Analysis of the Amount of Polyphenols, Flavonoids and Assessment of the Antioxidant Capacity of Frozen Fruits

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The data from literature shows that frozen vegetal products preserve their nutritional qualities for a long time. Fruits have a high content of substances with antioxidant capacity in the body. There are many recent studies demonstrating the importance of antioxidant substances in neutralizing free radicals in the human body. In this study there were analysed phenolic compounds and flavonoids in eight different species of fruits, belonging to three families. The antioxidant capacity of the extracts was evaluated by: CUPRAC assay, ABTS method and FRAP method. Our results show that the studied fruits represent rich sources of compounds with antioxidant capacity.

Keywords: frozen fruits, phenolic compounds, flavonoids, antioxidant capacity

For a long time, the increased consumption of fruits and vegetables was considered very important in protecting humans against cancer, diabetes, heart and brain, vascular and neurodegenerative diseases. Presently, it is thought that the protective properties of these foods result from the presence of antioxidants that protect the cells and their structures against oxidative damages [1-3].

Oxidative damages are caused by reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radicals [4]. Antioxidants are thought to be highly effective in the management of oxygen species mediated tissue impairments. Many antioxidant compounds possess antiinflammatory, antiatherosclerotic, antiproliferative, antitumor, antimutagenic, anticarcinogenic, antibacterial or antiviral activities, to a greater or lesser extent [5, 6].

An important group of antioxidants are polyphenols and flavonoids. Flavonoids are essential factors in plants interactions with the environment, often serving as the first line of defense against ultra violet irradiation and pathogen attacks. Flavonoids are also major nutritional compounds in foods and beverages that have been found to have antioxidant activities and other health strengthening properties [7-16].

The content of active substances, including polyphenols and flavonoids, in fruits and vegetables [9-16] depends a lot on the different soil management practices [17, 18] on the cultivation area and varies with the types of pollutants resulted from poor management of waste [19-24], which are readily absorbed by water, air, soil, and plants. Due to the increased use of frozen fruits, the evaluation of these products in terms of their antioxidant content became a matter of great importance.

Experimental part

In this study the frozen fruits obtained from several fruits were investigated, after 1, 3 and 6 months of freezing period. We followed the composition of phenolic compounds and total flavonoid contents. Antioxidant capacity of the extracts was evaluated by using CUPRAC assay, FRAP method, and ABTS method.

Plant materials

There were studied the fruits of eight different species, from three families. After harvesting, fruits are spread in a single layer and frozen at temperatures between -35 and -40^0 C, using the quick freezing grain technique (IQF, individual quick frozen). Based on the size of the fruits, the exposure time at these temperatures varies from about 30 minutes (raspberries) to two hours (strawberries). After freezing, fruits are packaged in bulk, in boxes of ten kg and are stored in freezers at temperatures between -18 and -20°C. The term of validity of the frozen product is 18 months. Fruits frozen in this way were used to perform the analysis. The analysed fruits were defrosted and dehydrated after one month, three months and six months.

Studied fruits were: *Rubus idaeus (Fam. Rosaceae*), raspberry; *Fragaria ananassa (Fam. Rosaceae*), strawberry; *Rubus fructicosus (Fam. Rosaceae)*, blackberry; *Prunus cerasus (Fam. Rosaceae*), cherry; *Vaccinium vitis-idaea (Fam. Ericaceae*) lingonberry; *Vaccinium myrtillus (Fam. Ericaceae*), cranberry; *Ribes nigrum (Fam. Grosullariaceae*), black currant; *Ribes rubrum (Fam. Grosullariaceae)*, red currant.

Study of the bioactive compounds of plant extracts

The determination of total phenolic contents using Folin-Ciocalteu method

The alcoholic extract solutions, *tinctures*, were prepared by maceration in 70^0 alcohol, at room temperature (20°C) for 10 days. The residue was removed by decantation.

Total phenolic contents were determined by using the Folin-Ciocalteu reagen [25-28] by using gallic acid as standard, with some modifications. The extract solution (0.1 mL) containing 1000μg of extract was mixed with 46 mL distilled water in a volumetric flask and 1 mL Folin-Ciocalteu (Merck) reagent was added; the flask was thoroughly shaken. The mixture was allowed to react for 3 min and 3 mL aqueous solution of 2% Na_2CO_3 were added. At the end of the 2 h incubation at the room temperature, the absorbance of each mixture was measured at 765 nm, in Shimadzu UV-1700 Pharmaspec UV-Vis

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Spectrophotometer. The same procedure was also applied to the standard solutions of gallic acid, and a standard curve was obtained.

