Influence of chemical structure of alcohols on extraction and stability of anthocyanins

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Abstract

Introduction. The aim of this research was to evaluate the efficiency of the anthocyanin extraction from grape skins with different alcohols under different conditions such as temperature and pH.

Materials and methods. Grape pomace of red grapes of the Vitis Vierul variety, which are obtained after the production of wine by white way and are considered as winemaking waste, were studied. The mass concentration of the anthocyanins was measured by pH differential spectrophotometry and expressed in mg of equivalent cyanidin-3-glycoside per gram of dry matter. The rate constant and half-life of the anthocyanin degradation were calculated for a temperature of 60 ºC.

Results and discussion. The effect of pH and the chemical structure of the molecule of alcohol extractant on the efficiency of anthocyanin extraction and its spectral characteristics was studied. The number of hydroxyl groups in the alcohol used as extractant as well as the length of the hydrocarbon moiety had a key role in the process efficacy. In particular, for monohydric alcohols, the efficacy follows the order: 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol. On the other side, increasing the number of OH groups in the line ethanol > ethane-1,2-diol > propane-1,2,3-triol does not enhance the extraction performance. The spectral characteristics of extractions obtained with ethanol and polyhydric alcohols are similar, the absorption maximum is unclear and is in the range of 530–560 nm. When the length of the carbon chain of alcohol increases, the electronic absorption spectra are characterized by different intensity and a wide, indistinct absorption maximum. The thermal stability of anthocyanins in the extracts was determined by the rate constant of the anthocyanin degradation reaction and the half-life. Decreasing the pH of the extract leads to an increase in the thermal stability of anthocyanins. Ethanol is the best extractant in terms of the technological and economic efficiency of natural pigment extraction and for its application in the food industry. Anthocyanin half-life in ethanol is about 10 hours at 60 °C that indicates its suitability to be used in industrial processing.

Conclusions. The results obtained make it possible to evaluate the efficiency of the extraction of anthocyanins with alcohols from grape skins and their thermal stability.

Introduction

Food production and processing generate high amounts of waste and by-products containing valuable compounds and biological active substances, and can be turned into useful products (Sirohi et al., 2020). As example, there is a growing scientific interest towards raw materials with organic dye compounds, which have not only the ability to provide a stable color, but also antioxidant properties (Qin et al., 2021). Natural flavonoid pigments, particular anthocyanins, are related to such substances, although anthocyanins are easily degraded being effected of the environment factors (Enaru et al., 2021; Delgado-Vargas et al., 2000). To preserve their properties, modern methods of extraction with supercritical carbon dioxide and subcritical water could be used (Essien et al., 2020). However, as these methods require special equipment, improvements in the extraction of anthocyanins from plant raw materials, as well as their application in various industries (food, chemical, pharmaceutical, cosmetic) are still open issues.

From a chemical point of view, anthocyanin dyes are mono- or diglycosides, the aglycone in which are anthocyanidins – phenolic derivatives of 2-phenylchromene in the form of benzpyrylium salts, such as pelargonidine, cyanidin, delphinidin. The main aglycones also include their methyl ethers: syringidine (malvidin), peonidine, hirsutidine. The properties of dyes are exhibited by both aglycones and the corresponding glycosides (Loarce et al., 2021). A mixture of different anthocyanins gives fruits, berries and flower petals of various colors from red to blue (Konieczynski et al., 2021). The chemical structure of anthocyanins causes easy solubility in water and polar solvents, especially in acidic media.

It is known that anthocyanins are present in solutions in several tautomeric forms, depending on a number of factors: the acidity of the medium, the polarity of the solvent and its ability to form hydrogen bonds. In general, depending on the pH value, anthocyanin dyes exist in the form of a flavylium cation, a carbinol base, a chalcone or a quinoid form. In an acidic environment, anthocyanins are mainly in the form of a bright red cation of flavylium. As the pH shifts to an alkaline medium, the color turns purple as the quinoid form appears. The formation of colorless structures in the form of a carbinol base and a chalcone is also possible (Etxabide et al., 2021; Manzoor et al., 2021). In addition, they are sensitive to the conditions of technological processing and storage, namely, they may lose or change color under the action of high temperatures, enzymes, the presence of oxygen, and heavy metal ions (Loarce et al., 2021).

