CAPITALIZATION OF HYDROALCOHOLIC EXTRACTS FROM THE ARONIA FRUITS AS A SOURCE OF ANTIOXIDANTS FOR FUNCTIONAL FOODS

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Abstract: it has been demonstrated that the highest rate of extraction of antioxidant substances at a concentration of ethanol is 40–60% and a temperature of 45°C and tannins are–91.36mg/g, anthocyanin–5.64mg/g, phenolic compounds (Gallic acid)–24.85mg/g. It has obtained a hierarchy of antiradical activity (DPPH•), ranging from 61.44% to 40% ethanol concentration and the extraction temperature of 30°C up to 86.62% at the concentration of 80% ethanol extraction at 65°C. It was found that the highest values of antioxidant activity (HPSA) is observed in the extracts from aronie fruits at the extraction temperature 30°C and constitues 71.67%. The obtained results denote an important antioxidant activity of the aronia fruits extracts, which could be used in the manufacture of functional foods.

Keywords: Aronia fruits, hydroalcoholic extracts, temperature, antioxidant substances.

Introduction

The concept of sustainable development of society cover the full and effective use of material sources of natural origin. This issue is of extremely importance for the Republic of Moldova, being linked with the development of agriculture and food industry–strategic branches of national economy. The degree of processing of local organic raw material is small, domestic and the import food additives increases the cost of production [1]. Synthetic food additives have dubious nutritional qualities, being allergens, carcinogens, etc. Replacing them with harmless natural substances is a strategic global problem, linked to the maintaining of health and human gene pool.

Aronia Melanocarpa (Chokeberry) Elliot is a plant native from the North of America and due to the taste and curative qualities of the fruit has been cultivated over large areas in European and Asian countries. Plantations in Moldova occupy an area of 157.8 ha in the forest detours [2]. The fruits of aronia Melanocarpa Elliot differ by the content of phenolic compounds, organic acids, vitamins, minerals, carbohydrates [3]. It is a valuable source of food and pharmaceutical therapeutic virtues: vaso–protective hypertensive, antioxidant, chemoprevention, antiviral, anti–inflammatory, astro–protective, antimicrobial [4] and that is why it is welcomed in the daily food ration for strengthening the body and promoting a healthy lifestyle [5].

The purpose of research is to obtain extracts of aronia fruits with high antioxidant activity for the formulation of functional foods.

Materials and methods

To obtain aronia dried fruit extracts of indigenous origin were ground up and sifted powdered state. The powder obtained was subjected to the extraction in hydro–alcoholic medium using ethanol of different concentrations: 20, 40, 50, 60, 80 and 96%. The extraction was performed in solid–liquid ratio 1:18 for one hour in a dark place at temperatures: 30, 45 and 65°C. The extracts obtained were subjected to filtering and transferred to the packaging dark color. Samples of Aronia fruit extracts have been preserved in dark place at a temperature of 4 ± 1°C.

In the aronia fruit extracts were determined: index of total polyphenols, content of phenolic compounds (Gallic acid), the content of tannins and anthocyanins, antiradical activity (DPPH•) and antioxidant (HPSA) [6].

Index of total polyphenols (IPT) or D_{280} Index is a parameter describing the content of total phenolic compounds (tannins, phenolic acids, flavones, anthocyanins, etc.) in the extract. The principle of the method for determining the D_{280} index is based on the strong absorption of ultraviolet light by the benzenice kernels, characteristic to phenolic compounds, attaining a maximum absorption at wavelength λ = 275–280 nm.

The content of phenolic compounds is determined for phenolic compounds with reducing properties. The principle of the method for determining the Folin–Ciocalteu index: in the base and in the presence of phenolic compounds, mixture of fosfotungistic acids ($H_3PW_{l2}O_{40}$) and fosfomolibdic ($H_3PMo_{l2}O_{40}$) is reduced to the blue oxides of tungsten (W_8O_{23}) and molybdenum ($Mo_{12}O_{23}$). This blue colouration shows a maximum absorbance at λ =750 nm (visible spectrum). The colouration time, respectively of light absorption, is proportional to the total content of phenolic compounds. The content of phenolic compounds in the extracts is expressed in mg/mL.

