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TOTAL PHENOLICS OF FRESH AND FROZEN MINOR BERRIES AND THEIR ANTIOXIDANT PROPERTIES

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Abstract: *The aim of this work is revealing the minor berries biological abundance (bilberries and cowberry), fruits which can be found a lot in Bucovina's forests and are used at their real value. The biochemical characteristic studied in order to prove their strong antioxidant potential are the content of ascorbic acid, total polyphenol, antiradical activities, brute protein, humidity and dry substance of fresh and frozen fruits of bilberries (Vaccinium myrtillus L.) and Cranberries/lingonberry (Vaccinium vitis-idaea). The content of total phenolic compounds was measured in methanol fruits' extracts as well as their radical scavenging activity. Bilberries were highest in total phenolics and antioxidant activities, whereas cranberries contained the lowest quantity of phenolics, corresponding to 4.68 mg GAE/kg.It was studied the evolution of ascorbic acid during storage lengthy freeze fruit for 6 and 12 months at -25°C. In conclusion at the end of frozen storage period, ascorbic acid content and total poliphenols remained significantly unchanged compared with the values measured just after the freezing process.*

Keywords: *lingonberries, ascorbic acid, DPPH, frozen storage, loss, total poliphenols.*

1. Introduction

Bilberry and lingonberries are one of the most important shrubs that grow in our forests and mountain hill and mountain meadows, across the Carpathians, especially on shady slopes and humid forests. Berries have a more pronounced flavor than cultivated fruits, with sugar content higher than vitamins constitute a valuable food for human consumption.
The genus *Vaccinium* belongs to the family *Ericaceae* and consists of more than 150 species. Some plants of this genus bear edible fruits that have attracted the attention of the industry for the production of juices and jams. Like other fruits, bilberry contains micro- and macronutrients such as vitamins, minerals, and fiber; however, their biological properties have been largely attributed to their high levels of various phenolic compounds [1-3].
Dried blueberries give a tea; have been used as a folk remedy to fight against dysentery and diarrhea. Also, stalks and leaves are commonly used for tea, in order to reduce blood glucose and combating of diabetes, with similar effects to those of insulin. Bilberry anthocyanidins present in fruits develop a protective role against radiation are chemoprotective [4]. Many studies have shown a lower incidence of cancer as a result of dietary antioxidant that neutralizes free radicals multiple. Elements of phenolic constituents of

berries are designed to protect the body from oxidative stress caused by free radicals [5]. Eating cranberries or cranberry products based prevents urinary tract infections, the active principle is been identified type-A proanthocyanidins (PAC), [6]. Blueberries are commercialized in different ways mainly as fresh or frozen products [1].

Iwona Scibisz, 2007 investigate the effect of freezing and long-term frozen storage on total phenolic, total anthocyanin contents and antioxidant activity of highbush blueberries (*Vaccinium corymbosum* L. Accordind Iwona Scibisz, 2007 research, at the end of frozen storage period, antioxidant activity remained significantly unchanged compared with the values measured just after the freezing process [7].

Cranberries or blueberries red (*Vaccinium vitis-idaea)* is a shrub 10-40 cm, which grows at high altitudes in the mountain meadows and clearings. Cranberries are composed of water (88%), organic acids (including salicylate), fructose, vitamin C (high levels, i.e., 200 mg/kg of fresh berries), flavonoids, anthocyanidins, catechins, and triterpinoids [8]. Lingonberry (*Vaccinium vitis-idaea L.)* is rich in antioxidants such as vitamins C, A, and E (tocopherol) and especially polyphenols [9]. Anthocyanin pigments are responsible for the red color and are part of the complex antioxidants. Lingo berries contained only cyanidin based anthocyanin and three anthocyanins in order of elution (% proportion of individual anthocyanins) were found: cyanidin-3-galactoside (79%), cyanidin-3-glucoside (10%), and cyanidin-3-arabinoside (11%), [10].

Cranberries/lingonberry (*V. vitis-idaea)* are preserved well in fresh water at a temperature of 10-18°C and for a year, because they contain substances such as benzoic acid which are natural preservatives and can be eaten as sauce, jam, sorbet, juice and pickled [11]. It is also used in the preparation of a wine.