Promising raw materials for the production of anthocyanin extracts are winemaking waste from dark grapes. The amount of dyes in the extract depends on the grape variety and the method of wine production. Red grape pomace, which remain as waste in the production of wine by the white method, has a higher content of dyes. When the grapes ripen, the number of anthocyanins is constantly increasing. The content of anthocyanins in the skin can be from 3 to 6% of the dry weight of the skin during the ripening of the grapes, and from 0 to 500 mg/dm^3 in the pulp. The composition of anthocyanins depends on the grape variety and place of growth (Perestrelo et al., 2020).

The most widespread as extractants of anthocyanins from plant raw materials are water, solutions of ethyl alcohol, and alcohol-glycerol mixture (Kurambhatti et al., 2020; Popović et al., 2020). Typically the extraction is carried out with aqueous or aqueous-alcoholic solutions, acidified with mineral or organic acids, using operations of infusion, pressing, filtration and concentration in vacuum at a temperature 60°C. The disadvantages of these methods include the difficulty of evaporating the extract containing pectin, tannins and mineral or organic acids. In fact, during evaporation, the degradation of dyes occurs due to hydrolysis and polycondensation of anthocyanins with the formation of insoluble products.

In addition, the use of aqueous extracts of anthocyanins is not suitable in the manufacture of some perfumes and cosmetics based on hydrophobic compositions. The use of alcohols of different chemical structure will increase the thermal stability of the extracts due to obtaining anthocyanins without carbohydrates and resistance to microorganisms.

The aim of this work was to determine the effect of the number of hydroxyl groups and the carbon chain of the aliphatic alcohols of the extractants on the efficiency of anthocyanin extraction from red grape pomace, optical characteristics and stability of anthocyanins in the obtained extracts. The proposed protocols can open new routes for a by-products disposal by the recovery and recycling of valuable substances.

Material and methods

Materials

Vitis Vierul grapes were obtained from local farms in Ukraine. Grape pomace obtained after two days of fermentation in the production of wine according to the "white method", squeezing and freezing was used as raw material.

Extraction of anthocyanins

Extraction of anthocyanins was performed in conical flasks with reflux in a water bath with a temperature of 60±2°C under stirring. The extraction process was performed at module 10 for 20 minutes with the following extractants: methanol, ethanol, propan-2-ol, butan-1-ol, 2-methylpropan-1-ol, ethane-1,2-diol (ethylene glycol), propane-1,2,3-triol (glycerol). The extracts were cooled, filtered through a blue ribbon filter and adjusted to the initial level with fresh extractant solution (Pérez et al., 2021).

The efficiency of the extraction process was determined by the amount of extracted anthocyanins at the pH of the obtained extracts (5.2–6.7 depending on the alcohol-extractant) and with acidification of the extracts with hydrochloric acid to pH 2.9, because at this pH anthocyanins are almost 100% flavylium cation form.

Determination of the concentration of anthocyanins

pH-differential spectrophotometry was used to determine the concentration of anthocyanins. For this purpose, 2.5 cm^3 aliquots of the filtrate were placed into two volumetric flasks with a capacity of 50 cm^3 and filled up with a buffer solutions of pH=1 (0.025 M potassium chloride) and pH=4.5 (0.4 M sodium acetate), respectively. After stirring, the optical density of each solution was measured at wavelengths of 510 and 700 nm on a spectrophotometer Spekol-11 (Germany). Measurements of the optical density at 700 nm were performed to establish the amount of light absorption by impurities.

