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Storage effect on phenolic content and antioxidant activity in selected fruit extracts

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The objectives of this study were to investigate the influence of light and temperature on the content of phenolic compounds and its correlation with antioxidant activity in selected fruit extracts (strawberry (*Fragaria vesca*), blackberry (*Rubus fruticosus)*, raspberry (*Rubus idaeus)*, and sour cherry (*Prunus cerasus*)). The antioxidant capacity evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH▪) scavenging assays ranged from 90.87% to 96.50%. The content of target phenolic compounds showed high correlation with the antioxidant activity (r^2 = 0.829) and with the content of anthocyanins (r^2 = 0.995) of the investigated fruit extracts. Total phenol content was monitored in fruit extracts stored at 7 °C for 23 days and at room temperature (25 °C) for 90 days. All fruit extracts exhibited fluctuations in total phenol contents with an initial increase after 4 days, followed by a decrease at both storage temperatures. These changes were not significant during 23 days of storage and the investigated fruit extracts can be used as an easily accessible source of natural antioxidants in food and pharmaceutical supplements.

Keywords: Fruit extracts; antioxidant activity; phenolic composition; storage temperature.

INTRODUCTION

Many human diseases, like cancer, atherosclerosis and rheumatoid arthritis, as well as neurodegenerative diseases and ageing processes are caused by free radicals [1-3]. Epidemiological studies have indicated that frequent consumption of fruits and vegetables is associated with a lower risk of cardiovascular disease and cancer [4-8]. This protective role of fruits and vegetables had been attributed to their antioxidant compounds, such as phenolics, flavonoids, flavonols, anthocyanins, so natural antioxidants have gained increasing interest among consumers and scientific community [9].

Polyphenols are abundant micronutrients in our diet that have been credited with chemoprevention of diseases associated with oxidative stress. Over 8000 phenolic compounds have been identified from plant materials and they possess a wide spectrum of biochemical activities (antioxidant, antimicrobial, antimutagenic, anticancerogenic and ability to modify the gene expression) [10-16].

Fruits like strawberry, blackberry, raspberry and sour cherry are good sources of natural antioxidants and are traditionally used in diet [8]. Environmental factors like temperature, light and geographical area are very important for the development of red pigment in the fruit [16-20].

It has been reported that the freezing process decreased the total phenolic content and antioxidant capacity by 4-20 % in raspberries [21].

As antioxidant capacity is becoming an important parameter with respect to fruit and vegetable quality, it is of great interest to evaluate changes in antioxidant activity and total phenolic contents during storage of selected fruit extracts at elevated temperatures. This study was undertaken to investigate the effects of different temperatures on total phenolics and antioxidant capacity of extracts of raspberry (*Rubus idaeus)*, blackberry (*Rubus fruticosus)*, strawberry (*Fragaria vesca*), and sour cherry (*Prunus cerasus)*, originating from Serbia (Rasinski region).

EXPERIMENTAL

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) in free radical form was obtained from Sigma Chemical Co. (St. Louis, MO). Methanol, formic acid, gallic acid, catechin and quercetin were purchased from Merck Co (Germany). All reagents were of analytical grade.

Plant materials

Samples of strawberry, blackberry, raspberry and sour cherry were harvested in western Serbia (Rasinski region) at the commercial maturity stage. After harvest, the samples were immediately frozen and stored at -20 ^{o}C until analysis.

Preparation of extracts

Fruit extracts were obtained by grinding the berries (10 g) in 100 mL of methanol/water solution (70/30) at room temperature for 30 min. The mixture

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was stored at 7°C in the dark for 24 h and then centrifugated at 4000 rpm for 15 min.

Determination of total phenols, tartaric esters and flavonols

The total phenol content in the extracts was determined according to the Mazza and Miniati [9] procedure: 0.25 mL of extract and 0.25 mL of 0.1% HCl in 95% ethanol were mixed with 4.55 mL of 2% HCl and the absorbance was measured at 280, 320, 360 and 520 nm after incubation at room temperature for 5 min. Total phenol content was expressed as mg of galic acid equivalents /100 g of fruit (280 nm); tartaric esters - as mg catechin equivalent /100 g fruit (320 nm); flavonols - as mg quercetin equivalent/100 g fruit (360 nm).

Determination of monomeric anthocyanin

The total monomeric anthocyanin content in the fruit extracts was determined by the pH-differential absorbance method described by Guisti and Wrolstad [22]. Anthocyanins have maximum apsorbance at a wavelenght of 513 nm at pH of 1.0. The coloured oxonium form predominates at pH 1.0 and the colourless hemiketal form at pH 4.5. The pH-differential method is based on this reaction and permits accurate and rapid measurement of the total monomeric anthocyanins. Absorbance of the fruit extract was measured at 520 and 700 nm in potasium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5) after incubation at room temperature for 15 min. Anthocyanin content was expressed in mg of cyanidin-3-glucoside equivalent (Cygl)/100 g fruit using the molar extinction coefficient of cyanidin-3-glucoside of 26900 L/mol cm and molar weight of 449.2 g/mol.

