STUDIES REGARDING SOME SECONDARY METABOLITES OF SELECTED FOREST FRUITS DURING STORAGE BY FREEZING

Purcărea Cornelia*, Chiş Adriana*Timar Adrian*

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania, e-mail: prcneli@gmail.com

Abstract

Fruits in general and especially the forest fruits have a high content of biologically active substances which has been associated with health protection. To preserve the best quantitative and qualitative content of biologically active substances, conservation is trying in various ways. Storage by freezing is one of the best treatment. Forest fruits in this case does not suffer thermal heating and thus retain the best nutritional value, the higher content of antioxidant compounds, vitamins and enzymes.

The selected forest fruits for our study are: blueberries – cultivated or from wild bushes, blackberries and seabuckthorn fruits. The beneficial properties appear to be related to the antioxidant content of the fruit, particularly ascorbic acid, polyphenols, total flavonoids and anthocyanins, which may play a role in inhibiting reactions mediated by reactive oxygen species.

Key words: forest fruits, polyphenols, anthocyanins, vitamin C

INTRODUCTION

Berries are widely recognized for their nutritional quality and potential health benefits. Recent increasing interest in nutraceuticals and functional foods has led plant breeders to initiate selection of crops blackberries (Kafkas et al., 2006) and blueberries (Remberg et al., 2007), with higher contents of taste- and health-related compounds.

Blueberries (*Vaccinum myrtillus*) are species of plant belonging to the genus *Vaccinium*; which is a member of the sub-family *Vaccinioidiae*, of the family *Ericaceae.* Research studies have shown that blueberries are one of the richest sources of antioxidant phyto-nutrients, with higher levels of anthocyanin and phenolic contents than other fruit or vegetable (Prior et al, 1998). They have also shown the ability to slow the aging process (Mason et al, 2007).

Blackberry (*Rubus* sp.) fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contribute to its high antioxidant capacity and other biological activities. Blackberry phenolic compounds have protective effects on age-related neurodegenerative diseases and bone loss in vivo and can inhibit low-density lipoprotein and liposomal oxidation in vitro. Blackberry extracts have also exerted antimutagenic effects in vitro and in vivo by modifying

cell signaling pathways and suppressing tumor promotion factors. (Kaume et al, 2012).

The fruits of seabuckthorn (*Hippophae rhamnoides* L.) have been used as a drug by traditional Tibetan and Mongolian medicine since ancient times. In 1977, seabuckthorn was officially for the first time listed in the Chinese Pharmacopoeia by the Ministry of Public Health. Since 1985, meanwhile, medicinal research on seabuckthorn has received much attention in China. The great advances and demonstrations of its medicinal values have been seen in recent years (Xu Mingyu et al. 1991). *Hippophae* L. is a multipurpose wonder plant found in the Himalayan region which is beneficial both ecologically and economically. It shows multiple pharmacological and therapeutic activities such as antioxidant, immunomodulatory, antiinflammatory, anti-atherogenic, anti-stress, cardio protective and wound healing. Seabuckthorn has attracted attention world over due to its nutritional and medicinal values (Bhartee et al., 2014).

The role of dietary antioxidants, including vitamin C, vitamin E, carotenoids, polyphenols and anthocyanin in disease prevention has received much attention in recent years (Halliwel et al., 1995; Feris, 1994). It is also an important source of minerals. There are several factors affecting the nutritional value of these fruits, including variety, maturity, processing and storge conditions.

Small fruits like blueberries, blackberries, seabuckthorn constitute a good source of natural antioxidant substances. Extracts of these fruits act effectively as free radical inhibitors. It has been already demonstrated that a wide diversity of phytochemical levels and antioxidant capacities exist in these small fruits (Pantelidis eta al., 2006)

The aim of this study is to investigate health protecting components like: total polyphenols, monomeric anthocyanin and vitamin C, of selected forest fruits, immediately after harvest and after 3 and 6 month of storage by freezing.

MATERIAL AND METHOD

The experiments were performed in 2014-2015, at the Laboratory of Secondary Metabolits in Food Industry, of Faculty for Environmental Protection, University of Oradea.

