THE INFLUENCE OF STORAGE PERIOD ON VARIATION OF PHENOLIC CONTENT IN SWEET CHERRIES

INFLUENȚA PERIOADEI DE PĂSTRARE ASUPRA VARIAȚIEI CONȚINUTULUI DE FENOLI LA CIREŞE

MANOLE Carmen Gabriela [1,2], PARASCHIV M.[2,3], CONSTANTIN M.[4], BĂDULESCU Liliana[1]

e-mails: manolecarmen2000@yahoo.com, lilib_20@yahoo.com

Abstract. *The paper deals with bio-compounds preservation in soft fruits during storage in order to allow their consumption in off-season. The work addresses sweet cherries, the most popular consumed fruits in countries across the temperate regions like Romania, which the consumer can enjoy only in May-July period. Consequently, the aim of the study was to evaluate the influence of storage conditions on the yield of biologically active compounds. In this respect, the content of total phenols and flavonoids, and free radical scavenging activity have been analyzed in order to enable consumers to choose the most efficient storage method. The best characteristics cultivar offering the potential prospects for growers is also highlighted. Sweet cherries provided by 'New Star', 'Celeste' and 'Giant Red' cultivars were stored for 7 days in refrigerated and freezing conditions and then subjected to the extraction method with hydrochloric acid in methanol. Using Folin-Ciocâlteu method total phenols content of the extracts was determined. The flavonoid content was identified using an adapted method based on rutin as reference sample. The free radical scavenging activity (EC50) of the extracts was determined using stabile 2,2 diphenyl-1-picrylhydrazyl radical. The results showed that high phenols and flavonoids contents are in 'N Star' cultivar (0.144 g in fresh fruits and 0.129 g refrigerated ones) while the free radical scavenging activity is better conserved in frozen fruits from 'Celeste' cultivar (5.94 mg/mL).*

Key words: cherry varieties, biologically compounds, temperature variation

Rezumat. *Articolul prezintă date referitoare la conservarea bio-compuşilor în fructele moi pe parcursul depozitării, pentru a spori consumul lor în afara sezonului. Lucrarea are ca obiect de studio cireşele dulci, cele mai populare fructe consumate în țări din regiunile temperate precum România, de care consumatorul se poate bucura numai în perioada mai-iulie. În consecință, scopul studiului a fost de a evalua influența condițiilor de depozitare asupra randamentului compuşilor biologic activi. În acest sens, a fost analizat conținutul total de fenoli şi flavonoide şi activitatea antiradicalică pentru a permite consumatorilor să aleagă metoda cea mai eficientă de stocare. De asemenea, în lucrare se evidențiază şi cel mai bun cultivar cu caracteristici importante cu perspective potențiale pentru cultivatori. Cireşele dulci furnizate de soiurile 'New Star', 'Celeste' şi 'Giant Red' au fost depozitate timp de 7 zile în condiții de refrigerare şi congelare şi apoi supuse metodei de extracție cu acid clorhidric în metanol. Astfel, a fost determinat conținutul total de fenoli ai extractelor folosind metoda Folin-Ciocâlteu. Conținutul de flavonoide a fost identificat utilizând o metodă adaptată bazată pe o curbă de calibrare a*

rutinului. Activitatea antiradicalică (EC50) a extractelor a fost determinată utilizând un radical liber stabil de 2,2 difenil-1-picrilhidrazil. Rezultatele au aratat un conţinut ridicat de fenoli şi flavonoide în cultivarul 'N Star' (0,144 g în fructe proaspete şi 0,129 cele g refrigerate) în timp ce activitatea antiradicalică este mai bine evidenţiată în fructele congelate din soiul 'Celeste' (5,94 mg / mL).

Cuvinte cheie: varietăţi de cireşe, compuşi biologici, variaţia temperaturii

INTRODUCTION

In the past years, numerous research studies have been focused on plant micronutrients and their health benefits. These studies showed that a diet rich in fruits and vegetables containing various classes of phenols, which are key compounds in decreasing the risk of premature diseases and aging (Piljac-Zegarac *et al*., 2009; Patthamakanokporn *et al.*, 2008; Freeman *et al*., 2011; Rekhy and McConchie, 2014). Therefore, these foods may provide optimal fibres, minerals, vitamins and phytochemicals such as natural antioxidants (Palafox-Carlos *et al.*, 2011; Charanjit and Harish, 2001).