Determination of indices for anthocyanin pigment degradation, polymeric colour and browning

Indices for anthocyanin degradation of the fruit extracts can be derived using the pH-differential method described by Giusti and Wrolstad [22]. The absorbance at 420 nm of the disulphide-treated sample serves as an index for browning. The colour density of the control sample and the polymer colour of the disulphide-bleached sample are calculated as follows:

$$\text{Colour density} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A\lambda_{\max} - A_{700\text{nm}})]$$

The hue value is calculated as follows:

$$\text{Hue} = [(A_{420\text{nm}} - A_{700\text{nm}})/ (A\lambda_{\max} - A_{700\text{nm}})]$$

The ratio between polymerization color and colour density is used to determine the percentage of the colour that is contributed by the polymerized material.

Antioxidant activity

The antioxidant capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH▪) scavenging assays [1,3,12,15]. The antioxidant assay is based on the measurement of the loss of DPPH˙ colour by the change of absorbance at 517 nm caused by the reactions of DPPH˙with the tested samples. The reaction was monitored on a UV/VIS spectrophotometer. Reaction solution was prepared by mixing 2.5 mL of diluted fruit extract with 1 mL of methanolic DPPH solution. The solution was kept in dark at room temperature for 20 min. Scavenging capacity of DPPH• in percent (%) of each fruit extract sample was calculated from the decrease in absorbance according to the relationship:

$$\text{Antioxidant capacity (\%)} = (1 - A_{\text{sample}} - A_{\text{blank}}/A_{\text{control}}) \times 100$$

where A_{control} is the absorbance of the control sample, A_{blank} is the absorbance of the diluted fruit extract sample and A_{sample} is the absorbance of the diluted fruit extract sample with DPPH radical.

Statistical analysis

Three analytical replicates were obtained from each sample fruit extract. Measurements were averaged, and results are given as mean ± standard deviation (SD). The corelations between parameters were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor. One-way ANOVA was used to determine differences between measurements. Differences at $p < 0.005$ were considered to be significant.

RESULTS AND DISCUSSION

The quantity and the composition of phenolic compounds in fruits are influenced by genotype, storage conditions, extraction procedure, and environmental conditions. In red-coloured fruits, the phenolic content increases during the last ripening stage due to anthocyanins and flavonols accumulation, so it is very important to collect samples at equal ripening stages [11].

Fruit extracts show significant differences in anthocyanins content, since these pigments are responsible for the blue, purple, violet, and red colours of fruits. They are one of the major flavonoid classes. The major sources of anthocyanins in edible plants are the families *Vitaceae* (grape) and *Rosaceae* (cherry, plum, raspberry, strawberry, blackberry, apple, peach, etc.). Other plant families which contain anthocyanin pigments are *Solanaceae* (tamarillo and eggplant), *Saxifragaceae* (red and black currants), *Cruciferae* (red cabbage), and *Ericaceae* (blueberry and cranberry).

The concentrations of total phenols, tartaric esters, flavonols and anthocyanins in the investigated fruit extracts are presented in Table 1. Analysis of obtained results shows that the total phenol contents varied from 166.17 to 273.22 mg/100 g fruits; tartaric esters contents varied from 21.62 to 44.42 mg/100 g fruits; flavonols were between 9.21 and 24.42 mg/100 g fruits; and the monomeric antocyanins varied from 26.02 to 121.95 mg/100 g fruits.

Similar results for phenol content in raspberry, strawberry, and blackberry extracts have been reported by other authors [10,13]. The highest concentrations of total phenols, tartaric esters and flavonols were found in sour cherry extract, followed by blackberry, strawberry and raspberry extract.

Sour cherry extract also contained the highest concentration of monomeric anthocyanins, followed by the blackberry extract. Significantly lower monomeric anthocyanin contents were recorded in strawberry and raspberry extracts. Data presented by other researchers from neighbouring countries for raspberry, strawberry and blackberry are similar to the results for anthocyanin content we obtained [13,14].

Polymeric colour has the lowest value in blackberry (12.20%), followed by raspberry and sour cherry. A higher value of polymeric colour is determined in strawberry (27.03%).

The portion of anthocyanins in the total phenol content was evaluated by calculating the monomeric anthocyanins/total phenols ratio. Anthocyanins represent a significant portion in the total phenol content in sour cherry (0.61) and blackberry (0.37), following by strawberry (0.19). The portion of anthocyanins in raspberry (0.16) was considerably lower.

