Influence of ultrasound and extraction conditions on the intensity of extraction of anthocyanin pigment from berry raw materials

N Y Chesnokova1, L V Levochkina1, Y V Prikhod'ko1, A A Kuznetsova1 and P A Chebukin2

1 School of Biomedicine, Far Eastern Federal University, Vladivostok, 690000, Russia
2 Far Eastern Branch N.I. Vavilov Research Institute of plant industry, Vladivostok, 690025, Russia
3 E-mail: chesn_natali@mail.ru

Abstract. The effect of ultrasound and extraction conditions on the degree of anthocyanin pigment extraction from berry raw materials has been studied. It has been shown that the use of ultrasound for extraction allows increasing the anthocyanin pigment yield. The ultrasound-assisted water extraction at 25°C for 30 minutes increases the yield of pigment in aqueous solution by 22%. The increase in the ultrasound-assisted extraction temperature to 65°C reduces the extraction time down to 15 minutes. It has been shown that the use of ultrasound reduces the extraction degree in 96 % ethanol and in the ethanol/water/hydrochloric acid system and increases the amount of anthocyanin pigment into the solution containing water as an extraction agent. In this case, the use of the ultrasound-assisted extraction reduces the anthocyanin pigment extraction time to 20 minutes with the use of the 96 % ethanol as an extraction agent, and to 15 minutes in an aqueous solution. The study of the ultrasound-assisted extraction effect, as well as the frequency and power of ultrasonic vibrations on the anthocyanin pigment extraction intensity has shown that the indirect ultrasound-assisted extraction (ultrasonic bath) with the ultrasound exposure frequency of 35 kHz and the power of 80 W allows allocating the anthocyanin pigment to the fullest extent possible within 30 minutes. With the direct ultrasound exposure (ultrasonic homogenizer) with the frequency of 20 kHz and the power of 75 W, the extraction time with an aqueous solution is reduced to 15 minutes, and with 96 % ethanol to 20 minutes.

1. Introduction
Synthetic dyes are mainly used for food staining in the food industry. Synthetic dyes have a bright stable color, are easy to use; however, they can cause allergic reactions and have another pathological effect on the human body. Therefore, at present, there is growing interest in the use of natural dyes, especially for the production of baby foods. Unlike many synthetic dyes, natural ones are nontoxic and impart a natural color to the product [1]. Moreover, many of them have high antioxidant activity [2-4].

Plant raw materials such as flower petals, fruits, berries, vegetables and others serve as a source for natural dyes. Flavonoids are primarily and most widely represented by anthocyanins, and have the widest range of colors and shades.

Anthocyanins are a group of natural pigments that color plants in orange, red, magenta, or blue. The structure of anthocyanins represents phenolic compounds that are mono- and diglycosides and contain,
as aglycone-anthocyanidin, hydroxy- and methoxy-substituted flavylium salts (2-phenylchromenylium) [5, 6].

In addition to the coloring components, the extracts of anthocyanin pigments are composed of useful biologically active substances: vitamins, glycosides, organic acids, and trace elements. These compounds have many useful pharmacological properties: they reduce cholesterol levels, prevent the formation of blood clots, increase the blood vessels’ elasticity, accelerate the healing of wounds, have a positive effect on vision, and contribute to cancer prevention [7-11].

There are many methods for extracting anthocyanin pigments [12-17] from plant materials; however, the issue of improving methods and optimizing their isolation regimes remains relevant. The ultrasound-assisted extraction occupies a special place among the modern extraction methods, which allow accelerating the mass exchange processes in plant tissues, as well as increasing the yield of biologically active substances. This study is aimed at the research of the effect of ultrasound and extraction conditions on the degree of anthocyanin pigment extraction from berry raw materials.

2. Materials and methods

2.1. Materials
Frozen black currant berries (Ribes nigrum), cranberry tree (Viburnum opulus), red bilberry (Vaccinium vitis-idaea L.) and Chinese magnolia vine (Schisandria chinensis) were used as objects to isolate anthocyanin pigment.

2.2. Extraction
The anthocyanin pigment solutions were prepared by extracting crushed berry raw materials with distilled water, 96% ethanol or the 96% ethanol/distilled water/37% hydrochloric acid system (69/30/1 by volume) at the ratio 1:20 in the temperature range 25-100ºС for 5-60 minutes.

The ultrasound-assisted extraction of the anthocyanin pigment was performed in water and ethanol in the temperature range of 25-65°C for 5-60 minutes.

