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Development of a technology for obtaining a polyphenolic biologically active additive from grape pomace for a wide range of applications.

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Annotation. The research work considers the development of a technology for processing secondary raw materials of winemaking, which, due to preliminary preparation, passes into the category of enriched high-quality environmentally friendly grape raw materials. This study is carried out with the aim of obtaining concentrated high-quality polyphenolic biologically active extracts that can be used as a functional raw biomaterial for a wide range of products: healthy/sports nutrition, medical and preventive cosmetics, alcoholic and non-alcoholic drinks, and other types of special functional additives. The novelty of the development lies in the fact that at the initial stage, the enrichment of raw materials with target polyphenolic compounds is carried out using effective supercritical fluid CO_2 extraction, followed by the use of the obtained AGP** extracts in the form of a finished product - a phytopreparation with inherent functional properties.

1 Introduction

Currently, interest in the study of products of secondary processing of grape raw materials is constantly growing [1]. Firstly, due to the content of a rich composition of useful bioactive polyphenolic compounds, which are best suited for the creation of herbal medicines and various dietary supplements in the form of final products. Secondly, consumer interests are growing every day and new opportunities are emerging in the creation of innovative materials with proven improved properties of active ingredients. The task of the research is the development of a universal technology for waste-free processing of secondary grape raw materials. The goal is to obtain finished products by processing secondary grape raw materials in one cycle.

It is known that when exposed to a living organism, natural polyphenolic substances can reduce the risk of pathological diseases without causing side effects. While exhibiting antioxidant, hepato-, geroprotective and other types of bioactivities [2].

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**AGP – aqua-glycerol-propylene glycolic

This problem is widely studied in the modern world. And although a lot of research has been devoted to this topic, a particularly important task of processing secondary raw materials still remains - to determine the purity, safety of use and effective dosage of phytopolyphenolic compounds, as well as the creation of specialized finished forms of phytopreparations containing biologically active substances of a polyphenolic structure.

Whole grape pomace was chosen as the most capacious object of research. It is in any form: fresh, dried, fermented - a source of a large number of biologically active substances. Such substances include flavonoids of various structures, coloring, phenolic and polyphenolic compounds, organic acids, vitamins, amino acids, phytoncides, phytosterols, macro- and microelements, pectin substances and vegetable fiber [1-6, 9-11].

Along with these substances, it contains a number of unknown, little studied and under study compounds.

In studies, particular importance was attached to the fact that in fresh grape pomace, i.e. not yet subject to any external influences, there is the largest number of organic compounds in the native state. They are in the natural composition of the plant in an ideal form, taking into account all the specific functional purposes, harmoniously combined with each other for each class of substances. With the help of chemical analyzes, a convincing result was obtained, confirming the unchanged quality and the highest concentration of useful bioactive organic compounds in whole grape pomace.

It was envisaged to obtain effective productivity and high-quality end products - in the form of bioadditives of phytopreparations for various purposes.

Of great interest to the food and cosmetic industries are the coloring matter of grapes (see Fig. 1). The color of grape skins is due to the complex of anthocyanins. In grapes, the content of anthocyanins varies widely depending on the variety.

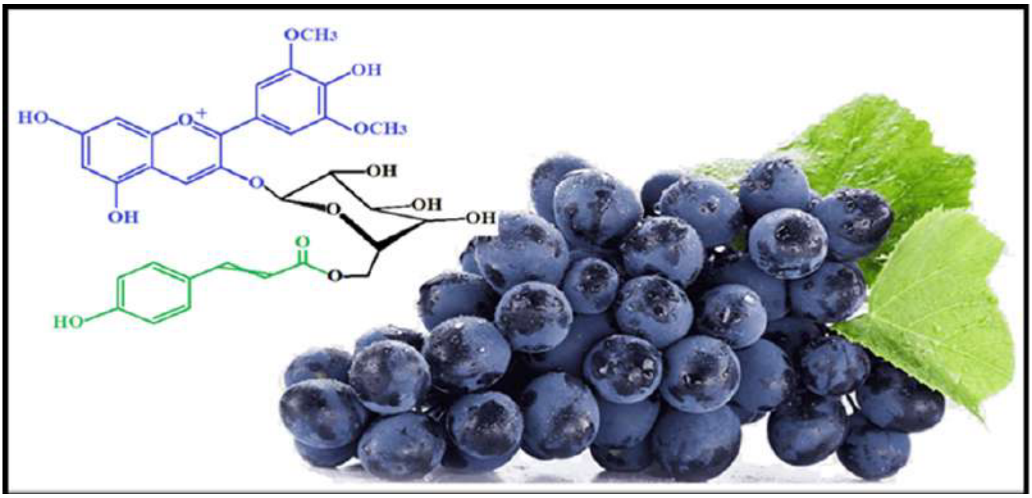


Fig. 1. Chemical structure of anthocyanin [Mv-3-(p-coumaroyl)glu] from grape skins responsible for its blue color.