For this study, blueberries-cultivated or from wild bushes, blackberries and seabuckthorn samples were taken. It was made 3 repetition for every sample.

Sample I – immediately after harvest
Sample II – after 3 month freezing
Sample III - after 6 month freezing

Samples preparation

For each sample it was made the alcoholic extraction: 10 g of each sample were mixed with 10 ml ethanol solution (50%), and after 30 minutes were filtered. Ethanol extracts were diluted than 1/10 with ethanol solution (50%) (Moigrădean et al., 2007).

Total Phenolic content

The total phenolic (TP) content was determined by using the Folin-Ciocâlteu (1927) colorimetric method developed by Singleton and Rossi (1965). A diluted extract (0.5 ml) or phenolic standard was mixed with 2.5 ml Folin- Ciocâlteau reagent and after 5 minutes 2.0 mL sodium carbonate (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. For the preparation of calibration curve 0.5 ml aliquot of 0.2, 0.4, 0.8 and 1.2 μM/ml aqueous gallic acid solution were used as the standard and expressed as mg of gallic acid equivalent (GAE) (Gergen, 2004).

Total Flavonoid compounds content

The total Flavonoid compounds content (FC) was measured with $AlCl_3$ colorimetric assay (Atanassova et al, 2011). The absorbance was measured at 510nm. As standard we used catechin.

Ascorbic acid (Vitamin C)

Ascorbic acid was extracted using metaphosphoric acid and the extract was titrate with iodine solution starch indicator (Kallner,1986).

Monomeric anthocyanin

Monomeric anthocyanin pigments reversibly change pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to color change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0 (Lee et al., 2005).

Determine absorbance of test portion diluted with pH 1.0 buffer and pH 4.5 buffer, at both 520 and 700 nm. The diluted test portions are read versus a blank cell filled with distilled water. Measure absorbance within 20–50 min of preparation.

Calculate anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where:

A = (A520nm – A 700nm)pH 1.0 – (A520nm – A700nm)pH 4.5;
MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside
DF = dilution factor established;
l = pathlength in cm;
ε = 26 900 molar extinction coefficient, in $lxmol^{-1}$ x cm^{-1}, for cyd-3-glu;
10^3 = factor for conversion from g to mg.
Finaly the results were expressed as milligrams of pelargonidin-3-glucoside equivalent, per 100 g fresh weight.

RESULTS AND DISSCUSIONS

Results obtained after performing analyses for health protecting component determination in selected forest fruits were content in table 1.

Table 1.

Calculated values for health protecting components of selected forest fruits

Fruits	Nr. sample	TP mg GAE/100g FW	FC mg CAT/100g FW	Vitamin C mg/100g FW	Monomeric anthocyanin Mg/100g FW
		mean±sd	mean±sd	mean±sd	mean±sd
Cultivated blueberries	I	119.8±0.38	22.72±0.65	43.4±0.95	87.57±1.23
	II	103.3±1.01 ***	20.8±0.36 **	42.3±1.15 ns	84.76±1.56 *
	III	93.3±0.91 ***	15.2±0.21 ***	36.1±0.51 ***	83.6±1.1 **
Wild bush blueberries	I	200.46±1.97	16.6±0.3	54.4±3.29	289.16±2.1
	II	192.5±2.11 **	15.7±0.45 **	55.9±0.26 ns	286.83±1.7 ns
	III	175.4±3.17 ***	13.6±0.12 ***	51.3±0.95 ns	285.2±3.12 ns
Blackberries	I	224.1±2.98	29.65±0.54	30±0.52	275.76±1.65
	II	199.9±0.60 ***	27.8±0.87 **	28.1±0.7 ***	274.43±2.04 ns
	III	167.7±0.96 ***	22.2±0.67 ***	24±1.5 ***	273.96±2.3 ns
Seabuckthorn	I	191.6±1.35	1.71±0.04	450.55±2.35	-
	II	178.25±2.63 ***	1.68±0.03 ns	424.1±9.8 ***	-
	III	159.16±1.31 ***	1.47±0.02 ***	379.45±0.96 ***	-