2.3. Shooting UV spectra of solutions
The relative content of the pigment was determined by the magnitude of the optical density of the extracts on the SHIMADZU UV-1800 spectrophotometer (Japan) in the wavelength range of 400-800 nm.

2.4. Ultrasound-assisted extraction
Sonorex RK100H ultrasonic bath (Bandelin, Germany) was used for indirect ultrasound-assisted extraction. Direct ultrasound-assisted extraction was performed in a Sonopuls Vitasoros homogenizer (Bandelin, Germany). Samples were processed using an ultrasonic bath at the exposure frequency of 35 kHz and the power of 80 W, using an ultrasonic homogenizer - 20 kHz and 75 W for 5 – 60 min.

2.5. Determination of anthocyanin pigment content
The content of anthocyanins in solutions was determined in accordance with the method described in the paper of Ivanova et al. [18]. To determine the content of the anthocyanin pigment, the samples were diluted with an ethanol/water/37% hydrochloric acid system (69/30/1), and their optical density was measured at a wavelength of 540 nm. The content of anthocyanins in solutions was calculated by the formula:

\[ C = 16.7A_{540d}, \]

where \( d \) is the dilution factor, \( A_{540d} \) is the optical density of solutions at a wavelength of 540 nm, and \( C \) is the anthocyanin content mg/liter, expressed as malvidin-3-glycoside equivalent.
Experiments to determine the content of anthocyanin pigment in solutions were carried out in series, and each series was repeated at least three times. The average of three parallel measurements was taken as the determination result.

3. Results and Discussion

3.1. The effect of ultrasound and extraction conditions on the anthocyanin pigment extraction intensity

The effect of ultrasound and the isolation conditions on the yield of anthocyanin black currant pigment was studied in order to optimize the extraction process. The dependence of the degree of anthocyanin black currant pigment selection on the isolation method and the extraction temperature is shown in figure 1.

![Figure 1](image_url)

**Figure 1.** The dependence of the degree of anthocyanin black currant pigment extraction on the isolation method and the extraction temperature.

The presented results show that the extraction method and temperature affect the yield of the anthocyanin pigment. The use of ultrasound to extract anthocyanin pigment from berry raw materials at a room temperature increases the isolation thereof. At the temperature of 25 °C, its content in the extract increases by 22% compared with the extraction with an aqueous solution under similar conditions. This is probably due to the fact that under the action of ultrasonic waves and cavitation effect produced, the mass transfer is intensified and the access of solvent to the plant cell becomes easier. At the same time, the collapse of cavitation bubbles near the cell walls leads to the destruction of the cell wall, contributing to more intensive penetration of the solvent inside the cell [19].

Increasing the extraction temperature to 65°C contributes to an increase in the yield of the anthocyanin pigment, both in the ultrasound-assisted extraction and in the extraction using aqueous solution. At this temperature, the amount of the isolated pigment that was extracted with an aqueous solution is higher than with ultrasound-assisted extraction and equals 2.99 and 2.88 mg/ml, respectively. Increasing the temperature to 100°C slightly increases the yield of the anthocyanin pigment. Under these conditions, the yield of the pigment extracted with an aqueous solution and ultrasound is 3.19 and 2.99 mg/ml, respectively.

The dependence of the anthocyanin pigment isolation rate on the extraction time was studied in order to determine the optimum extraction conditions since the extraction duration can significantly affect the yield of anthocyanin pigment. The dependence of the isolated anthocyanin pigment on the method, duration and the extraction temperature are presented in figures 2 and 3.
Figure 2. The dependence of the anthocyanin pigment yield on the extraction method, duration and temperature.

Figure 3. The dependence of the anthocyanin pigment yield on the extraction method, duration and temperature.

It follows from figure 2 that an increase in the extraction time has a different effect on the yield of the anthocyanin pigment and depends on the isolation method. The maximum yield of the anthocyanin pigment is observed when it is extracted with an aqueous solution at the temperature of 65°C for 30 minutes, and is 2.99 mg/ml. The increase in the extraction duration also leads to an increase in the yield of the anthocyanin pigment extracted with an aqueous solution and ultrasound at the temperature of 25°C. Its content in the solution is 2.16 and 2.78 mg/ml, respectively.

