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STUDY OF ANTHOCYANIN CONTENT IN RED GRAPE JUICE CONCENTRATE OBTAINED BY MICROWAVE DEHYDRATION

Actuality. *Anthocyanins, as representatives of polyphenols with antioxidant properties, have a wide range of protective biological properties that attract vitamin P.*

Aim of the study. *To investigate the composition and content of polyphenolic compounds, in particular anthocyanins, in red grape juice concentrate obtained by microwave dehydration.*

Material and methods. *The content of anthocyanins, flavonols and ascorbic acid was determined in juices from red grapes, actinidia and apples. The composition and content of polyphenolic compounds in red grape juice concentrate was determined using HPLC. Alcohol-free grape juice concentrate was obtained using a dehydration method developed by us using electromagnetic energy.*

Research results. *It was found that red grape juice contains a significant amount of anthocyanins, unlike juice from actinidia fruits and apples. The HPLC method determined the content of anthocyanins in red grape juice concentrate at 115,3 mg% which is more than 65% of the content of all polyphenolic compounds. Catechins and catechol-like compounds account for less than 25% of the content of all polyphenolic compounds.*

Conclusion. *Studies have found that due to the high content of anthocyanins, red grape juice concentrate obtained by microwave dehydration can be recommended as a dietary supplement for pathological conditions caused by oxidative stress.*

Key words: *grapes, anthocyanins, polyphenolic compounds.*

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ДОСЛІДЖЕННЯ ВМІСТУ АНТОЦІАНІНІВ У КОНЦЕНТРАТІ СОКУ ЧЕРВОНОГО ВИНОГРАДУ, ОТРИМАНОГО МЕТОДОМ МІКРОХВИЛЬОВОЇ ДЕГІДРАТАЦІЇ

Актуальність. *Антоціаніни, як представники поліфенолів з антиоксидантними властивостями, володіють широким спектром захисних біологічних властивостей, приманних вітаміну Р.*

Мета дослідження *– дослідити склад і вміст поліфенольних сполук, зокрема антоціанінів, у концентраті соку червоного винограду, отриманого методом мікрохвильової дегідратації.*

Матеріал і методи. *Уміст антоціанінів, флавонолів і аскорбінової кислоти визначали в соках із червоного винограду, актинідії та яблук. Склад і вміст поліфенольних сполук у концентраті соку червоного винограду визначали за допомогою ВЕРХ. Безспиртовий концентрат виноградного соку отримували за допомогою розробленого нами методу дегідратації, з використанням електромагнітної енергії.*

Результати дослідження. *Встановлено, що сок червоного винограду містить значну кількість антоціанінів, на відміну від соку із плодів актинідії і яблук. Методом ВЕРХ визначено вміст антоціанінів у концентраті соку червоного винограду в 115,3 мг%, що становить понад 65% умісту всіх поліфенольних сполук. На долю катехінів і катехолподібних сполук припадає менше 25% вмісту всіх поліфенольних сполук.*

Висновок. *Дослідження встановили, що завдяки наявності високого вмісту антоціанінів концентрат соку червоного винограду, отриманого мікрохвильовим методом дегідратації, може бути рекомендований як дієтична добавка за патологічних станів, зумовлених оксидативним стресом.*

Ключові слова: *виноград, антоціаніни, поліфенольні сполуки.*

Introduction. Actuality. Oxidative stress is the pathogenetic basis of many diseases: atherosclerosis, myocardial dystrophy, type 2 diabetes, cancer, neuropsychiatric disorders (Moharram, Youssef, 2014; Ndrepepa, 2019; Ray et al., 2012). Oxidative stress also plays a crucial role in the pathogenesis of post-stress reactions (Arkelius et al., 2024; Lambert, 2009).