Sweet cherries are one of the most popular consumed fruits across the temperate regions, in several areas being the first fruit of the season (Prvulović *et al.*, 2011). In addition, they are natural sources of antioxidants being part of human diet for several years (Melichàĉovà *et al.*, 2010).

In Romania, Prunus avium L. is cultivated in home gardens, farms and professional orchards, and have a celebration day. According to Food and Agriculture Organization of United Nations, in 2013 Romania has registered a cultivated area of 7078 hectares, and a production of 80477 tones. Local people use sweet cherries both as fresh and frozen fruits, jams, compote or different types of beverages. Amongst other bioactive compounds, sweet cherries are rich in sugars and organic acids, and are considered a major source of phenolic compounds, responsible for the colour and sensory properties (Kelebek and Selli, 2011). Most often, biological properties differ depending on growing conditions, the harvest year, cultivar and phenological phase of fruit maturation (Ferretti *et. al.*, 2014).

Considering these aspects, over the years numerous research regarding the identification and exploitation of fruit phenolic compounds were conducted (Gonçalves *et al.,* 2004; Asănică *et al.*, 2004, Jakobek *et al.,* 2007; Liu *et al*., 2011; Ballistreri *et al*., 2013; Cao *et al*., 2015). They found that the storage temperature has a high influence on phenolic content. Thus, this content decreases when the temperature is around 1-2 °C and rises when the storage temperature is around 15 +/- 5 °C. Moreover, analysing different cherry species (*Prunus avium*, *Prunus cerasus*, *Prunus pseudocerasus* and *Prunus tomentosa*) they proved that inter-species variability of phenolic compounds was greater that of intra-species one. Also, the results of their research suggest that climatic conditions have a significant influence on the yield of these compounds, especially in cultivars

'Burlat', 'Saco', 'Summit', and 'Van' grown on the mountainsides of the Etna volcano (Sicily, Italy).

Due to the pleasant taste and appearance of the fruit but also for the significant amounts of compounds with free radical scavenging properties, the demand is continuously increasing (Naderiboldaji *et al.*, 2015). As the fruits are perishable and have a short shelf life (up to 10 days in normal conditions), in some cases the consumers are not receiving them in optimal quality. Their shelf life is influenced by several factors (fig. 1), which influences both organoleptic and biochemical properties (Wani *et al*., 2014).

Fig. 1 Factors influencing shelf life of fresh sweet cherry (Wani *et al*., 2014)

This paper presents quantitative analysis on the phenolic and flavonoid contents, and anti-radical activity of several sweet cherries varieties grown in Romania. The reason of choosing these parameters for analysis is related to the fact that they ensure the quality of soft fruits, which begins to decline after harvesting. For most of fruits (crops), the environmental conditions (i.e. the storage temperature) are critical for ensuring the required level of health-promoting compounds *(*Karlund *et al.* 2014).

The goal of the study was to assess the influence of storage conditions on the yield of biologically active compounds in sweet cherries and on their anti-radical activity in order to enable consumers to choose the most efficient storage method, depending on the intended use.

MATERIAL AND METHOD

The biological material

The fruits provided from the three cultivars of cherries represented the biological material. Harvested at the end of May, the fruits from the Prunus avium L.

cultivars 'New Star' (N. Star), 'Celeste' and 'Giant Red' (G. Red) were kept in three different storage conditions according to table 1.

Table 1.

Storage conditions of sweet cherries

Varieties	Fresh	Refrigerated	Freezer (-85°C)
N. Star Celeste G. Red	Harvest day	7 days after harvest day	7 days after harvest day

The fruits were milled and subjected to the extraction method adapted after Cheel *et al.* (2007) with hydrochloric acid (HCl), 1% (v/v) in methanol (MeOH), for 30 minutes on ice bath. The used fruit/solvent extraction ratio was 1/5. The extracts were shaken and left at room temperature for 48 hours. Then, the extracts were filtered through Whatman paper and further subjected to the spectral analyses.

Methods

Using Folin-Ciocâlteu method Neményi *et al.* (2015) total phenolic content of the extracts was determined. After 45 minutes, the absorbance was measured at λ = 750 nm against a control sample, which was obtained by replacing the extract volume with distilled water. The results were quantified based on a calibration curve of gallic acid (Sigma purity) and were expressed as gallic acid equivalents (GAE). The linear regression curve was given by the equation (1):

$$\text{Abs} = 0.00968 + 0.000167857 \times \text{C gallic acid}, \text{ R} = 0.996, p < 0.05 \quad (1)$$

The results are showed as [g] GAE / 100 [g] fresh weigh (FW).

