RATIONAL USE OF THE SECONDARY RESOURCES OF THE VINEYARD AND WINEBRANDING INDUSTRY

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1. Introduction

The use of secondary resources is one of the main tasks in ensuring the productivity of processing industries. At the same time, it is fundamental, on the one hand, to mitigate the impact on the environment, and on the other, to obtain additional new types of products. This is especially relevant in industries engaged in the processing of vegetable raw materials, since the secondary production resources are of biological origin and can be the starting material for food production.

In grape processing, secondary resources are primarily the remnants of the generative plant organs (berries) — sweet and fermented husks of grapes, seeds, peels, etc. However, it is promising the use of vegetative organs such as vines and vine leaves. [1].

The leaves of red grapes in autumn are colored red, due to the anthocyanins presence. In France, for a long time (the first references refer to the 17th century), red vine leaves were used to treat swollen, painful feet. The peasants working in the vineyards, after the grapes were harvested, collected red leaves, insisted on grape alcohol and were used as compresses for the legs in winter, as well as consumed with a teaspoon inside. It is known, that France has the lowest incidence rate of varicose veins, which is largely facilitated by the use of red grape leaves as a medicine. Red grape leaves are officially listed in the 9th edition of the French Pharmacopoeia. The indications for their use are venous insufficiency, expressed by the symptom of «heavy legs», swelling, and the manifestation of fragility of the vessels and capillaries. [2–5]. It has been established, that the alcoholic extract of grape leaves, exhibits antioxidant activity, has a hepatoprotective and neuroprotective effect [6,7,8].

The main biologically active components of the leaves are catechins, flavonoids, tannins, malic, silicic, citric, tartaric and succinic acids and resveratrol. According to the literature, grape leaves contain polyphenolic compounds: hydroxy derivatives of stilbenes — resveratrol, viniferin, astringin, piceid; flavonoids — quercetin, isoquercetin; as well as phenol carboxylic acids, tannins, anthocyanins, lipids, sugars, terpenoids, vitamins; polyamines — putrescine, cadaverine, spermidine, spermine, agmatine and other components [9,10].

Resveratrol is a substance, belonging to the stilbene group. There are evidences of studying stilben-containing extract of grapevine pruning [11]. Resveratrol is phytoalexin, which is naturally produced by certain plants in response to stress, damage, fungal infection and ultraviolet radiation. It is found in the skin and stem of red grapes, raspberries, mulberries, plums, peanuts, blueberries and blueberries, red leaves of grapes, as well as in the roots and stems of the Japanese knot grass (Polygonum aviculare) [12,13].

In nature, there are two forms of resveratrol: cis-resveratrol and trans-resveratrol. Trans-resveratrol is more stable form. Under the influence of ultraviolet rays, it turns into possessing a large supply of energy, and therefore less stable, cis-resveratrol. Compared with the cis form (cis-3,4-5-trihydrostilbene), trans-resveratrol (trans-3,4-5-trihydrostilbene) has a greater biological and antioxidant activity [14].

The main property of dietary supplements, containing resveratrol and red grape leaf extract is the ability to strengthen the walls of the thinnest capillaries, reduce their permeability, restore their resistance, ensuring their normal functioning and improving blood microcirculation. Resveratrol also normalizes heart rhythm and blood pressure. Resveratrol lowers cholesterol and triglycerides in plasma. It also prevents lipoprotein oxidation, which prevents the possibility of its deposition on the walls of blood vessels, thereby reducing cardiovascular diseases risk. Resveratrol has antibacterial and anti-inflammatory effects, stimulates natural defense mechanisms, improves blood circulation in the tissues [1,15,16,17].

According to researchers, most resveratrol is found in grapes growing in cold climates. This substance helps the grapes to survive in winter. A large amount of resveratrol in trans form forms in plants in response to stressful situations.