Total Phenolic content - Results of the determination of TP content are shown in Tabel1. Evaluating our results we can say that the analysed fresh fruits are good source of polyphenols, between 119.8 minim, in case of cultivated blueberries, and 224.1 maxim value expressed in mg of GAE (gallic acid equivalents)/100 g fresh weight (FW) –in case of blackberries. Similar results for blueberries were obtained by Sellapan et al. 2002. In their study TP ranged from 261.95 to 929.62 mg/100 g FW. The average values

for TP in case of blackberries was similar with values obtained by Wang and Lin, 2000.
After 6 month of freezing the total polyphenol content decreased very significantly, however large amounts of polyphenols are found in all the analysed samples.
Total Flavonoid compounds content - As in case of total polyphenol determination, blackberries are those that have the highest FC content of 29.6 mg CAT% followed by cultivated and wild bush blueberries and seabuckthorn had the lowest contents in flavonoid compounds, 1.71 mg CAT/ 100 g fresh weight. Similar results were presented by Henning, 1981 (142–435 mg/ kg) in case of blackberries, and by Cho et al, 2005 (from 173 mg/kg to 328 mg/kg), in case of blackberries.
Ascorbic acid (Vitamin C) – The highest content in vitamin C was found in seabuckthorn, 450 mg% after harvest and 380 mg% after 6 months freezing, follow by wild bush and cultivated blueberries, and the lowest vitamin C content in blackberries - an average of 30 mg% immediately after harvest and 4mg% after 6 months of freezing. Similar avarege values for vitamin C content in seabuckthorn were found by Gutzeit et al, 2008 (400 mg%)
Monomeric anthocyanin - The anthocyanin pigment content decrease was insignificant during the 6 months of freezing, remaining at very close level to those of immediatley after harvest. In case of cultivated blueberries the average decrease was 4.4%, and 1.36% for wild bushes blueberries. The lowest level of decreas was observed in case of blackberries (0.65%). The average values for monomeric anthocyanin content in blueberries ranged from 87 to 289 mg/100 g of fresh berries. Similar results are presented also by Sellapan et al. 2002; Vollmannová et al, 2009.
The results shown that all the fruits taken in to study can be stored at low temperature without alteration. The bioactive compounds level it was quite stabile. The same results were found by Timar, 2014, for other species of berries.

CONCLUSIONS

Analyzing the results obtained for the studied samples it can be concluded:

Blackberries and wild blueberries contained the largest amount of total phenolic compounds and flavonoids.

Seabuckthorn was the fruit with the highest content of vitamin C, about 10 times higher than in other analyzed fruits.

Anthocyanin pigments were present in blueberries and blackberries, the highest amount being in the wild blueberries and blackberries.

Cultivated blueberries found in supermarkets throughout the year have a lower content of antioxidant compounds than wild bush blueberries or blackberries.

In the performed analysis it was observed that even after 6 months of freezing, the content of antioxidant compounds decreases, however, their content remains quite close to the baseline, immediately after harvest.

The analyzed fruits are seasonal fruits which can not be preserved fresh. For them to have the closest variant of fresh fruits throughout the year the one of best way for storage is freezing.