The flavonoid content was determined using a method adapted after Tuker et al. (2012) using the rutin as reference sample. The diluted extracts were mixed with sodium nitrite solution (NaNO2), 5% (w/v) in water. After 5 minutes was added aluminum chloride solution (AlCl3), 10% (w/v) in water, and after another 6 minutes sodium hydroxide solution, 1M NaOH, has been added, too. After 45 minutes, the sample's absorption was measured at the wavelength of 510 nm. The results were obtained based on the rutin calibration curve, having the equation (2):

$$\text{Abs} = -0.0068 + 0.000627455 \times \text{C rutin}, \text{ R} = 0.999, p < 0.05 \quad (2)$$

The free radical scavenging activity of the extracts was determined using stabile radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•), using an adapted method (Clarke et al., 2013). The inhibitory effect of DPPH was calculated using to the equation (3):

$$Inhibition\,(\%) = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100 \quad (3)$$

The results obtained were figured against the sample concentration to determine the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. IC50 (EC50) represents the level where 50% of the radicals were scavenged by sweet cherries extracts.

Bonferoni and Tukey tests were used for the comparison of means values for the bio-compounds content between groups, using Statistical Package for Social Science (SPSS version 21.0). The statistical significance for the probability value of difference $p < 0.05$ was considered. The obtained results were expressed as mean values ± standard error. For charts design, Microcal Origin version 6.0 software was used.

RESULTS AND DISCUSSIONS

Several studies on the influence of freezing temperatures on biochemical content of sweet cherries were realized by Kelley et al. (2006), Chaovanalikit and Wrolstad (2004) and Richman et al. (2007). They found that the degradation of phenolic compounds in frozen cherries is related to the presence of native enzymes, particularly polyphenol oxidase that is a temperature-dependent enzyme. Polyphenol oxidase can accelerate the degradation process in presence of chlorogenic acid, the major phenolic compounds in cherries. Considering different cultivars, statistical results show that in fresh stage are not significant differences. Belge et al. (2015) carried out similar studies on postharvest changes of cell wall of sweet cherry from 'Celeste' and 'Somerset' cultivars after storage at 0 °C.

The total phenolic content for fresh sweet cherries of all three cultivars is shown in Table 2. It can be seen that the maximum value was recorded by 'N. Star' cultivar the results being similar to those published by Serrano et al. (2009) for 'Cristalina' cultivar.

Table 2

Total phenolic content – fresh sweet cherries

Varieties	Mean g GAE/100g FW	Std. Error (SE)	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
N. Star	0.144	0.001	0.138	0.149	0.142	0.146
Celeste	0.126	0.002	0.119	0.133	0.123	0.128
G. Red	0.125	0.006	0.111	0.138	0.093	0.146

Values represent the mean of three replicates ± SE

In the case of refrigerated sweet cherries (see Table 3), the value of total phenolic content was well preserved only for 'N. Star' cultivar, and the value stays close to those found by Gil et al. (2006) for refrigerated strawberries. For the other cultivars, the phenolic content reduces during cold storage, for 'Celeste'cultivar the decrease being more significant.

Table 3

Total phenolic content – frozen sweet cherries

Varieties	Mean g GAE/100g FW	Std. Error (SE)	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
N. Star	0.114	0.002	0.106	0.122	0.110	0.116
Celeste	0.097	0.005	0.075	0.119	0.087	0.104
G. Red	0.108	0.002	0.101	0.115	0.106	0.111

Values represent the mean of three replicates ± SE

These results contradict those recorded by Kevers *et al.* (2007), who reported that phenolic content was generally stable during storage. Moreover, several authors reported an increase of phenolic compounds during cold storage and subsequent shelf-life (Bernalte *et al*., 2003).

The total flavonoid content for fresh sweet cherries of all three cultivars is shown in Table 4. Once again, 'N. Star' cultivar exhibits the maximum value of flavonoid content. The recorded values is in good correlation with data published by Prvulović *et al.* (2011).