The concentration of polyphenols, in studies of different grape varieties, averaged 4.75 g/kg of fresh berries [4-5]. The confirming results of the high content of polyphenolic substances make it possible to use grape processing products not only for enriching food products with biologically active substances, but also for cosmetic preparations [10-11].

The unique bioactive properties of phytopolyphenols contained in grape pomace are already used in the development of drugs [10-11]. The geroprotective and cardioprotective effect of polyphenolic compounds is due to the P-vitamin activity of the tannin-catechin and anthocyanin complex [3].

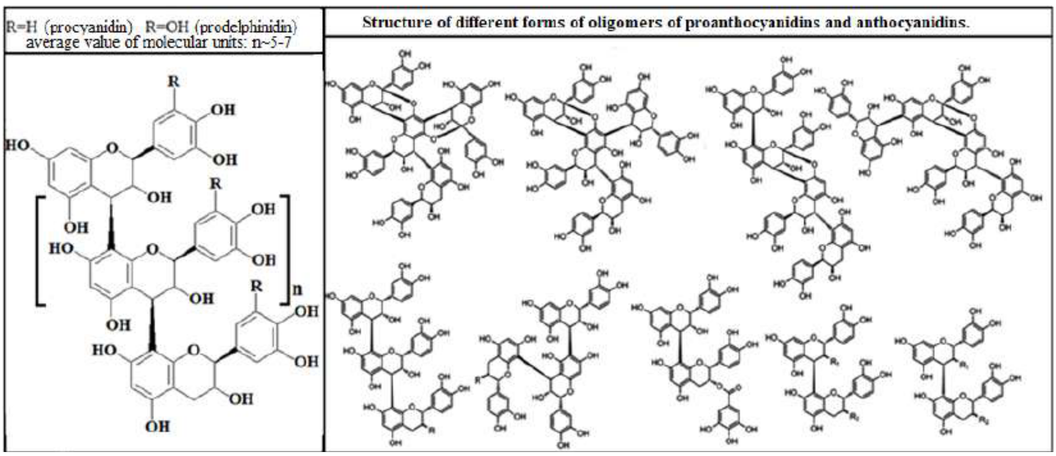
Polyphenolic compounds of red grape varieties in all viticulture products are represented by two main groups of substances of flavonoid and non-flavonoid structure. The monomeric units of these compounds are also able to condense with each other into oligomeric structures, forming chain and branched molecular systems (see Tab. No. 1).

Polymeric procyanidins contain seven or more catechin units. Oligomeric flavonoids - procyanidins consist of several (up to 7) fused catechin units (catechin, epicatechin, also from their dimers). The oligomeric and polymeric structures of flavonoids make up a large variable part of the total polyphenolic compounds in the composition of red grape varieties with biological activity.

Most researchers have determined that the protective and rejuvenating biological activity depends on both the concentration of polyphenols and their structure [10-11]. Bioavailability also depends on the structure, taking into account the dependence on the individual characteristics of the person. Thus, the need for further deeper clinical trials of the effect of polyphenolic substances from red grape varieties is obvious.

An average of 50 to 75% of the composition of phenolic substances passes into the extract. Of these, about 50% of anthocyanins from the total supply of these substances in grape pomace. About 30% of anthocyanins that are capable of being lost due to adsorption back into the pulp, when interacting with protein compounds, or during oxidation, etc. reactions [12-15].

Table 1. Oligomeric structure of proanthocyanidins.



Thanks to a special approach to the extraction of enriched grape pomace, described below, it is possible to extract polyphenolic biologically active substances without changes into a water-glycerol-propylene glycol extract without losing the quantity and quality of native substances [7,8].

2 Experimental part

The object of the study is the grape pomace of the Cabernet Sauvignon variety grown on the territory of the Republic of Moldova with the 2020 harvest. Fresh grape pomace was obtained directly after squeezing the juice on presses in the factory, then subjected to shock freezing at -45°C. Next, the pomace was dried by IR drying with infrared elements with unique spectral characteristics in the temperature range of 35-40°C. Grinded in a laboratory mill to a fine state.