Cranberry tea (leaves and stems) is effective action in combating diseases of the kidney and urinary tract. Cranberry juice, predominantly in the form of a juice cocktail drink with approximately 25% cranberry juice, has been the traditional choice of most women seeking to prevent lower urinary tract infections [8].

Hoda M. Eid et al., 2010 elucidate the mechanism of action of one of the berries of *Vaccinium vitis idaea,* to treat symptoms of diabetes. They findings indicate that quercetin and quercetin 3-O-glycosides are responsible for the antidiabetic activity of *V. vitis* crude berry extract [11]. The impact of bioprocessing on lingonberry flavour was studied by Viljanen, Kaarina, et al. 2014. Enzyme treatment is a potential tool to decrease naturally acidic flavour of lingonberry. Fermentation, especially with yeast, could be an interesting new approach to increase the content of natural preservatives, such as antimicrobial benzoic acid [12].

The antioxidant properties of red cranberry are reported in the literature, and red berries have the highest antioxidant activity among many other fruits [13-14]. Studies with lingonberry (*V. vitis-idaea*) also associated health-promoting effects such as the decrease in proliferation of cancer cells by the fruit extract [15-16]. From the research by Mane Carine, et al. 2011, the use of lingonberry extract as a dietary supplement may be considered in the future to improve the antioxidant activity in human health. Antioxidants in red cranberry composition have effects for the entire cardiovascular system and the property to restore normal levels of cholesterol [17].

The aim of this work was to determine total phenolic content (TPC), antioxidant activity (TAA) and investigate how freezing and long-term storage can affect the ascorbic acid content (AAC) in blueberries and lingo berry.

2. Materials and methods

2.1. Samples and storage conditions

Collection of fruits was carried out manually at the time of full maturity. Bilberry and cranberries was collected at the site Pojorâta. Fresh undamaged berries were frozen and stored at -20°C for twelve months. The fruits were defrosted and mashed before chemical analyses.

2.3. Chemical analysis

The determination of moisture and volatile matter content in kernel was effectuated according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103 °C.

Total ash composition was obtained by calcinations of 5g of sample at 600 °C for 240 min.

Total soluble solids (TSS) was assayed using the refractometric method, with an Abbe refractometer and corrected to the equivalent reading at 20^0C (AOAC, 1995).

Protein content was analyzed by Kjeldahl method.

Ascorbic acid determination was done on acid extracts of samples.

Extraction of ascorbic acid from samples

The extracts were obtained following Rodriguez-Saona and Wrolstad (2001) modified protocol: 4 gram of samples was extracted with 12 ml of acidified solutions (Perchloric acid and o- Phosphoric acid 1%) using a ceramic mortar and a pestle. The residue was re-extracted until the extraction solvents remained colorless (the total solvent volume was 50 ml).The extract was filtered through a Whatman no. 1 filter paper.

The extracts were kept at -20°C until further analysis.

Ascorbic acid separation, identification and dosage

The ascorbic acid in the samples was separated, identified and dosed in a HPLC SHMADZU system coupled with UV–VIS detector (DAD). ZORBAX - C18 column (5µm, 250x4,6) was used. The column was eluted in isocratic system with a mobile phase consisted of phosphate buffer pH = 3.5 (TFA): solution 0.02 m/l of monopotassium phosphate and orthophosphoric acid 10%, adjusted to pH = 3.5. At a flow rate of 0, 6 ml/min. The chromatograms were registered at 245 nm.

For ascorbic acid identification standard L-ascorbic acid (Sigma 99% standard L ascorbic acid) was used. For dosage of ascorbic acid in the samples, a calibration curve was constructed using dilutions of standard L-ascorbic acid in bidistilled water.

The content of total polyphenolic compounds in fruits methanol extracts diluted 1/10 was determined by Folin-Ciocalteu method. For the preparation of the calibration curve 0.5 mL aliquot of 0.2, 0.3, 0.4, 0.8 and 1.2 µM/mL aqueous gallic acid solution were mixed with 10.0 mL Folin-Ciocalteu reagent (diluted ten-fold) and 1.0 mL sodium carbonate (20.0%) and the volume made up to 10.0 mL with H_2O. The absorption was read after 2 h at 25°C, at 760 nm. Total phenols were determined as gallic acid equivalents on a dry weight (mg GAE g^{-1} FW).