All fruit extracts exhibited significant antioxidant activity (Table 1). The strongest radical scavenging activity is displayed by blackberry (94.80%), sour cherry (94.13%), raspberry (92.70%) and strawberry (90.87%).

Correlation coefficients of antioxidant activity and contents of selected phenolic compounds in the fruit extracts are shown in Table 2. These results show significant corrrelations between total phenolics and monomeric anthocyanins content (r^2 = 0.995). There are strong correlations between antioxidant activity and total phenol content (r^2 = 0.829), antioxidant activity and anthocyanin content (r^2 = 0.792), flavonols and total phenols (r^2 = 0.909), flavonols and tartaric esters (r^2 = 0.893), and flavonols and anthocyanins (r^2 = 0.928) in the investigated fruit extracts (Table 2).

One group of selected fruit methanol extracts was kept at 7°C during 57 days, simulating a household situation where the fruit products are stored in the refrigerator. Another group of fruit methanol extracts was stored at room temperature (25°C), exposed to sunlight during 90 days.

Table 1. Concentrations of total phenols, tartaric esters, flavonols, monomeric anthocyanins (mg/100 g fruit), polymeric color and antioxidant activity (%) of the investigated fruit extracts.

	Strawberry extract	Blackberry extract	Raspberry extract	Sour cherry extract
Total phenols	178.07 ± 3.62	230.00 ± 1.092	166.17 ± 2.07	200.01 ± 0.80
Tartaric esters	21.62 ± 0.08	24.81 ± 2.23	21.71 ± 0.85	44.42 ± 2.45
Flavonols	9.21 ± 0.11	18.06 ± 2.83	9.73 ± 0.42	24.42 ± 2.38
Monomeric anthocyanins	33.20 ± 0.20	84.16 ± 2.82	26.02 ± 2.80	121.95 ± 1.37
Polymeric colour	27.03 ± 0.68	12.20 ± 0.32	20.01 ± 0.80	21.14 ± 0.72
Antioxidant activity	90.87	94.80	92.70	94.13

Table 2. Correlation coefficients (r^2) of antioxidant activity, total phenols, tartaric esters, flavonols and anthocyanins assays of the investigated fruit extracts.

	DPPH•	Total phenols	Tartaric esters	Flavonols	Monomeric anthocyanins
DPPH•	1.000	0.829	0.254	0.607	0.792
Total phenols		1.000	0.658	0.909	0.995
Tartaric esters			1.000	0.893	0.682
Flavonols				1.000	0.928
Monomeric anthocyanins					1.000

Table 3. Changes in the total phenol content of the investigated fruit extracts (mg GAE /100g fruit) at 7°C during 23 days.

Storage	Strawberry extract	Blackberry extract	Raspberry extract	Sour cherry extract
1st day	198.07 ± 3.62	231.50 ± 1.09	166.17 ± 2.07	273.22 ± 4.38
4th day	196.71 ± 3.06	230.13 ± 2.65	165.27 ± 3.10	272.24 ± 3.25
23rd day	192.58 ± 2.31	227.47 ± 2.13	158.22 ± 2.10	270.56 ± 1.59

Table 4. Changes in the total phenol content of the investigated fruit extracts (mg GAE /100 g fruit) at 25°C, exposed to sun light, during 90 days.

Storage	Strawberry extract	Blackberry extract	Raspberry extract	Sour cherry extract
1st day	198.07 ± 3.62	231.50 ± 1.09	166.17 ± 2.07	273.22 ± 4.38
4th day	191.62 ± 3.08	224.52 ± 2.23	160.12 ± 2.81	272.15 ± 2.45
23rd day	188.25 ± 2.11	211.28 ± 2.83	156.99 ± 2.42	270.78 ± 2.38
57th day	186.15 ± 2.20	200.12 ± 2.82	142.02 ± 2.80	259.19 ± 1.37
90th day	168.02 ± 168	198.50 ± 3.32	138.30 ± 1.80	232.43 ± 1.72

The changes in the total phenol contents of these samples are shown in Tables 3 and 4. During 23 days of storage at 7°C the changes in the total phenol concentrations were observed for all fruit extracts. The initial phenol contents of all samples increased after 4 days of storage. This increase in the phenol content in the first days of refrigerated storage was also observed by other authors [18,19]. This initial increase in the phenol content was followed by a decrease in the total phenol values at the end of storage (Table 3, Fig. 1).

