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Study of features of the biochemical composition of red vine leaves of autochthonous varieties in Russia

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Abstract. One of the fields of processing industries' activities is the use of secondary resources. The use the vegetative parts of grape plants may become an important component in solving this task. Such vegetative parts, first of all, include red grape leaves, which provide a large reserve of antioxidants and other biologically useful substances. The Russian Research Institute of Brewing and Wine Industry has carried out the detailed study of the features of the biochemical composition of red vine leaves of autochthonous varieties cultivated in the Rostov region of Russia. Cold winters are considered to be the major stress for the grape plants. Under these conditions, leaves accumulate large amount of biologically active substances, including trans-resveratrol, which provide significant advantage compared with the harvest from grapes cultivated in areas where the plants are not protected during winter. Comparative studies on the biochemical composition of red vine leaves of autochthonous and European varieties were conducted, including on the use of bioassay systems in vitro. It was found that extracts of red vine leaves of autochthonous varieties have a marked effect on the rate of glutathione reductase and pyruvate kinase reactions that are demonstrating their angioprotective and energizing properties. The increase in the rate of the catalase reaction indicates the manifestation of antioxidant properties. The technology of CO_2– and highly concentrated hydrophilic extracts production from red vine leaves that preserves biologically active compounds to the maximum extent possible. The extracts are used for the manufacture of soft drinks that have the venomotor action and may be applied in the process of the manufacture of fat products with extended shelf life, as well as the main raw material for the preparations with the pronounced angioprotective effect.

1. The relevance of studies

At the beginning of the XXI century one of the main issues in the life of processing industries is the use of secondary resources. At that, the main factors are the desire to mitigate the impact on the environment, on the one hand, and to receive new products additionally, on the other hand.

This trend is of topical interest in the industries engaged in the processing of agricultural raw materials, since the so-called waste products are of biological origin and may be the initial material for the production of feed and, in some cases, food products.

In the production of wines the main wastes are sweet and fermented grape pomace, yeast and racking sludges, grape seed and others. One of the new directions is the use of vegetative parts of the vine plant of a seasonal nature. These primarily include red vine leaves, which have a large reserve of antioxidants and other valuable substances.

Thus, the improvement of existing waste treatment processes and development of new ones are topical directions in the utilization of secondary resources. Special attention should be paid to obtaining knowledge-intensive products, including dietary (nutritional) supplements.

2. Red vine leaves as a natural source of bioactive compounds

In recent years, a lot of healthcare products, which include native biochemical compounds from leaves of the red vine varieties, appeared in the global pharmaceutical market. Indications for their use are venous insufficiency manifested itself by the symptom of "heavy legs", oedema, a manifestation of blood vessel and capillary fragility.

Brown colour of vine leaves is caused by the autumn cessation of continuous synthesis of chlorophyll, the main green pigment for photosynthesis,. After that, the leaves change their colour and fall off. Before they fall, the plant aims to recover from them all the nutrients and stock up on them in its trunks and roots for winter, it is especially important for the vine growing on poor soil. Red colour is given to leaves by pigment anthocyanin, which usually begins to synthesize just in autumn, when chlorophyll is decomposed. One of the numerous hypotheses on this subject states that anthocyanin protects weak and vulnerable leaves from sunlight, allowing the plant to hold them out as long as possible, so that the plant can recover from them as much nutrients as possible [1]. This is confirmed by observations: usually there are more red leaves in the cold clear autumn.

The main biologically active components of the leaves are catechins, flavonoids, tannins, malic, silicic, citric, tartaric and succinic acids, and resveratrol.

Resveratrol is a polyphenol of stilbene group. In nature, there are two forms of resveratrol: cis-resveratrol and trans-resveratrol. Trans-resveratrol is a more stable form. Under the effect of ultraviolet rays it has transformed into cis-resveratrol possessing a large store of energy, and, therefore, less stable. In comparison with

the cis-form (cis-3,4-5-trihydrostilbene), trans-resveratrol (trans-3,4-5-trihydrostilbene) possesses greater biological and antioxidant activities [2].

Like all of polyphenols, resveratrol is a powerful antioxidant, activity of which exceeds that of vitamin E. Resveratrol exhibits estrogenic activity by binding to the same receptors as estradiol (one of the main human estrogens) do. Despite the fact that the hormonal activity of resveratrol is significantly less than that of estradiol, its content in the organism can be so high that it will act much greater than estradiol do in physiological concentrations [2].

