Concentrates of polyphenols from grape raw materials and their functional properties

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Abstract. The work presents the possibility of obtaining polyphenol concentrates from raw materials (pomace, seeds, cane, vines, leaves) of Crimean grapes of white varieties Aligote, Rhine Riesling, Rkatsiteli and grapes of red varieties Muscat Hamburg, Cabernet-Sauvignon, by vacuum concentration of alcoholic extracts of grape raw materials. An experimental assessment on the qualitative and quantitative structure of polyphenols, the basic functional ingredients of biological activity of grapes has been given. It has been established that by concentration of phenolic substances, the concentrates are distributed in the following sequence: seeds of grapes of white and red varieties, stalks of grapes of white and red varieties, pomace of grapes of red varieties, leaves of grapes, vine of grapes, pomace of grapes of white varieties. In grape concentrates, anthocyanins, flavones, flavan-3-ols, oligomeric and polymeric procyanidins, oxycinnamic and oxybenzoic acids and stilbenoids have been identified. The maximum quantitative evaluation of stilbenoid concentration obtained in grape concentrates was up to 5.0 g/L, which is by order of magnitude greater than in red wines. The antioxidant activity indicator (AOA), estimated in vitro in grape seed concentrate, ranged from 180.0 to 250.0 g/l, which is by order of magnitude greater than in red grape wines.

1. Introduction
Recently there has been a surge of scientific interest in the healing properties of grapes due to the biologically active properties of grape polyphenols, localized in the skin, pulp, grape seeds, vine, stalks of grape bunch. Grape polyphenols are extracted from raw materials during alcoholic fermentation, on-skins fermentation or from grape pomace, vine, leaves, seeds as food extractants, determining the antioxidant status and biological activity of grape processing products.

Most of the total polyphenols in the production of red wines remain in the pomace which leads to significant losses of grape polyphenols, valued at the market in the range of 2.0-2.5 USD per 1 gram.

The possibility of deeper extraction of total polyphenols from pomace and other secondary raw materials of industrial grape processing are being implemented in innovative products containing polyphenols as the target component of biological activity. Such products, produced in different countries, include also Russian alcohol-free food concentrates of grape polyphenols “Enoant”, “FENOKOR” (manufactured by RESSFOOD LLC, the Republic of Crimea) [1, 2]. Grape food concentrates are powerful antioxidants that produce vasodilative, anti-inflammatory, antimicrobial, hypoglycemic, antiatherogenic, anti-stress effects and normalize hemoglobin levels [3, 4].
The characteristics of grape raw materials (pomace, seeds, cane, vines, leaves) by technological stock, quantitative and qualitative composition of polyphenols - the main functional ingredients of biological activity of products of grape processing have been previously estimated. The analysis of the received data shows that in aqueous-alcoholic extracts of grape raw materials the whole spectrum of polyphenols peculiar to red grape wines (anthocyanins, flavons, flavan-3-ols, oxyccinnamic and oxybenzoic acids, stilbenes, oligomeric and polymeric procyanidins) is presented, at the same time, the quantitative content of polyphenols exceeds by many times their concentration in wine [5].

2. The purpose of the study
The purpose of our study was to obtain experimental samples of concentrates from grape raw materials, to study qualitative and quantitative composition of polyphenols in the obtained concentrates as well as to assess the antioxidant activity indicator in experimental samples of these concentrates.

3. The object of the study
For the purpose of experimental production of alcoholic extracts from grape raw materials (pomace, seeds, vines, cane, leaves) and concentration of polyphenols from them, to determine qualitative and quantitative composition of the phenol complex, 29 samples of raw materials of grape varieties were prepared: Aligote, Rhine Riesling, Rkatsiteli, Muscat Hamburg, Cabernet-Sauvignon, from various Crimean grape-growing areas: western piedmont-littoral and piedmont area. Samples of grape raw materials were harvested in the autumn-winter period of 2018 and 2019 and during the harvest season of 2019.

