

Use of Red Grape Leaves Extracts in Food Production

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Abstract—One of the most promising areas for the development of the viticulture and wine industry is the use of secondary resources. A new element in the solution of this problem can be the use of the vegetative parts of the vine-growing plant, namely, the red leaves, which have a large supply of antioxidants and other valuable biological substances. Cold winters of the northern regions of Russian viticulture are the strong stress for the grape plant. In these conditions, a large number of biologically active substances, including trans-resveratrol, accumulate in the leaves, which are advantageously different from the raw materials, obtained from the regions of uncontaminated viticulture. The main purpose of the work was to study the biologically active compounds composition of red leaves of various grape varieties, as well as the development of ways to use extracts of red grapes leaves in the food products production. The introduction of a highly concentrated hydrophilic extract of red leaves into a non-alcoholic drink on a grape basis increases the content of biologically active compounds. It is proposed to use the extract of red grapes leaves in the production of fat and oil products, which, when stored, are subject to oxidative and hydrolytic processes. Indicators of oxidative and hydrolytic damage of margarine emulsion with the addition of red grape extract are on average 1.5 times lower than in the control sample. Extract from red grape leaves is an alternative to the currently used green tea hydrophilic extract.

Keywords— grapes red leaves, antioxidant capacity, fats oxidative stability, carbon dioxide extraction.

I. INTRODUCTION

Currently, one of the main issues in the life of the processing industries is the use of secondary resources. At the same time, the main factors are the desire on the one hand to mitigate the impact on the environment, on the other - to get additional new products.

This trend is especially relevant in the sectors, involved in the processing of agricultural raw materials, since secondary production resources are of biological origin and can be the starting material for the production of feed and food products [1].

In the production of wine products, the main waste is sweet and fermented grape squeezes, yeast and glue sediments, grape seeds and others. One of the new directions is the use of vegetative parts of the grape plant, which has a seasonal character. These primarily include grape red leaves, which have a large supply of antioxidants and other valuable substances [2-6].

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

Like all polyphenols, resveratrol is a powerful antioxidant, whose biological activity exceeds vitamin E. Resveratrol displays estrogenic activity by binding to the same receptors as estradiol (one of the main human estrogen). Despite the fact that the hormonal activity of resveratrol is significantly lower than that of estradiol, there can be so much in the body, that it will act much more than estradiol in physiological concentrations [7-9].

The extract of the grapes red leaves has an effect on the vascular wall of the veins and helps to eliminate its disturbances. Red leaves of grapes have long been used in France for the treatment of swollen, painful legs. Today, their use in this area is scientifically sound.

The main task in the production of biologically active additives from natural plant raw materials is the production of native compounds without subjecting them to temperature or chemical effects.

A high-quality extract from the red leaves of grapes without negative thermal effects, and at the same time the maximum possible yield of biologically active substances can be achieved by introducing subcritical CO₂ technology. Liquefied carbon dioxide, due to the deep penetration into plant cell organoids, completely removes the lipophilic fraction from the raw material, while the solvent itself completely evaporates without leaving traces in the extract.

After depressurization during the extraction process, an "explosion" occurs - the process of carbon dioxide transition from the liquid state to the gaseous state, which leads to the rupture of the cell walls. The "exploded" ground oil-cake, left after CO₂ extraction, contains a large number of hydrophilic biologically active compounds (polyphenols, resveratrol, vitamins, amino acids, etc.). These compounds, due to the deep destruction of plant cells, almost completely turn into a highly concentrated hydrophilic extract (HCHE), prepared from the ground oil-cake of grapes red leaves.

Rostov region belongs to the zone of concealed winemaking and is located north of other cultivation zones of grapes in Russia. According to researchers, resveratrol is found most in grapes, growing in a cold climate. This substance helps to survive the grapes in the winter. More resveratrol in the trans form is produced in plants in response to stressful situations. As mentioned above, trans-resveratrol has more biological activity than cis-resveratrol. Cold winters of the Rostov region can be considered a strong stress for a grape plant, since the air temperature can drop to -10 °C. Proceeding from this, it was suggested that grape varieties grown in a colder climate contain more trans-resveratrol than grapes grown in milder climates.

