Development of a food antioxidant additive technology out of grape pomace

V V Sadovoy¹,², T V Voblikova³, A V Morgunova² and A V Permyakov³

¹ North Caucasus Federal University, 56, 40 years of October Ave., Pyatigorsk, 357500, Russia
² Belgorod University of Cooperation, Economics and Law, 36, Goleneva Stavropol st., 355008, Russia
³ Yaroslav-the-Wise Novgorod State University, 41, B. St. Petersburgskaya str., Veliky Novgorod, 173003 Russia

E-mail: vsadovoy@yandex.ru

Abstract. The use of red grape pomaces to develop food antioxidant additive technology is theoretically justified. As a result, it was proposed to use polar solvents for sugar extraction, titratable acids and other organic compounds made from grape pomace. Using the methods of mathematical planning, we studied the influence of extraction time on the process results (τ), treatment temperature (t), active acidity (pH) and concentration of common salt (C NaCl) in the solution. With the use of artificial intelligence, the optimal parameters of the investigated factors were established. The precipitate was separated from the solution, ground and dried at a temperature of 70-80°C. As a result, the technology of producing a food antioxidant additive, which had a dark cherry colour and a powdered consistency without a pronounced odour, was developed. The antioxidant properties of the developed food additive have been studied on the test (no additives) and experimental (with additives) lipid samples. After processing butter at 37°C for 28 days, the test sample was found to have a lower peroxide number than the control number (0.033 compared to 0.055 mmol of active oxygen / kg), which confirms the antioxidant properties of the food additive, enriched flavonoids.

1. Introduction

In the Russian Federation, the average annual grapes crushing is 250 thousand tons. In the Stavropol Territory there are 18 organizations of primary winemaking, which have production capacities for processing 120–130 thousand tons of grapes per year. However, grape pomace is used to feed domestic cattle or is exported to the fields as organic fertilizer. The main reason for the irrational use of secondary raw materials for grape processing is the lack of efficient technology. Modern theories of nutrition place great emphasis on the biologically active components of foodstuffs, which determine the therapeutic and therapeutic-prophylactic action [1; 2]. Of particular interest is the development of a technology for processing grape extracts from red grapes, which contains a large number of biological antioxidants that reduce the activity of oxidation processes of radicals. The biological membranes are oxidized mainly by unsaturated fatty acids that are part of the phospholipids. At the initiating step (in the alpha position), a double bond between the carbon atoms leads to the deprotonation (H •). The C-H bond is also weakened in adjacent carbon atoms, contributing to the
deprotonation. The carbon radical undergoes regrouping and is combined with oxygen to form a peroxide radical capable of separating the hydrogen atom from another unsaturated fatty acid. [4]

Phenolic compounds (with an aromatic ring) are the most numerous group among natural and synthetic antioxidants. As a result, a negative charge shifts to the hydroxyl group, which promotes the deprotonation from the OH group and the formation of phenoxy radical isomers. Consequently, antioxidants flavonoids reduce the formation of peroxide radicals [9, 10].

2. Formulation of the problem
As a result of numerous studies it was found that red grapes, used regularly in the diet, beneficially affect the cardiac and circulatory systems of a human. It turns out that the polyphenols (flavonoids) contained in it (flavonoids) significantly reduce the probability of thrombus formation. Red dry wine and red grapes (wine itself and grape pomace) contains powerful antioxidants – quercetin, resveratrol, epicatechin, catechin, epicatechin gallate, rutini and others. The purpose of the present research was to develop a technology for the production of antioxidant food additives from red grape pomace enriched with flavonoids and purified from sugar (alcohol), tartaric and other compounds easily extracted with polar solvents.

3. Materials and methods
The object of research was grapes pomace obtained from red grape sort “levokumsky ustoichiviy” (“levokumsky resistant”), which had grown in ZAO Zarya in the Levokumsky district of the Stavropol Territory. Extraction of sugar, tartaric acids and protein from grapevine was conducted on the laboratory extractor-modification ES-8110. The temperature regulation during the extraction was carried out with the water thermostat (model 1TG-0-03). During the experimental studies we used ingredients and materials allowed for use by the state sanitary epidemiological surveillance.

The pH value was determined in aqueous extracts by the potentiometric method at pH-340. The protein content (in parts by weight) was determined by the Kjeldahl method; ashes - by incineration in a muffle furnace followed by calcination of a portion of the mineral residue; the mass fraction of moisture at a chemical analyzer modification OHAUSMB 45.Determination of the sugar quantitative content was carried out by photocolorimetry, interaction in the alkaline condition of the sugar carbonyl groups with potassium ferrocyanide [2, 6]. The mass concentration of the titrated acids in the test solution was determined by sodium hydroxide titration (concentration 0.1 mole / dm3) in the presence of phenolphthalein [5, 12].

