CHANGES IN ANTIOXIDANT CAPACITY OF FRUIT EXTRACTS DURING STORAGE

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1. INTRODUCTION

The antioxidant capacity of fruit extracts is predetermined by their chemical composition and content of phenolics [1, 2]. Plant phenolic composition depends on the plant species and parts, and is extremely diverse [3, 4]. Some phenolic molecules are specific for particular plant families. In plus, they are highly unstable substances which easily transform into various reaction products during technological processes and storage with partial or complete loss of antioxidant properties [5-8]. Previously it was shown that during 72 hours of storage the aqueous extracts from fruits of medicinal plants lost their antioxidant effectiveness by 4…35% [9]. The aim of this study was to determine the stability of antioxidant capacity of fruit alcohol extracts during storage.

2. MATERIALS AND METHOD

Collected fruits of blackcurrant (*Ribes nigrum L*), hip (*Rosa cinnamomea L.*), hawthorn (*Crataegus oxyacantha L.)*, chokeberry (*Aronia melanocarpa Elliot*), bird cherry (*Padus avium Mill.*) and strawberry (*Fragaria ananassa Duch*) were immediately frozen and stored at -18^{o}C. The frozen fruits were homogenized with 2 volumes of 70% ethanol-water mixture, centrifuged at 14000 rpm for 15 min, and filtered. Obtained extracts were stored at +5±1^{o}C.

The total phenolics were determined by Folin-Ciocalteu method and reported as gallic acid equivalent in mg per g on dry residue [10].

Antioxidant capacity was evaluated by measurement of the peroxyl radical scavenging activity. *In vitro* assay is based on the degree of inhibition of potassium iodide oxidation by antioxidants that scavenge peroxyl radicals, generated from thermal degradation of 2,2'-azobis(2-amidinopropane)-dihydrochloride [11, 12]. Antioxidant capacity was calculated as gallic acid equivalent (GAE) in μM per g on dry residue of extract.

HPLC method was applied for quantitative evaluation of phenolic compounds. Detection was conducted with diode array detector ("Jasco") at 220-540 nm. Column was Zorbax Eclipse XDB C8, 5 μm, 4.6x150 mm. The mixture of acetonitrile and trifluoracetic acid solution (0.05%) was used as mobile phase with linear gradient of acetonitrile from 4 to 40% during 20 min, followed by return to initial conditions and column equilibration per 5 min. The flow rate of mobile phase was equal to 1.5 ml/min. Injection volume of extract samples was 10 μl. The calibration curves were obtained by injecting the solutions of standard compounds (Sigma-Aldrich). The standart compounds were detected at following wavelengths: 280 nm – gallic acid, protocatechuic acid, oligomeric proanthocyanidins, catechins; 328 nm – chlorogenic and other hydroxycinnamic acids; 360 nm – quercetin and quercetin glycosides; 528 nm – anthocyanidins.

3. RESULTS AND DISSCUTIONS

The total phenolic content in initial extracts was highest in hip and hawthorn extracts, followed by blackcurrant and chokeberry extracts (tab. 1). Extracts of bird cherry and strawberry contained the lowest quantity of phenolics, corresponding to 31.5 and 24.2mg/g. The most complex compositions of phenolics were identified in extracts of hawthorn and bird berry (tab.1). In dark-colored fruit (black currant, chokeberry, bird cherry) the anthocyans dominated, and constituted 42.1…71.7% from total phenolics. The most inconsistent results were observed by examining the hip extract. On the one hand the extract was characterized as the best in total phenolic content but on the other side, only three individual components were separated out of the ten investigated in frame of this research.

