30. Kalinina, I. V. Issledovanie kachestva obogashchennyh vidov hleba v processe hraneniya [Text] / I. V. Kalinina, N. V. Naumenko, I. V. Feklicheva // Vestnik YUUrGU. – 2015. – Vol. 3, Issue 1. – P. 36–44.
31. Goryacheva, A. F. Sohranenie svezhesti hleba [Text] / A. F. Goryacheva, R. V. Kuz'minskiy. – Moscow: Legkaya i pishchevaya promyshlennost', 1983. – 240 p.
32. Market Research Reports and Technical Publications [Electronic resource]. – Available at: https://www.bccresearch.com/
33. Murav'eva, M. Razroznennye nanotekhnologii budut ob'edi-nyat' [Text] / M. Murav'eva // V mire nano. – 2010. – Issue 6. – P. 12.
34. U nanotekhnologiy v Rossii est' budushchee [Electronic resource]. – ROSNANO. – Available at: http://www.rusnano.com/about/press-centre/75823
35. Aleshkov, A. V. Pishchevaya promyshlennost' – industriya innovaciy [Text]: monografiya / A. V. Aleshkov. – Habarovsk: RIC HGUEHP, 2016. – 188 p.
36. Ma-Hock, L. Generation and Characterization of Test Atmospheres with Nanomaterials [Text] / L. Ma-Hock, A. O. Gamer, R. Landsiedel, E. Leibold, T. Frechen, B. Sens et. al. // Inhalation Toxicology. – 2007. – Vol. 19, Issue 10. – P. 833–848. doi: 10.1080/08958370701479190

UDC 664 : [663.26 : [641.1 : 613.31]
DOI: 10.15587/1729-4061.2017.108992

RESEARCH OF EXTRACTION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM GRAPE POMACE BY SUBCRITICAL WATER

V. Sukmanov
Doctor of Technical Sciences, Professor
Department of technological equipment for food production and commerce
Poltava University of Economics and Trade
Koval str., 3, Poltava, Ukraine, 36014
E-mail: Sukmanovvaleri@gmail.com
A. Ukrainets
Doctor of Technical Sciences, Professor
Rector*
E-mail: ukrainets@nuft.edu.ua
V. Zavyalov
Doctor of Technical Sciences, Professor
Department of Processes and Apparatus for Food Production*
E-mail: zavialov@nuft.edu.ua
A. Marynin
PhD
Problem research laboratory*
E-mail: andrii_marynin@ukr.net
*National University of Food Technologies
Volodymyrska str., 68, Kyiv, Ukraine, 01601

Досліджено процес вилучення біологічно активних речовин з виноградних вичавок субкритичною водою. Ідентифіковано наявність галової кислоти і фурфуролу. Встановлено вплив технологічних параметрів процесу екстрагування (розмір фракції сухих виноградних вичавок, температура, тиск, гідромодуль) на антиоксидантну активність екстрактів, вихід сухих речовин, загальний вихід поліфенолів, винно-кислих сполук, редукуючих речовин. Визначено раціональні параметри даного процесу

Ключові слова: виноградні вичавки, біологічно активні речовини, екстрагування, субкритична вода, антиоксидантна активність

Исследован процесс извлечения биологически активных веществ из виноградных выжимок субкритической водой. Идентифицировано наличие галловой кислоты и фурфурола. Установлено влияние технологических параметров процесса экстрагирования (размер фракции сухих виноградных выжимок, температура, давление, гидромодуль) на антиоксидантную активность экстрактов, выход сухих веществ, общий выход полифенолов, винно-кислых соединений, редуцирующих веществ. Определены рациональные параметры данного процесса.

Ключевые слова: виноградные выжимки, биологически активные вещества, экстрагирование, субкритичная вода, антиоксидантная активность

1. Introduction

According to the UN FAO (Food and Agriculture Organization of the United Nations), the world production of grapes is more than 76 million tons per year. The production of grapes in Ukraine in recent years has been growing, and in 2016 exceeded 390 thousand tons [1].

As a result of grape processing, up to 20 % of waste grape pomace (GP) is produced, which has a rich polysaccharide complex and contains a considerable amount of phenolic

substances and lignin. The pomace consists of 37...39 % (of the total mass) of the grape's skin; 15...34 % – pulp particles, 1.0...3.3 % – stem rests, 23...39 % – seeds. The moisture content of the pomace depends on the quality of pressing and varies from 50 to 60 %.

Up to 60 % of the mass of dry GP are polysaccharides [2], which are a valuable raw material for the production of biologically active substances (BAS) with high antioxidant properties, including polyphenols, gallic and tartaric acids and their salts, etc.[3].

The technological reserve of grape polyphenols in Ukraine exceeds 500 tons of total polyphenols per year. In this way, the polyphenols of grapes in their native form are mainly flavonoids – poorly water-soluble substances. At the same time, their distribution in GP corresponds to: 12...20 % in the grape skin, 1 % in the pulp, 60 % in the seeds, 19...24 % in the grape stems [4].

Progress in studying the methods of extraction and analysis of the biological activity of grape polyphenols is associated with their widespread use in the food, pharmaceutical and cosmetic industries [5]. When extracting BAS from vegetable raw materials, traditional extraction methods (maceration, remaceration, percolation, repercolation) are used widely [6]. For the purpose of their intensification, various methods of electro- and magnetic-pulse treatment of extractable raw, the use of centrifugation and ultrasonic treatment have been developed [7, 8]. In these technologies, the target product isn't extracted completely, the processes are time-consuming, the contents of ballast substances (high-molecular compounds, pectins, mucus, proteins, etc.) are too high and the process is labor-intensive. In most methods, there are significant losses of the extractant in the diffusion and evaporation. The methods used to intensify these processes partially eliminate the above-mentioned drawbacks, and some of them substantially reduce the quality of the extracts obtained.

Application of modifiers in liquid extraction (cosolvents) can change the qualitative (and, possibly, quantitative) composition of the extracts obtained due to the polarity of the extractant.

Since the 90s of the last century, active development of methods for sub- and supercritical fluid extraction has begun [9, 10].

Solvents with a low boiling point are used as extractants – liquefied gases: carbon dioxide, hexane, propane, ammonia, methane, ethylene and some other compounds with low critical temperatures. At the first stages of development of subcritical extraction technologies, CO_2 extraction was the most widespread. However, it must be borne in mind that carbon dioxide is one of the main greenhouse gases, like methane, ozone, nitrogen oxides and so on. In addition, some fluid substances, such as methane, are toxic. The principles are developed, which "green" (environmentally friendly) extraction methods, including, subcritical water (SCW) extraction, must conform to [11].

SCW is water in a liquid state at a pressure of up to 21.8 MPa in the temperature range between the usual boiling point (100 °C) and the critical temperature (374 °C).

The most effective method of extracting plant raw materials in the food and pharmaceutical industries is currently the SCW extraction [12–14]. The terms used for the description of SCW: "subcritical water", "high-temperature water", "superheated water", "pressurized hot water", "pressurized low polarity hot water".

Advantages of using SCW as a solvent:

– combination of properties of gases at high pressure (low viscosity, high diffusion coefficient) and liquids (high ability);

– combination of negligible interfacial tension with low viscosity and high diffusion coefficient;

– high sensitivity of the solvent ability to pressure or temperature changes;

– simplicity of separation of SCW and dissolved substances when the pressure is released;

– SCW penetrates into porous structures more easily in comparison with traditionally used liquid solvents.

