

# The Influence of Storage on the Total Phenols Content and Antioxidant Activity of the Cranberrybush (*Viburnum opulus L.*) Fruits Extract

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*The fluctuations in the total phenolic content and antioxidant activity of the ethanolic extract of Viburnum opulus fruits were studied. The total phenolic content was determined using the Folin-Ciocalteu assay. To determine the antioxidant activity, the 2,2'-azinobis-3-ethyl-benzthiazino-6-sulphonic acid (ABTS) test was applied. Results indicate that no correlation can be made between the total phenol content and the antioxidant capacity of the extract during refrigerated storage.*

*Keywords: total phenols, antioxidant activity, Viburnum opulus*

Cranberry (*Viburnum opulus L.*) belongs to the *Caprifoliaceae* family; its common name in English are European cranberrybush or snowball tree. This plant is widely distributed especially in the Eastern Europe countries and in Turkey, and is generally used as an ornamental plant. The cranberrybush fruit is edible [1] and well known for its biological properties, such as anti-inflammatory, antibacterial and antinociceptive, being traditionally used for the treatment of menstrual, stomach and kidney cramps [2-4].

The research on cranberrybush fruits are limited.

Phenolic compounds are secondary metabolites, synthesized in plants. They possess various biological properties such as antioxidant, anti-inflammatory, anti-aging, antiapoptotic, anticarcinogenic, anti-atherosclerotic capacity, cardiovascular protection as well as inhibition of angiogenesis and cell proliferation activity. Most of these actions are attributed to their reducing capacity [5].

European cranberrybush fruits are reported as being a good source of flavonoids [6]. Flavonoids, a family of polyphenolic compounds, are an important group of antioxidants.

The purpose of this study was to monitor the changes in the phenolic content and antioxidant capacity of Cranberrybush fruit ethanolic extract during refrigerated storage.

## Experimental part

All chemicals and reagents were of analytical grade and were purchased from Merck Germany and were used without further purification.

The cranberrybush fruits were harvested in October 2010, in Cluj-Napoca, Romania and kept frozen.

The frozen cranberrybush fruits were crushed in a mortar. 5 g of fruits were transferred to an Erlenmeyer flask, 200 mL of solvent (water or 96% ethanol) and 0,2 mL concentrated HCl were added and the mixture was stirred for 1 h at room temperature and then filtered. The residue was twice washed with 10 mL of solvent. The filtrate was quantitatively transferred to a 250 mL volumetric flask and made up to 250 mL with solvent. The extract was stored in the dark, at 4°C (refrigerator), well capped to avoid evaporation. At regular time intervals, samples were

removed from the refrigerator and analysis was conducted immediately.

The total phenol content of the extract was determined using the Folin – Ciocalteu method [7].

A sample of 0.5 mL of extract was mixed with 3 mL Folin-Ciocalteu reagent and kept 5 min in the dark. Then, 2.4 mL of 0.7 M sodium carbonate solution were added. After 2 h in the dark, the absorbance of the resulting mixture was measured at 760 nm (using an UV-Vis Perkin Elmer Lambda 35 double beam spectro-photometer), against a blank sample containing only the reagents and 0.5 mL distilled water.

Using gallic acid (GA) as standard, total phenolic content was expressed as g GA equivalent / kg of frozen fruits. Data reported were done in triplicate (n=3).

The antioxidant activity was determined using the 2,2'-azinobis-3-ethyl-benzthiazino-6-sulphonic acid (ABTS) test [8].

ABTS (360 mg) was dissolved 100 mL distilled water. In order to activate the ABTS cation radical form, 100 mL solution (2.45 mM) of potassium persulfate in distilled water was added and the resulting mixture was allowed to stay 24 h in the dark. A portion of the resulting solution (20 mL) was diluted with distilled water to an absorbance between 0.4 – 0.7, which was measured at a wavelength of 734 nm. To 6 mL of diluted ABTS solution were added 0.1 mL water (reference sample) and to another portion of 6 mL ABTS were added 0.1 mL extract (test sample). The absorbencies were read after 15 min of storage in the absence of light. The differences between the reference sample absorbance and the test sample were calculated and were used for determination of antioxidant capacity.

The antioxidant activity was expressed in ascorbic acid equivalents (AAE) using a calibration curve of the standard.

## Results and discussions

Its composition and the high content of substances having antioxidant activity make the fruits of European cranberrybush unique [9].

