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THE FREEZING IMPACT ON ANTIOXIDANT COMPOUNDS OF SOME BERRIES

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Abstract

A healthy diet includes the consumption of fresh fruits. Forest fruits are known as an important source of bioactive compounds such as phenolics, vitamins, carotenoids, anthocyanin. All those compounds have important benefits for human health. Six types of forest fruit were investigated in this study regarding the content of some bioactive compounds. The parameters were determined in fresh state and after freezing at -18^{0}C and -80^0 C, respectively. Total phenolic content ranges from 442 in white currants to 2,385 mg GAE/100g in black currants. Flavonoids content ranges from 110 in white currants to 420 mg QE/100g in black currants. The vitamin C content ranges from 10 mg% in blackberries to 138 mg% in blackcurrants. The anthocyanin content shows the greatest variability among the tested fruit, from 18 mg cianidyn-3-glucozid/100g FW in red currant to 547 in blackberries. Freezing at -18^{0}C is appropriate to preserve the content of investigated bioactive compounds.

Key words: currants, raspberry, mulberry, blackberry, bioactive compounds

INTRODUCTION

Fruits are an important part of a healthy diet. They are rich in phytonutrients and generally low in calories. Red fruits, among them forest fruit, are known as an important source of antioxidants as phenolic acids, flavonoids and vitamins. Flavonoids, compounds which include flavonols, flavones, flavanols, flavanones, isoflavonoids and anthocyanins as plant secondary metabolites have many biological functions, which are important for human health. (Meskin et al, Editors, 2008, Jideani et al, 2021) Those minor constituents are defined as food components that have an impact on physiological or cellular activities in the humans or animals that consume such compounds (Walia et al., 2019).

We can name a lot of commercial berry species consumed all around the world such as blackberry (*Rubus* sp.), bilberry (*Vaccinium myrtillus* L.), blackcurrant (*Ribes rugrum* L.), chokeberry (*Aronia melanocarpa* (Michx.) cranberry (*V. macrocarpon* Ait.), bayberry (*Myrica* sp.), raspberry (*Rubus ideaus* L.), black raspberry (*Rubus occidentalis* L.), strawberry (*Fragaria ananassa* Duch.), highbush blueberry (*V. corymbosum* L.), maqui

(*Aristotelia chilensis*), murtilla (*Ugni molinae* Turcz.) and calafate (*Berberis microphylla* G. Forst.) (Pena-Sanhuenza et al, 2017. Studies on cultivars from different geographical regions found red and black currant, raspberries, blackberries, gooseberries, cornelian cherries to be rich in phenolics, organic acids, microelements, vitamin C and also antocyans: Diaconeasa et al, 2019, Petrișor et al, 2013, in Romania, Ersoy et al, 2013, in Turkey, Pantelidis et al, 2007, in Greece. Those components are responsible for the health benefits of fruit consumption including anticarcinogenic effect (Li et al, 2016, Gordon and Derek, 2012).

Fruits are consumed mostly in fresh state for a scanty period of time, but they are preserved and sold in frozen state all year long. The present study focuses on the determination of some bioactive components of selected forest fruits and also to their preservation in different thermic conditions.

MATERIAL AND METHOD

Material

The tested material comprises six cultivated berry types codded as follows.

Currants: White (*Ribes niveum*) – WC, Red (*Ribes rubrum*) – RC and Black (*Ribes nigrum*) - BC

Berries: Raspberry (*Rubus idaeus*) – RB, Blueberry (*Vaccinium myrtillus*) – BB, Mulberry (*Rubus fruticosus L*) – MB

All samples where homogenised and split in three portions. The first was used for the determination in fresh state of the investigated parameters and the others two portions were frozen at -18^0C and -80^0 C, respectively. The experiments were conducted in the food control laboratory of the Food Engineering Department, Environmental Protection Faculty, Oradea.

Methods

The extraction technique is very important for all bioactive compounds determination (Garcia-Salas et al, 2010), so in order to have proper comparisons, for the present study experiments a unique procedure was applied on all tested samples for every determined parameter. Total phenol content (TPC) and flavonoids (FL) were determined using the same alcoholic extract, using methanol/water solvent 1:1. Homogenised blended fruit ware sonicated at 20°C for 30 minutes, then centrifuged for accurate separation. The reaction mixture contains appropriate diluted extract, Folin-Ciocâlteu reagent and natrium carbonate 7.5%. After 2 hours the absorbance was read at 765 nm. Gallic acid, from 0 to 250 mg/l was the used standard and the results were expressed as mg GAE/100g FW (Petcovics et al,2014, Singleton and Rossi, 1965).