With the Folin-Ciocalteu method we measured the OH groups of the samples taken into study in alkaline conditions (adjusted with sodium carbonate). The absorbance at the 765 nm wavelength increases proportional with the number of OH groups of the anthocyanins.

The calibration curve (fig.1) was obtained with a solution of known concentration of gallic acid (20-100 ppm), and the concentration of polyphenol extracts was calculated from the regression equation and expressed as mg gallic acid equivalents (GAE)/100 g dry sample [9, 13, 25-28].

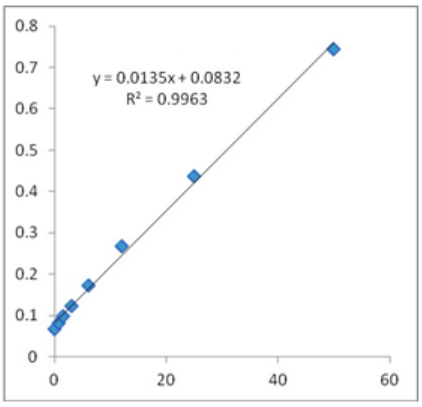

Fig. 1. The calibration curve made with gallic acid for Folin-Ciocalteu method in alcoholic medium

The determination of total flavonoid contents

The content of total flavonoids was determined by using a colorimetric method which has been described previously [25, 26, 29]. 1 mL of the sample (containing 0.1 mg/mL dry weight) is mixed with 4 mL water and placed in a 10 mL volumetric flask.

First there were added 0.3 mL ground $NaNO_2$ 5%, after five minutes 0.3 mL $AlCl_3$ 10% and after 6 min, 2 mL of NaOH 1M. The volumetric flask is filled to the mark with distilled water. The solution is mixed in the volumetric flask and it is read the absorbance at 510 nm in the Shimadzu UV-1700 Pharmaspec UV-Vis Spectrophotometer. The calibration curve (fig. 2) shall create its standard using quercetin (QE) [29, 30].

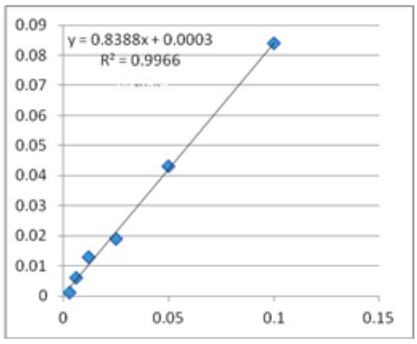

Fig. 2. Calibration curve made with quercetin in alcoholic medium (surroundings, environment)

Study of the antioxidant capacity. FRAP method (Ferric Reducing Antioxidant Power)

FRAP method is a simple spectrophotometric method that assesses the antioxidant power of the studied samples, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid *p*H. The stock solutions included: 300 mM acetate buffer; 270 mg $FeCl_3 \cdot 6\ H_2O$ dissolved in 50 mL distillated water; 150 mg TPTZ and 150µL HCl, dissolved in 50 mL distillated water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL $FeCl_3 \cdot 6\ H_2O$ solution and 5 mL TPTZ solution. Trolox was used as a standard solution, the calibration curve was made for concentrations between 0-300µM, having the correlation coefficient $R^2 = 0.9956$ and the regression equation ($y = 0.0017x + 0.0848$), where *y* represents the absorbance detected at 595 nm. The results are expressed as µmol Trolox equivalents (TE)/ 100µL extract [26-34].

CUPRAC assay (Cupric Ions (Cu^{2+}) Reducing Power)

In order to determine the cupric ions (Cu^{2+}) reducing antioxidant capacity the method proposed by Karaman et al. (2010) was used with slight modifications [32]. To this end, 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanolic neocuproine solution ($7.5x10^{-3}$ M) and 0.25 mL CH_3COONH_4 buffer solution (1 M) were added to a test tube, followed by mixing with the plants extracts. The total volume was adjusted to 2 mL with distilled water and thoroughly mixed. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank, 30 min later. Increased absorbance of the reaction mixture indicates increased reduction capability [35-37].

ABTS Method (Determination of Antioxidant Capacity using the $ABTS^{\cdot+}$ Radical Cation)

The test method of antiradical capacity, with the application of ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) is known and widely used to determine the antioxidant activity of substances of whatever nature. Based on the given method, it was determined the antioxidant activity of both pure substances, and antioxidant complexes. The $ABTS^{\cdot+}$ radical is generated by the oxidation of ABTS with potassium persulfate and is reduced by the addition of hydrogens atom.