In the conditions of our country, the use of red grape leaves is particularly effective due to special climatic conditions. First, part of the vineyards is located in the area of sheltered viticulture, and therefore, to improve wintering conditions before the shelter, as a rule, ripe leaves are purposefully cut off, which is practiced primarily in the Rostov region. Secondly, the grape plant resists shock from the cold better, producing increased amounts of resveratrol, which makes red leaves a very valuable raw material in terms of the content of biologically active substances.
2. Materials and Methods

The All-Russian Scientific Research Institute of Brewing, Non-Alcoholic and Wine Industry, together with the Institute of Medicinal and Aromatic Plants of the Russian Academy of Sciences, conducted studies on the physicochemical composition and biological activity of red grape leaves. After the harvest were selected samples of red autochthonous and European grape varieties grown in the Rostov region — Krasnostop Zolotovsky, Tsimlyansky Black, Golubok, Cabernet Sauvignon, Saperavi.

Dry extracts from red grape leaves of different grape varieties were prepared under laboratory conditions at the All-Russian Research Institute of Medicinal and Aromatic Plants according to the method, described in the Pharmacopoeia of the Russian Federation OFS.1.4.1.0018.15

A comparative study of dry extracts of different red grape leaves varieties enzymatic activity, as well as antistax venotonic and chemically pure resveratrol, was carried out in accordance with the methods, described in RF patents No. 2181890. 2001, № 2181891. 2002, No. 2181892. 2002.

The study of the acute toxicity of red grape leaves variety Cabernet Sauvignon was carried out in accordance with GOST R53434–2009 «Principles of Good Laboratory Practice» and «Guidelines for the Experimental Study of New Pharmacological Substances» 2005.

3. Results and discussion

It has generally recognized, that most medicinal herbal preparations are safe to use. However, every year there are more and more publications about negative adverse reactions caused by taking herbal medicines for several reasons: raw materials quality, method of preparation and chemical composition of the selected BAS, the choice of doses, the method and duration of their use, etc. [18]. To make sure in feedstock safety, the Scientific Center for Biomedical Technologies of the Russian Academy of Medical Sciences conducted a study of the acute toxicity of red leaves of Cabernet Sauvignon grapes. The results of the studies indicate that when administered once to the stomach of mice, the extract of red grape leaves is a low-toxic substance and belongs to the fourth class of toxicity according to the Hodge and Sterner toxicity classification.

In selected samples of red grape leaves were determined the moisture content, the content of extractive substances, the content of phenolic compounds, the antioxidant capacity and resveratrol mass concentration. The amount of phenolic compounds were recalculated to rutin. As it known, rutin is an organic compound from the group of flavonoids, quercetin-3-rutinoside, which has a powerful angioprotective effect. The research results are summarized in Table 1.

From the obtained results it can be seen, that the moisture content of analytical samples of plant materials varies from 8.35 % to 9.91 %. Samples of Saperavi, Golubok, and Cabernet Sauvignon are characterized by a high content of extractive substances. Red leaves of Cabernet Sauvignon variety have the highest content of phenolic compounds — 2.85 %. The quantitative determination of resveratrol in autochthonous and European grape varieties confirms the assumption, that under extreme cultivation conditions, there is a greater accumulation of resveratrol as a protective substance. The highest content of resveratrol is observed in autochthonous grape varieties. The maximum content of resveratrol was observed in the autochthonous variety Golubok — 170.5 mg/kg.

In order to select the most promising grape varieties with respect to venotonic and antioxidant actions, had been conducted researches to study the biological activity of extracts of red grape leaves of using glutathione reductase, pyruvate kinase and catalase biotest systems. Chemically pure resveratrol and antistax venotonic drug were used as reference agents. The data are presented in Figure 1.

As a result of the research it was found, that extracts of red grape leaves of all varieties increase the speed of the above reactions. The extract of red leaves of the Cabernet Sauvignon grape variety has rather high indicators, while the area of plantings considerably exceeds the area of other varieties planting. Thus, it is advisable to use red leaves of Cabernet Sauvignon grapes as a raw material for the production of preparations with a targeted venotonic action, as well as for food products production with increased physiological value.