These advantages are the result of changes in the physicochemical properties of water in the subcritical state:

– the dissociation constant increases by almost 2 orders of magnitude;

– the pH decreases from 7.0 to 5.5;

– the relative permittivity decreases 1.5–2.0 times;

– the heat of vaporization is reduced by a factor of 1.5;

– the specific heat increases 2.0–2.5 times;

– the density of water decreases 1.3–1.5 times;

– the dynamic viscosity decreases by a factor of 6–7;

– the surface tension decreases 2–3 times;

– the coefficient of self-diffusion increases by an order of magnitude;

– the ion product of water increases depending on the pressure 50–2,000 times [12].

These circumstances, as well as the low cost of SCW, make it possible to classify the SCW extraction process as the most effective and promising one, from the existing at the present time.

In the process of SCW extraction, quite complex physical and biochemical transformations take place. Autocatalyzed hydrolysis of polysaccharides occurs in the conditions of GP extraction. Catalysts are acids present in the raw material: tartaric, formic, acetic, etc. These acids are formed as a result of chemical transformations under high temperature during the extraction itself. These acids can have a significant effect on the processes. Various acids are intermediate or final products of reactions that occur during subcritical extraction. Consequently, the acidity is an important indicator that characterizes the extraction process from the point of view of the formation of simple sugars from polysaccharides.

Currently, the SCW extraction of BAS from various plant and algae species in the food, cosmetic and pharmaceutical industries is considered as the most innovative technology [13, 14].

The main obstacles to the full use of GP are high humidity and the need for speedy processing to eliminate the development of molds and prevent spoilage. GP begins to deteriorate after 2–3 days, and at high humidity (85–90 %) and elevated temperatures (25–40 °C), the storage period is 8–12 hours. Consequently, the dry matter of GP is the raw material for BAS extraction. The influence of drying regimes on the preservation of BAS should be taken into account [15].

The relevance of the studies is determined by a number of circumstances. A significant part of grape processing and winemaking enterprises in Ukraine now require technological and technical modernization and the proposed technologies should be innovative and highly efficient. The chemical composition of the processed raw materials depends significantly on the soil-climatic conditions and varietal characteristics of grapes, as evidenced by the numerous data on the biochemical composition of GP given in the literature [3]. These circumstances lead to the need to study the processes

of GP extraction from those varieties of grapes that are common in the regions of the location of processing enterprises, subject to modernization.

2. Literature review and problem statement

Grapes are rich in BAS, but their content in different parts of the fruit (pulp, skin, stems, seeds) is different [3, 5].

Nowadays, the effectiveness of using SCW for the extraction of various BAS from vegetable raw materials, including from grapes and GP, is beyond doubt. SCW provides more complete extraction, there is a selectivity of the process in relation to different target products and the ecological purity of the extract obtained [5, 12, 13, 16].

Comparison of the stability of anthocyanins extracted from the grape skin by the subcritical extraction together with ultrasound with the results obtained by microwave extraction showed that the effect of temperature and the resistance of anthocyanins are different [17].

Using the parameters of the extraction process 2 MPa, 121 °C with a process duration of 2 hours ensure a high yield of phenols and monosaccharides from the grape skin of Cabernet Sauvignon, Merlot, and Shiraz.

A number of works are aimed at comparative studies of BAS extraction from GP using both SCW and SCW in combination with ethanol at their different percentages [19]. When using a water-methanol mixture (60:40) as an extractant, for the extraction of eleven anthocyanins of the grapes, the following process parameters are optimal: 100 °C for 5 min.

To accelerate the extraction process and increase the yield of anthocyanins from GP of red grape Sunbelt, it is recommended to use a solvent of a mixture of SCW and 70 % ethanol. The total number of anthocyanins extracted at 100 °C was 450 mg/100 g dry pomace [21].

When extracted from the Pinot Nero grape skin and seeds by the SCW in the semi-continuous mode (10 MPa, 80–120 °C, 2–5 ml/min for 2 hours), the yield of total polyphenols increases with increasing temperature and decreasing solvent consumption.

Thus, with an increase in temperature from 80 to 120 °C, the yield of total polyphenols from the skin increased from 44.3±0.4 to 77±3 mg/g and seeds – from 44±2 to 124±1 mg/g [22].

Dynamic extraction (flow rate 1.2 ml/min, 120 °C, pressure 80 bar and extraction time 30 min) of BAS (anthocyanins and phenolic compounds) from red grape skin was carried out with a mixture of SCW and ethanol at a ratio of 1:1. Compared with traditional dynamic liquid extraction, the yield is 3 times higher for anthocyanins; 7 times for the total amount of phenolic compounds and 11 times for flavonols. In addition, the best results were obtained by using grape skin dried for 24 hours at 40 °C. It is noted that ethanol accelerates sharply the extraction of anthocyanins and phenols, probably because it denatures cell membranes and facilitates the solubilization of these compounds [23].

The results of tannin extraction from grape seeds showed that the continuous mode is more effective in comparison with static conditions [24].

The studies showing that the use of SCW allows increasing the yield of the total amount of polyphenols 2 times compared to extraction with 70 % ethanol should be noted [25]. The studies of SCW extraction of GP have confirmed the high efficiency of this process. Thus, the extraction at 140 °C and a pressure of 11.6 MPa provided a high yield of polyphenols, flavonoids and antioxidant activity of the extract [26].

It should be emphasized that in most of the studies reviewed, the possibility of selective extraction of individual BAS from GP and obtaining rational process parameters for each of the studied BAS constituents was not investigated. The effect of flavonoids is mainly due to the antioxidant activity of these compounds. At the same time, the antioxidant activity is considered as a function not of individual groups of flavonoids, but of all polyphenols, including tannins (the tannin-catechin complex). Therefore, if flavonoids are considered as an antioxidant additive, then it is advisable not to separate them from tannins, but to use them together with the latter.

When extracted with organic solvents, the proportion of phenolic substances in the resulting extract is substantially higher and they are represented mainly by non-toxic substances. In the SCW extraction of polyphenols, its small selectivity with respect to these compounds is manifested. This inhibits isolation, separation and purification of polyphenolic substances from SCW extracts. The observed significant increase in their yield due to tannins testifies to the expediency of isolating and using not individual fractions of polyphenols, but the entire tannin-catechin complex. The results of studies of the extraction of total polyphenols from grapes and GP of red and white grape varieties using microwaves and SCW (100–120 °C, 5–30 min), and the use of a mixture of water with (0–2.5 %) sodium carbonate are of interest. The rational parameters for extraction from grapes are 100 °C with an extraction time of 8 min without the addition of sodium carbonate. When extracting polyphenols from GP, it is recommended to use 2.5 % sodium carbonate. The yield of total polyphenols increases with additional use of microwaves in the extraction process [27].

The content of BAS, the chemical profiles of phenolic compounds and anthocyanins, as well as the antioxidant properties of the extracts differ depending on the place of cultivation, time of harvesting, growth environment, and also on the method of extraction [3].

Most methods of SCW extraction of polyphenols are developed for GP from red grape varieties, because the total amount of polyphenols in red grape varieties is higher compared to white varieties. Except for the absence of anthocyanins in GP from white grape varieties, there are no significant differences in the content of BAS [28].

The extracted polyphenols have a fairly wide range of biological and physico-chemical properties, but to date, there is no single extraction procedure that could be considered optimal for all the samples studied [29]. Comparison of the extracts obtained from the GP of Albariño grape variety, collected from 12 wineries located in Galicia (Spain), confirmed the high efficiency of SCW extraction.

Differences in the content of BAS in the extracts were noted: the content of gallic acid ranged from 0.06±0.00 to 0.17±0.03 mg/g dry substances, catechin – from 1.73±0.28 to 4.13±0.39 mg/g, total polyphenols expressed in mg of gallic acid/g dry extract – from 29.1±3.4 to 42.5±1.8, total flavonols expressed in mg catechins/g dry extract – from 10.4±1.7 to 20.4±0.7, total flavonoids in mg catechins/g dry extract – from 19.8±0.0 to 43.5±4.5 [30].

