

THE TREATMENT EFFECT ON THE ANTIOXIDANT ACTIVITY OF ARONIA PRODUCTS

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In the last decade, agricultural producers in Serbia have been massively reoriented to aronia (*Aronia*) cultivation. Due to low and inadequately organized purchase, the accumulation of large amounts of aronia forced the producers to organize and start processing. The most common and most represented products of aronia in our market are cold pressed juice, pasteurized sweet syrup and liqueur. Processing can affect a decrease of the bioactive components concentration, as well as the reduction of beneficial effects on the human organism. The antioxidant activity of cold pressed aronia juice, pasteurized sweet aronia syrup and liqueur obtained in double processing of aronia (candied aronia) was studied using the DPPH (1.1-diphenyl-2-picrylhydrazyl radical) test. The cold pressed juice has shown a higher antioxidant activity compared to the pasteurized sweet syrup. It was noticed that the antioxidant activity of liqueur from candied aronia was preserved but markedly decreased. So far, fresh aronia has mainly been used for the liqueur production, but candied aronia has been proved as an appropriate raw material, too. The liqueur obtained in that way could be applied in food and beverage industry as a substitute for synthetic antioxidants, food colors and aromas.

Keywords: Aronia, liqueur, syrup, antioxidant activity

Introduction

The genus *Aronia* belongs to the family *Rosaceae* and includes two types of shrubs: *A. melanocarpa* and *A. arbutifolia*, black and red aronia, respectively. Today, it is often cultivated in Russia and the Baltic countries, as well as in Poland and Germany where there is developed industrial processing of this fruit [1]. *Aronia* originates from the eastern parts of North America and it is about 90 - 180 cm high, with red stems and red-black berry fruits. Fruits of aronia are similar to blueberries, resistant to low temperatures, frost and winter. It can survive the temperatures below -47 °C and this is the reason why it has been named Siberian blueberries [2].

Aronia is one of the richest natural sources of polyphenols and anthocyanins [3 - 5]. These compounds (polyphenols and anthocyanins) are powerful antioxidants *in vitro* [6, 7] and they can be the protection against many degenerative diseases [8 - 10].

The aronia extract is used as an ingredient in foods, beverages, pharmaceuticals and cosmetics, primarily as a natural herbal antioxidant replacing synthetic antioxidants [11]. In addition to the antioxidant activity, aronia also exhibits anti-inflammatory, antidiabetic, antimutagenic, immunomodulatory, antiproliferative and anticancer effects [10 - 12].

A recent study suggests that aronia can also be used for combating obesity. Anthocyanins and proanthocyanidins

from aronia exhibit a significant inhibitory effect on pancreatic lipase, and thus through a complex mechanism, indirectly act to reduce the energy intake and reduce obesity [13].

The antioxidant activity of berry fruits, including aronia, is still an actual topic although it has been quite studied. In most previous studies of the antioxidant activity, by using various tests such as ORAC (the method which has been developed and standardized by Ghiselli and Glazer [14, 15] and which basically measures the antioxidative inhibition of radical peroxide by the reaction mechanism) [16] followed by the DPPH test (*in vitro* method) [17, 18] it was confirmed that aronia is one of the best antioxidant of berry fruits.

Practically, fruit liqueurs, in addition to ethanol, sugar and water, can be produced with one or more fruit types. However, fruit with rich aroma, color and acid such as sour cherries, currants, blueberries, raspberries, blackberries, strawberries and more recently aronia are more suitable for the liqueur production. The content of alcohol in liqueurs is in the range from 15 to 40% vol., and the recommended content in fruit liqueurs is 30 to 40%. The content of the extract in sweet liqueurs should be at least 220 g/dm³. In traditional medicine, liqueurs have been known as healthy drinks, excellent for immunity. Lately, the popularity of the liqueurs has been noticed because consumers are increas-

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ingly turning to traditional products of recognizable taste and proven quality. Many cocktails are attractive due to the color and taste that are mostly derived from liqueurs used for the preparation [19].

According to the Food Guidelines for Americans [20], the moderate consumption of wine, beer and other alcoholic beverages may have a positive effect on the human health. Phenolic compounds are main antioxidants in alcoholic beverages such as wine and liqueurs; they can resist the pro-oxidant activity of alcohol to a certain extent [21]. As the awareness grows, and the need of consumers to consume high-quality food products which, in addition to their nutritional value have a functional value, there is a growing tendency in the food industry to add natural extracts of plants rich in bioactive compounds to the products. These bioactive compounds or phytochemicals act positively on health due to the antioxidant activity. It is recommended to consume food antioxidants as part of optimal nutrition in order to enhance the antioxidant protection of the organism and prevent the development of cardiovascular and other chronic diseases [22].