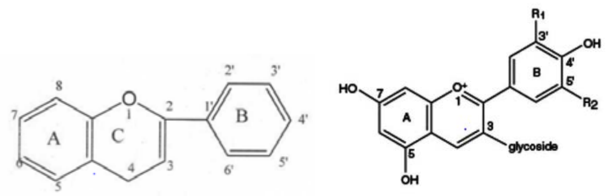
The cause of oxidative stress in humans and animals is an excessive reaction of the central nervous system, an excess of pro-oxidant factors in the tissues (metals with variable valence, active genes responsible for the biosynthesis of oxygenase enzymes) (Gorchakova et al., 2012; Maksjutina et al., 2012; Yahfoufi et al., 2018).

To protect against oxidative stress, the body has a large number of antioxidant systems, represented by antioxidant enzymes (superoxide dismutase, heme oxygenase I, glutathione peroxidase, catalase), endogenous liposoluble (squalene) and water-soluble (uric acid, thiol compounds) (Bacchetti et al., 2019; Joseph et al., 2016; Makarenko, Levitsky, 2016; Moharram, Youssef, 2014).

Exogenous (food) antioxidants also play a significant role in the antioxidant defense of the body, including ascorbic acid (vitamin C), bioflavonoids (vitamin P), tocopherols (vitamin E), carotenoids (β-carotene, lycopene), and polyphenolic compounds (Gorchakova et al., 2022; Kudrickaja, 1990; Liga et al., 2023; Lis, Bartuzi, 2023; Mishhenko et al., 2020; Yahfoufi et al., 2018).

Among food polyphenolic compounds, a special place is occupied by anthocyanins, a significant amount of which is found in berries, fruits, and red grapes (Liu et al., 2021; Mattioli et al., 2020).

Fig. 1 shows the chemical structure of anthocyanins.



Flavan Anthocyanidin

Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3

Fig. 1. Chemical structure of anthocyanins

Eight mechanisms of biochemical actions of anthocyanins have been identified, which determine its therapeutic and prophylactic effectiveness.

1. Direct effect on reactive oxygen species (ROS) with the formation of low-activity anthocyanidin radicals (Garcia, Blesso, 2021).

2. Formation of inactive complexes with metals of variable valence (Fe, Cu, Cr), which are a source of ROS formation (Fedenko et al., 2022; Stote et al., 2023).

3. Activation of the biosynthesis of antioxidant enzymes through the action on the corresponding genes of the nuclear factor N_2f2 (Festa et al., 2023).

4. Anti-inflammatory effect due to inhibition of the formation of the nuclear factor NF-kB, which activates genes responsible for the biosynthesis of pro-inflammatory factors (interleukins IL-1, IL-6, TNFα, proteases, phospholipases A_2) (Aboonabi, Aboonabi, 2020; Ngamsamer et al., 2022; Zhao, Yuan, 2021).

5. Hypotensive effect due to activation of the endothelial enzyme e-NOS, which forms NO (Horie et al., 2019).

6. Estrogenic effect similar to that of soy isoflavones (Horie et al., 2019).

7. Antidysbiotic effect due to activation of phagocytosis, reduction of lipopolysaccharide receptor (TLR-4) expression, formation of phenolic acids in the intestine, which have antibacterial properties and reduce the penetration of bacteria through the intestinal wall (Ayvaz et al., 2023; Borges et al., 2007).

8. Anticancer effect of anthocyanins in in vitro experiments on cancer cell cultures (de Arruda Nascimento et al., 2022).

It has been established that anthocyanins are not produced in the human and animal body and therefore they must be consumed with food containing these compounds (Ayvaz et al., 2023). It is believed that the daily human need for anthocyanins is 50 mg (Gonçalves et al., 2021).

The aim of the study. To investigate the composition and content of polyphenolic compounds, in particular anthocyanins, in red grape juice concentrate obtained by microwave dehydration.

Materials and research methods. We used red grapes of the Moldova variety to obtain juice and concentrate. Grape juice was obtained by pressing. Juice dehydration was carried out by the following electromagnetic method developed by us (Burdo et al., 2025).