To extract the maximum amount of phenolic compounds, anthocyanins and achieve a high antioxidant activity of the extracts when processing GP from red grape varieties Cab-

ernet Sauvignon, Merlot, Petit Verdot, Syrah, Tempranillo and Tintilla, a water-ethanol mixture (1:1) is suggested. The parameters of the extraction process were 120 °C, 90 bar, a flow rate of 5 g/min and extraction time of 90 min.

Products containing total polyphenols of grapes are characterized by a synergy of the antioxidant activity. In this connection, it is obvious that when creating biologically active products from grapes, it is preferable to obtain total polyphenols dissolved in the liquid phase.

In organic substances, the main role is played by three types of interactions:

1) dispersive, emerging due to the interatomic forces of attraction;

2) intermolecular dipole-dipole interaction;

3) intermolecular hydrogen interaction.

The Hansen parameter represents the solubility parameter in the form of three components, taking into account the energy from dispersion forces among molecules, the energy from dipole forces among molecules and the energy of hydrogen bonds among molecules.

This parameter should be used to describe and predict the properties of the solvent, predict the solubility of the analyte and determine the optimum conditions for subcritical extraction [32–34].

For today, there are several models that have been successfully used when describing the process of SCW extraction of BAS [35–37]. The most promising model is the one constructed using renormalization-group methods. This model allows taking into account the heat and mass transfer properties and the content of BAS in each of the components of a multicomponent polydisperse extractant – skin, seeds, stems, pulp [38].

In different grades of grapes, the content of BAS and their quantity are different. Based on this, the research on the extraction of BAS from grape and GP is focused on the determination of rational process parameters exclusively for the raw material being studied. Thus, it should be noted that the process of SCW extraction from the GP of the table grape Moldova, which is widespread in Ukraine, the Republic of Moldova, in a number of European countries, has not been investigated at present.

3. The aim and objectives of the study

The aim of the research was to study the process features of the SCW extraction of GP and the yield of various target products – BAS.

To achieve the aim, the following objectives have been accomplished:

– to study the influence of SCW extraction process parameters (liquid-solid ratio, temperature, pressure) and the size of the fraction of dry GP on the yield of various target products – BAS;

– to determine the rational parameters of the SCW extraction process and the influence on the yield of various target products – BAS.

4. Used raw materials, equipment and methods of studying the extraction process and the resulting target products

The raw materials. The table grape variety Moldova (producer – Republic of Moldova) was used for obtaining the GP. The average weight of the bunch is up to 350 grams. The berries are large (2.5×1.9 cm), oval, dark purple with a thick wax bloom. The skin is thick, dense, strong. The pulp is fleshy, crispy.

The berries along with the stems were crushed on a juicer and pressed to the humidity of the industrial pomace – 55 %. To analyze the initial characteristics, the GP was analyzed with the determination of the most significant groups of compounds according to conventional methods [39].

The target products in this study were: dry substances, the total content of polyphenolic compounds (tannin-catechin complex), reducing substances, tartaric compounds, gallic acid, furfural, as well as the antioxidant activity of the extracts obtained.

Method of obtaining dry grape pomace. Drying of the original pomace at 75±2 °C to a constant weight was carried out in porcelain bowls placed in a drying cabinet ТРЦ02 ТП-1. The residual moisture of the pomace after drying was 4–7 (% abs.). The resulting agglomerates were crushed to a fraction passing through a 3 mm sieve. Samples in the form of powder were stored in a package that provides protection from the moisture of the environment at room temperature in a dark place.

Experimental equipment and area of experimentation. SCW extraction of GP was carried out in a high-pressure reactor with technical and operational characteristics: the nominal volume of the working chamber was 0.68 l, the temperature recording accuracy, ±0.5 °C, and the sensitivity of the pressure transducer was ±1 MPa [40].

The range of variation of process parameters: the ratio of raw materials and extractant (water) with the liquid-solid ratio – 1:5 and 1:10. The temperature was varied from 100 to 160 °C with a step of 10 °C and was maintained with a regulator with an accuracy of ±1 °C. The exposure time is 30 min, 60 min and 90 min. The repetition of the experiments at each point was triple. The time was calculated after reaching the set temperature.

The pressure level (P) equals 12 MPa, which provides subcritical conditions and a high yield of the extracted substance. This was established based on the thermodynamic properties of water described by the differential equations of thermodynamics of the International System of Equations of 1997 (Formulations IF-97). These equations describe the properties of water in the sub- and supercritical fields. The subcritical field is described by fundamental equations for the Gibbs energy [41].

Method of preparation of samples and determination of the yield of dry extractive substances (DS). To prepare the sample, the GP was weighed, recalculated to an absolutely dry substance (matter) and, in accordance with the accepted liquid-solid ratio, was poured with a portion of degassed distilled water. The sample was kept then under continuous stirring for 45 minutes. Thus, the subsequent extraction process was not affected by the diffusion of water deep into the dry plant material, which would be limited to the extraction process in the initial period of time. The filtration of the extract was carried out on a Buchner funnel. The yield of the dry extract was determined by evaporating the exact volume of the extract (50 ml) at 105 °C under vacuum on a rotary evaporator IR-1M with a temperature regulator ERA-M to a constant weight.

Method for evaluation of polyphenols extraction (tannin-catechin complex). The total content of polyphenolic compounds (tannin-catechin complex) (Ph) was determined in terms of gallic acid by the Folin-Ciocalteu method [5].

Method for evaluation of extraction of reducing substances (RS). Determination of the yield of reducing substances (RS) was carried out using the Feling's reagent [42].

Method of furfural identification. To identify furfural from the extraction products, the nuclear magnetic resonance (NMR) method was used. The investigations were carried out on a multifunction spectrometer of *'Bruker' Avance II 400 MHz*: 50 ml of the substance (furfural) were placed in an ampoule with a solvent (deuterated water or deuterated chloroform). The sample was placed in a strong homogeneous magnetic field H_0 and exposed to radiation with a frequency ν: ^1H – 400 MHz, ^{13}C – 100 MHz at a temperature of 293 K. Chemical shifts (ppm) of the model furfural and obtained extract were compared with chloroform of $CDCl_3$ as the model.

Method of gallic acid identification. Gallic acid was identified by NMR: 50 ml of the substance (gallic acid) were placed in an ampoule with a solvent (deuterated water or deuterated chloroform). Recording parameters of spectra: chemical shifts (ppm) are given for D_2O, ^1H – 400 MHz, ^{13}C – 100 MHz, temperature 293 K.

Method for evaluation of the content of free organic acids in terms of tartaric acid. The determination of the titratable acidity (A) was carried out by alkylmetric titration with phenolphthalein as an indicator [43].

Evaluation of the extracts antioxidant activity. The antioxidant activity of the studied extracts and the kinetics of inhibition of free radicals were studied by the spectrophotometric method DPPH with the construction of a calibration chart for the free radical DPPH. The antioxidant activity of the extract was determined by the formula:

$$AA\% = \frac{[DPPH]_0 - [DPPH]_t}{[DPPH]_0} \cdot 100, \quad (1)$$

where $[DPPH]_0$ – concentration of radical *DPPH* in the set moment of time t=0 sec; $[DPPH]_t$ – concentration of radical *DPPH* determined on spectrophotometer after 30 sec.