The study of the influence of the anthocyanin pigment extraction duration at 5 and 30 minutes showed an increase in its yield when extracted with an aqueous solution at 25°C and 65°C by 10% and 8%,
respectively. Ultrasonic exposure at the temperature of 25°C increases the anthocyanin pigment yield by 7%.

With indirect ultrasound-assisted extraction with an aqueous solution at 65°C, the maximum isolation of the anthocyanin pigment is observed after 15 minutes of extraction, and is 2.96 mg/ml. The findings are consistent with the works of Espada-Bellido et al., Bonfigli et al. [20,21], which also show a reduction in the time of the anthocyanin pigment isolation using ultrasound-assisted extraction. Apparently, with this method, the extraction is accelerated due to the acoustic pressure of microflows, initiated by bubble explosions, and the sound pressure generated by the capillary effect, which accelerates the diffusion of the solvent into the cell walls and their further destruction, resulting in a more rapid isolation of anthocyanin pigment from the cells [22].

A further increase in the time of extraction with ultrasound at 65°C for 30 minutes slows down the anthocyanin pigment extraction process. The pigment content under these conditions is 2.89 mg/ml.

An increase in the isolation time to 60 minutes (figure 3) leads to a decrease in the yield of anthocyanin pigment, extracted with ultrasound at room temperature. At the same time, the amount of pigment with these methods and conditions of extraction is reduced by 6%. Apparently, in this case, an increase in the extraction duration leads to a partial destruction of the anthocyanin pigment.

Increasing the extraction temperature to 65°C and the extraction duration to 60 minutes leads to an increase in the anthocyanin pigment yield by 2% both when extracted with an aqueous solution and ultrasound. The heat effect and pressure created by using ultrasound contribute to the destruction of cell walls of plant raw materials and to fuller isolation of anthocyanin pigment into the environment.

The dependence of the anthocyanin pigment yield on the isolation object, method and temperature is shown in figure 4.

![Figure 4](image.png)

**Figure 4.** The dependence of the anthocyanin pigment yield on the isolation object, method and temperature.

The figure shows that all the studied berry raw materials are the sources of the anthocyanin pigment, and the pigment yield depends on the isolation method and temperature. The highest content of the pigment in all isolation methods was observed in black currant. In the berries of the magnolia vine, red bilberry and cranberry tree its content was much less. When extracting berries of currant, red bilberry and cranberry tree and magnolia vine with an aqueous solution at 25°C, the content of anthocyanin pigment isolated from these objects was 2.17, 0.60, 0.24 and 0.37 mg/ml, respectively.

Increasing the temperature to 65°C contributes to an increase in the extraction of the anthocyanin pigment with an aqueous solution in all the studied berries. This extraction method increases the yield
of pigment from berries of currant, red bilberry and cranberry tree and magnolia vine by 27%, 47%, 20% and 74%, respectively.

The ultrasound-assisted extraction contributes to the anthocyanin pigment extraction from berry raw materials such as currant and red bilberry, and its yield increases by 22% and 18%, respectively. The ultrasound-assisted extraction of berries of cranberry tree and magnolia vine leads to a slight increase in the yield of anthocyanin pigment.

Thus, it has been shown that the use of ultrasound for extraction allows increasing the anthocyanin pigment yield. The ultrasound-assisted water extraction at 25°C for 30 minutes increases the pigment yield by 22%. The increase in the ultrasonic treatment temperature to 65°C allows reducing the extraction time down to 15 minutes. The optimal conditions for the anthocyanin pigment extraction with an aqueous solution are the isolation thereof at 65°C for 30 minutes.

3.2. The effect of ultrasound and the nature of the extraction agent on the degree of extraction of the black currant anthocyanin pigment

The dependence of the degree of anthocyanin black currant pigment selection on the extraction method and the nature of the extraction agent is shown in figure 5. The quantitative content of anthocyanins in solutions is given in table 1.

Table 1. The content of anthocyanins in solutions.

| No. | Extraction agent                               | Anthocyanins content, mg/ml | Ultrasound-assisted extraction |
|-----|-----------------------------------------------|-----------------------------|--------------------------------|
| 1.  | Water                                         | 2.668 ± 0.082               | 2.864 ± 0.014                  |
| 2.  | Ethanol/water/hydrochloric acid system         | 3.193 ± 0.035               | 2.911 ± 0.065                  |
| 3.  | 96% ethanol                                    | 3.293 ± 0.032               | 2.947 ± 0.046                  |

Figure 5. The dependence of the degree of anthocyanin black currant pigment isolation on the extraction method and the nature of the extraction agent. 1. Extraction with 96% ethanol; 2. Extraction using ethanol/water/hydrochloric acid system; 3. Ultrasound-assisted extraction with 96% ethanol; 4. Ultrasound-assisted extraction using ethanol/water/hydrochloric acid system; 5. Ultrasound-assisted water extraction at 25°C; 6. Extraction with an aqueous solution at 25°C.