FL was determined using the spectrophotometric method based on the formation of chelated compounds between flavonons, flavonols and aluminum chloride in methanol. (Kroyer and Molnar, 2011, Bahorun et al, 2004). The absorption of the reaction mixture containing alcoholic extract, NaNO 25%, 3 $AlCl_3$ $6H_2O$ 10% and NaOH 1M was read at 510 nm against reagents blank. The used standard was quercetine 0-100 mg/L in methanol and results were expressed as mg QE/100g FW.

Vitamin C was extracted from fresh fruits with metaphosphoric acid and spectrophotometric determined by the Beltran – Orozco et al., 2009, method. Ascorbic acid reacts with 2:6-diclorphenol indophenol (DCPIP) which changes its colour from blue to colourless in acetate buffer (pH 4) environment. The reaction product was extracted in xylene and the absorbance read at 520 nm. Pure ascorbic acid between 0.1mg/ mL and 1mg/ml concentration was used for the calibration curve.

Anthocyanins were determined through AOAC Int. 88, 1269 (2005) pH differential method. Monomeric anthocyanin pigments reversibly change colour with a change in pH; the colour oxonium form exists at pH 1.0, and the colourless 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 cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to colour change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0. For all the samples we checked the appropriate dilution factor by diluting the test portion with pH 1.0 buffer, until the absorbance at 520 nm was between 0.2 and 1.4 AU.

The results represent the mean of two determinations at each sample for all tested parameters. The same methods were applied on fresh and on frozen samples, after gentle thawing at the refrigeration temperature of 4^0C.

RESULTS AND DISCUSSION

The experiment results are presented for each investigated parameter for fresh and frozen fruits in tables 1 to 4.

Looking at the results, we can see that all tested berries had significant, but different phenolic content, with a maximum value for blackcurrant. The content is higher than the one found by Petrişor et al, 2013 and Najda şi Labuda, 2013 for white currant (188 mg GAE/100g), red currant (from 95 to 237 mg GAE/100g), and blackcurrant (between 205 and 880 mg GAE/100g). But Pena-Sanhuenza et all, 2017 found higher phenolic content, up to 657 mg GAE/100g for white currant and up to 1,342 mg GAE/100g for the red ones. Anyway, all the comparisons are affected by the fact that the experimental design is different in terms of extract obtaining and experimenting protocol. The same reasoning is valuable for forest

berries, which in our experiment presents a lower content than the one reported by De Ancos et al. 2000 (1137-2112 mg GAE/100g) or Pena-Sanhuenza et all, 2017 (1280-2494 mg GAE/100g). However, all studies emphasise that blackcurrant are definitely the richest in bioactive compounds.

Table 1

Total phenolic content, mgGAE/100g FW

Sample	Fresh	Frozen -18˚C	Frozen -80˚C
	Mean		
	+/- Sd		
WC	442.47	455.575	536.8241
	11.09	23.21401	4.974431
RC	937.77	915.0453	979.9756
	22.76	47.73027	10.32006
BC	2384.94	2473.274	2635.945
	29.90	22.99532	10.22014
MB	1243.77	1187.81	989.51
	38.01	14.99	5.51
BB	1208.61	1115.66	1055.82
	18.34	6.11	26.03
RB	894.03	789.07	711.05
	18.36	15.55	8.05

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Table 2

Flavonoid content, mg QE/g FW

Sample	Fresh	Frozen -18˚C	Frozen -80˚C
	Mean		
	+/-Sd		
WC	110.56	51.67	64.44
	3.89	0.56	0.00
RC	249.44	151.11	170.00
	5.00	5.56	1.11
BC	420.56	321.11	332.22
	7.22	5.56	10.00
MB	296.67	276.67	218.89
	22.22	8.89	3.33
BB	322.78	305.56	248.33
	6.11	6.67	1.67
RB	180.00	168.89	119.44
	20.00	2.22	3.89

Flavonoids content present the same trend as TPC, with the lowest value for white currants (110.6 mg QE/g) and the highest for blackcurrants (420.6

mg QE/g). But freezing has very different impact on the tested fruits; for currants FL content drops up to 51% in the case of the black ones no matter the freezing temperature, meanwhile for raspberry, mulberry and blackberry there are no changes at -18^{0}C which practically preserves their FL content and the content drops until -33% at -80^{0}C. The limitation of the spectrophotometric method cannot detail those results as it could be accessible only through a chromatographic one. (Biswas et al., 2013).