Previous studies have shown that red leaves of different varieties of grapes growing in the Rostov region contain a large number of phenolic compounds, including resveratrol. It has been established that autochthonous varieties contain more resveratrol than European varieties [10, 11]. Also, a study was made of the biological activity of grapes red leaves, using glutathione reductase, pyruvate kinase and catalase biotest systems. It is shown that the grape red leaves exert an activating effect on the basic metabolic enzymes, thus manifesting the venotonic, antioxidant and adaptogenic properties [12].

The aim of the work was to study the biologically active compounds composition of red grapes leaves extracts under conditions of cover viticulture and their subsequent use in the production of certain food products.

II. MATERIALS AND METHODS

The grape red leaves were taken by hand from the vineyards of the Vedernikov Winery after the growing season and dried under a canopy to a moisture content of 8-10% (Konstantinovskiy district of the Rostov region 47° 41'N, 42° 24'E).

The dry extract was obtained by extracting the crushed grape red leaves with 24% alcohol solution at a temperature of 20 °C, based on 5 g of solvent per 1 of leaves. The extraction was carried out for 4 hours in 3 stages: 2 hours, 1 hour, 1 hour. After each stage, the miscella was decanted and the raw material was poured with a new portion of the solvent. The extract was concentrated on a rotary evaporator in vacuum at a temperature of 55 °C 15 times. Further evaporation was carried out in porcelain dishes in a water bath to constant weight. The resulting dry mass was ground into a powder.

The HCHE was prepared from the dry ground oil-cake of dried red leaves after CO₂ extraction, using subcritical extraction parameters, pressure of 65.5 atm. and a temperature of 25.5 °C for 2 hours. Next, the ground oil-cake with the destroyed cell structure was extracted in a Soxhlet apparatus. The weight of raw material in 1 kg was washed three times with 2 kg of the 70% water-alcohol mixture. The resulting miscella was concentrated on a rotary evaporator at a pressure of 0.19 atm. 10 times.

The qualitative and quantitative composition of microelements in the red leaves of the grapes was determined by X-ray fluorescence analysis. The intensities of the analytical lines of the elements were measured with a S4 Pioneer wave spectrometer (Bruker AXS, Germany).

The antioxidant capacity was determined by spectrophotometric method with use of the ABTS cation-radical spectrophotometer Carry 100 Bio (USA).

The mass concentration of amino acids was determined by high performance liquid chromatography (HPLC), using an automatic amino acid analyzer LC 3000 by Eppendorf-Biotronic (Germany).

The mass concentration of amino acids was determined by high-performance liquid chromatography using an automatic amino acid analyzer. Analysis of the amino acid content was performed on an amino acid analyzer of LC 3000 type by Eppendorf-Biotronic (Germany). The results were treated statistically using Student's criterion t-test.

III. RESULTS AND DISCUSSION

A. Studies of the Biochemical Composition of Grapes Red Leaves

After harvesting, red leaves of Cabernet Sauvignon, Saperavi, Krasnostop Zolotovskiy and Golubok varieties grown in the Rostov region were collected.

Previous studies have shown that red leaves of different varieties of grapes growing in the Rostov region contain a large number of phenolic compounds, including resveratrol. Table 1 presents data on the antioxidant capacity of the dry extract of red leaves of grapes of various varieties growing in the Rostov Region.

The highest antioxidant capacity is observed in a sample of a dry extract of Cabernet Sauvignon variety of red leaves - 95.4 mmol of trolox-eq/g. In the same sample, the highest content of phenolic compounds was found, 2.85%. [13]. The second one by the value of antioxidant capacity is a sample of the Golubok variety - 82.1 mmol trolox-eq/g. According to previous studies, the sample of the Golubok variety of red leaves extract contains the largest amount of resveratrol - 170.5 mg/kg, while both samples are capable of exerting a powerful activating effect on the actomyosin enzyme complex, which causes venotonic and adaptogenic properties [14]. Thus, it is advisable to use the red leaves of the Golubok and Cabernet Sauvignon varieties for obtaining extracts.