The concentration of anthocyanins in grape pomace was calculated as equivalent of cyanidin-3-glucoside in mass per gram of dry matter (AC, mg CG/g DM) according to (Wrolstad et al., 2001):

$$AC = (\Delta A \cdot M \cdot V \cdot F \cdot 10^3)/(m \cdot \varepsilon \cdot l) \quad (1)$$

where, ΔA – the difference absorption solutions at different wavelengths 510 and 700 nm and the corresponding pH values, $\Delta A=(A_{510} - A_{700})_{pH1}-(A_{510} - A_{700})_{pH4,5}$; M – molar mass of cyanidin-3-glucoside; V – volume of prepared solution, l; F – dilution factor; m – mass of absolutely dry sample, g; ε – molar extinction coefficient cyanidin-3-glucoside, $l \cdot mol^{-1} \cdot cm^{-1}$; l – optical path length of the cuvette, cm.

Determination of the thermal stability of anthocyanins

To determine the thermal stability of anthocyanins, the extracts were kept in a closed flask at 60 °C for 4 hours. Determination of anthocyanin content was performed every 60 minutes. To study the kinetics of the process of anthocyanin decomposition under the influence of temperature, the rate constant of anthocyanin degradation reaction (k, h^{-1}) and half-life ($t_{1/2}$, h) were calculated. The first-order reaction was used as a kinetic model of anthocyanin degradation (Mulyawantia et al., 2018). The reaction rate constant was calculated by the formula:

$$k = -\ln (c/c_0)/t \quad (2)$$

c – current concentration of anthocyanins in solution, mg/l;
c_0 – initial concentration of anthocyanins in solution, mg/l;
t – time, hours.

The half-life was obtained as:

$$t_{0,5} = \ln 2/k \quad (3)$$

Results and discussion

Influence of the number of hydroxyl groups in an aliphatic alcohol molecule on the efficiency of extraction

At the first stage, the goal was to investigate the influence of the number of hydroxyl groups in the aliphatic alcohol molecule on the extraction efficiency. Mono-, di- and trihydric alcohols were chosen as extractants. The process of extraction of anthocyanins was carried out at different pH, which was created by the introduction of hydrochloric acid. The results of the experiments are given in the Table 1.

Table 1

Effect of pH and the number of hydroxyl groups in the extractant molecule on the number of extracted anthocyanins

Parameter	Extractant					
	ethanol		ethane-1,2-diol		propane-1,2,3-triol	
pH	5.4	2.9	5.6	2.9	5.9	2.9
AC, mg CG/g DM	7.71		5.78		4.49	

The efficiency of the extraction follows the order propane-1,2,3-triol < ethane-1,2-diol < ethanol (Table 1). The worst results in the extraction of anthocyanins were obtained for extractants with the largest number of carbon atoms and hydroxyl groups in the structure. The amount of extracted anthocyanins by two-stage extraction for each alcohol is: propane-1,2,3-triol (glycerol) 4.49 mg/g; ethane-1,2-diol (ethylene glycol) 5.78 mg/g; while with ethanol it increases up to 7.71 mg/g. The latter value indicates that ethanol and ethylene glycol compared to glycerol have 1.7 and 1.3 times higher efficacy, respectively, due to the different ability to form hydrogen bonds, as well as the intermolecular interaction between solvent and anthocyanins.

The spectral characteristics of the extractions obtained with ethanol and polyhydric alcohols are presented in Figure 1.