The data obtained was analyzed in the applications Statistic v. 10 and the module Statistic Neural Networks. The experiments were conducted 3-5 times. Prediction of the chemical compounds’ molecular properties was carried out in Hyper Chem applications v. 8 [9].

4. Results and discussion
While contacting with air grape pomace quickly spoils and become moldy, alcohol turns into acetic acid, and the tartaric compounds are destroyed by propionic fermentation bacteria. That's why, it is expedient to process the pomace immediately after pressing by extracting sugar, tartaric acids and other compounds.

The diffusion phenomenon lies at the root of the tartaric acids and sugar extraction from grape pomace. The diffusion process consists in the penetration of solutions of different concentrations of solute into each other. The diffusion process consists in solutions of different solute fraction penetration into each other. Based on this, at the initial stage of research using computer simulation methods, molecular characteristics of flavonoids of quercetin, resveratrol, rutin, epicatechin, catechin and epicatechin gallate were investigated [7, 11]. When studying the distribution of charge density on the molecules surface and flavonoid structures, it was found that practically all the compounds under study had hydrophobic properties (deviation of the charges values from zero is small), although there are insignificant hydrophilic zones. In Figure 1 the surface of the charge density distribution and the
The structural formula of the catechin molecule are demonstrated. Evidence of the flavonoids hydrophobicity can be the value of total charge density which is summarized in Table 1.

![Figure 1](image1.png)

**Figure 1.** Molecule of catechin: a - the surface of the charge density distribution; b - is the structure formula.

**Table 1.** Total charge density of the studied flavonoid molecules.

| Characteristics | Flavonoids          |
|-----------------|---------------------|
|                 | Catechin | epicatechin-gallate | rutin   | resveratrol | quercetine | epicatechin |
| Total charge density, eV | 0.010    | 0.010               | 0.095   | 0.050        | 0.010      | 0.010       |

The data obtained indicate that it is expedient to use nonpolar organic solvents for flavonoids extraction, at the same time it is known that polar solutions are usually used to extract sugar (alcohol) and tartaric acid compounds. The temperature, the active acidity (pH), the duration of the process and the solution ionic strength (т, pH, τ, CNaCl) have a significant effect on the diffusion distribution of substances between the liquid phases. The extraction regimes investigation of sugar and tartaric compounds from grape pomace was carried out using.

The extraction regimes investigation of sugar and tartaric compounds from grape pomace was carried out using mathematical planning (uniforms rotatable plan) in a laboratory reactor. The effects of extraction time, treatment temperature, concentration of sodium chloride (NaCl) in solution and active acidity (pH) were studied on the process. Preparation of extracts with a given value of active acidity was carried out with a help of NaOH (sodium hydroxide). The degree of extraction is calculated by dividing the quantitative content of the substance in the extract with the total content of the extract in the system. The effects of extraction time, treatment temperature, concentration of sodium chloride (NaCl) in solution and active acidity (pH) were studied on the process help.

In each experiment of the plan, after extraction, the solids content and the recovered amount (% of the total mass of the residues) were determined in the solution, as well as the quantitative content of flavonoids in the solution and the degree of their extraction from the feedstock. The obtained results indicated a significant effect of temperature, active acidity (pH), extraction time and ionic strength of
the solution (NaCl concentration) on the extraction process, degree of flavonoids extraction and dry substances from grape pomace.

Based on the experiment results, a neural network model of a multilayer perceptron was developed to create an array of data using artificial intelligence, which served as the basis for optimizing the extraction regimes. The optimization was carried out taking into account the fact that it is necessary to extract the minimum amount of flavonoids from grape pomace and to get the other solids as much as possible. As a result of the array data analysis, the optimal parameters of chemical compounds recovery factors from grape pomace (7.9 – 8.2% of the total mass of the residues) soluble in polar solvents were revealed in laboratory conditions (Table 2), the extraction degree of flavonoids was insignificant and it was 0.5 – 2.3% of their total amount in the feedstock.

Table 2. Optimum parameters of grape pomace purification from sugar and tartaric compounds.

| Time (τ), min. | Temperature (t), °C | Active acidity (pH) | Concentration of NaCl in the solution (C_{NaCl}, %) |
|---------------|---------------------|---------------------|-----------------------------------------------|
| 20 – 25       | 85 – 90             | 7.2 – 7.4           | 0.3 – 0.5                                     |