Berries show the highest antioxidant activity, which does not depend on the total content of polyphenol, but also on the composition of the present polyphenolic compounds. Compared to other berries, aronia has the highest content of polyphenols. For example, the aronia juice has the quadruple higher antioxidant activity than wine, blueberry juice and black currant juice [23].

In the production of fruit liqueurs by maceration, biologically active substances diffuse into the water-alcohol base. Except for the type and quality of macerated fruit, the maceration quality and concentration of biologically active compounds are affected by numerous other maceration parameters such as maceration time, temperature and the alcohol concentration.

Ćujić and associates have confirmed that maceration is an efficient and simple technique for bioactive compounds from dried aronia extraction [24]. Anna Sokół-Łetowska and associates have investigated the composition and antioxidative activity of liqueurs from ten red fruit types of aronia [25]. In addition to the Scottish rosefruit (*Rosa spinosissima* L.), aronia liqueur has shown the most pronounced antioxidant activity. Using the Folin-Ciocalteu method and gallic acid for the standard curve, it was found that the content of reacting substances with Folin-Ciocalteu reagent in aronia liqueur is 329.2 mg GAE/100cm³. In case of aronia liqueur, according to DPPH test with the results expressed in Trolox equivalents, the antioxidant activity of the liqueur was determined before and after storage of 6 months, at the temperature of 15 °C and 30 °C. The activity reduction of about 50% was observed during storage at 30 °C. The storage of the liqueur at the temperature of 15 °C and the presence of sucrose has increased the stability of phenolic compounds in liqueurs. During the storage process, the change in color was observed to the least extent in aronia, Scottish rose and mahonia liqueurs. The highest amount of phenolic compounds, determined by HPLC method, was found in aronia liqueur (over 220 mg/100cm³) [25].

The liqueurs tested in this study have been made from fresh fruit. Having this in mind, it would be important to study the antioxidant activity of liqueurs prepared from candied aronia fruit. In that way, the impact of double fruit processing can be studied in two stages – primarily from fresh fruit to the candied product, and maceration into a liqueur after that.

Experimental

Material and Chemicals

- Liqueur from candied aronia obtained by candied aronia maceration (Karlito d.o.o. – Wholesale of dry and candied fruit) in grape brandy (Vinica Grković, Niš). The liqueur was obtained in the workshop of fruits and vegetables processing by the entrepreneur Nataša Vitošević within the program „Startup job” of the organization „Eneca” from Niš.

- Juice of aronia obtained by cold pressing of physiologically ripe fruit of aronia (no sugar added). Fresh aronia is grown on a farm in the Lukovo village (the municipality of Svrljig) located on the slopes of Stara Planina.

- Pasteurized sweet syrup of aronia obtained from aronia fruit grown in the Gabrovac village, municipality of Palilula, the City of Niš.

- 96% Ethanol (Zorka Pharma d.o.o. Šabac, Serbia)

- 1,1-diphenyl-2-picrylhydrazyl (DPPH radical) (Sigma Chemical Company - St. Louis, USA)

All other reagents used in this study were analytical grade of purity.

Antioxidant activity

DPPH-test

DPPH test is the most common used *in vitro* method for the effective determination of the antioxidant activity based on the exchange of hydrogen atoms or electrons between antioxidant molecules and DPPH radicals in the solution [26, 27]. Under the influence of reducing agents, DPPH radical changes the color from purple to yellow, which is the result of stable diamagnetic molecule hydrazine formation. The change in color is monitored spectrophotometrically [26, 28, 29]. Ethanol (96%) is used as a blank control.

The solvent was evaporated to dry in a drying oven at 105 °C. The dry matter was dissolved in ethanol and a series of analytical solutions (0.274-18.4 mg/cm³) were prepared. The ethanol solution of DPPH radical (1 cm³) in the concentration of 3×10⁻⁴ mol/dm³ was added to the analytical solution of liqueur, and the absorbance at 517 nm was measured immediately (the first sample) and after 20 minutes of incubation in the dark at 20 °C (the second sample). The absorbance at 517 nm of DPPH radical in ethanol (1 cm³ of DPPH in the concentration of 3×10⁻⁴ mol/dm³ added to 2.5 cm³ of ethanol) was measured as a control, as well as the absorbance of aronia liqueur (2.5 cm³ of liqueur added in 1 cm³ of ethanol) as a “blank”. Ethanol (96%) was used as a blank for all measurements.

The same procedure was applied for all samples. The only difference is in the analytical solutions preparation. In the case of cold pressed aronia juice, a series of analytical

solutions was in the concentration range of 0.028-14 mg/cm³, while the concentrations of pasteurized sweet syrup were in the range of 0.059-15 mg/cm³.