5. Results of studies of the influence of the process parameters of SCW extraction of GP on the yield of target products

Statistical processing of the results of experimental studies in the Microsoft Excel 2010 software package allowed obtaining regression dependences (2)–(9) of yields of dry substances (DS), polyphenols (Ph), reducing substances (RS) and acidity of the extract (A) on temperature and time of exposure (Table 1).

The results of regression analysis using the correlation coefficient (R), the determination coefficient (D), the standard deviation (σ), the Fisher criterion (F_{calc}>F_{table}) at the confidence interval of the model coefficients with the error level α=0.05 (95 % reliability level) showed the adequacy of the obtained equations.

The response surfaces for the investigated target products, corresponding to the obtained dependences, are presented in Fig. 1.

The main products of dehydration of sugars are furfural, formed from pentoses and uronic acids and oxymethylfurfural, formed from hexoses, mainly from glucose.

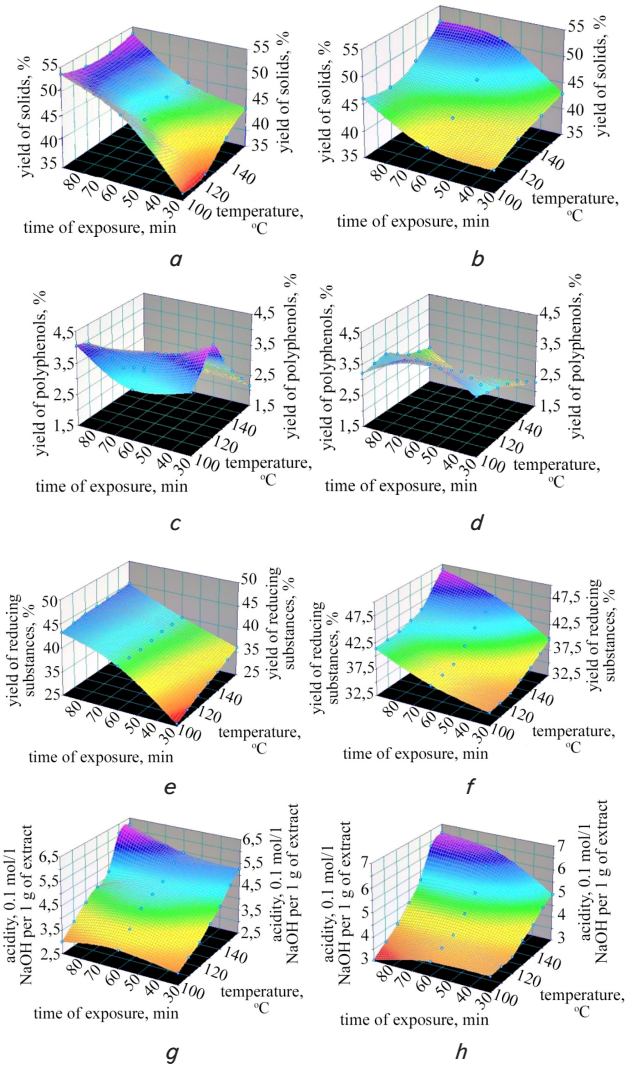

Fig. 1. Response surfaces for the investigated target products: *a* — the yield of dry substances on the working mass with the liquid-solid ratio of 1:5, *b* — the yield of dry substances on the working mass with the liquid-solid ratio of 1:10, *c* — the yield of polyphenols (tannin-catechin complex) with the liquid-solid ratio of 1:5, *d* — the yield of polyphenols (tannin-catechin complex) with the liquid-solid ratio of 1:10, *e* — the yield of reducing substances with liquid-solid ratio of 1:5, *f* — the yield of reducing substances with the liquid-solid ratio of 1:10, *g* — titrated acidity with the liquid-solid ratio of 1:5, *h* — titrated acidity with the liquid-solid ratio of 1:1

The results of identification of the extract for the presence of furfural by the NMR method of radiation with a frequency ν: 1H – 400 MHz, at a temperature of 293 K are shown in Fig. 2.

The results of identification of the extract with radiation at a frequency ν:

^{13}C – 100 MHz at a temperature of 293 K are, shown in Fig. 3.

The position of NMR signals (chemical shift) – the distribution of the electron density along the molecule – almost completely coincides with the standard spectrum on the obtained spectrum. Qualitative analysis of the compositions of the extracts obtained in the SCW medium showed that the extracted substance is indeed furfural.

Table 1

Dependencies of the yield of dry substances (DS), polyphenols (Ph), reducing substances (RS) and acidity of the extract (A) on temperature and time of exposure

The notation of the surface in Fig. 1	Dependencies	Correlation coefficient, R	No. of formulas
a	DS=14.649+ +0.103·t+0.36·τ–0.0014·t	0.987	(2)
b	DS=28.971+0.0745·t+ +0.0270·τ+0.0006·t·τ	0.950	(3)
c	Ph=–5.902+0.164·t+0.019·τ– –0.00019·t·τ–0.00069	0.906	(4)
d	Ph=7.086–0.021·t+0.002·t	0.881	(5)
e	RS=9.189+0.1071·t+0.3172·τ– – 0.00104·t·τ	0.992	(6)
f	RS=23.04+0.078·t+0.034·τ+ +0.00066·t·τ	0.969	(7)
g	A=3.281+0.0053·t– –0.0771·τ+0.000669·t·τ	0.971	(8)
h	A=0.281+0.0271·t– –0.0262·τ+0.000271·t·τ	0.972	(9)

*Note: * where t – the temperature of extraction, °C; τ – the time of exposure, min*

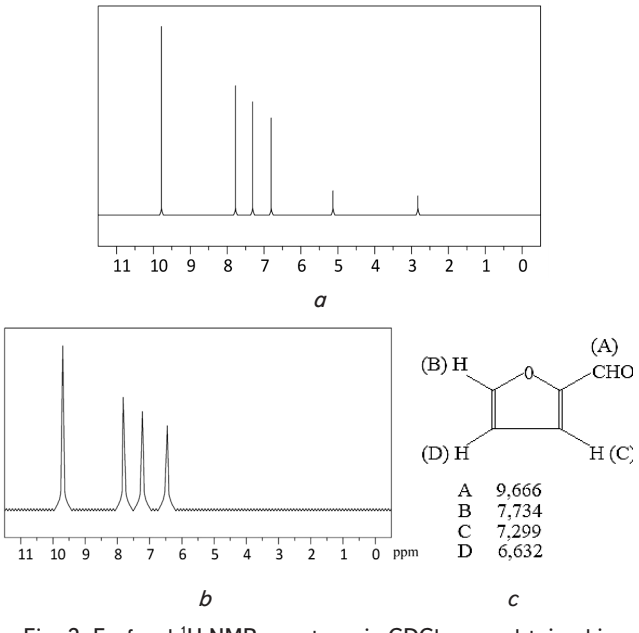

Fig. 2. Furfurol ^{1}H NMR spectrum in $CDCl_3$: *a* — obtained in the research; *b* — model; *c* — chemical shift, ppm

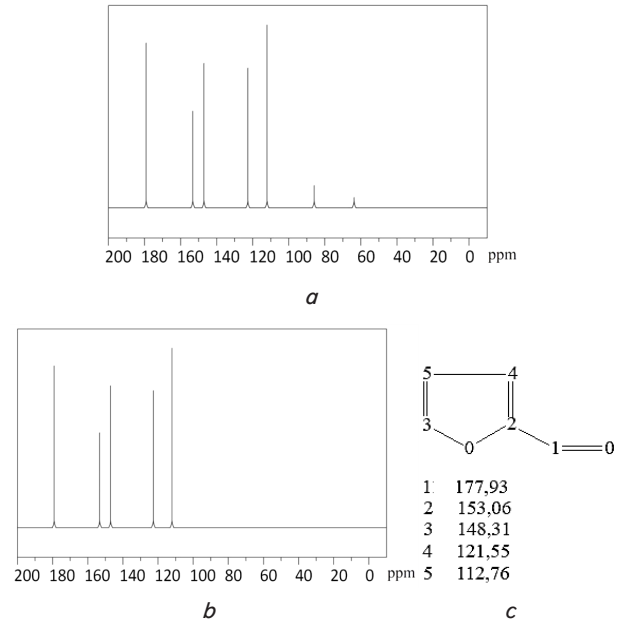

Fig. 3. Furfurol ^{13}C NMR spectrum in $CDCl_3$: *a* — obtained in the research; *b* — model; *c* — chemical shift, ppm

