, Microsoft Excel programs.

RESULTS AND DISCUSSION

Table 1 presents the data from the conducted studies of two genotypes (Siberian and Caucasian), grown and supplied by IPJZ - Troyan - differing in terms.

The data from Table 1 presents the studies carried out on the two genotypes sea buckthorn (Siberian and Caucasian) grown and provided by RIMSA - Troyan - differing in terms of ripening and form of the fruits.

Table. 1. Physico-chemical indicators of two genotypes of sea buckthorn (*Hippophae rhamnoides*. L.)

Genotype	Dry matter %	pH	L	a	b	mg GAE/ 100 g	µmol TE/ 100 g
Caucasian	9.25	3.24	55.94	31.46	65.59	55.00	579.17
Siberian	13.25	3.07	55.28	22.88	56.18	185.00	525.00

Data show that the measured dry matter has higher values for Siberian pepper - 13.25% as reported by (Lougas et al., 2006; Dhyani et al., 2007). Data are statistically distinct due to genotypic difference ($p<0.05$).

The total polyphenols defined in fruit Siberian sea buckthorn are 3 times more than the total content in the fruits of the Caucasian sea buckthorn.

Antioxidant activity was comparatively with similar values for both genotype due to the content of total polyphenols in fruits. The data were statistically distinguishable due to genotypes difference ($p<0.05$). A positive linear relationship with a high coefficient of determination $R^2 = 0.930$ between the total polyphenols and antioxidant activity in both genotypes of sea buckthorn. The data are presented in Figure 1.

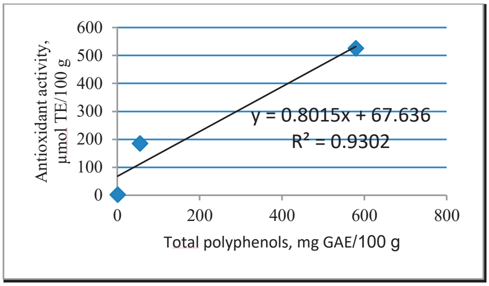

Figure 1. Linear relationship between the content of total polyphenols and antioxidant activity in the studied genotypes sea buckthorn (*Hippophae rhamnoides* L.)

The data from the conducted biochemical analyzes show that the fruits of the Caucasian sea buckthorn have higher values indicators of total sugars, sucrose and pectic substances. The fruits from Siberian genotypes have a higher percentage of invert sugar, total acidity, and tanning substances (Figure 2). The results were statistically distinguishable due to the difference of the genotypes selected raw materials ($p<0.05$).

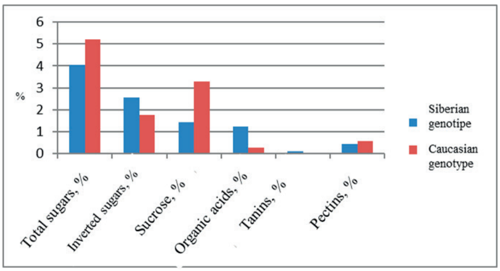

Figure .2. Biochemical indicators of genotypes of *Hippophae rhamnoides*. L.

The measured color characteristics in both genotypes have shown that indicator brightness of the color values is not close statistically distinguishable differences ($p> 0.05$). The predominant color tone is yellow for both genotypes, with the Caucasian genotype having higher values for this indicator and the measured red color component compared to the Siberian one.

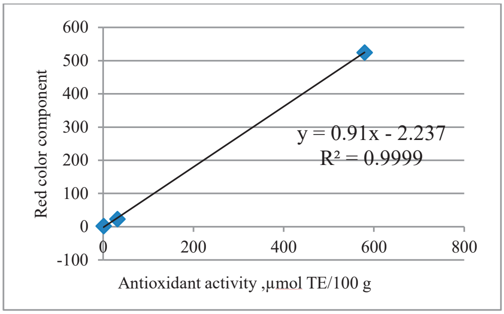

Figure 3. Linear relationship between antioxidant activity and a red component color genotypes *Hippophae rhamnoides* L.

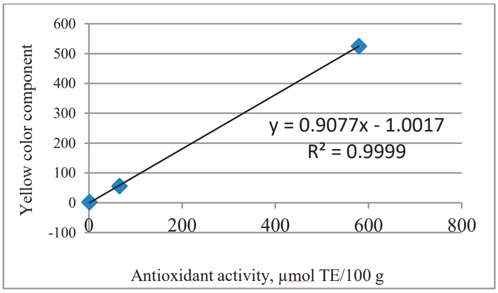

Figure 4. Linear relationship between antioxidant activity and a yellow component color genotypes sea buckthorn

Statistical processing showed that the values for both indicators were statistically distinguishable due to genotypes difference ($p < 0.05$). For both genotypes establish a positive linear relationship with a high coefficient of determination $R^2 = 0.99$ between antioxidant activity and pigments which impart yellow and red color in the fruit. The data are presented in Figures 3 and 4.

CONCLUSIONS

The biochemical composition ~~of~~ two genotypes sea buckthorn has been studied.

It was were reported higher values for dry matter and polyphenols in the Siberian genotypes.

The Caucasian fruits are distinguished by a higher percentage of invert sugar, total acidity and tanning substances.

Due to high nutritional value and its growing demand, it could be suggested that sea buckthorn berries might be explored for uses in different food commodities such as ready-to-serve beverage, squash, syrup, jam and jellies, etc. It is growing the variety of assortment list with a non-traditional raw material not well known to Bulgaria.

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