Our measurements revealed high contents of polyphenolics in *Viburnum opulus* fruits. The determined content was 4.22 g gallic acid equivalents / kg frozen mass, in accordance with literature data [10]. This content was

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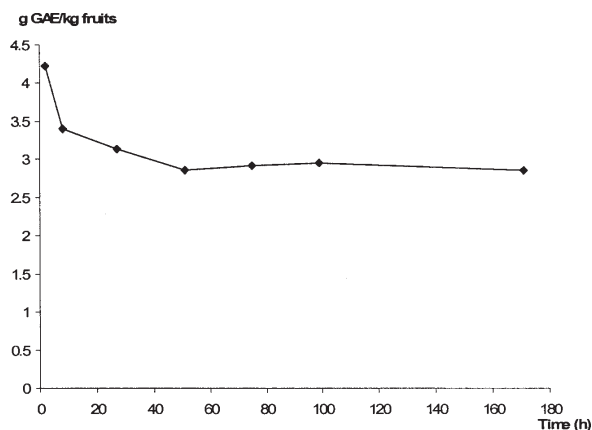


Fig. 1. Total phenolic content (Gallic Acid Equivalents) of cranberrybush fruits extract as a function of time

high compared with other fruits, for example: in plums, this content is 2.2 to 5 g GAE/ fresh mass (FW) [11], in apples the common content is between 0.6 to 2.1 GAE/kg FW [12].

During 171 h storage at 4°C fluctuations in the total phenolic content were observed, as shown in figure 1. In the first 51 h of refrigerated storage, a significant decrease of total phenolic content of the ethanolic fruit extract was observed. The initial rapid decrease in the phenol content of our sample was then followed by a slight increase of the value of total phenolics in the next 48 h.

Comparing these values with the phenolic composition of other fruits, our results are different from those of other studies concerning fluctuations in the phenolic content [13]. It is possible that during extract storage, new compounds are formed which significantly enhance the phenolic content or react with the Folin – Ciocalteu reagent. After approximately 3 days refrigerated storage, the phenolic compounds of the European cranberrybush fruits extract remain stable during refrigerated storage.

The free radical scavenging capacity of the ethanolic extract of cranberrybush fruits using the free radical ABTS reaction was evaluated by measuring the absorbance of the sample at 734 nm. The antioxidant activity was calculated as a decrease in the absorbance value using the formula (1):

$$A = A_0 - A_1 \quad (1)$$

where:

A = the resulting absorbance

A<sub>0</sub> = absorbance of the ABTS solution without sample

A<sub>1</sub> = absorbance of the mixture containing the sample

The resulting absorbance values are converted in ascorbic acid equivalents (AAE) using a calibration curve of the standard.

The evolution of antioxidant activity in cranberrybush fruit extract during refrigerated storage is shown in figure 2. The initial radical scavenging capacity was 7.05 g AAE / kg frozen fruits, according to literature data [14]. In the first 24 h in refrigerated storage, a 16% decrease of the antioxidant capacity was observed, followed by a 22% increase in the next 24 h. This pattern in the variation of the antioxidant capacity (decrease – increase) can be observed during the whole storage period. Increases in the antioxidant activity for grape extracts during storage have been previously reported [15]. According to [15] the increasing activity may be explained by strong tendency of polyphenols to undergo polymerization reactions, the resulting oligomers possessing larger areas for charge delocalization. The decrease in the radical scavenging capacity results when the degree of polymerization

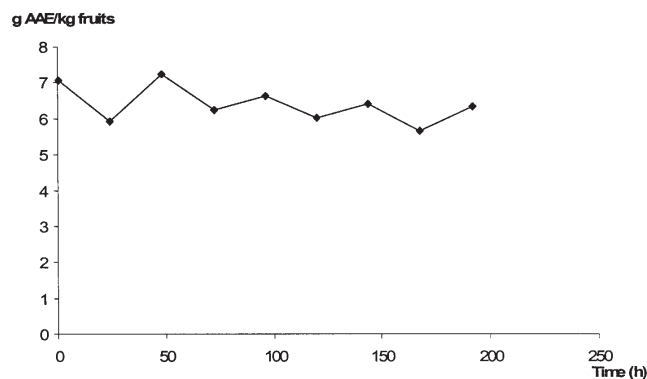


Fig. 2. Antioxidant activity (Ascorbic Acid Equivalents) of cranberrybush fruits extract as a function of time

exceeds a critical value, due to the increase of a molecular complexity and steric hindrance which reduce the capacity of hydroxyl groups to react with the radicals. A slightly tendency of decreasing the antioxidant activity of cranberrybush fruit ethanolic extract can be observed, the value remaining rather stable at 4°C.

No correlation between the total phenolic content (evaluated by the Folin – Ciocalteu method) and the antioxidant capacity (determined by ABTS test) of our sample was observed.

## Conclusions

Our study was focused on the influence of the refrigerated storage in the fluctuations in the total phenolic content and antioxidant activity of the ethanolic extract of *Viburnum opulus* fruits. The results indicate that the total phenolic content exhibited lower variations in comparison to the antioxidant capacity.

Due to the very high content of polyphenolic substances observed in the European cranberrybush fruits, these could be a valuable source of important substances for human nutrition and health.

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