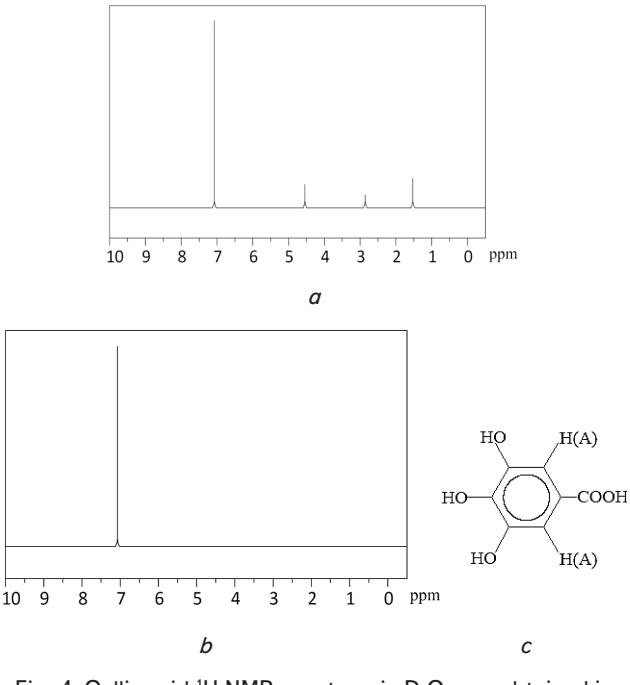

Fig. 4. Gallic acid ^{1}H NMR spectrum in D_2O: *a* — obtained in the research; *b* — model; *c* — chemical shift, ppm: D(A) 7.09

For the extraction of tannins during the SCW extraction, an extract obtained at 100 °C, liquid-solid ratio of 1:10 and exposure time of 30 min was used.

The results of identification of the extract with radiation at a frequency ν: ^{1}H – 400 MHz, at a temperature of 293 K are shown in Fig. 4 and with a frequency ν: ^{13}C – 100 MHz at a temperature of 293 K – in Fig. 5.

The obtained spectra almost completely coincide with the model one, which indicates that the isolated substance is indeed gallic acid.

On the spectra (Fig. 4, 5), additional peaks are observed, which does not correspond to the resonance of furfural and gallic acid atoms.

This is due to the presence of a small amount of impurities. For the same reason, the height of the peaks is somewhat less than the model one.

The yield of gallic acid from the original tannins was 15 %.

The experimental results of the inhibition kinetics of free radicals (percent of DPPH remaining in the steady state) with a 1:5 liquid-solid ratio are shown in Fig. 6, with the liquid-solid ratio 1:10 – in Fig. 7.

As a result of DPPH reduction with the antioxidant (GP extracts), the purple-blue color of DPPH in methanol decreased to yellow, the reaction was controlled by the change in optical density at a wavelength of 514 nm. Using the obtained

data, the antioxidant activity of the extract was determined by the formula (1).

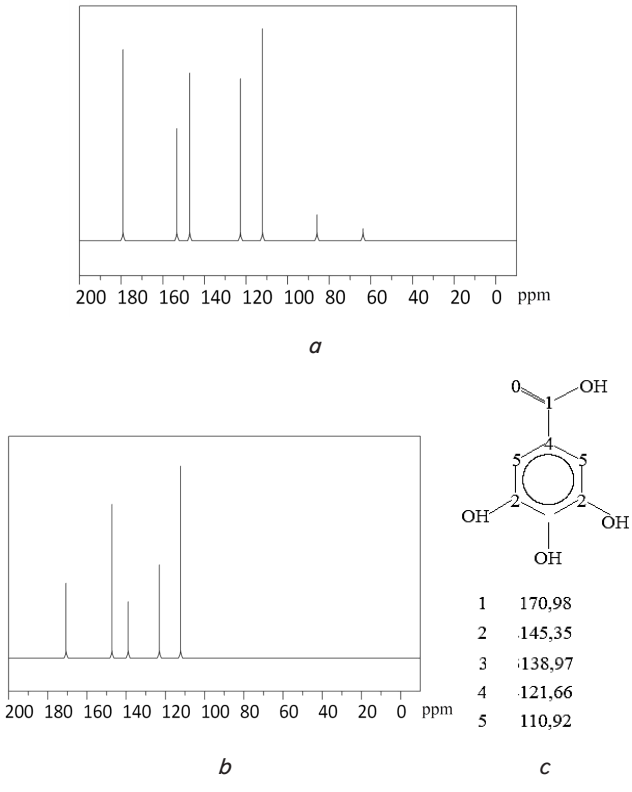

Fig. 5. Gallic acid ^{13}C NMR spectrum in D_2O: *a* — obtained in the research; *b* — model; *c* — chemical shift, ppm

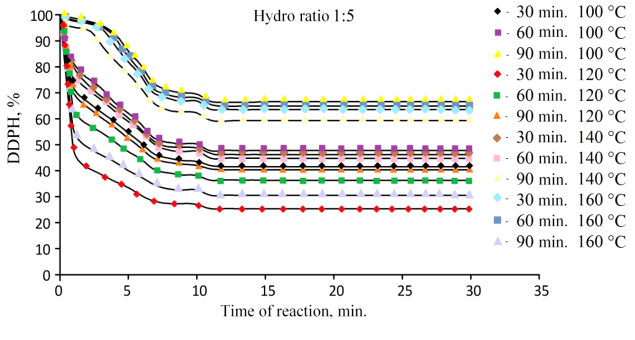

Fig. 6. Kinetic charts of interactions of GP extracts (liquid-solid ratio of 1:5) with the free radical DPPH

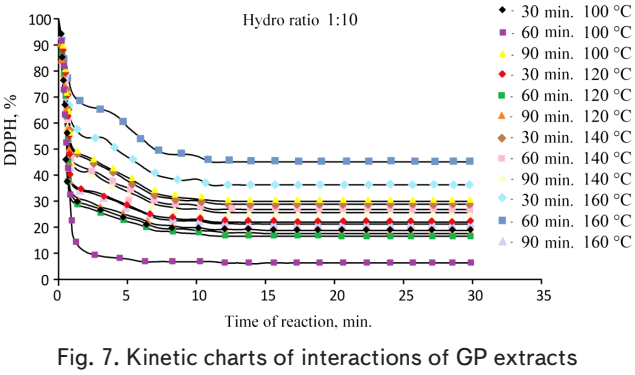

Fig. 7. Kinetic charts of interactions of GP extracts (liquid-solid ratio of 1:10) with the free DPPH radical

The antioxidant activity of GP extracts at liquid-solid ratios of 1:5 and 1:10 is described by the corresponding regression equations (10) and (11):

$$AA=117.1-0.45\cdot t+0.08\cdot\tau_1 (R=0.907), \quad (10)$$

$$AA=120.64\ 0.36\cdot t+0.03\cdot\tau_1 (R=0.881), \quad (11)$$

where t – extraction temperature, °C; τ – time of exposure, min.