The capacity of free radical neutralization was calculated according to the following equation:

$$\text{capacity of DPPH radical neutralization (\%)} = 100 - [(A_U - A_B) \times (100/A_K)]$$

where they are:

A_U - absorbance of sample (517 nm),

A_K - absorbance of control (517 nm). Control ethanolic solution of DPPH radical in the concentration of 3×10⁻⁴mol/dm³

A_B - absorbance of „blank“(517 nm). „Blank“– the ethanolic solution of the sample not treated by the DPPH radical solution.

The EC₅₀ values determined in this paper represent the concentration of the tested samples required to neutralize 50% of the initial concentration of DPPH radical [26, 30 - 34].

Results and discussion

The results of DPPH test of cold pressed aronia juice are shown in Figure 1. It is observed that the increase of the juice concentration affects the increase of free DPPH radical neutralization. Incubated samples showed a more expressed antioxidant activity because of the longer interaction time. The incubated samples have shown a higher antioxidant activity. For incubated samples EC₅₀ is 0.046 mg/cm³. In case of non-incubated samples EC₅₀ value was 3.75 mg/cm³. Cold pressed aronia juice is a very efficient antioxidant even in the concentration below 1 mg/cm³. It can be noticed that there is no significant change of the antioxidant activity of incubated samples above 1 mg/cm³.

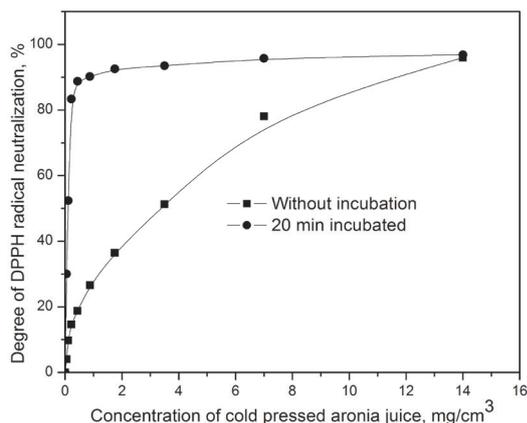


Figure 1. Antioxidant activity of cold pressed aronia juice

The results of the antioxidant activity of pasteurized sweet aronia syrup are shown in Figure 2. It is noted that the increase in the concentration of dry pasteurized aronia syrup leads to the increase of DPPH radical neutralization capacity. A higher antioxidant activity has showed the samples incubated for 20 minutes. The EC₅₀ values were 0.206 and 10.75 mg/cm³ for incubated and

non-incubated samples, respectively. There is almost no significant change of the antioxidant activity of incubated samples in the concentration above 1.75 mg/cm³. It indicates that pasteurized syrup of aronia is already a good antioxidant in this concentration. A more expressed antioxidant activity was observed for incubated samples, similar as in previous case.

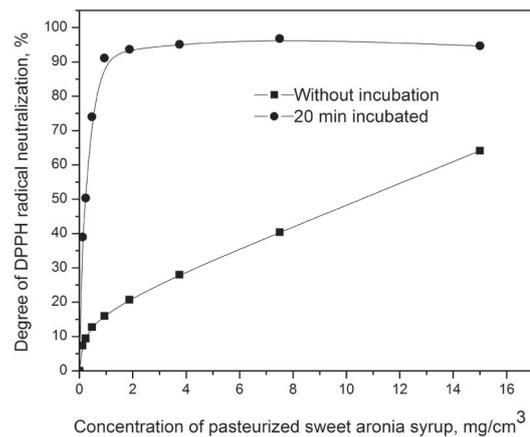


Figure 2. Antioxidant activity of pasteurized sweet aronia syrup

According to the obtained results, cold pressed aronia juice has shown to be a better antioxidant than pasteurized sweet syrup in both cases (with or without incubation).

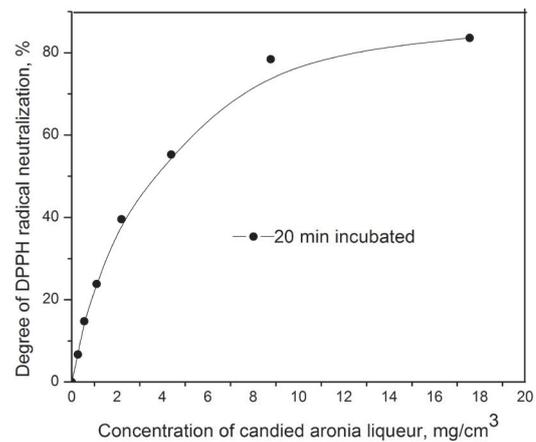


Figure 3. Antioxidant activity of aronia liqueur

The EC₅₀ value for cold pressed aronia juice was 0.046 mg/cm³ and 0.206 and 10.75 mg/cm³ for pasteurized sweet aronia syrup for incubated and non-incubated samples, respectively. A higher antioxidant activity of cold pressed aronia juice compared to pasteurized syrup could be a consequence of the production process and the presence of sucrose as a reducing sugar, since reducing agents reduce antioxidant compounds and their activity.