Table 1

| Grape variety name | Moisture, % | Extractive substances, % | Resveratrol mass concentration, mg/kg | AC, mmol of trolox eq/dm³ | Phenolic substances, % |
|--------------------|------------|--------------------------|--------------------------------------|--------------------------|------------------------|
| Cabernet Sauvignon | 8.47 ± 0.5 | 23.4 ± 1.2                | 104.2 ± 0.8                          | 95.4 ± 0.02              | 2.85 ± 0.08            |
| Krasnostop Zolotovsky | 9.91 ± 0.5 | 20.9 ± 1.2                | 164.5 ± 0.8                          | 75.8 ± 0.02              | 2.15 ± 0.08            |
| Saperavi           | 8.35 ± 0.5 | 27.5 ± 1.2                | 42.5 ± 0.8                           | 79.3 ± 0.02              | 2.14 ± 0.08            |
| Tsimlyansky Black  | 9.24 ± 0.5 | 16.0 ± 1.2                | 156.7 ± 0.8                          | 76.2 ± 0.02              | 2.15 ± 0.08            |
| Golubok            | 8.83 ± 0.5 | 23.6 ± 1.2                | 170.5 ± 0.8                          | 82.1 ± 0.02              | 2.24 ± 0.08            |

Figure 1. The effect of dry extract of red grape leaves of different varieties on the rate of enzymatic reactions.
Red grape leaves dry extract has a high biological activity, but it is not soluble in water, which makes it impossible to use it in this form to obtain drinks with high physiological value. In order to select the optimal modes of red grape leaves extracting, extracts were prepared using various technological methods: CO₂-extract, hydrophilic extract, dry extract.

The CO₂-extract had been obtaining under production conditions of «Biocevtica Ltd» on the EV-130 extractor using subcritical extraction parameters — pressure of 65.5 atm. and a temperature of 25.5 °C. Such parameters make it possible to obtain high-quality extract without negative heat exposure and at the same time to achieve the fullest yield of fat-soluble biologically active substances. In the process of extracting liquefied carbon dioxide turning into a gaseous form, breaks the membrane and organoids of the cells of the raw material, and extracts the lipophilic component. After CO₂-extraction, the so-called «blasted» meal, freed from the lipid fraction, remains, with a destroyed cell structure, which contains a large amount of valuable hydrophilic compounds. This meal was used in further work to obtain a hydrophilic extract. The extraction was carried out with a water-alcohol mixture, followed by concentration. Thus, three types of extracts were obtained from red grape leaves — dry extract, hydrophilic extract (HT) and CO₂-extract. Figure 2 shows the scheme for obtaining extracts of different nature from red grape leaves.

Were carried out comparative studies of red grape leaves extracts biochemical composition, obtained by various methods. Table 2 shows the results of the researches.

As can be seen from the table, HE contains more biologically active compounds as compared with extracts, obtained by other methods. In this sample, the highest polyphenols content, including resveratrol. In addition, tocopherol (vitamin E) is present in the HE, in contrast to the dry extract. This is explained by the fact, that the gap in the walls and organoids of the cells in the process of CO₂-extraction provides a more complete yield of biologically active compounds. The polyphenols, which are present in red grape leaves, have a powerful antioxidant effect, are almost completely transferred to the hydrophilic extract. They cause a high rate of antioxidant capacity (AC) in this sample.

It is known, that during CO₂-extraction, the lipophilic fraction is mainly extracted from plant materials. This explains the maximum concentration of fat-soluble tocopherol in the CO₂-extract. In addition, this sample has a high content of resveratrol. However, with high biological activity, the use of CO₂-extract in drinks production is difficult, as it is an oily product, insoluble in water.

In the analysis of red grape leaves dry extract biochemical composition, it was found, that the content of resveratrol and phenolic compounds in it is lower compared with other samples of extracts. At the same time, tocopherol is practically absent in the dry extract.

The results of the conducted studies allow us to conclude that the technological regimes for obtaining HE provide the greatest extraction of biologically active compounds from the raw materials compared to other methods of extraction. In addition, HE, unlike the dry extract, is highly soluble in water, which makes it the optimal biologically active additive in soft drinks production.