6. Discussion of the results of studies on the influence of the process parameters of SCW extraction of GP on the yield of target products

High yield of total extractives even at 100 °C and 30 min (31.9 % at the liquid-solid ratio of 1:5 and 39.4 % at the liquid-solid ratio of 1:10 can be explained by the following factors:

– the residual sugar content of unfermented grape pomace;

– partial hydrolysis of pectin substances, uronic acids and mucus.

Evidently, this process has already been actively proceeding during drying, when grape pomace was exposed to high temperatures for a considerable period of time. Due to its own organic acids, an acidic environment, that can catalyze the hydrolysis process is created. However, the yield of the dry extract and reducing substances from the initial cake was only slightly smaller – by 2–3 % (abs.), than from the dried one. This can be explained by the high saturation of free water in the cake with soluble substances and, above all, with sugars. A high concentration of soluble substances prevents the hydrolysis of polysaccharides and transition to a solution of other compounds.

In the extraction process at the liquid-solid ratio of 1:5, the same factors lead to a lower yield of the dry extract than at the liquid-solid ratio of 1:10. This is especially noticeable at the time of exposure of 30 minutes. As the liquid-solid ratio decreases, the concentration of the extract increases, which leads to the acceleration of thermal destruction processes at high temperatures.

It should be noted that at 160 °C, a considerable swelling of the extracted mass was observed. At this temperature, the liquid-solid ratio of 1:5 and the time of exposure of 90 min, free water was not visually observed in the mass after extraction. This indicates that the liquid-solid ratio of 1:5 is insufficient. At high temperatures, the yield of the dry extract is determined by the ratio of the two processes: formation and transition of water-soluble compounds to the extract and their decomposition under the influence of temperature and acidity. The main contribution to the increase in the extract yield is made by the process of hydrolysis of polysaccharides with their transition into soluble compounds (oligosaccharides, dextrins, monosaccharides). Hydrolysis of pectins is accompanied by the cleavage of acetic acid.

With increasing the time of exposure and temperature up to 140 °C, the yield of the extract increases. At 140 °C, the rates of hydrolysis of hemicelluloses and decomposition of monosaccharides are increased. In this case, strong organic acids are formed – formic and acetic, increasing the concentration of which leads to the acceleration of hydrolysis and decomposition of sugars. The decay of sugars also leads to the formation of oxymethylfurfural, furfural, humic substances, etc.

When washing the sweet pomace with hot water, the yield of the dry extract is up to 15 % of the mass of absolutely dry grape pomace. Wherein, the bulk of the extract are carbohydrates (mainly free monosaccharides) and acidic compounds. In the SCW extraction, the yield of the extract is at least 2 times greater. The increase in the extract yield is explained by the formation of water-soluble carbohydrates of polysaccharides, and a significant amount of polyphenolic compounds is also observed.

SCW extraction at 160 °C by the degree of conversion of plant material is comparable with the acid hydrolysis of wood. But with acid hydrolysis, the dissolution of plant components takes place under more stringent conditions: a temperature of up to 180 °C and the presence of sulfuric acid. Therefore, when using SCW, the formation of an undesirable product (primarily as a result of decomposition of carbohydrates) will be significantly less with a similar flowsheet and, accordingly, the yield of target products will be higher. In addition, in comparison with catalytic hydrolysis, there is no need to neutralize sulfuric acid to form a low-liquid, hardly recyclable waste – gypsum, polluted with organic compounds.

The process of separation of polyphenolic compounds (tannin-catechin complex) is the result of two opposing processes:

1) the transition of polyphenolic compounds into a solution;

2) the secondary transformations of polyphenolic compounds, leading to degradation or transition to an insoluble state and precipitation.

The yield of polyphenolic compounds during the SCW extraction significantly (the maximum yield is increased by a factor of 10) exceeds the amount of polyphenols, obtained by extraction with organic solvents and water, at temperatures up to 60 °C. Such a significant increase is the result of the transition of tannins and products of their hydrolysis into a solution under the influence of high temperatures and acidic medium.

A quantitative analysis of GP extract sugars was determined through the yield of reducing substances in terms of glucose.

For each liquid-solid ratio, the yield of reducing substances increases with increasing temperature and time of exposure. The yield for the liquid-solid ratio of 1:10 for the corresponding temperatures and time of exposure is much higher (by 20–30 %) than for the liquid-solid ratio of 1:5. This is explained by the high concentration of the solution in the second case. The high concentration in accordance with the laws of chemical kinetics leads to a significant acceleration of the decay reactions. The final products of the decomposition of sugars are humic substances that do not manifest a reducing activity.

For the liquid-solid ratio of 1:5, some increase in the yield of reducing substances with increasing temperature is observed. However, it is significantly lower than for the liquid-solid ratio of 1:10 and stabilized already at 120–130 °C. This is explained by the high rate of various chemical transformations of the formed sugars in solutions with a relatively high concentration of extracted substances.

With increasing temperature at the liquid-solid ratio of 1:10 to 120 °C, an insignificant increase in the yield of reducing substances is observed. At a temperature of 120–150 °C, there is a significant increase of sugars, which is explained by the hydrolysis of easily hydrolyzable polysaccharides (hemicelluloses, pectin substances, uronic acids, etc.). Above 150 °C, the amount of extracted reducing substances is stabilized practically at one level, therefore, operation under conditions of stationary extraction is not expedient. However, in the continuous process, it is possible to operate at higher temperatures with increasing the yield of reducing substances.

When analyzing the acidity of the extracts obtained in terms of tartaric acid, a high titratable acidity of the extracts was noted. At the liquid-solid ratio of 1:10, the acidity at 100–120 °C is higher for any time of exposure than at the liquid-solid ratio of 1:5. This is explained by the large dilution of the solution at 1:10, and, correspondingly, by the greater dissolving power. At these temperatures, the acidity function passes through a maximum in time. This is explained by the ratio of the degree of extraction of tartaric acid from the solid phase and its chemical transformations at elevated temperature.

At 140–160 °C, the picture is somewhat different. High acidity is present in the extracts obtained with the liquid-solid ratio of 1:5. This is explained by the hydrolysis of polysaccharides and decomposition of sugars with the formation of strong organic acids – formic and acetic, and also a number of weak ones – levulin, glucosoisosaccharin, xylose isosaccharin, lactic acids. With a smaller liquid-solid ratio, the concentration of sugars in the solution is greater, respectively, and the rate of decomposition is higher. The titrated acidity of the extract increases. With increasing the time of exposure, the degree of decomposition of sugars increases, therefore, the acidity also increases.

According to the formation and transformation of organic acids, the composition of the dry extract obtained by evaporation of water is changed. Wine and levulinic acids are low volatile. They almost completely remain in the dry extract. Formic and acetic acids, on the contrary, are volatile. Therefore, the acidity of the dry extracts obtained by evaporation is less than the acidity of the non-evaporated solutions.

The yield of the acids at relatively low SCW extraction temperatures (100–120 °C) is comparable with the amount of tartaric compounds (in terms of tartaric acid) obtained by washing of the sweet pomace with hot water.

When flushing with hot water, the extraction of tartaric acid compounds reaches 80 % and more of their content in raw materials. Thus, at 100–120 °C, it is possible to extract the tartaric acid almost completely.

At higher temperatures (>120 °C), the amount of organic acids formed was up to 2–3 times higher than with acid hydrolysis of wood at 160–180 °C. This is mainly explained by the way the process is organized. In the traditional hydrolysis of wood with dilute acid (percolation method), the hydrolyzate from the hydrolysis apparatus at the liquid-solid ratio of 1:10 is taken continuously, which significantly reduces the decomposition of the resulting monosaccharides. The experiments described were carried out under stationary conditions. It should be expected that with the percolation method of extraction, the amount of free acids will be reduced significantly. But in any case, at high-temperature extraction, a significant amount of formic and acetic acids will be formed. Therefore, it is necessary to provide for their extraction.