TABLE I. ANTIOXIDANT CAPACITY IN EXTRACTS FROM GRAPES RED LEAVES

Name of Grape Variety	Saperavi	Golubok	Krasnostop Zolotovskiy	Cabernet Sauvignon	Tsimlyansky Black
Antioxidant Capacity, mmol trolox-equiv/g	79.3	82.1	75.8	95.4	76.2

In the red leaves of the grapes, a high calcium content of 3.3% (from the total dry mass) was found; a significant concentration of silicon was 0.71%, magnesium - 0.42%, potassium - 0.25%. These elements play an important role in the vital activity of the body, are directly involved in the normalization of the cardiovascular system, and the metabolism of cells, the generation of vitamin C in the body, are part of the bone tissue.

B. Study of acute toxicity of grape red leaves

It is generally accepted that most herbal medicines are safe to use. However, every year there are more and more publications about negative side reactions caused by the use of herbal medicines and due to several reasons: the quality of raw materials, the method of obtaining and the chemical composition of the selected biologically active substances, the choice of doses, the method and duration of their use, etc. To ensure the safety of the raw materials, the Scientific Center of Biomedical Technologies conducted a study of the acute toxicity of red leaves of the Cabernet Sauvignon variety.

Male mice of the BALB/c line (strain) aged 2 to 3 months with a body weight of 20 g, bred in the nursery of the Andreevka branch, were selected as laboratory animals for studying acute toxicity. The duration of observations of laboratory animals was 14 days. In the course of the study,

their appearance, behavior, motor activity, reaction to external stimuli were monitored. The clinical picture of "acute" poisoning of laboratory animals was recorded, when the substance was administered at doses, close to the mean. The results of the studies show, that with a single injection into the stomach of mice, the extract of grapes red leaves of the Cabernet variety is a low-toxic substance and belongs to the 4th toxicity class according to the toxicity classification according to Hodge and Sterner.

C. Soft Drink on a Grape Basis Technology Development

It is known that polyphenols contained in the juice of red grape varieties have high antioxidant activity. Due to the small amount of polyphenols, the juice of white grape varieties has less biological value, but the volume of its production is much higher than the volume of juice, produced from red grapes. In order to create an economical product with predetermined biological properties, samples of a juice drink, made from white grapes with the addition of HCHE in different concentrations, were prepared. HCHE was added in amounts of 3%, 4% and 5% of the total volume. An increase in the dosage of the extract over 5% led to undesirable changes in taste characteristics.

In the samples of beverages studied, the qualitative and quantitative composition of amino acids was determined.

TABLE II. QUALITATIVE AND QUANTITATIVE COMPOSITION OF AMINO ACIDS OF DRINKS BASED ON GRAPE JUICE WITH ADDITION OF HCHE

Mass Concentration of Amino Acids, mg / dm ³	Samples Designation.			
	Control	Drink with addition of 3% HCHE	Drink with addition of 4% HCHE	Drink with addition of 5% HCHE
Asparagine	221	528	645	766
Tryptophan	398	418	466	502
Serin	682	1287	1455	1668
Glutamine	974	1411	1591	1772
Glycine	246	596	720	864
Alanin	290	416	496	543
Cysteine	Not detected	166	214	281
Valine	171	289	317	378
Methionine	41	179	209	235
Isoleucine	259	398	462	504
Leucine	538	607	620	680
Tyrosine	268	369	391	418
Phenylalanine	240	264	266	280
Histidine	629	635	634	636
Lysine	200	664	751	934
Arginine	572	783	811	887
Proline	944	946	948	954

As can be seen from Table 2, when applied to the grape juice of HCHE, the quantitative and qualitative composition of amino acids changes. In particular, cysteine is not detected in the control, whereas in all beverage samples this amino acid is present, and its amount increases with increasing dosage of HCHE. It is known that cysteine is one of the most powerful antioxidants, while its antioxidant effect is enhanced in the presence of vitamin C. In this regard, the simultaneous increase in cysteine and ascorbic acid increases the antioxidant properties of beverages, which is confirmed by the results of the studies.

Adding a hydrophilic extract from red grape leaves to white grape juice significantly increases the phenolic compounds mass concentration, including resveratrol, antioxidant capacity, and also leads to an increase in the mass concentration of vitamins PP, B6, B2, C [14]. Juice containing drinks, prepared with the introduction of HE has high antioxidant activity due to an increase in the content of polyphenols, resveratrol and cysteine. In addition, prepared drinks can exhibit venotonic properties, including by increasing the concentration of B vitamins and ascorbic acid.