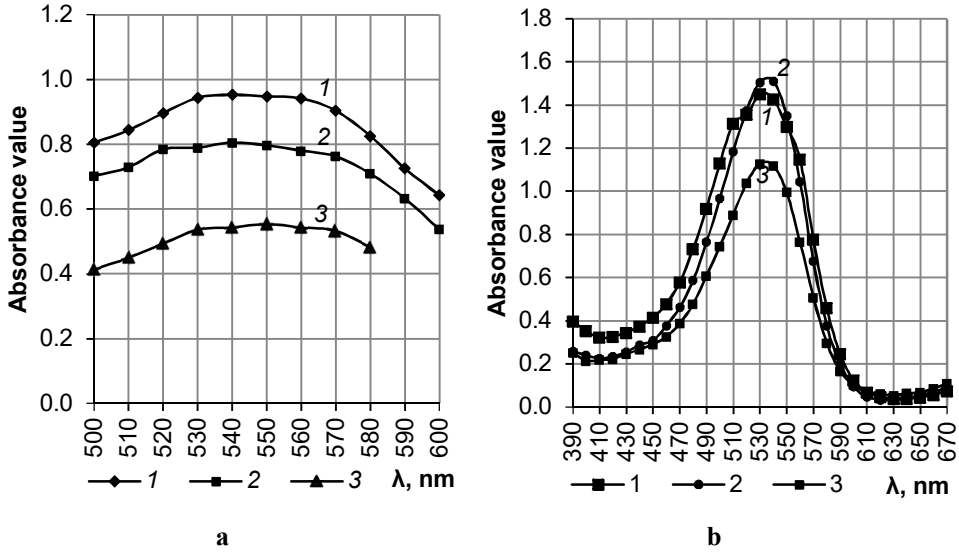

Figure 1. Absorption spectra of extracts from grape skins at:
a – pH 5.2 – 6.7; b – pH 2.9;
1 – ethanol; 2 – ethane-1,2-diol; 3 – propane-1,2,3-triol.

When comparing the spectral characteristics of the extractions obtained with ethanol and polyhydric alcohols, it should be noted that they are similar, the absorption maximum is not clear and is in the wide range of 530–560 nm, indicating a not bright red color with blue hues (Figure 1a). Provided that the introduction of hydrochloric acid is the cleavage of monosaccharides from the anthocyanin molecule to obtain the flavylium cation, the extracts were adjusted to pH 2.9 to obtain a more stable form of the natural pigment, namely – anthocyanidin. From Figure 1b it would be seen that the typical absorption maximum at 530–540 nm becomes clearer and more pronounced, and the value of the optical density increases by 1.5–1.8 times compared to the initial pH. The color of the solutions becomes bright red, which indicates the transition of the dye into the form of the flavylium cation. The results shown in Figure 1, b, show the advantages of using ethane-1,2-diol and ethanol in comparison with propane-1,2,3-triol in 1.4 times. Moreover, the use of ethane-1,2-diol is more effective than ethanol and allows you to obtain extracts with a clear maximum, which indicates the absence of a shade in the color.

Influence of the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins

It is known that anthocyanins are thermolabile compounds and degraded during heat treatment (Bakowska-Barczak, 2005). Temperature can have a negative effect not only on the color of dyes, but also changes their antioxidant properties (Martinsen et al., 2022). The exact mechanism of thermal degradation of anthocyanins is still unclear. However, it is known the formation of chalcones in the first stage of the process, the loss of glycosidic fragments and the formation of adiketone before the formation of final products, including coumarin derivatives, benzoic acid derivatives and threehydrobenzaldehyde (Reyes et al., 2007; Zhao et al., 2013). Therefore, it is important to investigate the ability of anthocyanins

to degrade when the extracts are kept at the temperature of their concentration with a decrease in pressure.

When studying the effect of the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins, it was found that the thermal stability increases as follows ethane-1,2-diol < propane-1,2,3-triol < ethanol (Figure 2, Table 2).