Table 4

Total flavonoid content – fresh sweet cherries

Varieties	Mean g RE/100g FW	Std. Error (SE)	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
N. Star	0.156	0.002	0.148	0.165	0.153	0.160
Celeste	0.114	0.000	0.113	0.116	0.114	0.115
G. Red	0.110	0.001	0.107	0.114	0.109	0.112

Values represent the mean of three replicates ± SE

In this work, by refrigerating the sweet cherries, the total amount of flavonoids decreases for all three-tested cultivars. The cultivar influence on total flavonoid accumulation during refrigeration appears to be significant. N Star cultivar is still showing the best performance, while the 'Celeste' registered the highest degree of reduction in total flavonoid content (28.94%) and the 'G. Red' lost the smallest quantity during the refrigeration (7.27%). The obtained results are summarized in Table 5.

Table 5

Total flavonoid content – refrigerated sweet cherries

Varieties	Mean g RE/100g FW	Std. Error (SE)	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
N. Star	0.129	0.006	0.103	0.155	0.117	0.135
Celeste	0.081	0.003	0.066	0.095	0.074	0.085
G. Red	0.102	0.001	0.098	0.106	0.101	0.104

Values represent the mean of three replicates ± SE

Analyzing the flavonoid content in frozen sweet cherries it was proved that, compared to fresh fruits, for all tested cultivars the values decreased. Compared to refrigerated fruits, it can be observed that 'Celeste' cultivar has a slight tendency of increasing the flavonoids content. The values are presented in Table 6.

Table 6

Total flavonoid content – frozen sweet cherries

Varieties	Mean g RE/100g FW	Std. Error (SE)	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
N Star	0.101	0.003	0.090	0.113	0.096	0.104
Celeste	0.083	0.000	0.082	0.085	0.083	0.084
G Red	0.094	0.005	0.072	0.117	0.084	0.101

As shown above, sweet cherries contain several phenolic compounds, important for consumer's health. These results are well correlated to Ferretti *et al.* 2010 investigation on antioxidant properties of fresh fruits, which were demonstrated using different approaches.

With regard to free radical scavenging activity, the obtained results for all three analyzed stages are presented in figure 2. Significant differences in free radical scavenging activity of the tested sweet cherry cultivars during conservation process can be observed, and similar phenomena were reported by Sen *et al.* (2016) analyzing 'Early Burlat', 'Napoleon', and '0900 Ziraat' sweet cherries cultivars.

Concerning storage conditions, the inhibition rate (EC 50) during all three analyzed stages decreased in the following order: fresh > frozen > refrigerated.

The inhibition rate (EC 50) of the sweet cherries extracts during all three analyzed stages was highlighted 'Celeste' cultivar with an average of 7.94 (fresh fruits), 5.94 (frozen fruits), and 4.35 mg/ml (refrigerated fruits). The values are presented in fig. 2.

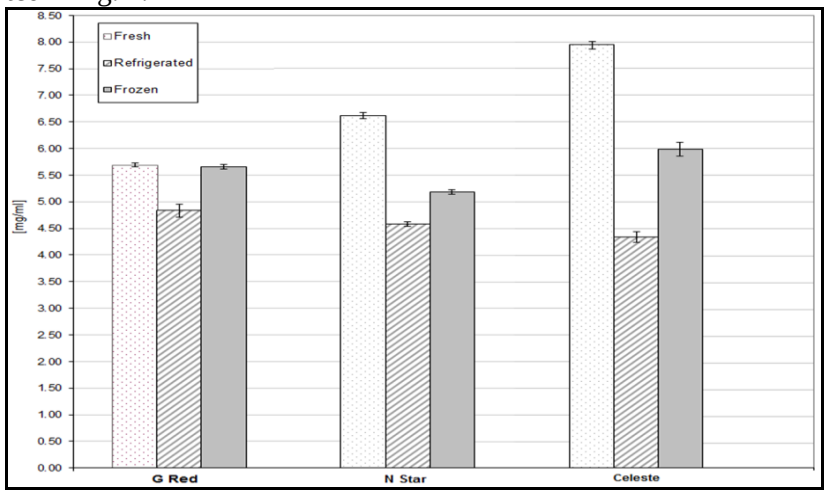

Fig. 2 Free radical scavenging activity during analyzed storage conditions

CONCLUSIONS

In this paper, the influence of storage conditions on the accumulation of biological active compounds in sweet cherries was highlighted.

1. It was found that postharvest factors such as storage could influence biological composition of sweet cherries.

2. For the studied cultivars, it was established that during postharvest storage, the ripening process advances in refrigerated stage, ranging the phenolic and flavonoid content of sweet cherries.

3. Moreover, the free radical scavenging activity of sweet cherries was well maintained during the storage period at -85 ºC in frozen stage.

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