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EFFECT OF LONG-TERM STORAGE OF FRUIT EXTRACTS ON ANTIOXIDANT ACTIVITY AND ANTOCYANINS CONTENT

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Abstract. In present article, we investigated the quality effect of long-term storage for ethanol/water extracts of local fruits. The quality parameters were based on antioxidant activity and anthocyanin content in extracts. Comparative analysis of antioxidant activity in the initial period and after one year of storage for technological extracts showed a decrease from 27 to 51% for different fruits. A quantitative comparison with anthocyanin degradation showed a positive correlation with DPPH activity degradation.

Keywords: *Fruits extracts, antioxidant activity, DPPH, anthocyanin, long storage, polyphenols.*

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1. Introduction

It is known that the fruit and vegetable consumption has positive health effects linked to prevention of disease due to their high content of bioactive compounds. Red fruits, such as berries, various cherries, grapes and pomegranates, have been characterized as such sources because they are rich in phenolic compounds such as phenolic acids, flavonoids and tannins (Cilla *et al*., 2011). Especially, pomegranate has popularity as a functional food due to the high content of bioactive components of the whole fruit, as well as its juice and extracts. There is a large amount of research that assigns them very important functions for the human organism (van Poppel & Goldbohm, 1995). These components possess antimicrobial and anti-inflammatory properties (Rao & Agarwal, 2000), can act as antioxidants (De Mello-Andrade & Fasolo, 2014), anticancer agents (5), antihepatotoxic agents (6) and can improve cardiovascular (7) health.

One of the important and largest groups of flavonoids - anthocyanins (ACNs) are presents in pomegranate juice as well as in juices from cherries and various berries. And together with hydrolysable tannins they constitute the most valuable bioactive compounds.

In this sense, beverage companies are currently making many efforts to formulate juices, which contain bioactive compounds from different fruits and offer value added products for the consumer (Siró *et al*., 2008). Nevertheless, usually these products are consumed over a short period set by the manufacturer (expiration date, also called shelf-life). During this time the product maintains the physicochemical characteristics that

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make it suitable for human consumption; however, currently this criterion does not include the antioxidant properties and how are they affected by one of the principal parameters in the market: the storage (Calderon-Hidalgo, 2007). Actually, few studies have addressed the evaluation of changes in the overall antioxidant properties and concentration of bioactive compounds of fruit beverages as affected by storage conditions (Del Caro *et al*., 2004; Piljac-Zegarac *et al*., 2009; La Cava & Sgroppo, 2015).

Extending knowledge in this field could lead to progress in technological processing methods for high-quality food production with effective biological activity. In this sense, we performed and analyzed the effect of long term storage on the bioactive compounds presented on commercial fruit extracts used for further blending various beverages.

2. Materials and Methods

Five samples of fruit 50% v/v ethanol-water extracts were kindly provided by Baku Craft Laboratory (Azerbaijan). Experiments were performed by two stages - initial as a control and next one year after, with storage conditions provided by supplier of the extracts. During the year the extracts were stored at room temperature in tightly closed glass vessels. The analyses were carried out over several days. Each sample was analyzed 3 times.

Antioxidant activity was measured by DPPH assay according to the method (Brand -Williams *et al*., 1995). The absorption of DPPH-methanol solution at 518 nm was adjusted to 0.5 that equal to 40 μM concentration. Absorbance changes were measured at the maximum on 518 nm within 20 minutes in a UV-Vis spectrophotometer (Jenway 7305). Concentrations were calculated from a calibration curve in the range between 1 and 10 μM of Trolox. All measurements were performed in 10 mm optical path cuvette.