2,2-Di (4-tert-octylphenyl)-1-pycrilhydra-zyl (DPPH) scavenging capacity assay

The method used for determining the antioxidant activity of berries extracts is based on scavenging 2,2-Di (4-tert-

octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The berries samples aliquot (0.5 mL) was added to freshly prepared DPPH reagent. After incubating for 5 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer. The control was conducted in the same manner, except that distilled water was used instead of sample.
The IC_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given.
The DPPH scavenging capacity assay value is calculated according to the formula:

$$IC_{50}\ \% = [1 - (A_{samples} / A_{control})] \times 100.$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample.

pH value was measured in macerate of edible fruits by direct insertion of electrode (Rimpapa, 2007).

Acidity was determined by titrating samples with 0.01M NaOH solution up to pH 8.2, and was expressed as citric acid per 100 g samples.

2.4. Statistical analysis
The correlation and linear regression analyses were performed using Microsoft Office Excel 2007. Statistical analysis was performed with Student's t-tests. Differences with P values of less than 0.05 were considered statistically significant. The experiments were performed in triplicates and repeated two times.

3. Results and discussion

The results obtained from proximate composition of berries samples were shown in Table 1.

Moisture content was higher in bilberry (*Vaccinium myrtillus L.)* (85.8%) and lower in cranberries (*Vaccinium vitisi claea L.)* (57.28%).

Ash content.This analysis showed ash content of 1.457% for cranberries and 1.331% indicating the presence of minerals and heavy metals.

Total soluble solids is specific to each specy, but it can be also used as an indicator for ripeness stage.
The potential effectiveness of fruit supplements in promoting various aspects of health depends upon their botanical and chemical composition and on the concentrations of active ingredients that they contain [18].

Ascorbic acid content in this study the wild species *V. myrtillus* had the highest amount of vitamin C (404.7 mg/kg FW). Similar amounts of vitamin C were observed in Cranberries species *Vaccinium vitisi claea L.* (361.42 mg/kg FW), respectively. Rop O., 2010 et al. reported lower values in different cultivars of fruit European cranberrybush that ranged from 1.01 to 1.64 grams/kg FM, [19].

Total phenols. Comparison between different fruits showed broad variations in both phenolic content and antioxidant activity as estimated by the scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. Bilberry identified to have high phenolic content and high antioxidant activity.
This is in line with the findings of Milivojevic J. et al. 2012, which examined and quantify important taste- and health-related compounds in *Vaccinium* berries.
However, other authors have measured total polyphenol contents in in wild bilberry and 2 commercial blueberry cultivars and quoted a range from 3.87 mg

GAE/g FW to 1.50 mg of GAE/ g fresh weight, depending on the cultivar [20]. Due to its high contents of polyphenolics ranged from 6.80 to 8.29 grams of gallic acid/kg FM, as recently reported by Rop O., 2010, for fruit of European cranberrybush [19].

DPPH scavenging capacity assay

The antioxidant capacities of cranberry juice and three extracts isolated from frozen cranberries containing anthocyanins, water-soluble and apolar phenolic compounds, was evaluated by Caillet, S et al., 2011. According to this, the juice exhibited a much lower antioxidant activity, compared to the cranberry extracts, especially when compared with the extract containing water-soluble compounds which the extraction conditions were similar to those used to obtain the juice [16].

The pH and acidity. In a Table 1 results showed that measured pH value was highest in Bilberry, and furthermore decreased in Cranberries.

Rimpapa et al., 2007 reported that fruit acidity depended directly on the concentration of aliphatic and aromatic organic acids in the fruits [18].

Table 1

Concentrations of moisture, ash, TSS%, brute protein, ascorbic acid, TP, IC_{50}, pH and titrable acidity of minnor berries

Samples	**Moisture %**	**Ash %**	**^{1}TSS %**	***Brute protein* % S.U.**	**^{2}AA mg/kg *FW**	**^{3}TP (mg GAE /kg)**	**$^4IC_{50}$**	**pH**	**^{5}TA (g of citric acid/100g**
Bilberry *Vaccinium myrtillus L.*	85.8	1.331	6.8	5.72	404.7	5.87	0.33	3.12	0.55
Cranberries *Vaccinium vitisi claea L.*	57.28	1.457	15.3	3.7	361.42	4.68	0.52	2.72	1.94

*FW – fresh weight of fruits

Abbreviations: ^{1}TSS-Total soluble solids (^{0}Brix), ^{2}AA-Ascorbic acid mg/kg, ^{3}TP mg GAE/kg – total polyphenols expressed as gallic acid equivalents, $^4IC_{50}$ = Free Radical Scavenging Activities, ^{5}TA-Titrable acidity

The contents of the basic nutritional compounds (moisture, total soluble solids, protein) was higher in *Vaccinium* fruit, but not statisticaly significant (table 2).