The ascorbic acid content was determined by spectrophotometric method. For this purpose, a solution of grapes juice and concentrate in 0,01 M HCl was prepared. To 1 ml of juice or concentrate solution, 1 ml of 1 mM $FeCl_3$ i 1solution and 1 ml of 3 mM $[K_3[Fe(CN)_6]$ solution was added and after 5 minutes the extraction was measured at 693 nm on a spectrophotometer Shimadzu UV-1240-mini.

The ascorbic acid content was determined of a calibration curve using ascorbic acid solutions.

The content of bioflavonoids (vitamin P) was determined by spectrophotometric method. For this purpose, solutions of actinidia juice or concentrate in ethanol were prepared. 1 ml of juice solution (concentrate) was mixed with 0,5 ml of 5% solution of $AlCl_3$ in ethanol, 2,5 ml of ethanol was added and the extinction at 410,5 nm was measured on a spectrophotometer Shimadzu UV-1240-mini.

To calculate the vitamin P content, a similar reaction was performed with a quercetin solution.

Analysis of polyphenol content and composition by HPLC (Levitsky et al., 2012; Khodakov, 2013).

The analysis was carried out on a Prominence LC-20 liquid chromatography system from Shimadzu (Japan), which consists of the following functional modules: a DGU-20A3 dispenser, an LC-20AD pump module, an SIL-20AC autosampler-refrigerator, an SPD-20AV photometric detector, a CTO-20A column thermostat, a Thermo Scientific Syncronisa column (aQ reversed-phase filler with an attached $C_{18}(CH_2)_{17}CH_3$ group, length 250 mm, internal diameter 4,6 mm, sorbent grain size 4,4 mm.

HPLC conditions:

1. Composition of the mobile phase: component A – methanol, component B – 1% solution of phosphoric acid in deionized water.
2. Chromatography mode – gradient.
3. The mobile phase speed is constant at 0,5 ml/min.
4. The temperature of the acetone column is 40 °C.
5. Injection volume − 5 µl.

Identification of substances in the extracts was carried out by comparing the retention time and spectral characteristics of the studied substances with similar characteristics of polyphenol standards (Khodakov, 2013).

The spectral characteristics of the studied compounds were determined by the height of the peaks of these substances on the chromatograms at wavelengths of 255, 286, and 350 nm compared to the height of the peak at a wavelength of 225 nm.

Research results and their discussion. Table 1 presents the results of determining the content of ascorbic acid (vitamin C), flavonols (vitamin P), anthocyanins in juices from actinidia, apples and red grapes.

The content of vitamins C and P was determined by spectrophotometric methods, and the content of anthocyanins – by HPLC. As can be seen from these data, the highest content of vitamins C and P is found in juice from actinidia, and the lowest – in juice from apples. Anthocyanins were found only in red grapes.

Table 2 presents data on the composition and content of polyphenolic compounds in red grapes, namely in juice and in concentrate using the HPLC method (fig. 2 and 3).

Table 1

Content of ascorbic acid, flavonols and anthocyanins in juices from actinidia fruits, apples and red grapes (mg%)

Product	Ascorbic acid	Flavonols	Anthocyanidins
Actinidia	364,00	6,25	0
Apples	1,20	0,64	0
Red grapes	50,00	0,84	27,30

Table 2

Composition and content of polyphenolic compounds in red grape juice and its concentrate (mg%) according to HPLC data

Polyphenol	Juice	Concentrate	Main representative
Total number	59,70	177,05	
Phenolic acids	3,10	4,81	chlorogenic acid, gallic acid
Catechins	3,20	16,30	Catechin
Catechin-like	20,20	34,50	
Flavonols	0,77	1,67	rutin, myricetin
Flavanones	0,12	0,13	–
Flavanoides	0,45	3,89	–
Flavones	0,01	0	–
Anthocyanins	27,30	115,29	delphinidin, malviedin, cyanidin
Isoflavones	0	0	–
Unidentified polyphenolic compounds	4,66	27,22	–