Because of the small amount of polyphenols, the juice of white grape varieties has a lower physiological value, but its production volumes significantly exceed the red grape juice production. In order to create an economical product with desired

### Table 2

| Sample Name       | Antioxidant capacity, mmol Trolox-eq/dm³ | Mass concentration of phenolic compounds, in terms of gallic acid, g/dm³ | Mass concentration of resveratrol, g/dm³ | Mass concentration of tocopherol, mg/dm³ |
|-------------------|-----------------------------------------|------------------------------------------------------------------------|------------------------------------------|-----------------------------------------|
| CO₂-extract       | 2.02 ± 0.1                              | 2.01 ± 0.1                                                             | 0.22 ± 0.01                              | 45.9 ± 2                                |
| Hydrophilic extract | 13.5 ± 0.6                              | 4.46 ± 0.1                                                             | 0.44 ± 0.01                              | 0.2 ± 0.01                              |
| Dry extract       | 0.17 ± 0.008                            | 1.11 ± 0.1                                                             | 0.04 ± 0.002                             | traces                                  |
The original oil, before the addition of CO₂-extracts, was analyzed by the main indicators of oxidative damage — this is the peroxide value and the anisidine number. Organoleptic studies of oil showed the absence of extraneous smells and tastes, i.e. the oil was completely impersonal to taste, had no smell and met the requirements of GOST 1129–2013 («Sunflower oil. Technical conditions») for the refined deodorized sunflower oil. The results of physicochemical studies of oil samples — initial and after storage for 6 months are presented in Figure 3.

The results of the research indicate that the samples of sunflower oil No. 1–4 with the addition of CO₂-extracts obtained from the secondary resources of the wine-growing industry, after 6 months of storage in room conditions, did not have discrediting taste characteristics and smells. At the same time, they were distinguishing by a weak herbal smell of varying degrees of intensity due to the extracts introduced into the oil. The control sample had a rich and bitter taste. When comparing the physicochemical parameters of the oil samples with CO₂-extracts and the control sample, it was found that after 6 months of storage for all experimental samples, the indicators of oxidative damage — peroxide and anisidine numbers — are better than for the control sample.

No less urgent is the question of increasing the shelf life of margarine emulsions. Oxidative spoilage of margarines, like vegetable oil, is caused by the accumulation of low-molecular compounds, peroxides, aldehydes, free fatty acids, ketones, etc., in them, which leads to a sharp deterioration in the taste properties of the product. Margarines retain their quality well at low temperatures and withstand storage in conditions of positive temperatures rather poorly. In this regard, the study of the kinetics of oxidation of margarine emulsions during storage at different positive temperatures is a very urgent task.

Currently, in the production of margarine emulsions, imported hydrophilic green tea extract is used as a dietary supplement that prevents oxidation and increases shelf life. To determine the optimal supplement for margarines, CO₂-extract from white grape seeds and CO₂-extract of grapes husks without red grape seeds were selected as a lipophilic additive, as well as red grape leaf HE and imported hydrophilic green tea extract as a water-soluble additive. Under laboratory conditions of the Moscow branch of the Moscow branch of All-Russian Scientific Research Institute of Fats, water-emulsions of the «margarine» type were prepared with a fat content of 82.0.

In the fatty phase, were added CO₂-extract from white grapes seeds and CO₂-extract of grapes husks without red grape seeds. A hydrophilic extract of red grapes leaves and a hydrophilic extract of green tea were added to the aqueous phase of the emulsion. All additives are introduced in the amount of 0.05% by weight of the product. As a control, a margarine emulsion was prepared without extracts addition. Samples were stored at room temperature for 30 days and at 5 °C for 180 days. The research results are presented in Table 4.