We registered a good correlation between the content of phenolics and the antioxidant potential. In our study, the long-term frozen storage of bilberry and cranberries did not induce significant changes in vitamin C content (figure 1).

Table 2

Univariate analysis of biochemical indicators studied

Groups	*Average*	*Standard deviation*
Moisture	71.54	20.17
Ash	1.395^{**}	0.09
TSS	11.05	6.01
Protein	4.71	1.43
AA	383.06	30.60
TP	5.275^{*}	0.84
pH	1.525	1.69
TA	1.245	0.98

* indicates significant differences at $p < 0.05$
** indicates significant differences at $p < 0.01$

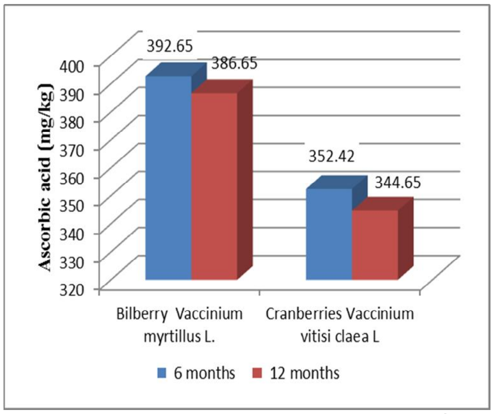

Fig. 1 Ascorbic acid concentrations of bilberry and cranberries during frozen storage

Iwona Scibisz and Marta Mitek [2007] showed similar antioxidant activity losses for frozen highbush blueberries stored for 6 months at -35°C. Comparing the evolution of the ascorbic acid of the two *Vaccinium* fruits, one can say that after 12 month of storage at -20ºC the values of this index was reduced by 4.46% in bilberries and by 4.64% in cranberries.

In cranberries, after 12 month of frozen storage, the poliphenols content content decreased by 11.53% (figure 2).

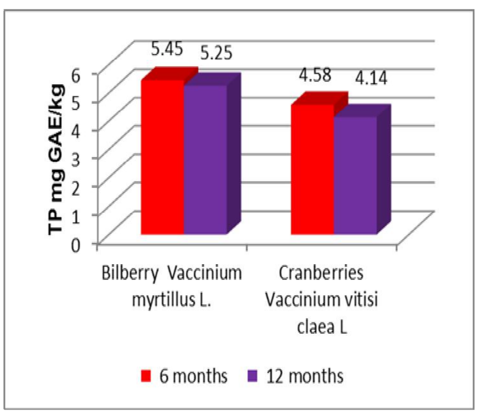

Fig. 2 Total phenols of of bilberry and cranberries during frozen storage

Pearson correlation coefficients were calculated to reveal the relationship between the vitamin C, total polyphenols content and time of freezing. The correlation value for the parameters ascorbic acid and total phenols measured in the selected fruits is 0.984, showing that there is strong correlation between these two parameters (table 3).

Table 3
Correlation matrix for the variables measured in *Vaccinium* fruits:
TP (mg GAE/100g) and AA (mg /kg)

	Time	***AA***	***Losses AA***	***TP***	***Losses TP***
Time	1				
AA	-0.165	1			
Losses AA	**0.980**	-0.086	1		
TP	-0.305	**0.984**	-0.243	1	
Losses TP	0.870	0.119	**0.948**	-0.052	1

4. Conclusions

Vaccinium fruits are rich in antioxidant activity. The contents of polyphenols, ascorbic acid and the antioxidant activity are significantly higher in bilberry *Vaccinium myrtillus L.*

During frozen storage, the biggest losses of ascorbic acid were recorded in cranberries.

The evolution of the poliphenols concentration in the two types of *Vaccinium* fruits, in all cases, greater decreased during frozen storage.

The work should contribute to the popularization of this minor berries as a promising fruits in human nutrition and the high antioxidant activity that guarantees the best nutritional results.

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