Table 3

Vitamin C content, mgQE/g FW

Sample	Fresh	Frozen -18˚C	Frozen -80˚C
	Mean		
	Sd+/-		
WC	22.03	92.88	97.34
	4.1	2.02	4,11
RC	53.5	119.0	122.58
	3.02	5.32	5.05
BC	138.2	144.92	162.83
	7.03	6.9	3.02
MB	23.11	14.45	27.11
	2.2	3.03	3.21
BB	10.01	14.02	11.78
	0.92	2.11	2.11
RB	24.31	51.05	31.09
	2.08	0.96	3.08

The Vitamin C content is very different for the tested samples, from a minimum of 10.7 mg/100 g in blackberry to 132.7 in blackcurrant. Studies in the field present similar results in red currant (46 – 52.9 mg/100g) or blackcurrant, from 99 to 126 mg/100g (Ersoy et al, 2018, Petrisor et al, 2013)

The evolution of vitamin C content after frosting shows an unexpected rise of the experimental values for the majority of the tested samples at both temperatures. This situation can be explained by the fact that only the fruit juice contains vitamin C and, after defrosting, the samples contain a different ratio of juice/seed than in fresh state. This reasoning is sustained by/ but the fact that fruits with high seed content present the maximum “rise of Vitamin C content, white currant (260%) and raspberry (132%)

Table 4 shows as expected, no anthocyanin content for white currant, low content for red currant and significant content for black currant and raspberries. Black currant, but especially mulberries and blackberries are a rich source of anthocyanin.

Table 4

Anthocyanin expressed as cianidyn-3-glucozid mg/100g FW

Sample	Fresh	Frozen - 18˚C	Frozen -80˚C
	Mean		
	+/-Sd		
WC	0		
RC	18.45	17.78	18.64
	0.42	0.55	0.14
BC	160.21	156.09	167.29
	8.45	3.13	0.20
MB	355.52	358.19	287.04
	3.05	0.38	3,39
BB	548.96	598.44	550.81
	0.89	0.97	3,03
RB	154.66	104.66	114.12
	0.77	3.17	0.44

Those results are consistent with the ones found by Horbonicz et al, 2008 (10 - 60 mg/Cy 3-glu /100 g FP for raspberries and 12-19 mg/Cy 3-glu /100 g FP for red currant) and Petrişor et al, 2013 for red currant (20,5 – 44,5 mg/Cy 3-glu /100 g FP or black currant 166,8 – 298,2 mg/Cy 3-glu /100 g FP, but lower than reported by Pena - Sanhuenza et al, 2017 (1.4 mg/Cy 3-glu /100 g FP white currant, up to 52 mg/Cy 3-glu /100 g FP in raspberries and 165 to 411 mg/Cy 3-glu /100 g FP for red currant).

All comparison regarding bioactive compounds in the investigated fruits should take into account the great number of cultivated species and the different cultivation conditions. As for the calculation itself ε is between 26900 and 34300 L x mol^{-1} x cm^{-1}, depending on the used solvent (Giusti and Wrolstad, 2001). The 26900 L x mol^{-1} x cm^{-1} value was used in the present study because it is the recommended one by AOAC method for the pH differential method of anthocyanin determination (AOAC Official Method 2005.02, 2005).

For currants, no matter the frozen temperature, the influence is negligible -3.63% and +4.42%. For the berries the influence is more significant, until -20% for mulberries but the fact that rise of the content was found can be due to experimental design or to the lack of similar fruit/seed ratio between fresh and frozen fruits like in the case of vitamin C.

CONCLUSIONS

The tested Forrest fruits are characterised by high, but very different bioactive compounds content. White currants show the lowest TPH and FL content and blackcurrant is 5.4 and 3.8, respectively, times higher. The darker the fruit is, the highest phenolic content is observed. As for Vitamin

C, blackberries has the lowest content and again black currants the highest, but the difference is much more significant, by a factor of 13 tested fruits. Even if white currants are not in discussion, the anthocyanin content shows the highest variability among the tested bioactive compounds. Thereby black currants have a 30 times higher cianidyn-3-glucozid content then red currant.

Freezing at -18ºC practically does not affects TPC, FL or anthocyanin content; the effect is more obvious at -80ºC, but not significant. Therefore the usual domestic conservation by frosting is enough for maintaining the antioxidant content of forest fruits. The influence of frosting on Vitamin C content should be studied on fruit juice only in order to avoid experimental errors.

Anyway it is obvious that none of the investigated forest fruit is great at all investigated parameters even if generally, the darker the fruit is, the highest the bioactive compounds content is.

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