The concentration of anthocyanin in the samples was measured using the differential spectrophotometry pH protocol described (Lee *et al*., 2005). Briefly, each extract sample was split into two equal portions. One part was treated with 0.1 N. HCl, bringing to pH 1 and the second was adjusted to pH 4.5 with subsequent measurements of absorption at 510 nm and 700 nm. The absorption difference ΔA calculated by the formula (1)

$$\Delta A = (A_{510} - A_{700})_{pH1} - (A_{510} - A_{700})_{pH4.5} \quad (1)$$

where A_{510} is absorbance at 510 nm and A_{700} is absorbance at 700 nm. The anthocyanins concentration was calculated using the extinction coefficient ε = 26900 l × mol^{-1} × cm^{-1}. The resulting data were processed using the Origin Pro 9 software package. Pearson's coefficient was obtained using a linear function fit to the data points as shown below.

3. Results and Discussion

A comparative analysis of changes in the antioxidant activity of the fruit extracts was carried out by registering the inhibition of the DPPH free radical in samples with a shelf life of 1 year and the control. The measurements results are shown in Figure 1. The straight line on the graph indicates 50% inhibition of the DPPH radical and with respect to this parameter we compare the activity of the samples. Analysis of the data shows that the extract from feijoa fruits is least exposed to changes in DPPH inhibition and results in 24% decrease from initial activity, approximately the same result can be observed in

sour cherry extract - 27%. The extracts of blackcurrant, pomegranate and cornelian cherry show us a drop in activity of 45%, 43% and 50%, respectively.

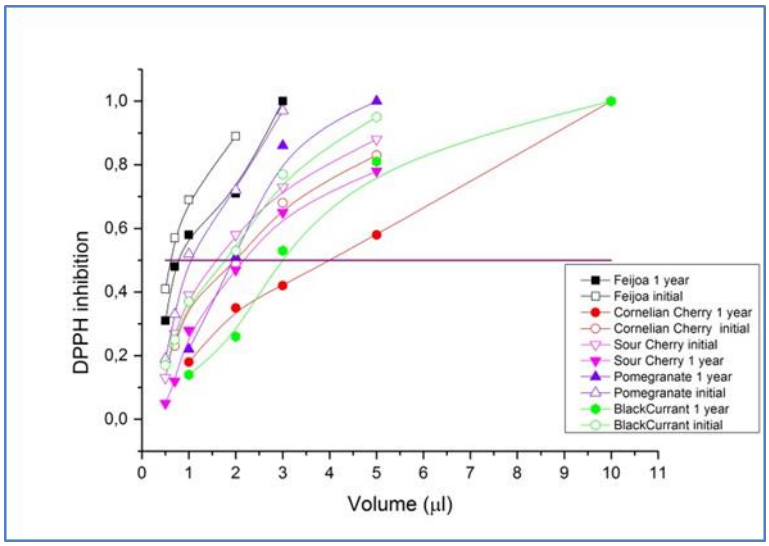

Figure 1. Dependence of DPPH radical quenching upon the volume of different fruit extract samples. Straight line represents of IC_{50} for 6.8 µM of Trolox equivalent

Based on our previous experience in wine research (Agalarov & Gasanov, 2018), we measured the concentration of anthocyanins in fruit extracts before and after one year of shelf storage and the data is shown in Table 1. From these results it can be seen that anthocyanins in feijoa and sour cherry extract are the least exposed to degradation. Next, according to the degradation level of the anthocyanins, there are extracts of black currant, cornelian cherry and pomegranate.

Table 1. Concentration of anthocyanin in fruit extracts and percentage of degradation of anthocyanin and antioxidant activity

Extract	**Anthocyanin concentration**	**Anthocyanin concentration**	**Percentage of antocyanins degradation**	**Percentage of degradation of DPPH activity**
	mg/ml	**mg/ml**		
	Initial	**1 year**		
Feijoa	115	106	0,08261	0,27
Sour Cherry	589	511	0,13243	0,31
Black Currant	1300	728	0,44	0,39
Cornelian Cherry	520	247	0,525	0,49
Pomegranate	570	278	0,51228	0,51

Figure 2 shows a graph of the dependence of the percentage of degradation of anthocyanins upon the degradation of 50% activity of DPPH inhibition. We could fit the data with a linear function Y = 1.92 * X – 0.418 and find fairly strong correlation between these two parameters with Pearson's coefficient r = 0.95.