4. Materials and methods

4.1 Modes of extraction and concentration of polyphenols from grape raw materials
To clarify the modes of extraction of polyphenols from grape raw materials, pomace, seeds, cane, leaves were filled with water-alcoholic extractant with a volume fraction of ethyl alcohol of 50 %, at a ratio of solid phase: liquid 1:3 to extract the total amount of polyphenols, including stilbenoids, and the vine, prior to extraction, was pre-dispersed on a Bosch AXT Rapid 2000 shredder to particles sized 2-11 mm. For the intensification of extraction, the suspension was treated with ultrasound (vibration frequency 35 kHz), time of ultrasound exposure - 45-60 min. Ultrasound processing was stopped after reaching the equilibrium concentration of polyphenols. The extract was separated by flowing down on a sieve. The equilibrium concentrations of polyphenols in alcoholic vine extracts were determined in triplicate by the HPLC method on the chromatograph of Agilent Technologies system (model 1100) with a diode matrix detector. The equilibrium concentrations of polyphenols were recorded after 45 days of extraction at a temperature of 10-15 °C. Concentration of alcoholic extracts of grape raw materials was carried out on the rotary evaporator by distillation of alcohol under vacuum at 40-50 °C, residual pressure 0.1-0.2 atm up to ethanol content, not more than 0.5% of ethyl alcohol volume fraction. The coefficient of evaporation of the obtained alcoholic extracts was 2.7-3.0 for white grape varieties Aligote, Rhine Riesling, Rkatsiteli (cane, pomace, seeds, leaves, vine); 1.9-2.1; 1.7-2.5; 18-22; 27-42 respectively, and for red grapes of the varieties Muscat Hamburg, Cabernet-Sauvignon (cane, pomace, seeds, leaves, vine) 2.4-3.1; 2.2-2.5; 2.2-3.2; 16-31; 34-45 respectively.

4.2 Determination of the mass concentration of phenolic substances by Folin-Ciocalteu method
The mass concentration of phenolic substances in extracts was determined by the colorimetric method according to P 4.1.1672-2003 [16]. The colorimetric method for determining phenolic substances is based on the fact that the Folin-Ciocalteu reagent when added to the studied product oxidizes phenolic groups, while restoring in a compound of blue color, the optical density of which is determined on the photocolorimeter. The intensity of coloration is proportional to the concentration of phenolic substances.
The control procedure was as follows. With a pipette with the capacity of 1 ml, 1 cm$^3$ of the tested solution was placed in a volumetric flask with the capacity of 100 cm$^3$ and brought to the mark with distilled water at a temperature of (20±0.5) °C. In each flask with a measuring pipette with a capacity of 1 ml, 1 ml of Folin-Ciocalteu reagent, and with measuring pipette with a capacity of 10 ml - sodium carbonate solution was added. It was adjusted to the mark with distilled water at a temperature of (20±0.5) °C and stirred.

After 30-40 minutes, the optical density of the solutions was measured in 10 mm cuvette, at a wavelength of 670 nm against control solution. The value of mass concentration of phenolic substances C, mg/l, by gallic acid, was determined by means of a graduation chart, using for calculation the following formula:

$$C = K \times M \times V,$$

where $K$ - dilution factor;
$M$- amount of phenolic substances found according to the calibration chart, mg;
$V$- volume of tested solution, ml.

Plotting of the calibration curve was carried out according to [6].

4.3 Determination of qualitative and quantitative composition of polyphenols in grape raw material concentrates and antioxidant activity indicator

The qualitative and quantitative composition of polyphenols in grape concentrates was determined by HPLC method using Agilent Technologies chromatographic system (model 1100) with a diode matrix detector. To separate substances, a Zorbax SB-C18 chromatographic column with a size of 2.1×150 mm, filled with silica gel with a bonded octadecylsilyl phase with a sorbent particle size of 3.5 μm was used. The chromatography was performed in a gradient mode. The eluent composition: solution A - methanol, solution B - 0.6% aqueous solution of trifluoroacetic acid. The eluent composition during chromatography was changed according to the following scheme (according to the content of component B): 0 min - 8%; 0-8 min - 8-38%; 8-24 min - 38-100%; 24-30 min - 100%. Eluent flow rate was 0.25 ml/min. The sample volume was 1 μl. The chromatographs were recorded at the following wavelengths: 280 nm for gallic acid, (+)-D-catechin, (-)-epicatechin and procyanidines, 313 nm for oxycinnamic acids derivatives, 371 nm for quercetin and 525 nm for anthocyanins. Identification of substances was carried out by comparing their spectral characteristics and retention times with similar characteristics of standards. Spectral characteristics of individual substances were obtained using the literature data [7].