The extract of red vine leaves has effect on the vascular wall of veins and helps to eliminate their malfunction. For a long time red vine leaves have been used in France for the treatment of painful swollen legs. Today, their use in this field is scientifically justified.

The main task in the manufacture of dietary supplements from natural plant materials is to extract native compounds without subjecting them to severe thermal or chemical exposure.

To obtain a high-quality extract of red vine leaves without negative thermal effect and at the same time to achieve the highest yield of biologically active substances is possible by means of adoption of the subcritical CO_2 technology. Due to deep penetration of CO_2 into the plant cell organelles, liquefied carbon dioxide fully extracts the lipophilic fraction from the raw, while the solvent completely evaporates leaving no its trace in the extract.

After depressurization during the extraction process a cell "explosion" happens resulting in rupture of the cell walls. The "exploded" extraction cake remaining after CO_2 extraction contains a large amount of hydrophilic biologically active compounds (polyphenols, resveratrol, vitamins, amino acids and others). These compounds, due to the deep breakdown of plant cells, is almost completely moved into a highly concentrated hydrophilic extract prepared from the extraction cake of red grape leaves.

3. Studies of biochemical composition of red vine leaves from autochthonous vine varieties of Russia

3.1. Studies of the qualitative characteristics of red vine leaves of different varieties

After the grape harvest, red vine leaves of Krasnostop Zolotovskii, Golubok, Cabernet Sauvignon and Saperavi varieties growing in Rostov region were collected. Krasnostop Zolotovskii and Golubok are native varieties for the given region.

Previous studies have shown that Krasnostop Zolotovskii has the highest technological reserve of dyes as compared with other red vine varieties. Golubok is considered as a dye grape since its colourants are contained not only in the skin, but also in the flesh of berries.

Rostov region refers to the area of sheltered viticulture and is located further north than other areas of vine cultivation in Russia, on 48° north latitude. Researchers generally agree that the most of resveratrol is found in vines growing in colder climates. This substance helps the vine to survive in winter. A larger amount of resveratrol in the form of trans-isomere is generated in plants in response to stress. As noted above, trans-resveratrol has a greater biological activity as compared with cis-resveratrol. Cold winters of Rostov region can be considered as a major stress for vine plants. On this basis, it has been suggested that vines grown in cooler climates contain more trans-resveratrol as compared with vines grown in the mild climate.

In order to select a vine variety that has optimal biochemical composition to obtain the dietary supplement, at the initial stage of research a work was carried out to determine chemical composition and evaluate quality of raw materials. In vine leaves it has been identified the following compounds: sugars (approximately 0.2%), inositol, quercetin, tannins, carotene (approximately 0.2%), choline, betaine, aloxuric bases, tartaric, malic and protocatechuic acids.

Studies to determine the content of extractives, total polyphenols and resveratrol in red leaves of different varieties of vine have been conducted. Their findings are presented in Table 1.

The content of extractives in the leaves of red vines ranges from 31.1 to 35.7%. The maximum content of phenolic compounds was found in the leaves of Krasnostop Zolotovskii, the highest content of resveratrol was found in the autochthonous grape varieties: Golubok and Krasnostop Zolotovskii. Quantitative determination of resveratrol in the autochtonous and European vine varieties confirms the assumption that under extreme conditions

Table 1. Content of extractives, total polyphenols and resveratrol in red vine leaves.

Name of vine variety	Content of phenolic compounds, %	Resveratrol content, mg/kg of dry extract	Extractives, %
Saperavi	11.6	42.5	35.5
Cabernet Sauvignon	11.6	104	35.7
Krasnostop Zolotovskii	11.8	164	31.1
Golubok	11.4	170	35.1

Table 2. Comparative analysis of biological activity of the extract of red vine leaves with the use of enzyme bioassay systems.

Name of vine variety	Rate of glutathione reductase reaction, μM/min per 1 mg of protein	Rate of pyruvate kinase reaction, μM/min per 1 mg of protein	Rate of catalase reaction, μM/min per 1 mg of protein
Control	3.97	78.1	1.88
Saperavi	4.11	77.8	2.47
Cabernet Sauvignon	4.56	99.2	2.29
Krasnostop Zolotovskii	4.06	81.8	2.59
Golubok	4.76	113.0	2.35

for cultivation of the vine plant a greater accumulation of resveratrol as an additional reserve substance takes place.

