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INTENSIFICATION OF ANTHOCYANIN EXTRACTION BY PULSE ELECTRIC DISCHARGE

Physical methods for intensifying the extraction process are used to accelerate mass transfer in the "solid-liquid" system in order to more complete and faster extraction of dyes. The use of electric pulsed discharges, under the influence of which there is an intensive mixing of the mixture of plant materials and extractant, thinning or complete disappearance of the diffusion wall layer of the cell, and an increase in the convective diffusion coefficient, is promising.

The article is devoted to the study of the process of extraction of dyes from frozen pomace of red grapes of the Vitis Vierul variety. The improvement of extraction was carried out due to the pretreatment of plant materials with a pulsed electric discharge. The degree of anthocyanins extraction was calculated according to the pH-differential method with measurement of the optical density of the obtained extracts on a Spekol 11 device. As a comparative experiment, we used the extraction of coloring matter from grape pomace with an aqueous solution of 1% by weight hydrochloric acid at pH 2.0 and a temperature of 60°C in several stages until the exhaustion of raw materials to determine the maximum possible amount of extracted anthocyanins.

The optimal conditions for the process were chosen: extraction module, duration and number of stages. It was established that the optimal processing time for a raw material:extractant ratio of 1:100 is 30 s, for 3:100 and 5:100 – 60 s. When using a one-stage extraction (hydromodule 20 duration 30 s) with an aqueous solution with the addition of citric acid, the yield of anthocyanins is 51%, in three stages – 77%. The destruction of anthocyanins during storage of extracts at temperatures of 3 and 20ºC for three and six days and the contamination of hoods with products of erosion of metal electrodes were investigated. It was established that when extracts are stored in a dark room at 3ºC, anthocyanins are destroyed by 18% in 6 days, and by 57% at 20ºC.

Key words: *extraction, anthocyanins, grape pomace, electrical impulse discharge.*

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ІНТЕНСИФІКАЦІЯ ЕКСТРАГУВАННЯ АНТОЦІАНІВ ІМПУЛЬСНИМ ЕЛЕКТРИЧНИМ РОЗРЯДОМ

Для прискорення масообміну в системі «тверде тіло – рідина» з метою більш повного і швидкого вилучення барвних речовин, використовують фізичні способи інтенсифікації процесу екстракції. Перспективним є застосування електроімпульсних розрядів, під впливом яких відбувається інтенсивне перемішування суміші рослинної сировини і екстрагенту, витончується або повністю зникає дифузійний пристінний шар клітини і зростає коефіцієнт конвективної дифузії.

Стаття присвячена дослідженню процесу екстрагування фарбувальних речовин із заморожених вичавок червоного винограду сорту Vitis Vierul. Інтенсифікацію екстракції проводили за рахунок попередньої обробки рослинної сировини імпульсним електричним розрядом. Ступінь вилучення антоціанів розраховували за pH-диференційною методикою з вимірюванням оптичної густини одержаних екстрактів на приладі Spekol 11. У якості досліду-порівняння використовували екстракцію барвних речовин з вичавок винограду водним розчином з 1% соляної кислоти від маси при pH 2,0 і температурі 60°С в декілька стадій до виснаження сировини для визначення максимально можливої кількості екстрагованих антоціанів.

Обрано оптимальні умови для проведення процесу: модуль екстракції, тривалість і кількість стадій. Встановлено, що для співвідношення сировина:екстрагент 1:100 оптимальним часом обробки є 30 с, для 3:100, та 5:100 – 60 с. При використанні одностадійної екстракції (гідромодуль 20 тривалість 30 с) водним розчином з добавкою лимонної кислоти вихід антоціанів складає 51%, за три стадії – 77%. Досліджено руйнування антоціанів при зберіганні екстрактів при температурах 3 і 20°С протягом трьох і шести діб та забруднення витяжок продуктами ерозії металевих електродів. Встановлено, що при зберіганні екстрактів у темному приміщенні при 3°С відбувається руйнування антоціанів за 6 діб на 18%, а при 20°С – 57%.

Ключові слова: *екстракція, антоціани, вичавки винограду, електричний імпульсний розряд.*

Formulation of the problem

Recently, interest in anthocyanins, as dyes of natural origin with complex action, has been increasing due to the development of the market for organic products. Anthocyanins are natural pigments found in cell vacuoles and tissues of flower and fruit petals that give them their bright red, pink, purple and blue colors. According to the chemical structure, anthocyanins belong to flavonoids, which are mono- or diglycosides, in which anthocyanidins are hydroxy- and methoxy-substituted 2-phenylchromenes in the form of benzpyrylium salts [1]. Anthocyanin dyes are obtained synthetically and by extraction from various plant materials, including winemaking waste.