### Table 5

| Indicator Name                          | Sample Name                  | Control | Drink with addition of 3% HE | Drink with addition of 4% HE | Drink with addition of 5% HE |
|----------------------------------------|------------------------------|---------|-----------------------------|----------------------------|----------------------------|
| Mass concentration of phenolic compounds, mg gallic acid/dm³ | 169 ± 2                      | 375 ± 5 | 442 ± 5                     | 505 ± 5                     |
| Mass concentration of resveratrol, g/dm³  | traces                       | 15.4 ± 0.8 | 17.8 ± 0.8                  | 22.2 ± 0.8                  |
| Mass concentration of vitamin PP (nicotinic acid), mg/dm³ | 1.2 ± 0.1                    | 2.1 ± 0.1 | 2.4 ± 0.1                   | 2.7 ± 0.1                   |
| Mass concentration of vitamin B6 (pyridoxine), mg/dm³ | 3.7 ± 0.2                    | 4.6 ± 0.2 | 4.9 ± 0.2                   | 5.2 ± 0.2                   |
| Mass concentration of vitamin B2 (riboflavin), mg/dm³ | 1.9 ± 0.2                    | 6.3 ± 0.2 | 7.7 ± 0.2                   | 9.9 ± 0.2                   |
| Mass concentration of vitamin C (ascorbic acid), mg/dm³ | 20 ± 1.2                     | 25 ± 1.2 | 26,6 ± 1.2                  | 27.9 ± 1.2                  |

Today, the problem of increasing the shelf life of fat and oil products is relevant. In the vegetable oil that has been stored for a long time, there appears a rancid taste, a greasy, oliphic taste and smell. In this regard, were conducted studies, aimed at increasing the shelf life of vegetable oils.

To obtain a CO₂-extract from grape seeds at the winery of the Krasnodar Territory, were selected samples of white grapes husks (Aligote) and red varieties (Cabernet Sauvignon). From the husks of white grapes and part of the red varieties husks, the husks (Aligote) and red varieties (Cabernet Sauvignon). From the Krasnodar Territory, were selected samples of white grapes prototype of CO₂-extracts of separately grape seeds and separation conditions of «Biocevtica Ltd» (Limited Liability Company), seeds;
Figure 3. Physicochemical indicators of oil samples after 6 months of storage

Research of extracts of different nature effect on the margarine emulsions (ME) oxidative stability