3.2. Studies of biological activity of red vine leaves of different varieties using bioassay systems in vitro

Comparative studies of bioactive properties of the samples of dry residue of red leaves from different vine varieties were carried out. For this purpose we used *in vitro* glutathione reductase and pyruvate kinase bioassay systems, which allowed to identify biologically active compounds directly influencing the activation of glutathione reductase and pyruvate kinase enzymes, respectively, in the samples taken. Obtained results are shown in Table 2.

Glutathione reductase restores glutathione performing antioxidant defense of thiol enzymes. Due to localization of glutathione reductase near the zymophores of thiol enzymes glutathione reductase restores autogenic glutathione in the right place at the right time that is necessary to maintain activity of the enzymes of tissue respiration, glycolysis, biosyntheses of nucleic acids and neurotransmitters, and enzymes of the actomyosin complex, which have direct impacts on maintaining venous tone. Pyruvate kina se, a glycolytic enzyme, transfers energy-rich phosphate residue from phosphoenolpyruvate to ADP, thus replenishing the ATP fund, and replenishing the fund of pyruvate, an important endogenous metabolite. The pyruvate kinase test allows to reveal pyruvate kinase activators making rising effect on the activity of pyruvate kinase and contributing the maintenance of the required rate of glycolysis, the most important metabolic process of living organisms.

Data presented in Table 2 show that *in vitro* samples of Saperavi and Krasnostop Zolotovskii varieties have a little rising effect on the rate of glutathione reductase reaction and virtually have no direct effect on the rate of pyruvate kinase reaction.

Samples of Golubok and Cabernet Sauvignon varieties have a more pronounced significant rising effect on the rate of glutathione reductase reaction and significantly increase the rate of pyruvate kinase reaction.

Samples of extracts of red vine leaves of all varieties examined significantly increase the rate of catalase reaction thus exhibiting antioxidant properties.

3.3. Studies of chemical composition of the extracts of red vine leaves obtained using various techniques

Our previous studies have shown that dry extracts of red vine leaves possessed high biological activity. However, the dry extract obtained by extraction with alcohol water mixture followed by evaporation of the extractant in the vacuum evaporator is not soluble in water. This hamper their use in the manufacture of beverages.

With the aim to select the optimum mode of extraction of red vine leaves, extracts were prepared using different techniques: CO_2-extraction, obtaining highly concentrated hydrophilic extract (HCHE) and dry extraction.

Table 3 presents the results of comparative studies of biochemical composition of the extracts of red vine leaves obtained by different techniques.

Table 3. Comparative analysis of biochemical composition of extracts from red vine leaves.

Sample name	Antioxidant capacity, mM trolox-eq/dm3	Mass concentration of phenolic compounds, mg/dm3, in equivalent of trolox	Mass concentration of resveratrol, mg/dm3	Mass concentration of tocopherol, mg/dm3
CO_2-extract	16.7	4067	442	45.9
HCHE	13,460	16,370	444	0.2
Dry extract	202.3	543.5	220	Trace amount

As it is seen from the table above, HCHE contains more biologically active components as compared with extracts obtained with other techniques. This sample has the highest content of polyphenols including resveratrol. Besides, HCHE contains tocopherol (vitamin E) contrary to the dry extract.

Polyphenols, which present in HCHE, have a powerful antioxidant effect that results in a high amount of antioxidant capacity in this sample, 13,460 mM trolox-eq/dm^3.

It is known that under CO_2-extraction mainly lipophilic fraction is produced from the vegetable raw material. This explains the maximum concentration of fat-soluble tocopherol in the CO_2-extract. Moreover, in this sample it was defined a high content of resveratrol. However, along with its high biological activity, the use of CO_2-extract in the beverage industry is difficult as it represents an oily product, which is not soluble in water.

Findings of these studies allow to make a conclusion that processing methods of obtaining HCHE provide the greatest recovery of biologically active compounds from the raw material in comparison with other extraction methods. Furthermore, HCHE, unlike CO_2-extract and dry extract, is soluble in water.

3.4. Comparative analysis of biochemical composition of alcohol-free beverages produced with addition of HCHE in various concentrations

It is known that polyphenols, which are contained in the juice of red grape varieties, have high antioxidant activity. Due to the low concentration of polyphenols, the juice of white grapes has less biological value, but its production volumes are far superior as compared to production of juice from red grapes. In order to create a cost-effective product with desired biological properties, samples of beverage based on the juice of white grapes with the addition of HCHE in different concentrations were prepared. HCHE were added in amounts of 3%, 4% and 5% of the total volume.