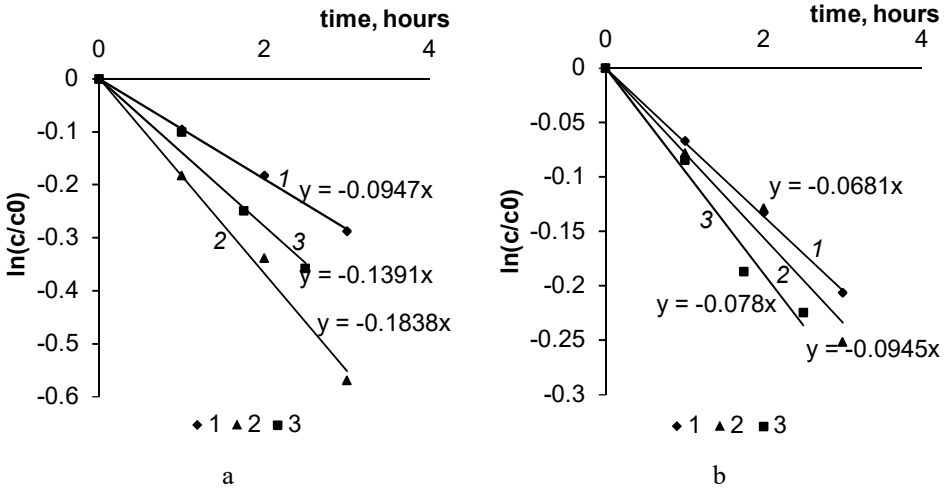

Figure 2. Kinetic curves of destruction of anthocyanins in extractants at:
a – initial pH of solvent; b – pH = 2.9: 1 – ethanol; 2 – ethane-1,2-diol; 3 – propane-1,2,3-triol.

Table 2

The effect of pH and the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins

Parameter	Extractant					
	ethanol		ethane-1,2-diol		propane-1,2,3-triol	
pH	5.4	2.9	5.6	2.9	5.9	2.9
k, h^{-1}	0.0947	0.0681	0.1838	0.0779	0.1391	0.0945
$t_{1/2}$, h	7.32	10.18	3.77	8.89	4.98	7.33

The results of the reaction rate constant of the decomposition of anthocyanins are given in Table 2 and they evidence the stability of anthocyanins in the form of the flavylium cation (pH 2.9). The decrease in the decomposition reaction constant of anthocyanins when moving to a more acidic environment was for: ethanol by 1.4 times, ethane-1,2-diol by 2.4 times, propane-1,2,3-triol by 1.5 times, with better stability of anthocyanins observed in ethanol at both pH values. It can be assumed that with an increase in the number of hydroxyl groups in an alcohol molecule, the ability to form intermolecular hydrogen bonds also increases, which negatively affects the stabilization of anthocyanins. The use of ethanol at pH 2.9 is 12% more effective than ethane-1,2-diol, and 23% more effective with propane-1,2,3-triol.

Influence of the length and structure of the carbon chain of monatomic alcohols on the amount of extracted anthocyanins

Further studies were aimed at determining the influence of the length and structure of the carbon chain of monohydric alcohols on the number of extracted anthocyanins – Table 3.

The largest amount of extracted anthocyanins was obtained when low molecular weight alcohols were used as extractants, which are able to penetrate better into the cells of grape pomace. Among alcohols of normal structure, butanol extracts 1.5–2 times less anthocyanins. Alcohols of the isostructure extract a smaller amount of anthocyanins due to the spatial structure of their molecule. Methanol and ethanol extract 2.4 and 3.0 times more anthocyanins compared to propan-2-ol. Ethanol extracts 2 times more anthocyanins than 2-methylpropan-1-ol.

Table 3

Influence of pH and structure of carbon chain of monohydric alcohols on the amount of extracted anthocyanins

Parameter	Extractant									
	methanol		ethanol		propan-2-ol		butan-1-ol		2-methylpropan-1-ol	
pH	6.7	2.9	5.4	2.9	5.75	2.9	5.6	2.9	5.2	2.9
AC, mg CG/g DM	6.34		7.71		2.60		4.04		3.94	

It was found that the efficiency of extractants increases as 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol (Figure 3b). When comparing the spectral characteristics of alcohol extracts with the linear structure of the carbon chain, we observe an increase in the number of anthocyanins in solution with a reduction in the carbon chain of the extractant: methanol – ca. 3 times, ethanol – 2.4 times compared to butan-1-ol. Extraction of anthocyanins by methanol occurs not only in cationic but also in quinoid form, as evidenced by the spectral characteristics of the extracts, which have a wide spectrum of absorption (Figure 3, a) and the highest maximum at pH 2.9 (Figure 3b). Alcohols of isostructure under these conditions showed better results than alcohols with the appropriate number of carbon atoms of normal structure. The absorption intensity of extracts with 2-methylpropan-1-ol is 2 times more than butan-1-ol.

Studies of the spectral characteristics of the obtained extracts (Figure 3a, b) showed that with increasing the length of the carbon skeleton of alcohol, the absorption spectra are characterized by different intensities, the absorption maximum is in the range of 520–560 nm.