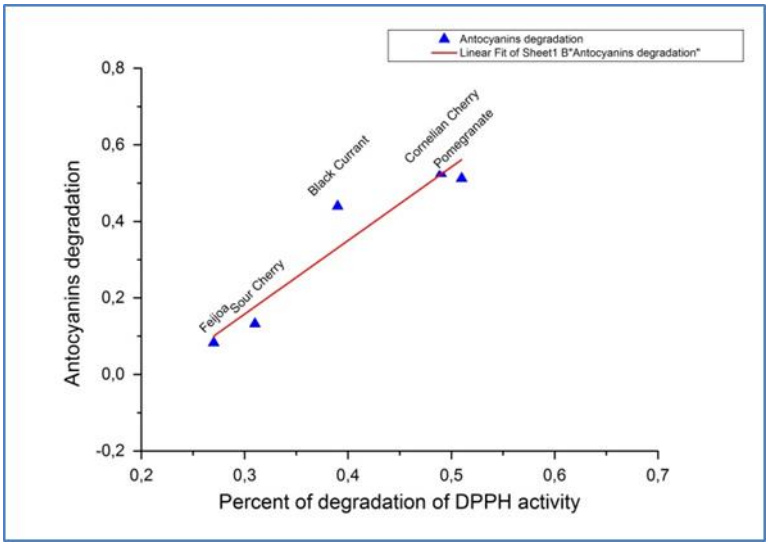

Figure 2. Dependence of the percentage degradation of anthocyanin on the percentage degradation of the DPPH activity of extracts. Red line - approximation by a linear function

Observed correlation between the decrease in antioxidant activity with the degradation of anthocyanins in fruit extracts during long-term storage we associate with action of various polyphenol oxidase enzymes normally presented in fruits. Indeed enzymatic browning because of polyphenol oxidases (tyrosinases) contributes to the color and taste quality of fruit.

Some studies have reported the inhibitory activities of longan (*Dimocarpus longan*) fruit's peel and seed extracts on tyrosinases. The ethanol extract of longan fruits peel at 100 mg/L suppressed the tyrosinase activity by approximately 23.6%. It appears that the antioxidant activity of longan fruits peel contributes to the polyphenol oxidase inhibitory action (Prasad *et al*., 2010).

Some researchers associate the observed effect of antioxidant activity with the presence of ascorbic acid in fruits. However, the presence of ascorbic acid and its derivatives leads to the degradation of polyphenols, including anthocyanins. Actually anthocyanins are rather unstable molecules that will subject of a number of degradative reactions. Polyphenoloxidase, peroxidase and glycosidase impact to destructive effect on anthocyanins (Singh *et al*., 2018). These enzymes may be native to the fruit tissue or their source may be the mold contamination (Nikolas *et al*., 1994). Adding ascorbic acid caused a significant and rapid decrease in stability after one week of storage at 30°C: 73% for acai fruits (*Euterpe oleracea*), 89% for carrot and 93% for blackberry. This negative effect of ascorbic acid on anthocyanins agreed with previous work (De Rosso & Mercadante, 2007; Del Pozo-Insfran *et al*., 2004; Zozio *et al*., 2011). This decrease was immediate, since the absorbance of the açai fruits, blackberry and carrot samples tended to stabilize after the first weeks of storage at 30°C.

A number of anthocyanin-rich sources have been investigated for their potential as commercial pigment extracts. The studies yielded results that demonstrate the relative stability of antioxidant activity and its correlation with anthocyanin degradation as natural markers. Our objective was to observe the effect of changes in antioxidant activity during long-term storage at room temperature. Our results indicate promising perspectives for the exploitation of the fruits extracts in the market and which showed a considerable levels of antioxidants capacity even after long term storage. Natural antioxidants provide

an effective alternative to the synthetic antioxidants traditionally used in the food industry.

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