D. The Application of the Extract of Grapes Red Leaves to Increase the Oxidative Stability of Margarine Emulsions

Oxidative damage to margarines, like vegetable oil, is due to the accumulation in them of low-molecular compounds, peroxides, aldehydes, free fatty acids, ketones, etc., which leads to a sharp deterioration in the taste of the product. Margarines retain their quality well at negative temperatures and poorly withstand storage in conditions of positive temperatures. At present, in the production of margarines, the

hydrophilic extract of green tea is actively used as a biologically active additive, which prevents oxidation and increases shelf life.

Together with the Moscow branch of the All-Russian Research Institute of Fats, a study was conducted aimed at studying the effect of HCHE of red leaves of grapes on the oxidative stability of margarine emulsion during storage at various plus temperatures in comparison with the hydrophilic extract of green tea.

In laboratory labs, fat-water emulsions of the "margarine" type with a mass fraction of fat of 82.0% were prepared with the following initial components:

- refined deodorized palm oil - 58%;
- refined deodorized coconut oil - 12%;
- refined deodorized sunflower oil;
- food emulsifier - 0.4%;
- water - 17.55%.

HCHE grape red leaves and hydrophilic green tea extract used in the industry to stabilize margarines were introduced into the aqueous phase of the emulsion in an amount of 0.05% by weight of the product. As a control, a margarine emulsion without extracts is prepared. Samples of margarine emulsions were stored for various positive temperatures for the purpose of subsequent investigation of the fat phase isolated from them in terms of oxidative and hydrolytic damage. Data on the oxidative changes in the fat phase isolated from margarine emulsions are given in Table 3.

TABLE III. STUDY OF DIFFERENT NATURE EXTRACTS INFLUENCE ON STABILITY OF MARGARINE EMULSIONS

Sample Name	Indicator Name (for the fat phase isolated from margarine)		
	Acid Number, mg KOH/g	Peroxide Number, mmol ½ O/kg (meqv O/kg)	Anisidine number, c.u.
Initial Margarine Emulsion - before Storage	0.37± 0.1	1.09± 0.01	1.15± 0.2
30 days storage at a temperature of 20 ± 2 °C			
Margarine Emulsion without Extracts	0.66± 0.1	8.31± 0.01	4.04± 0.2
Margarine Emulsion. + HCHE of Grape Red Leaves	0.40± 0.1	2.52± 0.01	2.60± 0.2
Margarine Emulsion + Hydrophilic Extract of Green Tea	0.42± 0.1	2.60± 0.01	3.37± 0.2
180 days storage at a temperature of 5 ± 1 °C			
Margarine Emulsion without Extracts	0.71± 0.1	9.34± 0.01	2.12± 0.2
Margarine Emulsion. + HCHE of Grape Red Leaves	0.42± 0.1	4.83± 0.01	2.00± 0.2
Margarine Emulsion + Hydrophilic Extract of Green Tea	0.42± 0.1	6.19± 0.01	1.84± 0.2

The results of the maintained studies showed that margarine emulsions with the addition of extracts have better resistance to oxidation processes. Hydrolytic processes in

samples with the introduction of extracts also proceed more slowly in a control sample of margarine.

HCHE from grape red leaves positively affects the stability of the margarine emulsion to hydrolytic processes, restraining the latter at the level of the hydrophilic extract of green tea. As a result of this, the acid number of the fat phase isolated from the margarine sample with the addition of HCHE is on average 1.5 times lower for the control sample. For a sample with the addition of HCHE from red leaves of grapes, a lower peroxide value is characteristic compared to the control one regardless of temperature and storage time. The anisidine number of this sample was also below the control and during storage for 30 days at a temperature of 20 ± 2 °C is much less than that of a sample with green tea extract.

According to the organoleptic parameters of margarine emulsions with the addition of extracts at the end of the entire storage period at the investigated temperatures, they did not have discoloring flavors and smells, remained practically impersonal. The control sample stored under the same conditions was characterized by a salty aftertaste and a slight bitter aftertaste indicating that hydrolytic and oxidative damage occurred.