Calculation of the quantitative content of individual components was performed with the use of calibration charts of dependence of the peak area on the concentration of the substance plotted on the solutions of individual substances. The anthocyanin content was determined expressed as malvidin-3-O-glucoside chloride, the caftaric acid content expressed as caffeic acid, the content of polymeric and oligomeric procyanidines was calculated expressed as (+)-D-catechin. All determinations were made in triplicate. As standards, gallic acid, caffeic acid, (+)-D-catechin, malvidine-3-O-glucoside chloride, dihydratequercetin chloride, isoquercytrin (FlukaChemieAG, Switzerland), and trans-resveratrol, (+)-epicatechin, sirenic acid (Sigma-Aldrich, Switzerland) were used.

The antioxidant activity indicator in the experimental concentrate samples was determined using the amperometric method on the device “Tsvet Yauza – 01AA” in concentration units of the standard antioxidant – “Trolox –C” by GOST P 54037 [8,9].

5. Discussion of the results

Concentrates of polyphenols have been obtained from alcoholic extracts of pomace, seeds, cane, vines, leaves of grape varieties Aligote, Rhine Riesling, Rkatsiteli, Muscat Hamburg, Cabernet Sauvignon.
The sum of phenolic substances according to Folin-Ciocalteu in grape polyphenol concentrates (g/l) from pomace of four studied grape varieties (Aligote, Rhine Riesling, Muscat Hamburg, Cabernet Sauvignon) was 25, 22, 45 and 50 g/l respectively; in seed concentrates - 75, 82, 104 and 96 g/l; in cane grape concentrates - 29, 18, 13 and 23 g/l; in leaves concentrates - 47, 41, 46 and 67 g/l; in vine concentrates - 46, 22, 33 and 34 g/l. In tables 1, 2 by the example of white grape Aligote and red grape Cabernet-Sauvignon, the experimentally obtained characteristics of qualitative and quantitative composition of polyphenols are given, derived at extraction from different raw materials and the antioxidant activity index expressed as standard antioxidant trolox.

**Table 1.** Characteristics of qualitative and quantitative composition of polyphenols, value of antioxidant activity in concentrates white grape Aligote

| Components                     | pomace | seeds | vines | cane | leaves |
|--------------------------------|--------|-------|-------|------|--------|
| Total Anthocyanins, mg/l       | 0      | 0     | 0     | 0    | 0      |
| Flavan-3-ols+ Flavones, mg/l    | 1045.9 | 4505.9| 2758  | 7614 | 44512.8|
| Total flavonoids, mg/l          | 1045.9 | 4505.9| 2758  | 7614 | 44512.8|
| Total hydroxybenzoic acids, mg/l| 1304.3 | 1542.1| 17    | 73   | 14.0   |
| Total oxycinnamic acids, mg/l   | 13.5   | 22.0  | 597   | 323  | 2066.3 |
| Trans-resveratrol, mg/l         | 0      | 0     | 53    | 631.8| 18.5   |
| ɛ-viniferin, mg/l               | 0      | 0     | 291   | 3847.0| -      |
| Unidentified stilbens, mg/l     | 0      | 0     | -     | 521.5| -      |
| Total stilbens, mg/l            | 0      | 0     | 345   | 5000.3| -      |
| Oligomeric Procyanidins, mg/l   | 3575.3 | 7724.9| 4631  | 12035| 1731.1 |
| Polymeric Proanthocyanidins, mg/l| 43964.8| 152903.9| 37024 | 41784| 20239.6|
| Total phenolic substances (by HPLC), g/l | 49.9 | 166.70| 45.35 | 66.86| 69.67     |
| Antioxidant capacity (trolox), g/l| ±0.22 | ±2.90 | ±0.22 | ±0.22| ±2.68   |