ABTS method or TEAC (Trolox Equivalents Antioxidant Capacity) is based on the ability of antioxidants to reduce the life of the cation radical ($ABTS^+$), a green -blue chromophore that absorbs at 734 nm, compared to Trolox. $ABTS^+$ is produced by reacting stock solution ABTS - 2,2 Azinobis-(3 ethyl 6 sulfonic acid) (7 mM) with potassium persulfate (2.45 mM) for 12-16 h. In order to study the antioxidant activity, $ABTS^+$ solution is diluted with ethanol until an absorbance of 0.70 ± 0.02 to 734 nm is obtained. After the addition of 100µL of sample to 2900 µL $ABTS^+$ solution, the mixture was monitored spectrophotometrically at 734 nm. The calibration curve was made with the Trolox standard [9, 12-14, 29, 31]. The calculation formula is the same as for the DPPH test. The reduction of the extinction values (% Inhibition) of the DPPH solution is calculated according to the equation:

% Inhibition = (AbsDPPH- AbsSAMPLE)/AbsDPPH x100

Results and discussions

Bioactive compounds of plant extracts

The total amount of polyphenols, determined by the Folin-Ciocalteu method, is the highest in *Ribes nigrum*, followed by *Rubus idaeus* and *Fragaria ananassa*, but we also obtained high values in case of other species introduced to the study, as it can be seen in figure 3.

The total amount of flavonoids is highest in *Vaccinium vitis-idaea*, followed by *Vaccinium myrtillus* and *Rubus fructicosus*. Also the other studied species contain high values of flavonoids, as shows in figure 4. A slight decrease may be observed over the time, in case of most species, but this decrease is insignificant.

Antioxidant capacity

Following the antioxidant capacity, increased values were obtained, in case of all studied species. The obtained

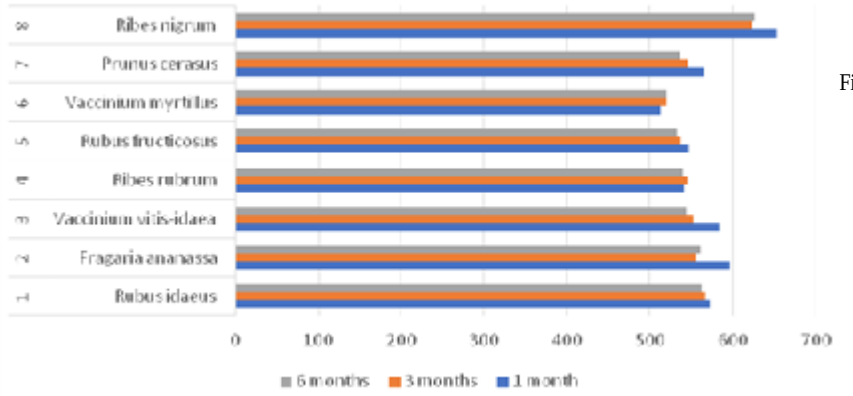

Fig. 3. Total polyphenolic content (mg GAE/100DW), after 1, 3, and 6 months of freezing period

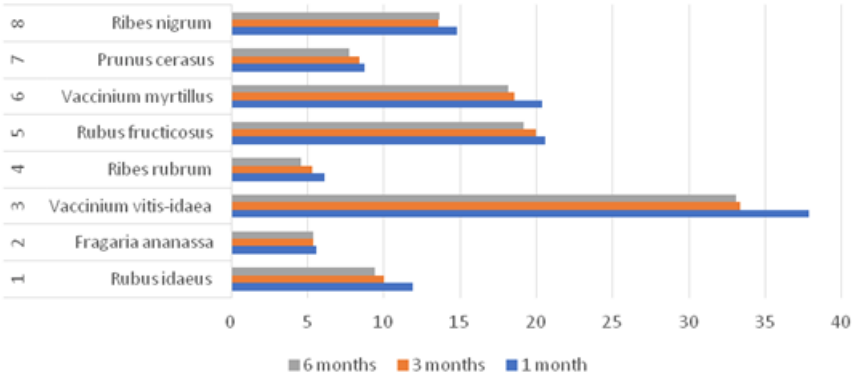

Fig. 4. Total flavonoid content (mg QE/100 DW) after 1, 3, and 6 months of freezing period