At the end of the extraction process, the precipitate was separated from the liquid fraction, ground (~ 30 to 50 microns) and dried at 70-80°C to a water content of not more than 8%. As a result we obtained an antioxidant powdered additive of dark cherry color, odorless, enriched with flavonoids. The antioxidant activity was determined due to the change in the lipid oxidation rate by the peroxide number, which characterizes the accumulation of lipid decomposition products, which is inversely proportional to the activity of the antioxidant [10, 11]. Lipid model system was unsalted butter "Krestijanskoe" with moisture mass fractions of 25% and fat of 72.5%. This choice is explained by the short shelf life of this product (up to 10 days at a temperature of no higher than 8 °C), which makes it possible to visually simulate the lipid oxidation inhibition with the help of antioxidants contained in the food additive. In unsalted butter “Krestijanskoe” food antioxidant additive was added in an amount of 3% per 100 g of the model system (from the fact that the quantitative content of flavonoids in the system should be approximately 0.15%) and placed in a thermostat at 37 °C. At the same time the we were preparing control sample without the additive, which was stored with the prototype under the same conditions. The results of the studies are presented in Table 3 and in Figure 2.

Table 3. Accumulation of primary oxidation products in model lipid samples

| Sample | Storage length, days |
|--------|----------------------|
|        | 0       | 7 | 14 | 21 | 28 |
|        | Peroxide number, mmol of active oxygen / kg |
| 1      | Unsalted butter “Krestijanskoe” (control) | 0.007 | 0.019 | 0.035 | 0.042 | 0.055 |
| 2      | Unsalted butter “Krestijanskoe” with the food antioxidant additive (experiment) | 0.007 | 0.013 | 0.018 | 0.022 | 0.033 |

The results of the experiment (Table 3, Figure 2) indicate that after processing the developmental prototype had a peroxide number lower than the control sample (0.033 compared to 0.055 mmol of active oxygen / kg). Thus, the conducted research confirmed the antioxidant properties of the developed food additive.
5. Conclusions

1. Studies of the molecular characteristics of flavonoids of grape squeezers have confirmed the antioxidant properties of these compounds, and polar solutions are useful for extracting wine acids and sugars.

2. Optimal technological regimes (t = 85-90°C, t = 20-25 min, pH = 7.2-7.4, NaCl concentration in the solution = 0.3-0.5%) were found to extract sugar and tartaric compounds from the berry grape skin, providing insignificant losses of flavonoids (0.5 - 2.3% of the total amount in the feedstock).

3. The technology of food antioxidant additive is developed, consisting in extracting wine acids and sugar by extraction from grape pomace, separating the liquid fraction, grinding and drying the precipitate.

4. Experiments on lipid model samples confirmed the antioxidant activity of the developed food additive.

References

[1] Abramova Zh I 1985 Human and antioxidant substances (Leningrad: Science)
[2] Barybina L I, Beloysova E V, Voblikova T V, Statsenko E N et al 2019 Multicomponent meat products for sports nutrition J. Journal of Hygienic Engineering and Design 28 81–84
[3] Barybina L I, Voblikova T V, Beloysova E V, Nagdalian A A et al 2018 Usage of Vegetable Stuff in Dry Sausage Production J. Research Journal of Pharmaceutical, Biological and Chemical Sciences 9 4 1536 –1540
[4] Georgievsky V P 1990 Biologically active substances of medicinal plants (Novosibirsk: Science)
[5] GOST 25555.0-82 Products of processing of fruits and vegetables. Methods for determining titratable acidity
[6] GOST 8756.13-87 Products of processing of fruits and vegetables. Methods for determination of sugars
[7] Morgunova A V, Ismailov I S and Tregubova N V 2018 Technology Development of Protein-Fat Emulsion and Its Use in Food Production J. Research Journal of Pharmaceutical, Biological and Chemical Sciences 9 6 658–662
[8] Sadovoy V V, Selimov M A, Shchedrina T V and Nagdalian A A 2016 Usage of biological active supplements in technology of prophylactic meat products J. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7 5 1861–1865
[9] Sadovoy V V, Shchedrina T V and Selimov M A 2017 Biologically active composition for regulation of lipolysis process in the organism under obesity Problems of Nutrition [In Russian - J. Voprosy pitaniia] 86 (6) 74–83
[10] Sadovoy V V, Shchedrina T V, Selimov M A and Nagdalian A A 2017 Nutritional supplement for control of diabetes J. Journal of Excipients and Food Chemicals 8 2 31–38
[11] Voblikova T, Mannino S, Barybina L, Sadovoy V et al. 2019 Immobilisation of bifidobacteria in biodegradable food-grade microparticles J. Foods and Raw Materials 7 (1) 74-83

[12] Voblikova T, Permyakov A, Rostova A, Masyutina G and Eliseeva A 2020 Study of Fatty-acid Composition of Goat and Sheep Milk and Its Transformation in the Production of Yogurt Biological Resources Development and Environmental Management : International Applied Research Conference (Murmansk: Murmansk Marine Biological Institute) 742–751