The objective of the experiment was to carry out degreasing (removal of the lipid-wax fraction from the whole grape pomace) to enrich the raw materials with target polyphenolic compounds. To obtain the maximum yield in full, the removal of "ballast substances" - lipid-wax fat-soluble compounds from the prepared sample - enriching the meal.

Extraction was carried out on a pilot plant for supercritical fluid CO_2 extraction. Sample extraction, in the amount of 500 g, was carried out at a pressure of 20, 25 and 35 MPa, at a temperature of up to 50°C, for 90 minutes. During the extraction process, selective SCF-CO2 fat-soluble extracts were obtained (in two separators: lipid-wax [M=48.75 gr.] and wax [M=21.25 gr.] fractions). The best sample at 35 MPa with the highest yield was selected. Fat-free meal 430g. The total yield of the fat-wax fraction averages 14%.

On a SHIMADZU GCMS-QP2010 SE gas chromatograph with a mass spectrometric detector, the substances shown in Table 2 were found in the studied sample of grape pomace.

Enriched meal was subjected to amino acid analysis on a gas chromatograph with a built-in automatic analyzer T339M (Mikrotechna, Praha). The interpretation of the results of the identified amino acid composition is shown in Table 3.

For the extraction of polyphenolic substances from enriched meal, an aqueous solvent was used. Distilled water, natural glycerin, lactic acid and 1,2-propylene glycol >99% pure were used in the experiment. When preparing an extract of polyphenols, meal (M=0.3 kg) was infused in an acidified water-glycerol-propylene glycol solution (M=1.5 kg) for several days under vacuum at a temperature of 45°C. Next, ultrasonication was applied to intensify the extraction process. The solution was prepared in the proportion by weight: water – glycerol – propylene glycol (1:1:2). A saturated extract was obtained, filtered through a filter element under vacuum. From this amount, 1 liter of a pure solution of ruby polyphenols weighing 1.11 kg was obtained.

Anthocyanin complex analysis was performed on a Shimadzu LC-20 GC system, on a C-18 Grace Rocket 53×7 mm, 1.5 µm column. For results see Table 4.

3 Results and discussions

Previously developed technologies for the production of extracts and phytopreparations from grape pomace were carried out by various methods. The developed technology in the whole scope of work was carried out for the first time, without noted analogues in world practice. The task was to ensure that the final products remained identical in terms of the qualitative and quantitative composition of the target polyphenolic compounds, in comparison with the initial composition of fresh whole grape pomace. Further, in this form, phytopreparations were used as a dietary supplement in the production of cosmetics, sports and functional nutrition. Before their intended use, an experimental evaluation of their biological activity was carried out.
During the study, a number of specific analyzes were made:

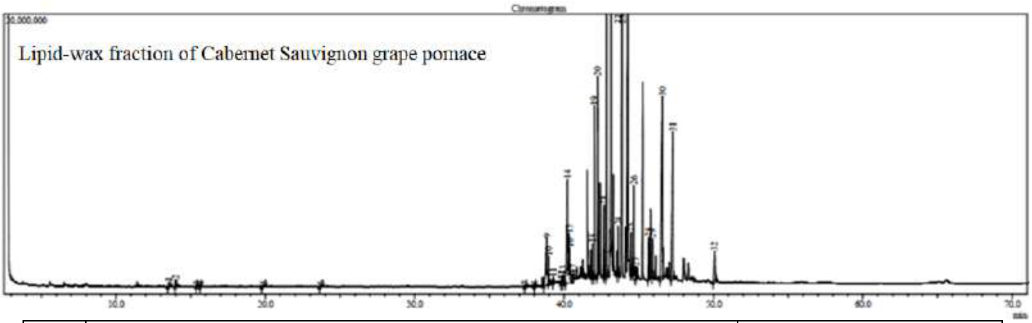
- chromatographic
- physical and chemical
- microbiological
- amino acid analysis of meal
- determined antioxidant / antiradical activity.