Determination of tannins content is that all the phenolic compounds are oxidized by Folin–Ciocalteu reagent. The colour intensity is proportional to the content of phenolic compounds. The colour intensity is determined by the spetro photometer at λ=750 nm, and the content of phenolic substances is determined by the calibration curve.

Determination of total content of anthocyanin from extracts is achieved by correlating the difference of colouring intensity with the variation of pH. The method is based on the following principle: in the acidic environment there is a balance between coloured and colourless forms of anthocyanines; this balance depends on the pH (0.6 and 3. 5) and the variation of intensity between two colouring pH values is proportional to the content of anthocyanins. Measure the absorbance (optical density) λ=520 nm at both pH values. Formula for calculating is deducted from a calibration curve and the results are expressed in mg/g.

Determination of antiradical activity by using the free radical DPPH•. The spectrophotometric method with free radical DPPH• (2.2–diphenyl–1–picrylhydrazyl) is based on the radical absorbance reduction in the presence of antioxidants. DPPH• is characterized as a stable radical, due to delocalization of the unpaired electrons on the whole molecule. The unpaired electron delocalisation causes the violet colour, forming an absorption band with a maximum located at about 520 nm. In this work the researches were performed at λ = wavelength of 515 nm [7].

Determination of antioxidant activity (HPSA), the method for determining the capacity of inhibition of hydrogen peroxide is estimated by titration of the substitution

method (analytical solution does not react directly, so it turns into a chemical combination that can then be titrated with a solution of known concentration).

Results and discussions

The total content of polyphenols from the aronia varies between 25.59 and 88.0. The highest value was recorded at 45°C and the alcoholic strength of 60%. The amount of polyphenols extracted differ both the temperature of extraction and the concentration of alcohol used. Thus, the polyphenols from aronia extract are extracted better at a temperature of 45°C and the concentration of 50–60%. The amount of polyphenols extracted at 45°C with the ethyl alcohol solution of 60% is 2.28 times higher than that extracted using distilled water and 2.65 times higher than that extracted with ethyl alcohol of 96%. Favorable extraction is alcohol with the concentration of 50%.

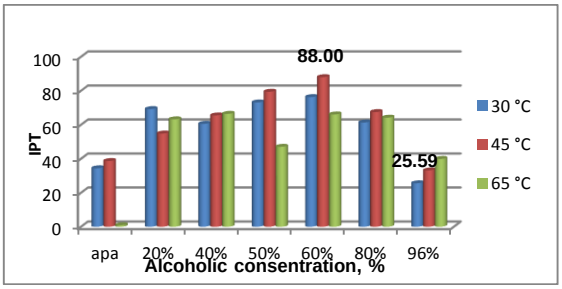

Fig. 1. Dependence of total polyphenols index depending on the temperature and different alcohol concentrations.

The total content of tannins varies between 15.56mg/g and 91.36mg/g. The highest value was recorded at 45°C and alcoholic concentration of 40%. Another similar value close to the maximum concentration of 60% (same temperature). And in the case of the sample without alcohol, just the temperature of 45°C, may be allowed a runtime error. The amount of tannins extracted at 45°C with ethyl alcohol solution of 40% is 1.27 times higher than that extracted at a temperature of 30°C and 1.5 times higher than that extracted at a temperature of 65°C.

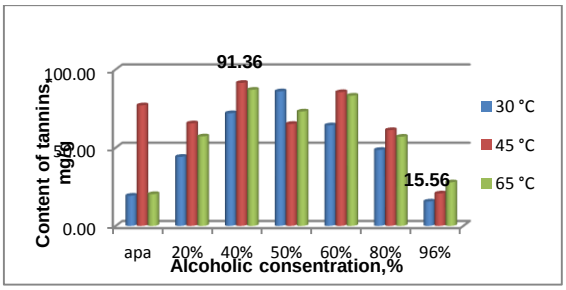

Fig. 2. Dependence of tannins content depending on the temperature and concentration of various alcohol.

Analyzing the data obtained was determined the content of anthocyanins of the three varieties of temperature: 30 and 65^{0}C. The values ranged between 0.60–5.64 mg/g.