| No. | Sample Name | Indicator Name | Acid number, mg KOH/g | Peroxide value, mmol ½O/kg (mEq O/kg) | Anisidine number, cu |
|-----|-------------|----------------|----------------------|--------------------------------------|----------------------|
| n/n | Initial ME — before putting it into storage |               | 0.37 ± 0.1           | 1.09 ± 0.01                          | 1.15 ± 0.2           |
|     | 30 days of storage at a temperature of 20 ± 2 °C |               | 0.66 ± 0.1           | 8.31 ± 0.01                          | 4.04 ± 0.2           |
| C   | ME without extracts |               | 0.39 ± 0.1           | 2.37 ± 0.01                          | 3.02 ± 0.2           |
| 1   | ME + CO2-extract of white grape seeds |               | 0.45 ± 0.1           | 2.63 ± 0.01                          | 2.16 ± 0.2           |
| 2   | ME + CO2-extract of grapes husks without red grape seeds |               | 0.40 ± 0.1           | 2.52 ± 0.01                          | 2.60 ± 0.2           |
| 3   | ME + hydrophilic extract of red grape leaves |               | 0.42 ± 0.1           | 2.60 ± 0.01                          | 3.37 ± 0.2           |
| 4   | ME + hydrophilic green tea extract |               | 0.42 ± 0.1           | 2.60 ± 0.01                          | 3.37 ± 0.2           |
|     | 180 days of storage at a temperature of 5 ± 1 °C |               | 0.71 ± 0.1           | 9.34 ± 0.01                          | 2.12 ± 0.2           |
| C   | ME without extracts |               | 0.41 ± 0.1           | 3.23 ± 0.01                          | 2.04 ± 0.2           |
| 1   | ME + CO2-extract of white grape seeds |               | 0.45 ± 0.1           | 3.14 ± 0.01                          | 1.62 ± 0.2           |
| 2   | ME + CO2-extract of grapes husks without red grape seeds |               | 0.42 ± 0.1           | 4.83 ± 0.01                          | 2.00 ± 0.2           |
| 3   | ME + hydrophilic extract of red grape leaves |               | 0.42 ± 0.1           | 6.19 ± 0.01                          | 1.84 ± 0.2           |
The results of the studies showed that margarine emulsions with the addition of CO2-extracts of white grape seeds and husks without red grapes seeds have better resistance to oxidative processes during storage at different positive temperatures compared to the model emulsion — control. Hydrolytic processes in samples with the introduction of extracts also proceed more slowly than in the control sample of margarine. HE from red grape leaves has a positive effect on hydrolytic processes, restraining the latter at the level of the hydrophilic green tea extract used in the margarine industry, as a result of which the acid value of the fatty phase isolated from the sample of margarine No. 3 is, on average, 1.5 times lower than for control sample No. 5 (the calculation was made as an arithmetic average for 2 storage temperatures). For the hydrophilic extract from red grape leaves, a lower peroxide value of the fatty phase of margarine is characteristic compared with control No. 5, regardless of temperature and storage time. Anisidine number of sample No. 3 was lower than control No. 5, and when stored for 50 days at a temperature of 20 ± 2 °C, 1.3 less than the sample No. 4 with green tea extract. The organoleptic characteristics of margarine emulsions No. 1–4 at the end of the entire storage period at the studied temperatures did not have discrediting tastes and smells, remained practically impersonal, with the exception of sample No. 3, which had a slight taste due to the extract. The control sample No. 5, which was stored in the same conditions, was characterizing by a mellow flavor and a weak bitter aftertaste, which indicates the occurrence of hydrolytic and oxidative damage. In general, it can be concluded that researched CO2-extracts can serve as an alternative to the imported hydrophilic green tea extract currently used in the margarine industry. Fats and oils are widely used for roasting various foods. When frying products for a long time in the same deep fat, there is a significant change in the oxidative stability of oils, which has a negative impact on the products quality and safety. Studies have been conducted to determine the possibility of using CO2-extracts as substances that enhance the vegetable oils oxidative stability that are exposed to many hours of high-temperature processing, restraining the latter at the level of the hydrophilic green tea extract currently used in the margarine industry. The control sample was an oil without added extracts. Sampling frequency — every 3 hours. Indicators of oils oxidative deterioration — acid, peroxide and anisidine numbers were determined. In addition, to assess the oils oxidative stability, an additional indicator was used — the extinction coefficient, characterizing the accumulation of diene compounds in oils. The research results are summarized in Table 5.

As can be seen from the results of studies, in oil samples with the introduction of CO2-extracts, the indicators of oxidative damage are better compared with the control sample. At the same time, the acid number of all oil samples in the process of thermostating increased very little, i.e. the breakdown of triglycerides was minimal. The best results were obtained for a sample with a CO2-extract of sea buckthorn seeds, however, it should be noted that the cost of sea buckthorn seeds is much higher than the cost of grape seeds.

The increase in the indicators characterizing the secondary oxidation products is stable in the process of the entire temperature control. The increase in the peroxide value occurs during the first 5 hours of temperature control, after which the active destruction of the unstable primary occurs and the accumulation of secondary oxidation products begins to rapidly increasing the anisidine number. At the same time, the anisidine numbers in oil with the addition of CO2-extracts increase more slowly, than in the control sample. An increase in the intensity of the absorption band at a wavelength of 232 nm indicates an increase in the content of conjugated diene chromophores and correlates with indicators of fat thermal oxidation degree. The magnitude of the extinction coefficient for samples with the introduction of CO2-extracts is lower than in the control sample. In general, there is a clear tendency to increase the oxidative stability of sunflower oil samples with the introduction of CO2-extracts when exposed to high temperatures for a long time.

4. Conclusion
Summarizing the research results, we can conclude that the obtained food additives are innovative products, which include valuable plant raw materials. In addition, when they are received, used production wastes, that are related to the secondary resources of the viticulture and wine industry, which is very important now. The introduction of red grape leaves and grapes husks extracts production will expand the range of manufactured food products with high antioxidant properties.

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