The studies have shown that the extraction method and the medium used for extraction have significant impact on the degree of extraction of anthocyanin pigment from berry raw materials and the content of anthocyanins therein. It can be seen from the presented results that the anthocyanin pigment is extracted to the fullest possible extent with 96% ethanol and the ethanol/water/hydrochloric acid system. The quantitative content of anthocyanins in solutions under the given extraction conditions is also the highest and amounts to 3.293 and 3.193 mg/ml, respectively. Using water as an extraction agent
reduces the extraction degree four times. In addition, it can be seen from the presented spectra that the use of the ethanol/water/hydrochloric acid system and 96 % ethanol as extraction agents contributes to the appearance of a rather pronounced additional peak, which disappears in the anthocyanin pigment aqueous solution.

In an experimental fashion, it has been revealed that the degree of ultrasound-assisted extraction of anthocyanin pigment depends on the nature of the extraction agent. The studies have shown that the ultrasound-assisted extraction with 96 % ethanol and the ethanol/water/hydrochloric acid system reduces the anthocyanin pigment extraction 1.1 and 1.3 times, respectively. The content of anthocyanins in these solutions also decreases and amounts to 2.947 and 2.911 mg/ml, respectively. Apparently, the use of 96 % ethanol and the ethanol/water/hydrochloric acid system contributes to the maximum extraction of anthocyanin pigment from cells, and the use of ultrasound in this case contributes to the pigment destruction.

On the contrary, in solutions containing water as an extraction agent, the use of ultrasound increases the anthocyanin pigment extraction degree 1.3 times compared with the relevant system without ultrasound.

Moreover, it can be seen from the presented UV spectra that the absorption maximum varies depending on the solvent present in the system. It was established that when using water as an extraction agent, the absorption maximum was observed at a wavelength of 510 nm. When using 96 % ethanol and the ethanol/water/hydrochloric acid system as the extraction agents, the absorption maximum shifts to 550 nm. There is a bathochromic shift, in the form of the spectral band shift when the polarity of the solvent changes and the color of the extracts changes from red to violet.

The dependence of the anthocyanin pigment yield on the extraction time is shown in figure 6.

![Figure 6. The dependence of the anthocyanin pigment yield of the extraction time.](image)

It can be seen from the presented data that the extraction duration differently affects the anthocyanin pigment yield and depends on the isolation method and the extraction agent nature. The fullest extraction of the anthocyanin pigment is observed upon its extraction with 96 % ethanol. The maximum yield of the anthocyanin pigment is achieved after 25 minutes of extraction, and is 3.15 mg/ml. Direct ultrasound-assisted extraction with 96 % ethanol slightly reduces the yield of anthocyanin pigment, while its maximum release is achieved in 20 minutes of extraction, and is 2.97 mg/ml.
The use of water as an extraction agent as well as the ultrasound-assisted extraction with an aqueous solution significantly reduce the yield of anthocyanin pigment. The pigment yield with these extraction methods is reduced by 30% and 25%, respectively. At the same time, as noted earlier (figure 2), the maximum anthocyanin pigment yield is achieved by ultrasound-assisted extraction with an aqueous solution in 15 minutes, and is 2.37 mg/ml.

Thus, when extracting the anthocyanin pigment, its extraction with 96% ethanol and the ethanol/water/hydrochloric acid system is the most efficient one. The use of ultrasound leads to a decrease in its extraction in 96% ethanol and the ethanol/water/hydrochloric acid system. The ultrasound-assisted extraction in an aqueous solution helps increasing the yield of pigment. The use of ultrasound to isolate the anthocyanin pigment reduces the anthocyanin pigment extraction time to 20 minutes with the use of 96% ethanol as an extraction agent, and to 15 minutes in an aqueous solution.

3.3. The effect of the frequency and power of ultrasonic vibrations, as well as the method of ultrasound-assisted extraction on the yield of anthocyanin black currant pigment

The dependences of the anthocyanin pigment yield on the ultrasound-assisted extraction method, as well as the frequency and power of ultrasonic vibrations are presented in figures 7 and 8.