**Table 2.** Characteristics of qualitative and quantitative composition of polyphenols, value antioxidant activity in concentrates red grape Cabernet-Sauvignon

| Components                     | pomace | seeds | vines | cane | leaves red |
|--------------------------------|--------|-------|-------|------|------------|
| Total Anthocyanins, mg/l       | 150.0  | 0     | 0     | 0    | 98.9       |
| Flavan-3-ols + Flavones, mg/l   | 1382.3 | 5810.0| 986.0 | 6900.1| 26032      |
| Total flavonoids, mg/l          | 1532.3 | 5810.0| 986.0 | 6900.1| 26130.9    |
| Total hydroxybenzoic acids, mg/l| 981.3  | 1179.2| 90.3  | 419.0 | 80.9       |
| Total oxycinnamic acids, mg/l   | 40.9   | 32.8  | 192.6 | 707.2 | 2335.7     |
| Trans-resveratrol, mg/l         | 5.6    | 0     | 6.7   | 601.5 | 15.2       |
| ɛ-viniferin, mg/l               | -      | -     | 95.9  | 3654.0| -          |
| Unidentified stilbens, mg/l     | -      | -     | -     | 601.1 | -          |
| Total stilbens, mg/l            | -      | -     | -     | 4255.5| -          |
| Oligomeric Procyanidins, mg/l   | 4286   | 10415 | 2008.9| 11628.3| 5912.0    |
| Polymeric Proanthocyanidins, mg/l| 79229 | 199590| 35252 | 38936 | 47021     |
| Total phenolic substances (by HPLC), g/l | 86.2  | 217.0 | 38.6  | 62.66 | 81.6       |
| Antioxidant capacity (trolox), g/l| 83.28±2.03 | 250.77±6.96 | 22.02±0.46 | 16.15±0.35 | 182.2±3.04 |

The analysis of the received data has shown that in concentrates of grape raw materials, the whole spectrum of polyphenols peculiar to red grape wines (anthocyanins, flavonos, flavan-3-ols, oxycinnamic and oxybenzoic acids, stilbenes, oligomeric and polymeric proanthocyanidines) is presented. The maximum content of polyphenols is achieved in seed concentrates (217 g/l), while the most promising raw material for obtaining concentrates enriched with stilbenoids (trans-resveratrol and its derivatives) is vine, which allows one to obtain concentrates containing up to 5.0 g/l of stilbens, which is several orders of magnitude greater than the maximum content in the best red wines. This fact
confirms the well-known viewpoint that grapes, along with peanuts and Reynoutria japonica, are the main food source of stilbene substances [10], the most famous representative of which, trans-resveratrol, as evidenced by preclinical studies carried out in vitro and in vivo, has a positive effect on the inhibition of tumor growth of various etiologies, inducing cancer cell apoptosis, as well as on the inhibition of early invasion and cancer metastasis after surgery [11-14]. This fact testifies to perspective of the use of vine for the production of stilbene-enriched water-alcoholic extracts and concentrates for conducting further comprehensive studies on models in vitro and in vivo and justification of their use in the production of functional food products.

6. Conclusion
According to the results of experimental studies on the qualitative and quantitative composition of polyphenols, concentrates of grape raw materials contain polyphenols characteristic for red wines: anthocyanins, flavan-3-ols, flavones, oxybenzoic and oxyccinnamic acids, oligomeric and polymeric proanthocyanidins, stilbenoids. Oligomeric and polymeric polyphenols are concentrated in grape seeds and vines. Flavonoids, oligomeric and polymeric polyphenols contained in concentrates of grape seeds and leaves have high biological activity, which is reflected in the antioxidant activity indicator. Values of the antioxidant activity indicator (AOA) in grape seed concentrates, measured by the amperometric method in standard antioxidant trolox units ranged from 169.0 to 250.0 g/l. The pomace concentrates of red grape varieties such as Cabernet-Sauvignon also had high biological activity.

The antioxidant activity indicator (AOA) value for this grape variety amounted to 83.0 g/l which was 3.7 times higher than the biological activity indicator of white grape concentrates. The AAI value ranged 123.1 g/l in concentrates made from the leaves of white grape variety Aligote and between 182.2 g/l in concentrates made from grapes of red varieties Cabernet Sauvignon.

Conspicuous is the fact that the white and red grape concentrates contain large quantities of stilbenoids - trans-resveratrol and its derivatives, the total concentration of which is up to 5.0 g/l. Apparently, stilbenoids in grape vine concentrates determine the antioxidant activity indicator and, as a consequence, the biological activity of tested samples.

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