In the prepared beverages there were identified the following indicators: antioxidant capacity (AOC), the mass concentrations of phenolic compounds, resveratrol, vitamins and amino acids. White grape juice without adding HCHE was used as a control. Findings of performed analyses are presented in Table 4.

Table 4. Biochemical indicators of beverages on the base of grape juice with addition of HCHE.

Indicator name	Control	Beverage with addition of 3% HCHE	Beverage with addition of 4% HCHE	Beverage with addition of 5% HCHE
AOC, mM trolox-eq/dm3	311.6	639.3	1275	1293
Mass concentration of phenolic compounds, mg/dm3, in equivalent of gallic acid	69	373	414	505
Mass concentration of resveratrol, mg/dm3	0.01	0.1	0.3	0.9
Mass concentrations of vitamins, mg/dm3:				
Vitamin C	20	25	26	27
Vitamin B2	1.9	6.3	7	8.2
Vitamin B6	3.7	4.6	4.3	4.3
Vitamin PP	1.2	2.1	1.4	1.4

As can be seen from Table 4, the addition of HCHE of red vine leaves in white grape juice increases mass concentrations of biologically active substances. When adding 3% HCHE it was observed sharp increase in antioxidant activity (two-fold compared to the control), the mass concentration of phenolic compounds (2,2 twice as much), and the mass concentration of resveratrol (up to 10 times as much).

Increasing the concentration of HCHE by 1% resulted in a sharp increase in AOE and the mass concentration of resveratrol. When adding 5% HCHE, antioxidant capacity increases slightly compared to the previous sample, but the mass concentration of resveratrol significantly increases (90 times increase compared with the control). Increase in concentration of HCHE has had little effect on polyphenol content.

Adding HCHE in grape juice in an amount of 3% resulted in an increase in the mass concentration of all vitamins identified in the beverage samples. Vitamin B comlex play an important role in normalization of the cardiovascular system, nicotinic acid and vitamin C are strong antioxidants. In addition, ascorbic acid exhibits venotonic properties.

When determining amino acids in the prepared beverages, it was found that addition of HCHE resulted in changes in qualitative and quantitative composition of the beverages. In particular, cysteine was not found in control, whereas this amino acid was presented in all samples of the beverages, and its content increases with increasing HCHE dose. It is known that cysteine is one of the most powerful antioxidants, at that the antioxidant effect of cysteine is enhanced in the presence of vitamin C. It has been demonstrated previously, in beverages prepared using HCHE of red vine leaves, concentration of ascorbic acid increased significantly. Thus, simultaneous increase in cysteine and ascorbic acid increases the antioxidant properties of the beverages. It is evident that with increasing concentration of HCHE in beverages content of almost all amino acids increases.

When selecting the optimal dose of added HCHE we took into account biological value and organoleptic characteristics of the beverages. According to organoleptic evaluation, the beverage with concentration of 4% HCHE had the most complete and well-balanced taste, at that this sample possessed high antioxidant and venotonic properties.

4. Conclusion

The results of performed studies allow to make the following conclusions:

- on the basis of the content of extractives, phenolic compounds, resveratrol and the antioxidant capacity, red vine leaves of autochthonous varieties of Russia are promising vegetable raw materials to produce a comprehensive nutritional supplement with increased biological activity;
- extracts of leaves of red vine varieties like mostly Golubok and Cabernet Sauvignon have an activating effect on the glutathione reductase reaction, indicating the presence of structurally similar elements in the structure of biologically active compounds. Samples of extract of red vine leaves of all varieties significantly increase the rate of catalase reaction, thus exhibiting antioxidant properties;
- extracts of red vine leaves obtained by using different techniques vary significantly in their biochemical composition. To produce a beverage with predetermined properties it is recommended a highly concentrated hydrophilic extract (HCHE);
- juice beverages with addition of HCHE in an amount of 4% have a high antioxidant activity due to increased content of polyphenols, resveratrol, cysteine and vitamin C. Furthermore, these beverages may exhibit venotonic properties owing to increase in the concentrations of vitamin B complex and ascorbic acid. Increasing concentrations of HCHE does not result in a significant increase in the content of biologically active compounds.

It is developed a technology of production of the alcohol-free beverage on the base of white grape juice with addition of HCHE of red vine leaves.

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