The highest content of anthocyanins found in dried extract was determined for concentration of 60%, followed by 40%, at the optimal temperature of 45°C. The concentration of ethyl alcohol of 50% is optimal for all three temperatures, while the lowest values are recorded in fluid extract. At a temperature of 30^{0}C, the concentration of ethyl alcohol of 40% has been registered the lowest value, which was not taken into account, describing it as an error. The amount of anthocyanins extracted at 45^{0}C with ethyl alcohol solution of 60% is 9.5 times higher than that extracted using distilled water and 3.0 times higher than that extracted with ethyl alcohol of 96%. And compared to the temperature of 30^{0}C, the amount of anthocyanins extracted at 45^{0}C with ethyl alcohol solution of 60% is 1.25 times higher and 1.7 times higher than that extracted at 65^{0}C.

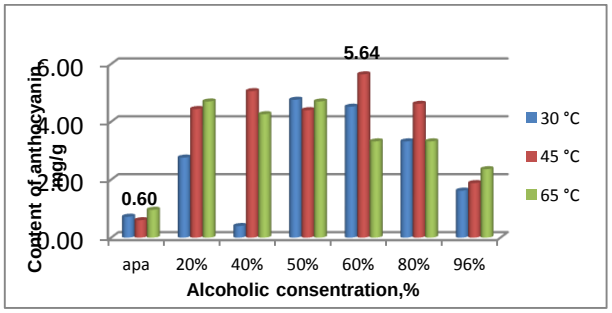

Fig. 3. Dependence of the anthocyanin content depending on the temperature and concentrations of various alcohol.

The highest content of phenolic compounds identified in aronia extract was determined for concentration of 60%, followed by 40 to 50%, at the optimal temperature of 45°C.

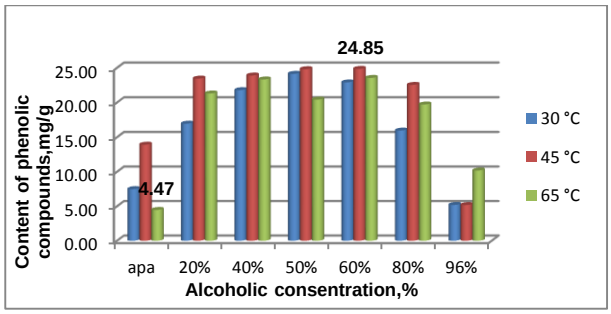

Fig. 4. Influence of temperature and alcohol concentration on the content of phenolic compounds.

Similar values can see the concentrations of 20, 40, 50 and 80% demonstrating the stability of phenolic compounds content at the ambient temperature of 45°C. The maximum value of 24.85 mg/g of phenolic compounds have been recorded at 45°C and alcoholic concentrations of 60% and a minimum of 4.47mg/g at a temperature of 65°C

aqueous extract. The amount of phenolic compounds extracted at a temperature of 45°C with ethyl alcohol solution of 60% is 1.79 times higher than that extracted using distilled water and 4.8 times higher than that extracted with ethyl alcohol of 96%. And compared to the temperature of 30°C, the amount of phenolic compounds extracted at a temperature of 45°C with ethyl alcohol solution of 60% is 1.1 times higher and 1.05 times higher than that extracted at 65°C. We can observe a gradual increase of the values of the aqueous extract optimal concentrations up to 40–60% but then to decrease, starting with 80% and 96% finished. This was noticed at all temperatures studied.

The antiradical activity determined by DPPH for aronia fruits reveals the fact that the highest values of 91.94–90.94% inhibition radical DPPH•, were recorded for the extracts obtained at temperatures of 45°C and 65°C with a concentration of 80 and 96% ethyl alcohol. A pronounced reduction of the radical DPPH• can be observed in the hydric sample at 65°C of 25.04% inhibition.