Grape pomace, consisting of skins, stems and seeds left after pressing grapes or re-fermented pulp in the production of wine, is a valuable source of raw materials for the extraction of such valuable components as polyphenols, lignin, aromatic, coloring, mineral substances, amino acids, vitamins, etc. The colorants are concentrated in the skin cells of the grape and can be extracted by extraction with a suitable solvent (ethanol, hot water or a mixture of water and alcohol) acidified with hydrochloric or organic acids and then concentrated in vacuum apparatuses at a temperature of 40–60°C [2, 3]. There are studies using other permitted organic solvents, such as glycerol, ethylene glycol and other alcohols [4], which allows to increase the yield and use the extracts as an ingredient in the production of cosmetic creams. A promising extraction method is the use of liquefied carbon dioxide, which increases the stability during storage and microbial contamination [5]. If it is necessary to isolate individual compounds, the extract is separated into components by the chromatographic method.

The wide introduction of such extraction technologies in the chemical, food, pharmaceutical and other industries is hampered by the slow speed of the processes, incomplete extraction of target components, the use of high temperatures and the destruction of extractable substances or the use of toxic solvents. To improve the efficiency of existing methods for extracting natural dyes and biologically active substances from plant materials, physical methods of intensification are used, based on the transfer of vibrations, pulsations, oscillations of various amplitudes, frequencies and intensities into the system [6]. Such extraction intensification methods are aimed at accelerating mass transfer in the “solid-liquid” system. Common pulse methods for processing plant materials are mechanical, hydraulic, electric pulse and magnetic pulse methods.

Therefore, the intensification of the process of extracting biologically active compounds, in particular dyes, from plant materials is an urgent problem that needs to be addressed.

Analysis of recent research and publications

As the production of extraction devices develops, more efficient methods for processing plant materials are being improved and developed. Thus, the use of the vortex extraction method based on intensive mixing with sharp blades of high-speed propeller mixers makes it possible to reduce the extraction time by increasing the phase contact surface, flow turbulence, and liquid pulsation.

During extraction with ultrasound, alternating pressure, cavitation and “sonic wind” are created, causing the destruction of cellular structures. This method accelerates the penetration of the solvent into the cells of the plant material and allows the extraction of thermolabile biologically active substances [6]. The composition of the extract, its profile and the concentration of biologically active substances are affected by the conditions of ultrasonic extraction: solvent composition, ultrasound power, time and temperature [7]. When using magnetic or electric pulse devices under the influence of a field of electromagnetic waves of high frequency, protein molecules coagulate, the size of the molecules of extractable substances decreases due to a decrease in hydration and, consequently, the diffusion of target components through the pores of cell membranes increases. An increase in the coefficient of internal diffusion is also achieved through the use of electroplasmolysis, which destroys protein-lipoid membranes and increases the wettability of the material as a result of a change in the electric potential of the surface.

The acceleration of the extraction process can be achieved under the influence of a pulsed current of high or ultrahigh frequency. Under the action of a high-voltage discharge leaving the battery in short periods of time, intensive mixing occurs, the near-wall diffusion layer thins, the cell membranes are partially destroyed, and the convective diffusion coefficient increases. At the moment of discharge, pulsating plasma cavities appear in the extractant, which, as a result of rupture, can accelerate the movement of the extractant into the middle of the cell [8].

An analysis of the literature data shows that physical methods for intensifying the extraction process using electrical pulsed discharges are promising and can be used to extract natural anthocyanin dyes.

Formulation of the research purpose

The aim of the work is to study the use of electric discharge nonlinear volumetric cavitation to intensify the process of extraction of natural anthocyanin dyes from red grape skin.

Presentation of the main research material

The traditional method of extracting anthocyanins with aqueous solutions acidified with mineral or organic acids with stirring can be improved by introducing a preliminary stage of processing plant materials or completely replacing long-term aqueous extraction with electric discharge processing.

Frozen pomace of red grape variety Vitis Vierul was used as a raw material for the isolation of anthocyanins. When they are frozen, enzymes are inactivated and biochemical and oxidative processes stop, which contributes to the stabilization of anthocyanin pigments.