Figure 7. The dependence of the anthocyanin pigment yield in water extraction from the frequency and power of the impact of ultrasonic vibrations.
Figure 8. The dependence of the anthocyanin pigment yield in alcohol extraction on the frequency and power of the impact of ultrasonic vibrations.

The presented results show that the ultrasound-assisted extraction method, as well as the frequency and power of the device have an impact on the anthocyanin pigment yield both at ultrasonic extraction with an aqueous solution (figure 7), and with 96% ethanol (figure 8).

The maximum yield of the anthocyanin pigment is observed when using an ultrasound bath with the exposure frequency of 35 kHz and the power of 80 W for ultrasound-assisted extraction with an aqueous solution and 98% ethanol for 30 minutes, and is 2.79 and 3.01 mg/ml, respectively. The use of an ultrasound homogenizer with a frequency and power less than that of an ultrasound bath reduces the yield of the anthocyanin pigment. However, its use reduces the duration of extraction of the anthocyanin pigment with an aqueous solution and 96% ethanol. The anthocyanin pigment is extracted to the fullest extent possible with the use of an ultrasonic homogenizer and an aqueous solution as an extraction agent in 15 minutes. The content of the anthocyanin pigment extracted with an aqueous solution at the frequency of 20 kHz and the power of 75 W for 15 minutes is 2.37 mg/ml. The use of 96% ethanol as an extraction agent with this extraction method reduces the extraction time to 20. The content of the anthocyanin pigment extracted with 96% ethanol at the frequency of 20 kHz and the power of 75 W is 2.98 mg/ml.

Thus, the method of ultrasound exposure, as well as the frequency and power of ultrasonic vibrations play a significant role in the intensity of extraction of anthocyanin pigment from berry raw materials. The ultrasound exposure frequency of 35 kHz and the power of 80 W allow the anthocyanin pigment to be isolated to the fullest possible extent within 30 minutes. Apparently, a more powerful effect of an ultrasonic wave through liquid leads to acoustic cavitation, which contributes to a more complete destruction of the cell walls and, accordingly, the release of the anthocyanin pigment into the environment. Reducing the exposure frequency to 20 kHz and the power to 75 W allows reducing the extraction time with an aqueous solution to 15 minutes, and to 20 minutes when using 96% ethanol.

4. Conclusion
Thus, it has been shown that the use of ultrasound for extraction allows increasing the anthocyanin pigment yield. The ultrasound-assisted extraction at the temperature of 25°C for 30 minutes increases
the anthocyanin pigment yield by 22%. The increase in the ultrasound-assisted extraction temperature to 65°C reduces the extraction time down to 15 minutes. The use of ultrasound reduces the anthocyanin pigment extraction degree in 96% ethanol and in the ethanol/water/hydrochloric acid system and increases the amount of its yield into the solution containing water as an extraction agent. In this case, the use of ultrasound-assisted extraction reduces the anthocyanin pigment extraction time to 20 minutes with the use of the 96% ethanol as an extraction agent, and to 15 minutes in an aqueous solution.

The use of indirect ultrasound-assisted extraction (ultrasound bath) with the ultrasound exposure frequency of 35 kHz and the power of 80 W allows the anthocyanin pigment to be isolated to the fullest extent within 30 minutes. The use of direct ultrasound-assisted extraction (ultrasonic homogenizer) with the exposure frequency of 20 kHz and the power of 75 W allows reducing the extraction time to 15 minutes with an aqueous solution, and to 20 minutes - with 96% ethanol.