The study compared the evaluation of data for several types of finished products in various formulations:

- product from the manufacturer Symrise, Actipone® Grape Seed (Organic) GW
- samples of hydroalcoholic grape seed extract produced at the factory of "Viorica-Cosmetic" JSC were studied,
- obtained water-alcohol extract of whole grape pomace according to a new method,
- from which the dry extract concentrate was developed and obtained,

- a special highly concentrated AGP extract has been developed

Table 2. Composition of the fat-wax fraction of the SFE-CO2 extract



#	Name of the composition component	%, mass
1	2,4-decadienal	0.42
2	Tetradecane	0.10
3	**Pentadecanoic acid C15:0**	**4.72**
4	Pentadecanoic acid ethyl ester	0.50
5	Phytol C20:2	0.17
6	**Composition of grape seed oil**	**12.79**
7	Stearic acid C18:0	0.91
8	Eicosanoic acid C20:1	0.56
9	Docosanoic acid (Behenic) C22:0	0.63
10	**Pentadecanoic aldehyde**	**2.25**
11	**Tetracosanol (lignoceryl alcohol) C24:OH**	**1.52**
12	**Octadecanoaldehyde**	**19.57**
13	**Octacosanol C28:OH**	**22.59**
14	**Tocopherol**	**3.89**
15	**δ-22-dihydrobrassicosterol**	**1.57**
16	**β-stigmasterol**	**1.39**
17	**γ-sitosterol**	**14.02**
18	**1,37-octatriacontadiene**	**8.58**
19	**Urs-12-en-28-al**	**2.26**
20	1-hentetracontanol C41:OH	0.68
21	1,3,12-nonadecatriene-1,14-diol	0.88
Components in an amount of more than 1% by weight are highlighted in bold.		

Table 3. Results of amino acid analysis of grape pomace meal.

#	Name of the amino component	*Raw, g/kg*	*Dry, g/kg*	*Nitrogen,g/kg*
1	Aspartic acid	4,5752	4,9731	0,5231
2	**Threonine**	**2,3271**	**2,5295**	**0,2973**
3	Serene	2,2898	2,4889	0,3316
4	Glutamic acid	9,9547	10,8203	1,0296
5	conditionally essential Proline	1,8434	2,0037	0,2437
6	conditionally essential Glycine	4,8880	5,3130	0,9908
7	Alanine	2,7226	2,9593	0,4650
8	**Valine**	**2,2433**	**2,4384**	**0,2914**

9	Cysteine	0,8124	0,8830	0,2058
10	**Methionine**	**0,3067**	**0,3334**	**0,0313**
11	**Isoleucine**	**1,9306**	**2,0984**	**0,2240**
12	**Leucine**	**3,0126**	**3,2746**	**0,3495**
13	Tyrosine	0,7438	0,8085	0,0625
14	**Phenylalanine**	**1,7557**	**1,9083**	**0,1617**
15	Aminobutyric acid	0,5613	0,6101	0,0828
16	**Lysine**	**3,6094**	**3,9233**	**0,7514**
17	**Histidine**	**1,9599**	**2,1304**	**0,5767**
18	**Arginine**	**2,1120**	**2,2956**	**0,7379**
19	Ammonia	1,6750	1,8207	1,4968
■	Σ Free amino acids	47,6485	51,7918	7,3561
■	Σ Metabolic nitrogen index	49,3235	53,6125	8,8528
■	Σ Non-essential amino acids	27,8299	30,2499	3,8520
■	Σ Essential amino acids	19,2573	20,9319	3,4212
■	Σ Immunoactive amino acids	25,4864	27,7026	3,2266
■	Σ Glycogen amino acids	19,0461	20,7023	2,8992
■	Σ Ketogenic amino acids	11,0521	12,0131	1,5491
■	Σ Proteinogenic amino acids	47,0872	51,1818	7,2732
■	Σ Sulfur-containing amino acids	1,1191	1,2164	0,2371
Essential amino acids are highlighted in bold.				

Table 4. Results of the analysis of the composition of the anthocyanin complex of the AGP extract

№	Components of the anthocyanin complex	Количество, мг/дм^3
1	Delphinidin-3-glycoside	1.831
2	Cyanidin-3-glycoside	0.158
3	Malvidol diglycoside	3.300
4	Petunidin-3-glycoside	2.207
5	Malvidin-3-glycoside	22.03
6	Peonidol-3-acetyl glycoside	2.163
7	Malvidol-3-acetyl glycoside	1.433
8	Peonidol-3-coumaryl glycoside	0.402
9	Malvidol-3-coumaryl glycoside	6.604
	Total Concentration	**40.128**

The results obtained prove the promise of the new technology with the possibility of implementation and scaling in industrial wineries.

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