Electric discharge processing (EDT) of raw materials in aqueous solutions was carried out on the Vega-6 laboratory facility, which was developed by scientists from the Institute of Pulse Processes and Technologies of the National Academy of Sciences of Ukraine (Nikolaev) and tested by scientists from the Kherson National Technical University [9, 10]. EDT is based on the phenomenon of a sharp increase in the hydraulic and hydrodynamic effects and the amplitude of the shock effect during the implementation of a pulsed electric discharge in a liquid under the condition of a maximum reduction in the pulse duration, the pulse front is as steep as possible, and the pulse shape is close to aperiodic. In the process of such electric discharge processing, the raw materials are simultaneously affected by physical and chemical factors: the direct effect of electrohydraulic action, as well as the products of water splitting formed in the working medium under the action of cavitation.

In order to determine the effectiveness of the use of electric discharge treatment in the extraction of anthocyanins from pomace of frozen grapes, the extraction was carried out by the action of an electro-hydraulic shock lasting 30 s in an aqueous solution at a temperature of 20°C in a neutral medium at pH 7.1, as well as with the addition of citric acid at pH 2.8 when studying the solvent:raw material ratio. The extraction was carried out in two stages of 30 seconds each with the introduction of fresh solvent in the second stage. As a comparative experiment, we used the extraction of colorants from grape pomace with an aqueous solution of 1% by weight hydrochloric acid at pH 2.0 and a temperature of 60°C in several stages until the exhaustion of raw materials to determine the maximum possible amount of extracted anthocyanins. The amount of anthocyanins (AC) was calculated after squeezing the extracts and filtering the extracts by the pH differential method with the measurement of optical density on a "Specol 11" device. The method consists in converting the amount of anthocyanins into the more common cyanidin-3-O-glucoside, the amount of which is calculated from the difference in the optical density of solutions at wavelengths of 510 and 700 nm and the corresponding values of the acidity of the extracts by 1.0 and 4.4 units pH (buffer solutions of potassium chloride and sodium acetate) [11].

It is known from the literature that the absorption curves of anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside) have characteristic maxima in the UV region at a wavelength of 280 nm and in the visible spectrum at 535 nm. In this work, the electronic absorption spectra of extracts obtained by electrodischarge treatment at pH 2.8 and 7.1 were plotted, and it was found that the absorption curve in a pH 7.1 medium has a shift of maxima towards long wavelengths at 355 and 590 nm, respectively. Therefore, the color shade of the extracts becomes more blue, which indicates the presence of anthocyanins in the quinoid form. Upon acidification of such a solution, the color is restored to dark red, which indicates the transition of the quinoid form of anthocyanins to the form of the flavylium ion. The amount of anthocyanins obtained at neutral pH of the medium was 5.21 mg/g of raw material, and when the extractant was acidified with citric acid to pH 2.8 – 6.61 mg/g, which is 40 and 51% respectively, in relation to the comparative experiment. Therefore, further studies of the effect of the extraction module on the amount of extractable anthocyanins were carried out at a pH of 2.8.

The results of extraction of anthocyanins using EDT, indicating the effect of the hydromodulus on the mass transfer process, are presented in Figure 1 and Table 1.

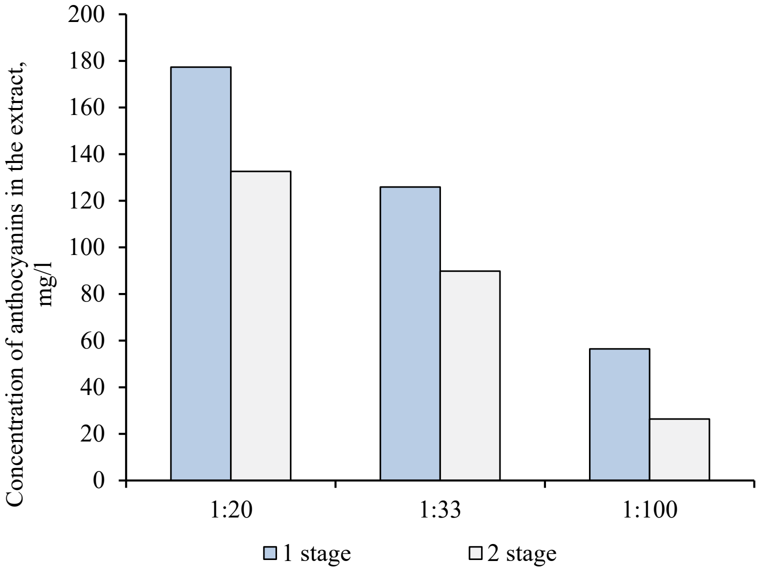

Fig. 1. Dependence of the concentration of anthocyanins in the extract on the extraction module

Table 1

The influence of the extraction module on the degree of extraction of anthocyanins