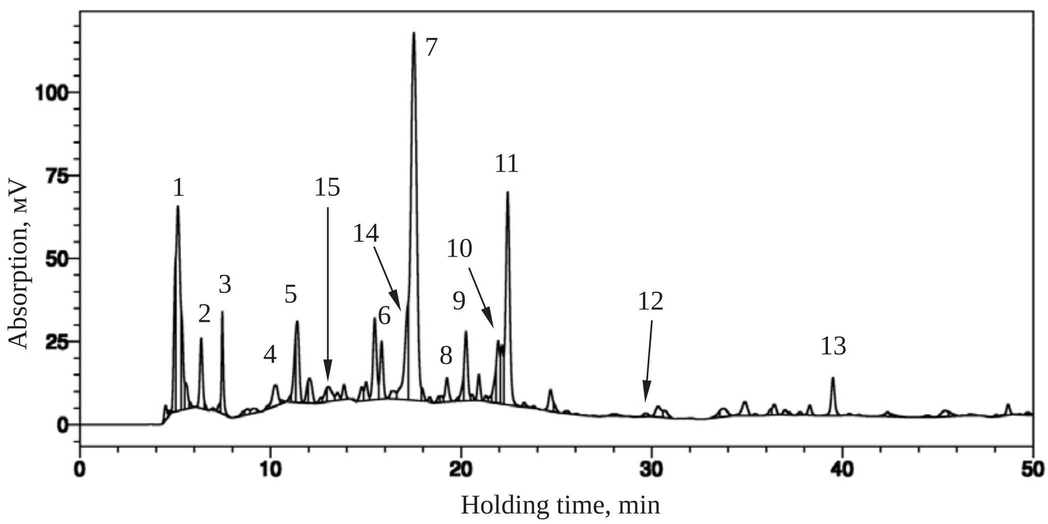


Fig. 2. Chromatogram of grape juice concentrate diluted with 70% ethanol in the ratio of 5,1 g of sample to 12 ml of ethanol solution, at λ = 255 nm. Polyphenols (4 – phenolic acids, 1, 2, 3, 5 – catechin-like, 6 – catechins, 7 – delphinidin-3-O-glucoside, 8 – cyanidin-3-O-glucoside, 9 – petunidin-3-O-glucoside, 10 – peonidin-3-O-glucoside, 11 – malvidin-3-O-glucoside, 12 – rutin, 13, 14 – anthocyanins, 15 – catechin)

