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INFLUENCE OF PRESERVING BY FREEZING ON SOME BIOCHEMICAL PARAMETERS IN FRUITS OF *RIBES NIGRUM* L. AND *RIBES RUBRUM* L.

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Abstract: *R. nigrum* and *R. rubrum* are species with remarkable potential of production. The quality and quantity of the chemical compounds present in the fruit collected from species of plants in the genus Ribes are influenced by genetic qualities, climatic conditions, procedures of cultivation, the way of harvesting and depositing the fruit etc. The results of our study show the fact that, in fresh state, the fruit harvested from *Ribes nigrum* present antioxidant capacity and content of anthocyanins superior to the fruit harvest from *Ribes rubrum,* while the content of water has slightly lower values in the *Ribes rubrum*. The analyses carried out after maintaining the biologic material to be analysed for nine months in the freezer underlines modifications of the biochemical parameters investigates. Thus, the content of total polyphenols was reduced by 16.03% in *Ribes rubrum* and by 17.09% in *Ribes nigrum.* The contents of anthocyanins and dry substance as well as the antioxidant capacity have registered values superior to those evaluated for the fruit in fresh state.

INTRODUCTION

Ribes nigrum L. (black currant) and *Ribes rubrum* L. (red currant) (Grosulariaceae family) are species of bushes found in culture on all the continents, mainly in the temperate area of the northern hemisphere. They grow spontaneously in Europe and Asia (Grădinaru and Istrate, 2004).

Currently, at international level and in Romania, there are concerns regarding the cultivation, research and valuing the fruit from the species mentioned for nutritional and therapeutic purposes.

The fruit of red currant and black currant are used fresh, frozen or processed in various forms (juice, syrup, compote, marmalade, jam, jelly, wine, liquor etc. (Pârvu, 2002).

The bioactive compounds in the fruit of red and black currant (simple carbohydrates, fibres, vitamins, phenolic compounds, organic acids, mineral salts etc.) (Maldin and Mladin 1992; Cionea et al., 2009; Milivojević et al., 2009; Nour et al., 2011; Cosmulescu et al., 2015) exhibit an extremely diverse biological activity, which confers them therapeutic value, being recommended for a balanced nutrition (Maldin and Mladin 1992; Pârvu, 2002; Tabart et al., 2012; Khoo et al., 2012; Burzo, 2015). The fruit of *R. nigrum* and *Ribes rubrum* are known as sourced of bioactive polyphenols (Moyer et al., 2002, Milivojevic et al., 2010, Vakula et al., 2015). The polyphenolic compounds (phenolic acids, flavonoids, anthocyanins, tannins) are secondary metabolites, synthesized in plastids and stored in vacuoles (Burzo, 2015) with a role in plants protection. The polyphenolic compounds have beneficial effects for the human health, with vital role in preventing and improving some cardiovascular or neuronal diseases, cancer, diabetes, eye diseases etc. These effects are due to their antioxidant, antibacterial, antiviral, anti-inflammatory and proliferative, cardio tonic properties etc. (Gosh and Konishi, 2007; Milivojevic et al., 2010; Tabart et al., 2012; Subash et al., 2014; Vakula et al., 2015).

The red and blue colours of the fruits of *Ribes nigrum* and *Ribes rubrum* respectively are due to the presence of anthocyanins, group of pigments soluble in water, located in vacuoles in form of glycosides, which present antioxidant potential (Bakowska- Barczak et Kolodziejczyk, 2011) and can be used as natural dyes in food industry (Nour et al., 2011).

The fruit of red and black currants are very perishable. Preserving them by various procedures modifies their content of bioactive compounds. One of the preserving methods the most used is freezing. The fruits of red currant and black currant are well suitable to freezing, the period to preserve them at the temperature of -18^{o}C being between 10 and 14 months (Pârvu, 2002; http://posdru.afiprofamilia.ro/).