The rates of incidence of the curves are characterized by the binding rate of free radicals (Fig. 6, 7). Consequently, with the liquid-solid ratio of 1:5, free radicals are most quickly bound by the extract of grape pomace, obtained in the SCW medium at a temperature of 120 °C and time of exposure of 30 min.

At the liquid-solid ratio of 1:10, a high rate of binding of free radicals was observed during the reaction with an extract of grape pomace, extracted in the SCW medium at a temperature of 100 °C and time of exposure of 60 min.

The results of the research revealed that the extracts of grape pomace obtained in the SCW medium have a high antioxidant activity from 33 % to 94 % in relation to stable DPPH free radicals. The extracts of grape pomace the most rapidly bind free radicals obtained at the liquid-solid ratio of 1:10, temperature of 100 °C and time of exposure of 60 min.

7. Conclusions

1. As a result of the conducted studies of SCW extraction of GP of Moldova grape variety, it was found that the temperature of the extraction process has the greatest influence on the yield of BAS from the GP. It is expedient to extract not separate fractions of polyphenols from the GP, but the entire tannin-catechin complex. The extracts obtained as a result of SCW extraction of GP are expedient to use for subsequent production of furfural and gallic acids. The use of dry 3 mm fractions of GP ensures the maximum yield of target products.

2. The maximum yield of dry matter during the SCW extraction of GP is provided by the following process parameters: T=150–160 °C, τ=90 min, P=12 MPa and liquid-solid ratio of 1:10. Rational parameters of SCW extraction of GP in the extraction of total polyphenols: T=100–110 °C, τ=60 min, P=12 MPa and liquid-solid ratio of 1:10. The extracts obtained with these parameters have a high antioxidant activity – 94.01 %. Rational process parameters of SCW extraction of GP during the extraction of reducing substances are: T=150–160 °C, τ=90 min, P=12 MPa and liquid-solid ratio of 1:10. These parameters provide extraction of up to 50 % of reducing substances. The high titrated acidity of the extracts obtained (6.649, 0.1 mol/l NaOH per 1 g extract, ml) is provided by extracting the GP with the following process parameters: T=150–160 °C, τ=90 min, P=12 MPa and liquid-solid ratio of 1:5.

References

1. World Vitiviniculture Situation. OIV Statistical Report on World Vitiviniculture [Text]. – Paris: International Organization of Vine and Wine, 2016. – 16 p.
2. Issa, G. (Jason) Chemical composition of composted grape marc [Text] / G. (Jason) Issa, A. F. Patti, G. (Jason) Issa, R. Smernik, K. Wilkinson // Water Science & Technology. – 2009. – Vol. 60, Issue 5. – P. 1265. doi: 10.2166/wst.2009.564
3. Wang, X. Chemical characterization and antioxidant evaluation of muscadine grape pomace extract [Text] / X. Wang, H. Tong, F. Chen, J. D. Gangemi // Food Chemistry. – 2010. – Vol. 123, Issue 4. – P. 1156–1162. doi: 10.1016/j.foodchem.2010.05.080
4. Hamatschek, J. Extraktion der Polyphenole von der Traubennahme biz zur Abfüllung unter besonderer Berücksichtigung der Entsaftung durch Dekanter [Text] / J. Hamatschek, O. Meckler // Mitteilungen Klosterneuburg. – 1995. – Vol. 45, Issue 3. – P. 75–81.
5. Xia, E.-Q. Biological Activities of Polyphenols from Grapes [Text] / E.-Q. Xia, G.-F. Deng, Y.-J. Guo, H.-B. Li // International Journal of Molecular Sciences. – 2010. – Vol. 11, Issue 2. – P. 622–646. doi: 10.3390/ijms11020622
6. Konichev, A. S. Tradicionnye i sovremennye metody ehkstrakciy biologicheski aktivnyh veshchestv iz rastitel'nogo syr'ya: perspektivy, dostoinstva, nedostatki [Text] / A. S. Konichev, P. V. Baurin et. al. // Vestnik MGU. Seriya «Estestvennye nauki». – 2011. – Issue 3. – P. 49–54.
7. Boussetta, N. Valorisation of grape pomace by the extraction of phenolic antioxidants: Application of high voltage electrical discharges [Text] / N. Boussetta, E. Vorobiev, V. Deloison, F. Pochez, A. Falcimaigne-Cordin, J.-L. Lanoisell // Food Chemistry. – 2011. – Vol. 128, Issue 2. – P. 364–370. doi: 10.1016/j.foodchem.2011.03.035
8. Galvan d'Alessandro, L. Ultrasound assisted extraction of polyphenols from black chokeberry [Text] / L. Galvan d'Alessandro, K. Kriaa, I. Nikov, K. Dimitrov // Separation and Purification Technology. – 2012. – Vol. 93. – P. 42–47. doi: 10.1016/j.seppur.2012.03.024
9. Kronholm, J. Analytical extractions with water at elevated temperatures and pressures [Text] / J. Kronholm, K. Hartonen, M.-L. Riekkola // TrAC Trends in Analytical Chemistry. – 2007. – Vol. 26, Issue 5. – P. 396–412. doi: 10.1016/j.trac.2007.03.004
10. Teo, C. C. Pressurized hot water extraction (PHWE) [Text] / C. C. Teo, S. N. Tan, J. W. H. Yong, C. S. Hew, E. S. Ong // Journal of Chromatography A. – 2010. – Vol. 1217, Issue 16. – P. 2484–2494. doi: 10.1016/j.chroma.2009.12.050
11. Chemat, F. Green Extraction of Natural Products: Concept and Principles [Text] / F. Chemat, M. A. Vian, G. Cravotto // International Journal of Molecular Sciences. – 2012. – Vol. 13, Issue 12. – P. 8615–8627. doi: 10.1002/9783527676828
12. Plaza, M. Pressurized hot water extraction of bioactives [Text] / M. Plaza, C. Turner // TrAC Trends in Analytical Chemistry. – 2015. – Vol. 71. – P. 39–54. doi: 10.1016/j.trac.2015.02.022
13. Zakaria, S. M. Subcritical Water Extraction of Bioactive Compounds from Plants and Algae: Applications in Pharmaceutical and Food Ingredients [Text] / S. M. Zakaria, S. Mazlina M. Kamal // Food Engineering Reviews. – 2015. – Vol. 8, Issue 1. – P. 23–34. doi: 10.1007/s12393-015-9119-x
14. Liang, X. Application of Sub-Critical Water Extraction in Pharmaceutical Industry [Text] / X. Liang, Q. Fan // Journal of Materials Science and Chemical Engineering. – 2013. – Vol. 01, Issue 05. – P. 1–6. doi: 10.4236/msce.2013.15001
15. Bondakova (Krivchenkova), M. V. Sovershenstvovanie sposobov polucheniya ehkstrakta vinograda s cel'yu ego dal'neyshego ispol'zovaniya pri proizvodstve kosmeticheskih izdeliy [Text] / M. V. Bondakova (Krivchenkova), E. V. Klyshinskaya, S. N. Butova // Novye himiko-farmacevticheskie tekhnologiy. – 2012. – P. 154–157.
16. Rajha, H. N. Extraction of Total Phenolic Compounds, Flavonoids, Anthocyanins and Tannins from Grape Byproducts by Response Surface Methodology. Influence of Solid-Liquid Ratio, Particle Size, Time, Temperature and Solvent Mixtures on the Optimization