References
[1] Cheynier V (2012) Phenolic compounds: From plants to foods Phytochemistry Reviews 11(2-3) 153-177
[2] Nems A, Peksa A, Kucharska A, Sokol-Letowska A, Kita A, Drozdz W and Hamouz K 2015 Anthocyanin and antioxidant activity of snacks with colored potato Food Chemistry 172 175-82
[3] Flanigan P M and Niemeyer E D (2014) Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (Ocimum basilicum L.) Food Chemistry 164 518-526
[4] Chiou A, Panagopoulou E, Gatzali F, De Marchi S and Karathanos V (2014) Antocyanins content and antioxidant capacity of Corinthian currants (Vitis Vinifera L. var. Apyrena) Food Chemistry 146 157-65
[5] Britton G 1986 Biochemistry of natural pigments (Cambridge: Cambridge University Press)
[6] Tanichev S S 1980 Antotsiany v plodakh i ovoshchakh [Anthocyanins in fruits and vegetables] (Moscow) 302
[7] Burton L J, Smith B A, Smith B N, Loyd Q, Nagappan P, McKeithen D and Odero-Marrah V A 2015 Muscadine grape skin extract can antagonize Snail-cathepsin L-mediated invasion, migration and osteoclastogenesis in prostate and breast cancer cells Carcinogenesis 36(9) 1019-27
[8] Jung H, Lee H, Cho H, Lee K, Kwak H K and Hwang K T (2015) Anthocyanins in Rubus fruits and antioxidant and anti-inflammatory activities in RAW 2647 cells Food Science and Biotechnology 24(5) 1879-86
[9] Mineo S, Noguchi A, Nagakura Y, Kobori K, Ohta T, Sakaguchi E and Ichiyanagi T (2015) Boysenberry polyphenols suppressed elevation of plasma triglyceride levels in rats J.of Nutritional Science and Vitaminology 61(4) 306-12
[10] Sorrenti V, Vanella L, Acquaviva R, Cardile V, Giofre S and Di Giacomo (2015) Cyanidin induces apoptosis and differentiation in prostate cancer cells International J. of Oncology 47(4) 1210-1303
[11] Mazewski C, Liang K and Gozalez de Mejia E 2017 Inhibitory potential of anthocyanin-rich purple and red corn extracts on human colorectal cancer cell proliferation in vitro J. of Functional Foods 34 254-65
[12] Chesnokova N Y, Levochkina L V, Prikhód’ko Y V, Kuznetsova A A and Vladykina T V (2018) Influence of polysaccharide functional groups on the extraction degree of blackcurrant anthocyanin pigment Pharm. Sci. & Res. 10(3) 659-61
[13] Chesnokova N Y, Levochkina L V, Kuznetsova A A and Zakharyan R A 2015 The use of anthocyanin of black currant and polysaccharides in the production of sweet dishes Biomedical and Pharmacology Journal 8(2) 697-703
[14] Jampani C and Rahavarao K (2015) Process integration for purification and concentration of red
cabbage (Brassica oleracea L.) anthocyanins. *Separation and Purification Technology* 141 10-6

[15] Liu S, Fu Y and Nian S 2014 Buffering color fluctuation of purple sweet potato anthocyanins to acidity variation by surfactants. *Food Chemistry* 162 16-21

[16] Valdes A, Vidal L, Beltran A, Canals S and Garrigos M C (2015) Microwave-assisted extraction of phenolic compounds from almond skin byproducts (prunus amygdalus) a multivariate analysis approach. *J. Agric Food Chem.* 63 5395-402

[17] Meneses M A, Caputo G, Scognamiglio M, Reverchon E and Adami R (2015) Antioxidant phenolic compounds recovery from Mangifera indica L., by-products by supercritical antisolvent extraction. *J. Food Eng.* 163 45-53

[18] Ivanova V, Dornyei A, Mark L, Vojnoski B, Stafilov T, Stefova V and Kilar F 2011 Polyphenolic content of Vranec wines produced by different vinification conditions. *Food Chemistry* 124 316-25

[19] Dumittrash P G, Bologa M K and Shemyakova T D (2016) Ultrazvukovaya ektraktsiya biologicheski aktivnykh soyedineniy iz semyan tomatov [Ultrasound-assisted extraction of biologically active compounds from tomato seeds]. *Electronic material processing* 52(3) 47-52

[20] Espada-Bellido E, Ferreiro-Gonzalez M, Carrera C, Palma M, Barroso C and Barberro G F 2017 Optimization of the ultrasound-assisted extraction of anthocyanins and total phenolic compounds in mulberry (*Morus nigra*) pulp. *Food chemistry* 219 23-32

[21] Bonfigli M, Godoy E, Reinheimer M A and Scenna N J 2017 Comparison between conventional and ultrasound-assisted techniques for extraction of anthocyanins from grape pomace. Experimental results and mathematical modeling. *Journal of food engineering* 207 56-72

[22] D’Alessandro L G, Dimitrov K, Vaucll P and Nikov L 2014 Kinetics of ultrasound assisted extraction of anthocyanins from Aronia melanocarpa (black choke-berry) wastes. *Chem. Eng. Res. Des.* 92 1818-26