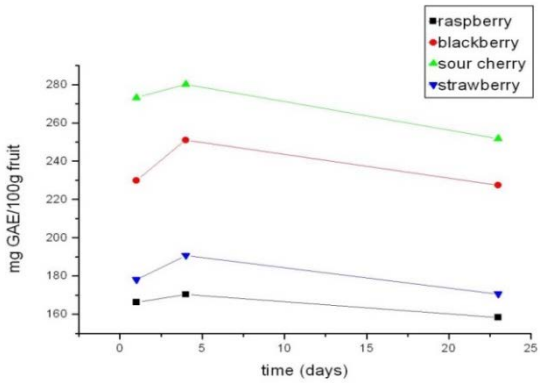

Fig. 1. Effect of refrigerated storage on the total phenol content in selected fruit extracts.

In the second group of fruit extracts stored at room temperature and exposed to sunlight, an initial increase in phenol content after 4 days was also followed by a decrease in these values at the end of storage (Table 4, Fig. 2).

The total phenol concentration decrease in selected fruit extracts after 23 days of refrigerated storage was 4.2% in strawberry extract, 4.8% in raspberry extracts, 7.85% in sour cherry extracts and 1.1% in blackberry extracts.

The total phenol concentration decrease in selected fruit extracts after 23/90 days storage at room temperature and light was 1.23%/5.65% in strawberry extract, 5.53%/16.77% in raspberry extracts, 0.89%/14.93% in sour cherry extracts and 8.1%/13.7% in blackberry extracts.

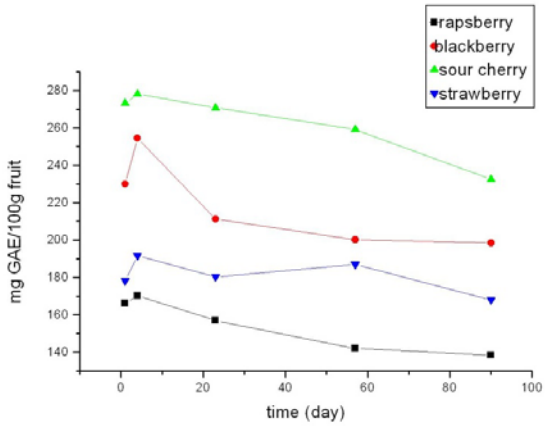

Fig. 2. Storage effect of sunlight and room temperature on total phenol content in selected fruit extracts.

Total antioxidant capacity decrease in selected fruit extracts after 90 days of storage at room temperature was from 4.08% to 4.82%.

Total phenols in fruit extracts kept in the dark at 7°C were not significantly different from those exposed to sunlight and room temperature ($p < 0.005$) after 23 days of storage.

CONCLUSIONS

The results of the present study showed that the investigated fruit extracts displaying the highest content of phenolic compounds exhibited the greatest antioxidant activity. The highest content of total phenols and monomeric anthocyanins, as well as the highest antioxidant activity was observed in sour cherry, followed by blackberry, strawberry and raspberry. The contents of phenolic compounds showed a good correlation with the total antioxidant activity of all investigated fruit extracts. The changes in the total phenol content were not significant during 23 days of storage in refrigerator or room temperature. This indicates that the selected fruits can be used as an easily accessible source of natural antioxidants and as possible food and pharmaceutical supplements.

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ЕФЕКТ НА СЪХРАНЕНИЕТО ВЪРХУ СЪДЪРЖАНИЕТО И АНТИОКСИДАНТНАТА АКТИВНОСТ НА ФЕНОЛИ В ИЗБРАНИ ПЛОДОВИ ЕКСТРАКТИ

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(Резюме)

Целта на това изследване е да се изследва влиянието на светлината и температурата върху съдържанието на фенолни съединения и тяхната корелация с антиоксидантната активност в избрани плодови екстракти от ягода (Fragaria vesca), къпина (Rubus fruticosus), малина (Rubus idaeus) и вишна (Prunus cerasus). Антиоксидантният капацитет е оценен използвайки 2,2-дифенил-1-пикрилхидразилов радикал (DPPH-3) и е в диапазона от 90,87% до 96,50%. Съдържанието на изследваните фенолни съединения показва силна корелация с антиоксидантната активност (r^2 = 0.829) и със съдържанието на антоцианини (r^2 = 0.995) на изследваните екстракти от плодове. Общото съдържание на фенол се наблюдава в екстрактите от плодове в продължение на 23 дни, съхранявани при 7 °C и 90 дни при стайна температура (25 °C). Всички плодови екстракти проявяват колебания в общото съдържание на фенол с първоначално увеличение след 4 дни, последвано от намаляване на общите фенолни стойности при двете температури на съхранение. Тези промени не са значителни по време на съхранение в продължение на 23 дни и изследваните екстракти от плодове могат да се използват като лесно достъпен източник на естествени антиоксиданти в хранителните и фармацевтичните добавки.