In the literature in the field, there are data regarding the effects of depositing by freezing on some bioactive compounds (polyphenols, anthocians etc.) and the antioxidant capacity in various fruit of berry type (Ancos et al., 2000; Ścibisz et al., 2007; Poiană et al., 2010) or in *Ribes nigrum* (Bakowska-Barczak et Kolodziejczyk, 2011; Oancea et al., 2014) but, regarding the *Ribes rubrum*, there is little information.

This paper has as purpose to underline some possible modifications of some biochemical parameters (water content, dry substance content, concentration of mineral elements, concentration of anthocians, concentration of total polyphenols and antioxidant capacity) in fruit of *Ribes nigrum* (black currant) and *Ribes rubrum* (red currant) at biological maturity, after preserving them by maintaining in the freezer, at a certain temperature, for a certain period of time.

MATERIAL AND METHOD

The plant material to be analysed is represented by fruit, harvested at biological maturity from specimens of *Ribes nigrum* and *Ribes rubrum*, coming from a particular crop (Vlădeni, Botoşani County).

The biochemical indicators by the experimental model were evaluated at fresh fruit (water contents, dry substance, total polyphenols, anthocians and antioxidant capacity) and frozen at the temperature of -18°C for nine months (apart from the indicators mentioned it was also evaluated the content of total mineral elements).

The dry matter and water contents are determined by the gravimetric method. This basically consists of evaluating the indicator by keeping the biological material at a temperature of 105°C to constant weight. The results are expressed in g of dry matter per 100 g of freshly analysed material. By difference, the amount of water contained in the biological material to be analysed is evaluated (Boldor şi colab., 1983).

The total mineral elements content was determined by assessing the calcinated residues at 550ºC. Keeping the sample to be analysed at the calcination temperature leads to the loss of organic substances and some of the volatile mineral substances. The results, representing the average of three consecutive determinations, are expressed in g of calcined residue/100 g of dry analysed material (Mănescu et al., 1978; Boldor et.al., 1983).

The content of total polyphenols was determined by the spectrophotometric method Folin - Ciocâlteu (Singleton and Rosi, 1965). The dose of total polyphenols is based mainly on the property of the compounds in this class to react in alkaline environment with the reagent Folin-Ciocâlteu, leading to a compound of blue colour. Color intensity evaluation is done at 765nm. The concentration of total polyphenols of the extracts is calculated by means of a calibration curve set in parallel and in the same conditions as the extracts, using a control solution of gallic acid. The results obtained, average of three parallel determinations, are expressed in mg/g equivalent of gallic acid.

Determination of anthocians content was done by the extraction of the biologic material to be analysed with an acidic alcoholic solution and measuring the absorption at the specific wave length of 515nm (Fuleki and Francis, 1968).

The free radical scavenging activity of the lyophilized powder was determined using the stable radical DPPH (2,4-dinitrophenyl-1-picryl hydrazyl) method as previously described (Seal, 2012). 200 μL of the tested sample were placed in test tubes and 2 mL of freshly prepared DPPH solution (60 μM) in methanol was added in each test tube and mixed. 30 minutes later, the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPHscavenged(%)=[(Ac−At)/Ac]×100; Ac - absorbance of the control and At - absorbance of the sample.

For the antioxidant activity we used a 3 mg/mL methanol extract solution. The results are the average of three determinations.

RESULTS AND DISCUSSIONS

The analysis of the experimental results regarding the content of water done on fresh fruit, harvested at biologic maturity from the species *Ribes nigrum* and *Ribes rubrum* respectively, indicates valued of 81.63 % and 84.68 % respectively) (table I), which confirms the data present in the literature in the field (Gherghi et al., 1973; Ekholm et al., 2007). Water, essential for the normal development of the metabolic processes, assures succulence and sweetness to the fruit. Different authors reported for other species values ranging from 80% to 90% (Mladin et Mladin, 1992; Ancos et al., 2000; Ekholm et al. 2007).