The lack of a clear maximum in the extracts can be explained by the fact that the extraction of anthocyanins occurs not only in the form of the flavylium cation, but also in the form of other structures.

When obtaining a stable form – flavylium cation, there is (Figure 3b) an increase in the absorption intensity for methanol at 540 nm by three times and a shift of the maximum by 10 – 15 nm towards long waves for alcohols of the isostructure and butan-1-ol. From Figure 3b, we can see the advantages of using methanol (2.7 times) and ethanol (2.3 times) in comparison with the alcohols of the isostructure. Thus, the extraction of anthocyanins depends on the polarity of the solvent and is associated with better diffusion properties of alcohols with lower molecular weight across plant cell membranes.

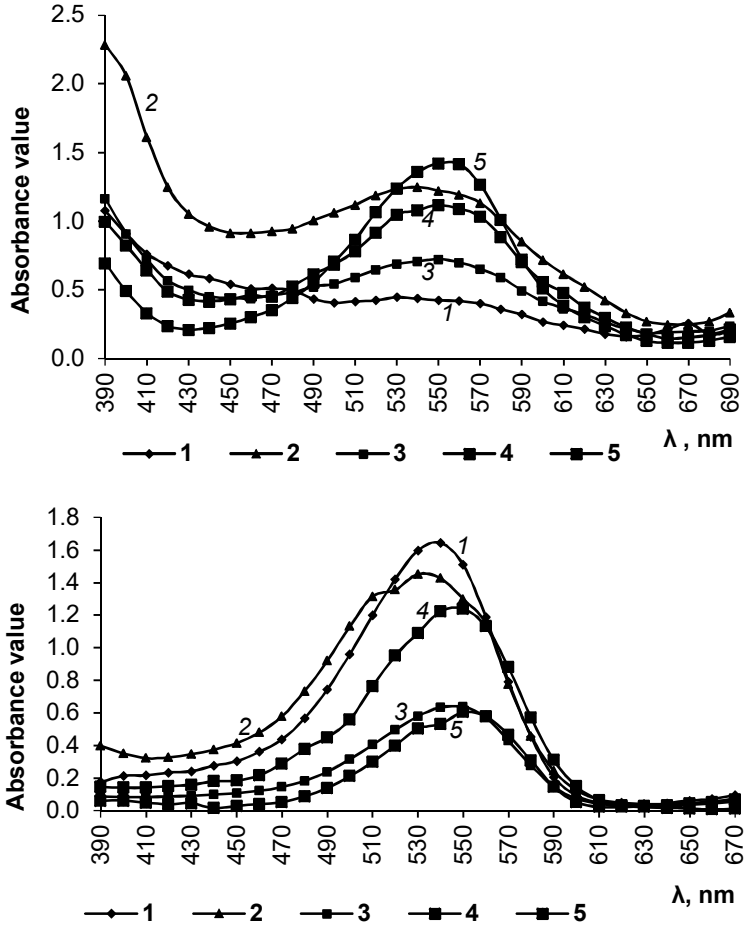

Figure 3. The spectrum of absorption of the extract from the skins of grapes at:
a – pH 5.2 – 6.7; b – pH 2.9;
1 – methanol; 2 – ethanol; 3 – propan-2-ol;
4 – butan-1-ol; 5 – 2-methylpropan-1-ol.

Influence of the length and structure of the carbon chain of alcohols on the thermal stability of anthocyanins

Determination of the influence of the structure of the carbon chain of monohydric alcohols on the thermal stability of anthocyanins is presented in Figure 4 and in Table 4.