Parameter	Hydromodule 100			Hydromodule 33			Hydromodule 20		
	1 stage	2 stage	total	1 stage	2 stage	total	1 stage	2 stage	total
Content AC, mg/g of raw material	5.64	2.64	8.28	4.20	3.00	7.20	3.94	2.95	6.89
Degree of AC extraction*, %	33.94	15.97	49.91	25.41	18.19	43.60	23.84	17.85	41.69

** The ratio of the amount of anthocyanins to the maximum value extracted in the comparative experiment*

It has been established that with a decrease in the hydromodulus by 3 and 5 times, the concentration of extractable anthocyanins in the extract increases by 2.6 and 3.7 times, respectively. But at the same time, the degree of extraction, and, accordingly, the degree of use of raw materials is reduced by 13–17%. In total, it has been calculated that a greater amount of dye is extracted from one gram of grape pomace at a hydromodulus of 100, and the concentration of anthocyanins in the extract is greater at a hydromodulus of 20, which is explained by an increase in the thickness of the raw material layer through which the water hammer formed by EDT passes. The efficiency of extraction at the first stage is due to a large number of readily available anthocyanins in the cells of the raw material, which are destroyed during freezing, and at the second stage – by the concentration gradient of anthocyanins in the raw material and solution, which is formed at the first stage. For further research, we choose hydromodule 20, since obtaining a more concentrated extract reduces the cost of the next step in obtaining the dye – evaporation of the solution.

To determine the influence of the driving force of the extraction process – the difference in the concentration of anthocyanins in the extract and raw materials – multi-stage processing was carried out using EDT for 30 seconds at each stage with a module of 20. The results are shown in Table 2.

Table 2

Results of multistage extraction with application EDT

Parameter	Number of stages					total
	1	2	3	4	5	
AC concentration in the extract, mg/l	166	123.5	88	66	45	97.7
Amount of AC, mg/g of raw material	3.32	2.47	1.76	1.32	0.90	9.76
Degree of extraction of AC by stages, %	34.0	25.3	18.0	13.5	9.2	100
Degree of AC extraction before the control experiment*, %	31.1	23.1	16.5	12.4	8.4	91.5

** control experiment – extraction of AC until exhaustion showed an AC value of 10.67 mg/g*

It has been established that during multi-stage EDT extraction (five stages) in an aqueous solution acidified with citric acid (pH 2.8), with each subsequent stage, the amount of anthocyanins extracted from the raw material decreases from 34% for the first stage to 9% for the fifth stage. The maximum amount of extracted anthocyanins compared to extraction to exhaustion was about 90%.

The duration of extraction is one of the important parameters of the process. It is known from the basic mass transfer equation that the amount of a substance that has diffused through a certain layer is directly proportional to the extraction time. However, it is necessary to strive for maximum completeness of extraction in the shortest possible time. Therefore, from an economic point of view, it is better to carry out extraction in three stages, while the degree of extraction of anthocyanins is 77%. With prolonged extraction, undesirable processes of obtaining free radicals and reactive oxygen species, decomposition of anthocyanins under the action of enzymes, as well as contamination of extracts with related compounds can occur.

To determine the optimal time for EDT, raw materials – frozen grape skins with extractant – were treated with water in different proportions for 30, 60, 90, 120 seconds at a temperature of 20°C (Figure 2).

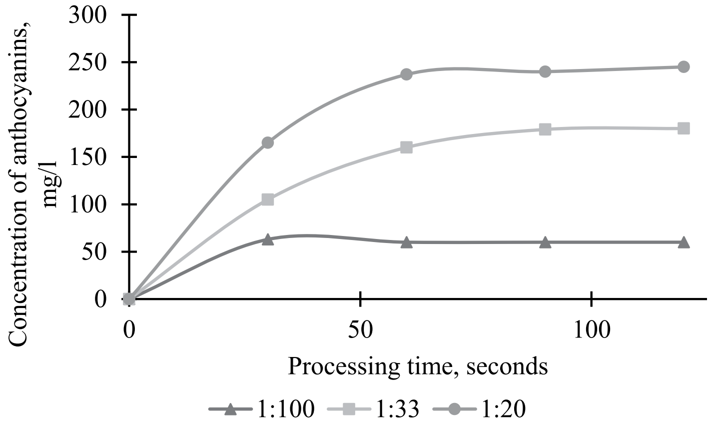

Fig. 2. The influence of the duration of electric discharge treatment on the extraction of anthocyanins at different hydromodule

The results presented in fig. 2, indicate that for the ratio of raw materials: extractant 1:100, the optimal processing time is 30 s, for 1:33 and 1:20 – 60 s. When this time is reached, the extraction process proceeds at a minimum speed or is already in a state of equilibrium with the process of destruction of anthocyanins. It should be noted that the maximum efficiency of alternating current extraction falls on the hydraulic module 20.