The dry matter content of fruits of the two species (table 1) is consistent with the data presented by Nour et al. (2011) in different species of *Ribes nigrum* (17.94- 23.17%) and *Ribes rubrum* (15.12 – 17.54%).

Regarding the total polyphenol content, we again notice the superiority of the black currant fruits that are over 3,5 times higher than those calculated for the red currants (table I).

Table I. Biochemical indicators for fruits harvested at biological maturity from *Ribes nigrum* and *Ribes rubrum*

The analyzed indicator (average value ± standard deviation; n=3)	Species	
	Ribes nigrum	*Ribes rubrum*

The water content (g %)	81.63±0.75	84.68±0.19
The dry matter content (g%)	18.36±0.75	15.31±0.19
The content of total polyphenols (mg/g)	30.67±0.05	8.8 ±0.15
The content of anthocians (mg%)	875.86±6.04	58.44±1.05
The antioxidant capacity (%)	65.7±1.13	72.7±0.42

Regarding the content of anthocians, our experimental results indicate the fact that the value of the indicator is about 15 times higher in the case of the fruit harvested from the species *Ribes nigrum*, comparing with those harvested from the species *Ribes rubrum* (table I).

In developing the experimental model, it was necessary to apply dilutions to the vegetal extracts carried out in order to determine the antioxidant capacity both in the case of the fruit of *Ribes rubrum* and in those of *Ribes nigrum*. The values mentioned in table I are for extracts that contain 5.04 mg vegetal material/ml for red currant and 1 mg vegetal material/ml for black currant. For this reason, the antioxidant capacity of the fruits harvested from Ribes nigrum is about five times higher than that harvested from the Ribes rubrum species.

The results presented in the literature in the field are in compliance with the data obtained in this study (Moyer et al., 2002; Pantelidis et al., 2007; Milivojević et al., 2010; Nour et al., 2011; Anisimovienė et al., 2013; Burzo, 2015). Some authors do not support the existence of a correlation between the content of anthocians and total polyphenols with antioxidant activity in the fruit of berry type (Moyer et al., 2002; Anisimovienė et al., 2013), but they mention that in the case of these fruits, anthocians and other compounds (tannins, stilbene, protoanthocyanidins, phenolic acids) participate in order to get the antioxidant capacity.

The research carried out by (Borges et al., 2010) underline the fact that the anthocians contribute to the increase of the total antioxidant capacity in proportion of about 73 % in the case of fruit of *Ribes nigrum* and 21% in the fruit of *Ribes rubrum.* In order to get the antioxidant activity, vitamin C also participates in proportion of 18% in *Ribes nigrum* and 47.5% in *Ribes rubrum*, but also other chemical compounds.

The results obtained after the determination of some biochemical indicators on fruit harvested from *Ribes nigrum* and *Ribes rubrum*, preserved by freezing at -18^{o}C for nine months, are presented in table II. The analysis of the results regarding the water content underlines the fact that the process of preservation by freezing led to the reduction of the value of the indicator by 1.01 % in the fruit of red gooseberry and by 4.26% in the fruit of black gooseberry. The content of dry substance presents amplitudes opposite to those presented in relation with the content of water (table II).

Decreasing of water content and implicitly, increasing othe dry smatter content of the frozen fruits of *Ribes rubrum* and *Ribes nigrum* is due to the formation of ice crystals which cause the alteration of the cytoplasm and the breaking of the cell walls and as a result some of the water content is released (Burzo, 1986).