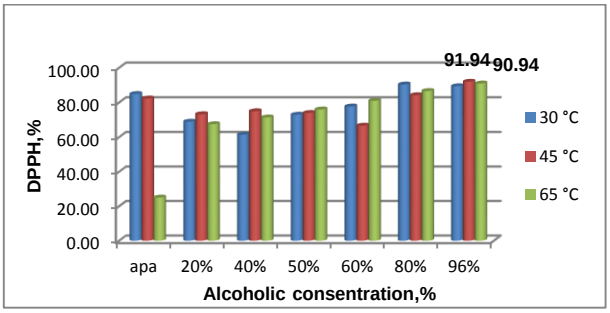

Fig. 5. Antiradical activity of hydroalcoholic extracts of aronia fruits analysed by the method of DPPH• radical reduction.

HPSA method has been obtained using the hierarchy of antioxidant activity ranging from 71.67 to 25%.

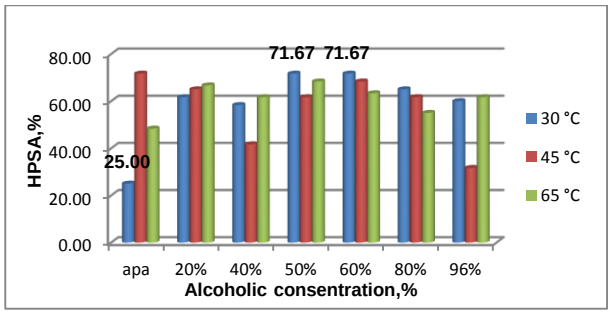

Fig. 6. Variation in antioxidant activity in dependence of temperature and alcoholic concentration.

The values obtained for the hydroalcoholic extracts of 50–60% at a temperature of 30°C on the evaluation of antioxidant potential were the highest of 71.67%, compared with the same concentration, but at temperatures of 45°C and 65°C at which has been registered an inhibition rate of H_2O_2 less. The smallest value, as in the case of the largest,

it was recorded at a temperature of 30°C and is 25% which corresponds to hydric extract. A better stability of the antioxidant potential is observed at a temperature of 65°C, where values between 48.33% and 68.33% no large fluctuations compared to those at 30 and 45°C.

Conclusions

Making a parallel between the dependency index of total polyphenols and phenolic compounds content depending on the temperature and alcoholic concentration, it can be said that the concentration influences to a higher degree on the aronia extract than the temperature. It was found, that with the increasing amount of alcohol added to the solvent increases the amount of tannins extracted up to a certain point, then starting with the concentration of 80% it decreases gradually. It was found that the anthocyanin content values are close to the levels 20, 40, 50 and 80% that proves their stability at a temperature of 45°C. Antiradical and antioxidant activity of aronia fruit reveals that the temperature and alcoholic concentration equally influence on them with some minor differences, as in the case of aqueous sample, so we can consider that aronia fruits have a high antioxidant and antiradical capacity regardless of conditions extraction.

References

1. Baerle, A. Studiu privind separarea şi stabilizarea coloranţilor antocianici din aronia Melanocarpa, Chişinău, 2006, p.43
2. Calalb, T. Structura şi compoziţia biochimică a fructelor de Aronia melanocarpa (Michx.) Elliot în vivo şi în vitro. Autoreferatul tezei de doctor habilitat în biologie. Institutul de Genetică şi Fiziologie a Plantelor al AŞM, 2010.
3. Jakobek, L., Seruga, M., Medvidovic–Kosanovic, M., et al. Antioxidant Activity and Polyphenols of Aronia in comparision to other berry species. In: Agriculture Conspects Scientificus, 2007, vol. 72, nr. 4, p.301–306.
4. Valcheva–Kuzmanova, S., Belcheva, A. Current knowledge of Aronia melanocarpa as amedicinal plant. In: Folia Med. (Plovdiv), 2006, vol. 48, nr. 2, p.11–17.
5. Calalb, T. Aronia melanocarpa Elliot tissue culture in vitro. In: Proceedings of the 8th Nation. Sympos. „Medicinal plants – present and perspectives", Piatra Neamţ, 2003, p. 40–43.
6. Ţârdea, C. Chimia şi analiza vinului. Edit. Ion Ionescu de la Brad, Iaşi, 2007
7. Blois, M.S. Antioxidant determinations by the use of a stable free radical, 1958, Nature, 181: 1199–1200.