It is known that, under the action of EDT, water molecules are rearranged and activated, free radicals and their recombination products are formed, and the pH level and electrical conductivity of water increase [10]. All this can lead to negative consequences – oxidation of the components of the extract, degradation of anthocyanins. To study this process, the extract obtained by electrodischarge treatment on a hydromodule 20 with the introduction of citric acid was stored for six days at a temperature of 3 and 20°C. The results presented in fig. 3 show that the loss of anthocyanins during storage of extracts at a temperature of 20°C for 3 days was 14%, and for 6 days – 57%. The loss of more than half of the AC can be explained by the high activity of the radicals formed at this temperature. When extracts were stored at a temperature of 3°C for 3 days, the loss of anthocyanins was 7%, and within 6 days – 18%.

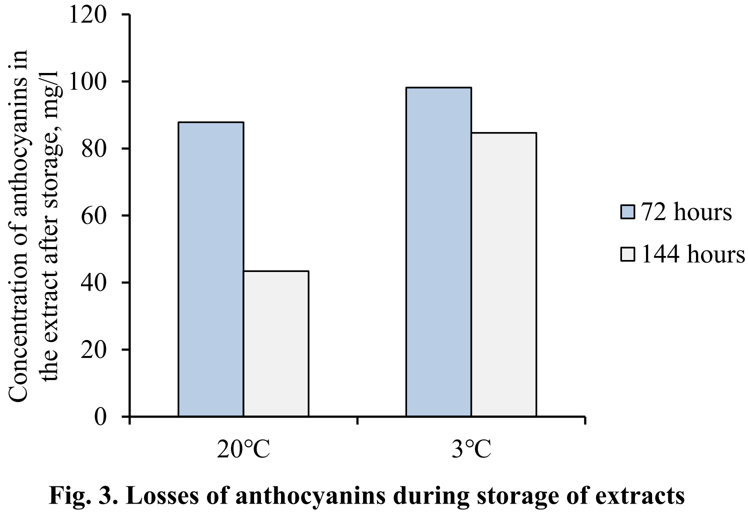

Fig. 3. Losses of anthocyanins during storage of extracts

The work also established contamination of extracts with erosion products of metal electrodes during EDT (Fig. 4). The results of determining the amount of total iron indicate an increase in its concentration by an average of 10 times in 60 seconds of treatment.

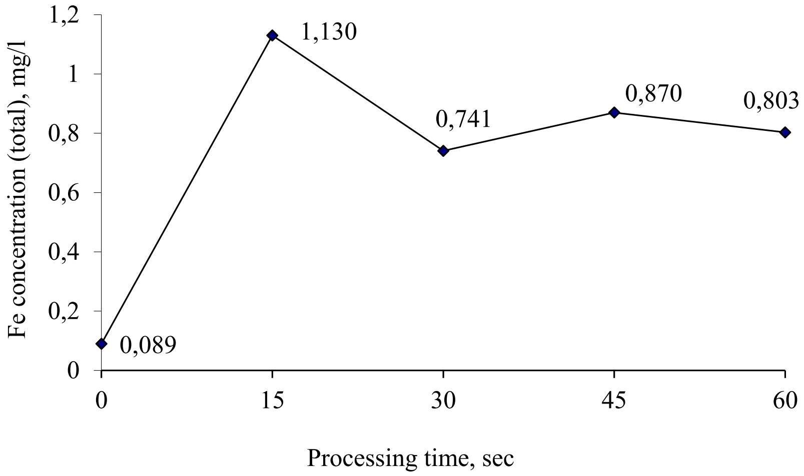

Fig. 4. Erosion of electrodes during electric discharge treatment

Thus, the method of processing grape pomace in aqueous solutions using a high-voltage pulsed high-frequency current has advantages over traditional methods of extraction with organic solvents. However, despite the short duration of the process and the high percentage of extraction of anthocyanins, during the extraction process, the extract is contaminated with erosion products of metal electrodes, which requires special measures to remove them.

Conclusions

In the course of the studies, the conditions for using EDT to intensify the process of anthocyanin extraction from grape pomace were established. When carrying out one-stage treatment with module 20 for 60 seconds, the yield of dyes is 51%, with three-stage treatment for 30 seconds, the degree of extraction of AC is 77%. The high degradation of AC is associated with the formation of free radicals during EDT and amounts to 18% when the extract is stored for 6 days at a temperature of 3°C. In addition, during processing, extracts become contaminated with electrode erosion products, which requires their purification. Further research will be aimed at improving the electrode material to intensify the anthocyanin extraction process.

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