Tabel II. Biochemical indicators for fruit harvested at biological maturity from *Ribes nigrum* and *Ribes rubrum*, preserved by freezing

The analyzed indicator (average value± standard deviation; n=3)	Species	
	Ribes nigrum	*Ribes rubrum*
The water content (g%)	78.16±0.89	83.53±0.58
- % compared to the fresh fruits	-4.26 %	-1.01%

The dry matter content (g%)	21.83±0.89	16.64±0.58
The content of total mineral elements (g%)	12.71	10.67
The content of total polyphenols (mg/g)	25.43±0.75	7.39±0.08
- % compared to the fresh fruits	-17.09	-16.03
The content of anthocians (mg%)	1093.6±2.95	81.68±0.25
+ % compared to the fresh fruits	+24.86	+40.07
The antioxidant capacity (%)	83.51	79.22
+ % compared to the fresh fruits	+27.10 %	+8.96

Regarding the total mineral content evaluated after freezing, we find values of about 16% more of the fruits harvested from the *Ribes nigrum* species compared to those harvested from the *Ribes rubrum* species (Table II). It is mentioned in the literature that the mineral elements present in red currant and black currant fruits are potassium, calcium, magnesium, phosphorus, sodium, chlorine, manganese, iron, zinc and copper (Ekholm et al., 2007; Nour et al., 2011).

The content of total polyphenols analyzed after the time when the fruits were preserved by freezing recorded a decrease of 16.03% in the fruits harvested from *Ribes rubrum* and 17.09% respectively in the ones harvested from *Ribes nigrum* (Table II). The results obtained by us are consistent with data present in the literature (Bakowska - Barczak et Kolodziejczyk, 2011). This behaviour may be explained by the slowing down of enzymatic processes due to the conservation and retardation conditions found and reported in the literature of fruit belonging to other fruit tree species such as *Rubus idaeus*, *Rubus fruticosus* or *Vaccinium myrtillus* (Ancos et al. 2000; Poiană et al., 2010).

By comparison with the fresh fruit, we notice an obvious increase of the content of anthocians after maintaining the biologic material to analyse at the temperature of -18^{o}C for nine months. Our results indicate values 24.86% and 40.07% higher in *Ribes nigrum* and *Ribes rubrum* respectively (table II). In the literature it is mentioned such a behaviour also at other intervals of time of preservation by freezing of the Ribes fruits (Oancea et al., 2014), but in the case of the fruit harvested from *Ribes nigrum* and maintained at the temperature of -20^{o}C for 9 months other authors notice the decease of the content of anthocians (Bakowska- Barczak and Kolodziejczyk, 2011). Changes in anthocyanins content during the period of preservation by freezing were reported also in fruit harvested from other species of shrubs with edible fruits, such as *Rubus idaeus*, *Rubus fruticosus*, *Lonicera caerulea*, *Vaccinium myrtillus* (Ancos et al., 2000; Poiană et al., 2010, Olteanu Z. et al., 2013).

Stability of anthocyanin content in fruit tissues during the freezing process depends on several factors, including the chemical composition of the fruit, the pH value, the organic acid content or the carbohydrate concentration.

Determination of antioxidant capacity after keeping fruits at -18^{o}C for nine months indicates the increase of the value of the investigated indicator by 27.10% in *Ribes nigrum* and by 8.96 in *Ribes rubrum* (tab. II). There is data in the literature showing that the temperature of freezing does not influence negatively the antioxidant capacity of the fruit harvested from certain bushes (Bakowska- Barczak and Kolodziejczyk, 2011).

These seemingly contradictory data on antioxidant capacity show us the multiple influences that the indicator can bear. It is genotype, variety, location, cultivation technique, maturation degree, season, storage conditions, processing conditions etc. (Skrankova et al., 2015).

CONCLUSIONS

The fruits and leaves of *Ribes nigrum* and *Ribes rubrum* are sources of biologically active compounds with depurative, sudorific, vitaminizing, remineralizing, healing, anti-rheumatic, antioxidant properties etc.

Analysis of fresh fruits from these species highlights high concentrations of total polyphenols and anthocyanins as well as important antioxidant activity. By comparison, the fruits harvested from *Ribes nigrum* have higher antioxidant capacity, higher anthocyanins content and slightly lower water content than those harvested from *Ribes rubrum*.

The conservation of black and red currants at -18 ° C for nine months caused, on the one hand, a slight reduction of total polyphenols and water and, on the other hand, the increase in anthocyanin content, dry matter content and antioxidant capacity.

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