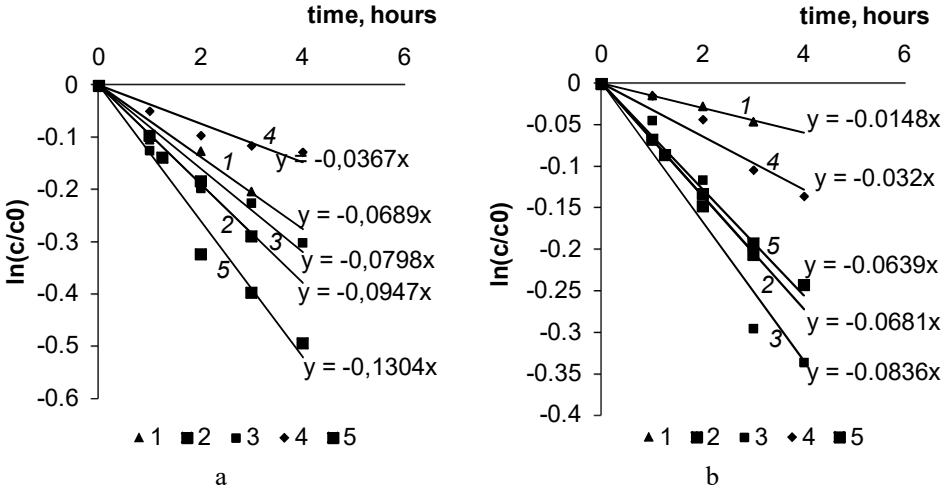

Figure 4. Kinetic curves of destruction of anthocyanins in extractants at:
a – initial pH of solvent; b – pH = 2.9: 1 – methanol; 2 – ethanol;
3 – propan-2-ol; 4 – butan-1-ol; 5 – 2-methylpropan-1-ol.

Table 4

Influence of pH and structure of carbon chain of monohydric alcohols on the amount of extracted anthocyanins

Parameter	Extractant									
	methanol		ethanol		propan-2-ol		butan-1-ol		2-methylpropan-1-ol	
pH	6.7	2.9	5.4	2.9	5.8	2.9	5.6	2.9	5.2	2.9
k, h^{-1}	0.069	0.015	0.095	0.068	0.084	0.080	0.037	0.032	0.130	0.064
$t_{1/2}$, h	10.06	46.83	7.32	10.18	8.29	8.68	18.89	21.66	5.32	10.85

It was found that the thermal stability increases in the series of 2-methylpropan-1-ol < ethanol < propan-2-ol < methanol < butan-1-ol (Table 4). The half-life of anthocyanins in butan-1-ol is the longest and is 18.89 hours, which is an order of magnitude higher than other tested extractants.

The stability of anthocyanins in the form of the flavylium cation (pH 2.9) is higher for all extractants: for propan-2-ol by 1.05 times, for ethanol by 1.4 times, for 2-methylpropan-1-ol by 2 times, for butan-1-ol by 1.15 times, for methanol by 4.7 times. The best thermal stability indicators were obtained for anthocyanins in methanol. Compared with the neutral medium, the stability in acidified methanol is 4.7 times higher, and the half-life is 46.83 hours. The second most effective storage of anthocyanins is butano-1-ol, in which the half-life of the dye is 21.66 hours. As evidenced in Table 4, the efficiency of extraction of

anthocyanins by ethanol is the highest 7.71 mg/g, but the half-life is less compared to methanol 4.6 times, and butan-1-ol by a factor 2. The use of alcohols of isostructure does not provide advantages when storing natural dyes.

Conclusions

1. The influence of hydrogen index value and structure of extractant alcohol molecule on anthocyanin extraction from Vitis Vierul red grape pomace was investigated and anthocyanin stability in extracts was determined by calculating the anthocyanid degradation reaction rate constant and half-life.
2. An increase in the efficiency of the use of extractants depending on the number of hydroxyl groups as: propane-1,2,3-triol < ethane-1,2-diol < ethanol and depending on the structure of the carbon fragment of monohydric alcohols as: 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol.
3. It was found that the stability of anthocyanins in the form of cation flavylium (at pH 2.9) is higher in the corresponding extracts compared to the stability of the extracts at pH 5.2–6.7. The best extractant in terms of the efficiency of extraction of natural pigment and use in the food industry is ethanol. The kinetic curves of anthocyanin degradation under the influence of temperature were constructed and the half-life was calculated, which is 10.18 hours in ethanol at a temperature of 60°C. The obtained results allow to evaluate the efficiency of extractants in the extraction of anthocyanins from grape pomace and their thermal stability.

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