Table 1. Content of phenolic compounds in fruit extracts, mg/g

Phenolic compound	Black currant	Hip	Hawthorn	Chokeberry	Bird cherry	Strawberry
Total phenolics	51.79	66.74	64.46	50.32	31.51	24.20
Anthocyans	37.15	--	1.52	21.17	19.94	7.93
Proanthocyanidins	--	--	22.11	--	0.34	--
Catechins	--	5.23	11.8	--	--	--
Rutin	0.29	--	0.51	0.46	0.35	--
Hiperoside	--	--	0.57	1.1	2.13	--
Quercetin and quercetin glycosides	1.08	0.43	0.91	2.15	1.27	0.43
Gallic acid and its derivatives	4.78	0.46	--	--	--	--
Chlorogenic acid	--	--	3.3	4.42	0.08	--

The antioxidant capacity of obtained fruit extracts varied in limits from 53.7μM GAE/g (strawberry) to 168.5μM GAE/g (black currant). The antioxidant effectiveness order of initial extracts was: black currant > hip > hawthorn > chokeberry > bird cherry > strawberry. Experimental data confirmed the strong correlation between total phenolic content and antioxidant capacity of initial fruit extracts (fig.1). Pearson's correlation coefficient was equal r^2=0.9794.

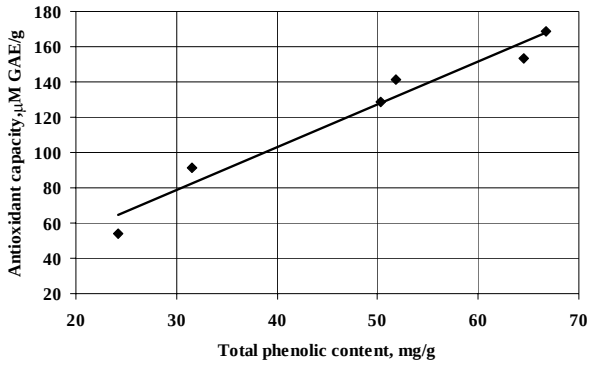

Figure 1. Correlation between total phenolic content and antioxidant capacity of fruit extracts

During 9 months storage of extracts the antioxidant capacity indices modified, but not always changed in the decreasing direction (fig.3). The same variation of antioxidant properties during storage we have observed also in chokeberry and plum jams [13]. Some fluctuations in the antioxidant capacity values can be explained by complex chemical composition of fruits extracts and a number of physical and chemical processes taking place in them. Significant impact on the stability of phenolic compounds in fruit extracts has a pH of medium and the presence of water-soluble antioxidants, in particular the content of ascorbic acid. It is known that the systems ascorbic acid-phenolic compounds are stable systems. Moreover ascorbic acid acts as reducing agent against to the phenolics [14]. Therefore, for a part of extracts (hip, hawtorn, chokeberry) which contain relatively large amounts of ascorbic acid, it was observed a raise in antioxidant capacity during storage as a consequence of phenolic compounds reduction by ascorbic acid at pH=3.5…4.0. Bird cherry and strawberry extracts were characterized by exponential decreasing of antioxidant capacity (fig.2). Pearson's correlation coefficients were equal -0.9375 and -0.9477, respectively for bird cherry and strawberry extracts. Analogical changes in antioxidant activity were determined during storage of frozen strawbwerry [15].

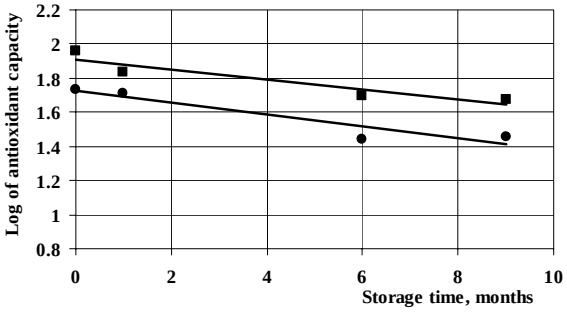

Figure 2. Changes in antioxidant capacity during storage of extract: ■- bird cherry; ●- strawberry.