REFERENCES

1. Atanassova M., S. Georgieva, K. Ivancheva, 2011. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy, 46, 1, 81-88.
2. Bhartee M.,, B.C. Basistha, and Sushen Pradhan, 2014- Seabuckthorn - A Secret Wonder Species: Review. SMU Medical Journal, Volume – 1, No. – 2, July, 2014, PP. 102 - 115, 2014.
3. Cho M.J., Howard L.R., Prior R.L., Clark J.R. (2005): Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. Journal of the Science of Food and Agriculture,85: 2149–2158
4. Feri B. 1994. Natural antioxidants in human health and disease. San Diego: Academic Press.
5. Folin O., Ciocâlteu V.,1927. On tyrosine and tryptofane determination in protein, Journal of Biological Chemistry, 24, p.627-650.
6. Gergen I.,2004- Analiza produselor Agroalimentare, Editura Eurostampa, Timisoara.
7. Gomez-Caravaca A.M., M. Gomez-Romero, D. Arraez-Roman,A. Segura Carretero , A. Fernandez-Gutierrez, 2006: Advances in the analysis of phenolic compounds in products derived from bees, Journal of Pharmaceutical and Biomedical Analysis 41: 1220–1234.
8. Gutzeit D Baleanu G, Winterhalter P, Jerz G., 2008- Vitamin C content in sea buckthorn berries (Hippophaë rhamnoides L. ssp. rhamnoides) and related products: a kinetic study on storage stability and the determination of processing effects . J Food Sci
9. Halliwell B, Murcia MA, Chirico S, Aruoma OI. Free radicals and antioxidants in food and in vivo: what they do and how they work. Crit Rev Food Sci Nutr 1995;35:7-20.
10. Henning W, Phenolics of fruit. XIV. 1981- Flavonol glycosides of strawberries *(Fragaria ananassa* Duch), raspberries (*Rubus idaeus* L) and blackberries (*Rubus fruticosus* L). Z Lebensm Untersuch Forsch 173:180–187.
11. Jiangsu New Medical College 1977. Traditional Chinese Medical Dictionary. Shanghai People's Medical Publishing House, Shanghai.

12. Kafkas, E.; Kosar, M.; Türemis, N.; Baser, K.H.C. 2006 - Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chemistry, v.97, p.732-736.
13. Kallner, A. 1986, Annals of the New York Academy of Sciences, 498, 418-423.
14. Kaume L., Howard L.R., Devareddy L., 2012 - The Blackberry Fruit: A Review on Its Composition and Chemistry, Metabolism and Bioavailability, and Health Benefits. J. Agric. Food Chem., 2012, 60 (23), pp 5716–5727.
15. 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 Vol. 88, No. 5. p. 1269-1278.
16. Mason, S, Bekhit, A, E, Sun, Bo, 2007-Nutritional Value and Antioxidants in Four Blueberry Varities.
17. Moigrădean D., Poiană M.A. Gogoasă I., Hărmănescu M, Gergen I, Lăzureanu A. 2007. The correlations between total antioxidant capacity and total polyphenols content established for tomatoes. Lucrări stiinłifice Medicină Veterinară, Timişoara Vol. XL, 486-489.
18. Pantelidis G.E., M. Vasilakakis, G.A. Manganaris , Gr. Diamantidis, 2006 - Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chemistry 102 (2007) 777–783.
19. Prior R. L,Cao, G, Martin A, Sofic E,McEwen J, O'Brien, C, Lischner, N, Ehlenfeldt M,,Kalt W, Krewer G,and Mainland C,M, Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity,and Variety of Vaccinium Species J. Agric. Food Chem. 1998, 46, 2686-2693.
20. Remberg, S.F.; Måge, F.; Haffner, K.; Blomhoff, R., 2007 - Highbush blueberries Vaccinium corymbosum L., raspberries Rubus idaeus L. and black currants Ribes nigrum L. - influence of cultivar on antioxidant activity and other quality parameters. Acta Horticulturae, v.744, p.259-266.
21. Sellapan S., Akoh C.C., Krewer G. (2002): Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. Journal of Agricultural and Food Chemistry, 50: 2432–2438.
22. Singleton V. L., Rossi J. 1965. A colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16,144-158.
23. Timar A., 2014 -Studies regarding the quality of berries during storage. Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară Vol. XIII/A, 2014.
24. Vollmannová A., T. Tóth, D. Urminská, Z. Poláková, M. Timoracká and E. Margitanová - Anthocyanins Content in Blueberries (*Vaccinium corymbosum* L.) in Relation to Freezing Duration. Czech J. Food Sci. Vol. 27, 2009, Special Issue, p.204-206.
25. Wang SY and Lin HS, 2000 -Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J Agric Food Chem 48:140–146.
26. Xu Mingyu Sun Xiaoxuan, Cui Jinhua, 1991. Present conditions and the future research on the seabuckthorn medicinal use. J. Water and Soil Conservation of China (5): 38.