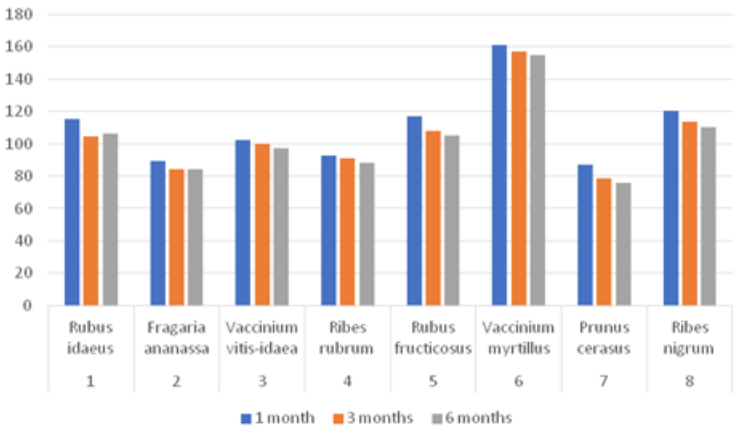

Fig. 5. Antioxidant capacity – FRAP method (mg TE/mL), after 1, 3, and 6 months of freezing period

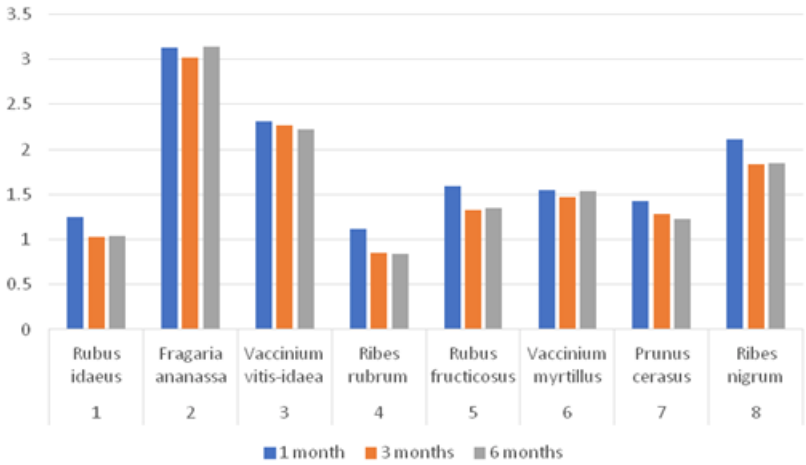

Fig. 6. Antioxidant capacity - CUPRAC assay (mM Trolox), after 1, 3, and 6 months of freezing period

results by those three methods of determining the antioxidant capacity, showed that the ethanol extracts obtained from the studied fruits, have shown a high capacity of reducing ABTS, FRAP and CUPRAC radicals, as are presented in figures 5-7.

The studied fruits contain significant quantities of antioxidant substances, presenting an antioxidant power as well, proven by other studies [38-40]. Vegetable and fruit consumption has been shown in epidemiological studies to be related to reduce risk of cancer and cardiovascular disease [10].

There was no direct relationship between total phenolics content and antioxidant activity, suggesting that the antioxidant activity is a result of combination of different compounds having synergic and antagonistic effects.

This study has proven that during freezing and maintaining fruits at very low temperatures, the total amount of polyphenols, flavonoids, and the antioxidant capacity are not affected.

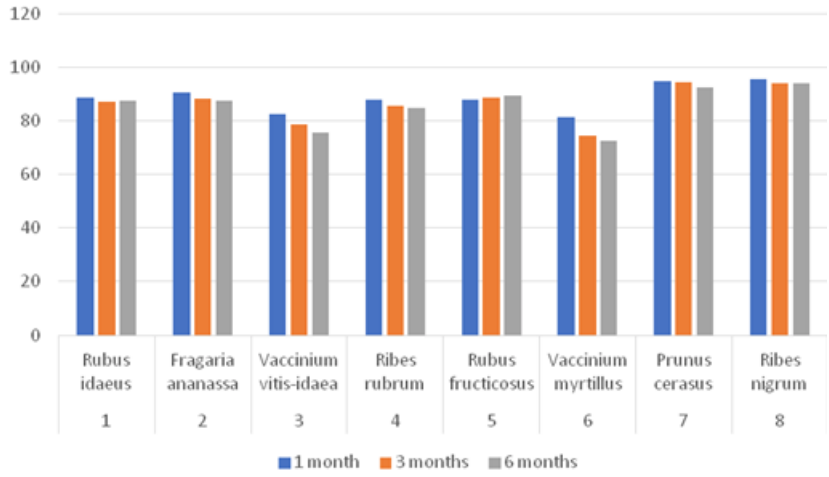

Fig. 7. Antioxidant capacity - ABTS method (%), after 1, 3, and 6 months of freezing period

Conclusions

The results showed that the studied fruits are rich sources of phenolic compounds, flavonoids and phenolic acids, with antioxidant capacity and reducing power. Generally, high levels of the phenolic compounds responsible for high total antioxidant capacity are found in studied fruits. Consumption of these fruits, fresh or frozen, can provide a good source of antioxidants and nutrients, with benefit to human health.

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