Changes in antioxidant capacity of extracts with high content of anthocyans and

proanthocyanidins such as black currant and chokeberry were more complicated, that can be explained by complex chemical compositions, speed of reaction of oxidation-reduction between phenolics and organic constituents of extract. Intermediate compounds or their complexes formed as a result of these chemical reactions may have different effects on the antioxidant capacity of extracts. Including into reactions the tannins (phenolics component that are considered superior antioxidants) and their eventual oxidation may lead to oligomerization by phenolic coupling and enlargement of the number of reactive sites [16]. This fact, apparently, also contributes to maintenance of the antioxidant capacity of extracts during storage. Within 9 storage months the loss of antioxidant capacity was from 2.9% for hips to 25.8% for black currant (fig.3). Thus, the antioxidant effectiveness order of extracts after 9 month storage was: hip > black currant > chokeberry > hawthorn > bird cherry > strawberry.

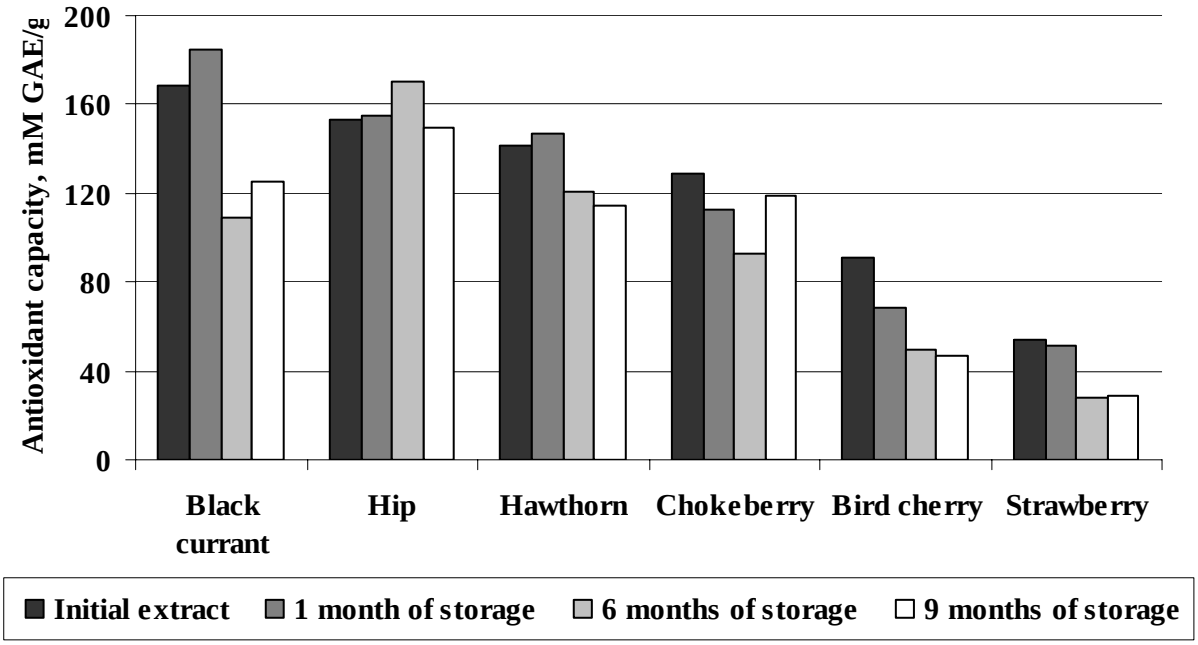

Figure 3. Changes in antioxidant capacity of fruit extracts during storage

4. CONCLUSIONS

The complex compositions of individual phenolics identified in the fruit extracts contributed differently to maintenance their antioxidant capacity during storage. Apparently, the high content of ascorbic acid in hip extract allowed keeping its phenolics from oxidation and protected antioxidant capacity of extract. Because of these multiple reaction of oxidation-reduction the antioxidant capacities of hip extract decreased only by 2.9%. The black currant extract with the highest content of anthocyans lost the antioxidant capacity more than other tested extracts. To conclude, the fruit extracts obtained using 70% water-alcohol solution were quite stable during long-term storage.

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