The results of the antioxidant activity of the liqueur from candied aronia are shown in Figure 3. It is noted that the increase in the concentration of liqueur leads to the increase of DPPH radical neutralization capacity. The antioxidant activity of incubated samples was the result of a longer exposure period to radical activity, while non-incubated samples have not shown the antioxidant activity. The EC_{50} value for aronia liqueur, for the incubated sample was 4.23 mg/cm^3 .

The EC_{50} values of cold pressed aronia juice, pasteurized sweet aronia syrup and aronia liqueur are shown in Table 1. It can be noted that cold pressed aronia juice has shown the highest antioxidant activity. Aronia liqueur prepared from candied aronia fruit has shown the lowest but still significant antioxidant activity. These results were expected since the activity of antioxidant components decreases during the production process (candy-ing and maceration) and during the long storage time. It is very important that antioxidant components of the liqueur were not completely degraded; therefore, they remain preserved in the product that can be used for different purposes. The obtained results can inspire further testing of the liqueur from candied aronia from the aspect of the efficient production process development in terms of antioxidant compounds preserving [24].

Table 1. The EC_{50} values of cold pressed aronia juice, pasteurized sweet aronia syrup and aronia liqueur

| Sample | EC_{50} values, mg/cm^3 | |
|-------------------------|------------------------------------|---------------|
| | Incubated | Non-incubated |
| Cold pressed juice | 0.046 | 3.75 |
| Pasteurized sweet syrup | 0.206 | 10.75 |
| Liqueur | 4.23 | - |

Conclusion

Based on the results obtained by DPPH test, it can be concluded that cold pressed aronia juice and pasteurized sweet aronia syrup have a more expressed antioxidant activity than liqueur from candied aronia. Cold pressed aronia juice is also a more pronounced antioxidant than pasteurized syrup, which can be explained by the absence of sucrose as an added reducing sugar and by the absence of a high temperature treatment. By studying the antioxidant activity, it has been shown that two-step processing of aronia, from fresh to candied product and then maceration to liqueur, affects the antioxidant activity of this increasingly popular fruit.

The aronia liqueur, obtained from candied aronia, is definitely the product worthy of attention. Except as a drink, it can be used as a natural supplement that can replace synthetic colors, aromas and synthetic antioxidants in food and beverage industry.

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Izvod

UTICAJ PROCESA PRERADE NA ANTIOKSIDATIVNU AKTIVNOST PROIZVODA OD ARONIJE

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Zadnjih desetak godina, poljoprivrednici u Srbiji su se masovno preorijentisali na uzgajanje aronije (*Aronia*). Nagomilavanje velikih količina aronije, nastalo usled niske otkupne cene i neadekvatno organizovanog otkupa, uslovalo je uzgajivače da se okrenu preradi. Najčešći i najzastupljeniji proizvodi od aronije na našem tržištu su hladno ceđeni sok, pasterizovani sirup i liker od aronije. Sam proces prerade može da utiče na smanjenje koncentracije bioaktivnih komponenti a samim tim i na smanjenje blagotvornog dejstva na organizam. U radu je ispitivana antioksidativna aktivnost hladno ceđenog soka od aronije (*Aronia*), pasterizovanog sirupa od aronije sa dodatim šećerom kao i likera dobijenog dvostrukim procesom prerade aronije (kandiranje i maceracija), primenom DPPH testa. Najizraženiju antioksidativnu aktivnost pokazuje hladno ceđeni sok od aronije bez dodatog šećera. Sok od sveže aronije je pokazao izraženiju antioksidativnu aktivnost u odnosu na pasterizovani sirup aronije sa dodatim šećerom. Dokazano je da se smanjila ali i očuvala antioksidativna aktivnost kod likera aronije dobijenog datim postupkom maceracije kandirane aronije u liker. Kako se do sada uglavnom koristila sveža aronija za dobijanje likera, prikazanim postupkom dobijanja likera od kandirane aronije, liker dobijen od kandirane aronije može naći sve veću primenu u prehrambenoj i industriji alkoholnih pića kao zamena sintetičkih antioksidanasa primenom biljnih antioksidanasa.

Ključne reči: aronija, liker, sirup, antioksidativna aktivnost