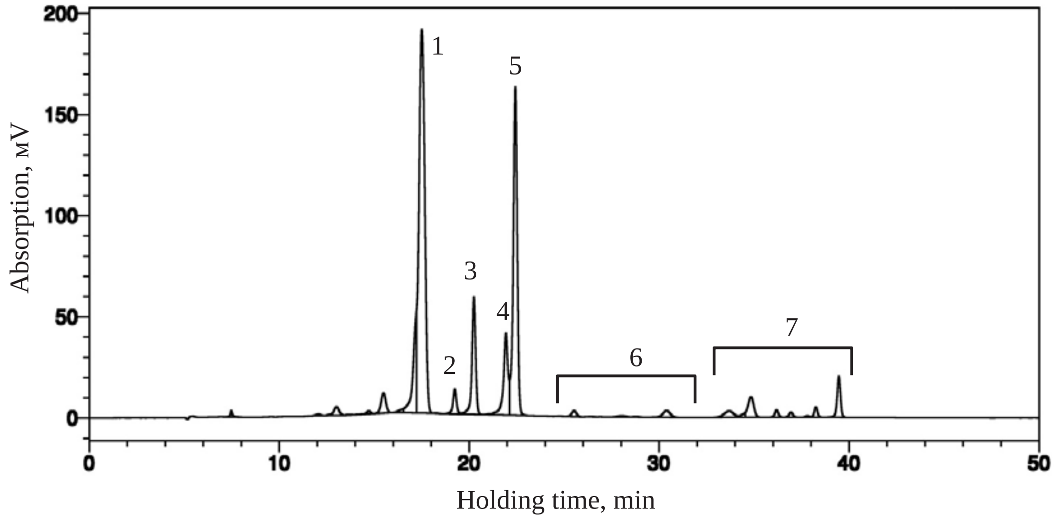


Fig. 3. Chromatogram of grape juice concentrate diluted with 70% ethanol in a ratio of 5,1 g of sample to 12 ml, at λ = 530 nm. Anthocyanins (1 – delphinidin-3-O-glucoside, 2 – cyanidin-3-O-glucoside, 3 – petunidin-3-O-glucoside, 4 – peonidin-3-O-glucoside, 5 – malvidin-3-O-glucoside, 6 – acetyl-glucosides of anthocyanins, 7 – caffeoyl-, coumaryl- and feiryl-glucosides of anthocyanins)

It can be seen that the total content of polyphenols in the concentrate is almost three times higher than in the juice.

The percentage content of polyphenol fractions in grape juice concentrate is presented in fig. 4.

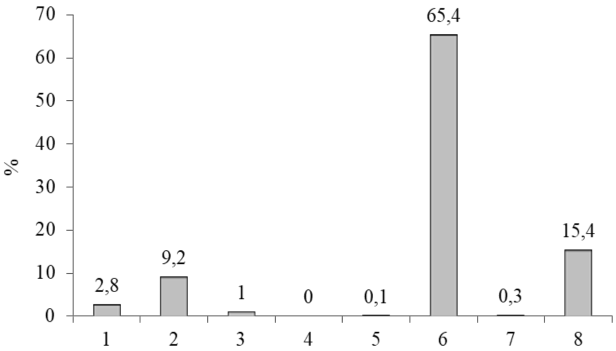


Fig. 4. The share of individual groups of polyphenols in the total content of polyphenols in red grapes (1 – phenolic acid, 2 – catechins, 3 – flavanones, 4 – flavones, 5 – flavonols, 6 – anthocyanins, 7 – resveratrol, 8 – unidentified polyphenols)

From these data it can be seen that anthocyanins account for 65% of the total polyphenols, catechins account for only 9,2%, and there are practically no fractions of isoflavones, flavones, and flavonols.

Existing data indicate the polyfunctionality of anthocyanins, ensuring the maintenance of human health in extreme conditions of existence. The physiological effects of anthocyanins are not limited to their antioxidant properties, they act on regulatory systems of cells that influence the processes of biosynthesis of anti-inflammatory factors, immune stimulants, the state of histo-hematological barriers and even the processes of carcinogenesis (Chen, Zhao, 2023; Paśko et al., 2009).

Unfortunately, anthocyanins are not synthesized in the human and animal body, nor in most plants. It is known that grapes, cherries, and some berry crops are producers of anthocyanins.

Anthocyanins can be considered as a vitamin similar to vitamin P, represented by bioflavonoids (more precisely, flavonols), the most active of which is quercetin (Liga et al., 2023; Paśko et al., 2009).

Characteristic properties of bioflavonoids are not only their antioxidant effect, but also their ability to inhibit the activity of hydrolytic enzymes (proteases, phospholipases) (Aboonabi, Aboonabi, 2020). The biological effects of bioflavonoids mainly relate to angioprotective processes, although they also have cardioprotective (Gonçalves et al., 2021) and hepatoprotective (Ngamsamer et al., 2022) properties.

One of the richest in flavonols is actinidia, but it is completely devoid of anthocyanins. Anthocyanins exceed flavonols in their physiological spectrum and therefore can be considered as vitamin P, the need for which must be met by consuming foods containing this compound.

We propose to use red grape juice concentrate for this purpose, 50 ml of which fully meets a person's daily needs.

Conclusions. Studies have found that due to the high content of anthocyanins, red grape juice concentrate obtained by microwave dehydration can be recommended as a dietary supplement for pathological conditions caused by oxidative stress.

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