Margarines obtained with use of HCHE red leaves of grapes are an innovative product which includes valuable vegetable raw materials. In addition, when manufacturing HCHE red leaves, production waste related to the secondary resources of the viticulture and wine industry is used, which is very relevant at the present time. The introduction of such product into production will allow expanding the range of produced fat-and-oil products with valuable biological properties.

IV. CONCLUSIONS

1. Red grape leaves growing in areas with a cold climate are a source of antioxidants (phenolic compounds and resveratrol). In the samples of red leaves of the varieties Golubok and Cabernet Sauvignon the greatest content of these compounds was noted.
2. Application of the CO₂-extraction technology during processing of red grape leaves makes it possible to obtain extraction cake with a high content of biologically active substances due to the destruction of the cellular structure during the extraction process. The HCHE obtained from such extraction cake is a valuable biological additive for use in the food industry.

3. The use of HCHE from the extraction cake after CO₂-extraction in the technology of soft drinks made it possible to obtain a drink with a high biological activity.
4. Margarine emulsions with the addition of HCHE have increased resistance to oxidative and hydrolytic processes during storage under conditions of positive temperatures. HCHE can serve as an alternative to the currently used hydrophilic green tea extract.

References

- [1] N.I. Maznev, Medicinal Plants: a Guide, Moscow, 1999.
- [2] M. Kosar, E. Küpeli, H. Malyer et al. "Effect of brining on biological activity of leaves of *Vitis vinifera*", Journal of Agricultural and Food Chemistry, vol. 55, no. 11, 2007, pp. 4596-4603.
- [3] K. Kotzabasis. M.D. Christakis-Hampsas. K.A. Roubelakis-Angelakis "A narrow-bore HPLC method for the identification and quantitation of free conjugated and bound polyamines", Analytical Biochemistry, vol. 214, no. 2, 1993, pp. 484-489.
- [4] M.G. Bezhuashvili. M.Yu. Meskhi. M.V. Bostoganashvili. M.L. Malaniya "Antioxidant activity of stilbene-containing extract in vitro experiments", Winemaking and Viticulture, no. 3, 2005, pp. 26-27.
- [5] D.D. Orhan. N. Orhan. E. Ergun "Hepatoprotective effect of *Vitis vinifera* L. leaves on carbon tetrachloride-induced acute liver damage in rats", Journal of Ethnopharmacology, vol. 112, no. 1, 2007, pp. 145-151.
- [6] J. A. Baur, K. J. Pearson, N. L. Price et al, "Resveratrol improves health and survival of mice on a high-calorie diet", Nature, p. 337.
- [7] S.H. Eo, H. Cho, S.J. Kim "Resveratrol inhibits nitric oxide-induced apoptosis via the nf-kappa b pathway in rabbit articular chondrocytes" Biomol Ther (Seoul), no. 5, 2013, p. 364.
- [8] Y.-J. Cho, J.-Y. Hong, H.S. Chun, S.K. Lee, H.-Y. Min "Ultrasonication-assisted extraction of resveratrol from grapes", Journal of Food Engineering, vol. 77, no. 3, 2006, pp. 725-730.
- [9] N. Orhan, M. Aslan, D.D. Orhan, "In-vivo assessment of antidiabetic and antioxidant activities of grapevine leaves (*Vitis vinifera*) in diabetic rats", Journal of Ethnopharmacology, vol. 108, no. 2, 2006, pp. 280-286.
- [10] L.A. Oganesyants, A.L. Panasyuk, E.I. Kuzmina, D.A. Sviridov, "Use of CO₂ extracts from secondary winemaking resources as a food additive for vegetable oils", Food Industry, no. 3, 2014, pp. 42-43.
- [11] A.L. Panasyuk. E.I. Kuzmina. D.A. Sviridov. T.E. Kostsova, "Deep processing of winemaking waste using carbon dioxide extraction", Food Industry, no. 8, 2014, pp. 17-19.
- [12] L.A. Oganesyants. A.L. Panasyuk. E.I. Kuzmina "Study of features of the biochemical composition of red vine leaves of autochthonous varieties in Russia", BIO Web of Conferences, vol. 5, no. 02018.[38th World Congress of Vine and Wine of OIV, Mainz, 2015]