Process [Text] / H. N. Rajha, N. E. Darra, Z. Hobaika, N. Boussetta, E. Vorobiev, R. G. Maroun, N. Louka // Food and Nutrition Sciences. – 2014. – Vol. 05, Issue 04. – P. 397–409. doi: 10.4236/fns.2014.54048

17. Liazid, A. Stability of Anthocyanins from Red Grape Skins under Pressurized Liquid Extraction and Ultrasound-Assisted Extraction Conditions [Text] / A. Liazid, G. Barbero, L. Azaroual, M. Palma, C. Barroso // Molecules. – 2014. – Vol. 19, Issue 12. – P. 21034–21043. doi: 10.3390/molecules191221034
18. Arnous, A. Comparison of methods for compositional characterization of grape (Vitis vinifera L.) and apple (Malus domestica) skins [Text] / A. Arnous, A. S. Meyer // Food and Bioproducts Processing. – 2008. – Vol. 86, Issue 2. – P. 79–86. doi: 10.1016/j.fbp.2008.03.004
19. Da Porto, C. Water and ethanol as co-solvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and a proposal [Text] / C. Da Porto, D. Decorti, A. Natolino // The Journal of Supercritical Fluids. – 2014. – Vol. 87. – P. 1–8. doi: 10.1016/j.supflu.2013.12.019
20. Liazid, A. Microwave assisted extraction of anthocyanins from grape skins [Text] / A. Liazid, R. F. Guerrero, E. Cantos, M. Palma, C. G. Barroso // Food Chemistry. – 2011. – Vol. 124, Issue 3. – P. 1238–1243. doi: 10.1016/j.foodchem.2010.07.053
21. Monrad, J. K. Subcritical Solvent Extraction of Anthocyanins from Dried Red Grape Pomace [Text] / J. K. Monrad, L. R. Howard, J. W. King, K. Srinivas, A. Mauromoustakos // Journal of Agricultural and Food Chemistry. – 2010. – Vol. 58, Issue 5. – P. 2862–2868. doi: 10.1021/jf904087n
22. Duba, K. S. Extraction of polyphenols from grape skins and defatted grape seeds using subcritical water: Experiments and modeling [Text] / K. S. Duba, A. A. Casazza, H. B. Mohamed, P. Perego, L. Fiori // Food and Bioproducts Processing. – 2015. – Vol. 94. – P. 29–38. doi: 10.1016/j.fbp.2015.01.001
23. Luquerodriguez, J. Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues [Text] / J. Luquerodriguez, M. Luquedecastro, P. Perezjuan // Bioresource Technology. – 2007. – Vol. 98, Issue 14. – P. 2705–2713. doi: 10.1016/j.biortech.2006.09.019
24. Garcia-Marino, M. Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction [Text] / M. Garcia-Marino, J. C. Rivas-Gonzalo, E. Ibanez, C. Garcia-Moreno // Analytica Chimica Acta. – 2006. – Vol. 563, Issue 1-2. – P. 44–50. doi: 10.1016/j.aca.2005.10.054
25. Antuon, I. D. Use of sub-critical water for the extraction of natural antioxidants from by-products and wastes of the food industry [Text] / I. D. Antuon // 14th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology. – Italy: University of Sassari Oristano, 2009. – P. 16–18.
26. Aliakbarian, B. Extraction of antioxidants from winery wastes using subcritical water [Text] / B. Aliakbarian, A. Fathi, P. Perego, F. Dehghani // The Journal of Supercritical Fluids. – 2012. – Vol. 65. – P. 18–24. doi: 10.1016/j.supflu.2012.02.022
27. Brahim, M. Optimization of polyphenols extraction from grape residues in water medium [Text] / M. Brahim, F. Gambier, N. Brosse // Industrial Crops and Products. – 2014. – Vol. 52. – P. 18–22. doi: 10.1016/j.indcrop.2013.10.030
28. Kammerer, D. Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis viniferaL.) by HPLC-DAD-MS/MS [Text] / D. Kammerer, A. Claus, R. Carle, A. Schieber // Journal of Agricultural and Food Chemistry. – 2004. – Vol. 52, Issue 14. – P. 4360–4367. doi: 10.1021/jf049613b
29. Escribano-Bailon, M. Polyphenol extraction from foods [Text] / M. Escribano-Bailon, C. Santos-Buelga; C. Santos-Buelga, G. Williamson (Eds.) // Methods in polyphenol analysis. – United Kingdom: The Royal Society of Chemistry, 2003. – P. 1–16.
30. Alvarez-Casas, M. Effect of experimental parameters in the pressurized solvent extraction of polyphenolic compounds from white grape marc [Text] / M. Alvarez-Casas, C. Garcia-Jares, M. Llompart, M. Lores // Food Chemistry. – 2014. – Vol. 157. – P. 524–532. doi: 10.1016/j.foodchem.2014.02.078
31. Otero-Pareja, M. Green Extraction of Antioxidants from Different Varieties of Red Grape Pomace [Text] / M. Otero-Pareja, L. Casas, M. Fernandez-Ponce, C. Mantell, E. Ossa // Molecules. – 2015. – Vol. 20, Issue 6. – P. 9686–9702. doi: 10.3390/molecules20069686
32. Hansen, C. Hansen solubility parameters [Text] / C. Hansen. – Boca Raton: CRC Press, 2007. – 544 p. doi: 10.1201/9781420006834
33. Srinivas, K. Optimization of Subcritical Fluid Extraction of Bioactive Compounds Using Hansen Solubility Parameters [Text] / K. Srinivas, J. W. King, J. K. Monrad, L. R. Howard, C. M. Hansen // Journal of Food Science. – 2009. – Vol. 74, Issue 6. – P. E342–E354. doi: 10.1111/j.1750-3841.2009.01251.x
34. King, J. W. Multiple unit processing using sub- and supercritical fluids [Text] / J. W. King, K. Srinivas // The Journal of Supercritical Fluids. – 2009. – Vol. 47, Issue 3. – P. 598–610. doi: 10.1016/j.supflu.2008.08.010
35. Haghighi, A. Subcritical Water Extraction [Text] / A. Haghighi, M. Khajenoori // Mass Transfer – Advances in Sustainable Energy and Environment Oriented Numerical Modeling. – 2013. doi: 10.5772/54993
36. Khajenoori, M. Proposed Models for Subcritical Water Extraction of Essential Oils [Text] / M. Khajenoori, A. Haghighi Asl, F. Hormozi // Chinese Journal of Chemical Engineering. – 2009. – Vol. 17, Issue 3. – P. 359–365. doi: 10.1016/s1004-9541(08)60217-7
37. Khajenoori, M. CFD modeling of subcritical water extraction [Text] / M. Khajenoori, E. Omidbakhsh, F. Hormozi, Asl A. Haghighi // The 6th International chemical Engineering Congress (IChEC). – Kish Island, Iran: Association of Chemical Engineers, 2009. – Available at: https://www.civilica.com/Paper-ICHEC06-ICHEC06_250.html
38. Sukmanov, V. Physical and mathematical modeling of extraction from grape marc by subcritical water [Text] / V. Sukmanov, L. Gaceu, V. Zakharevych, A. Marynin, I. Rogovyi, A. Farisieiev // Journal of EcoAgriToursim. – 2016. – Vol. 12, Issue 1. – P. 83–93.

39. Olennikov, D. N. Metodika kolichestvennogo opredeleniya gruppovogo sostava uglevodnogo kompleksa rastitel'nyh ob'ektov [Text] / D. N. Olennikov, L. M. Tanhaeva // Himiya rastitel'nogo syr'ya. – 2006. – Issue 4. – P. 29–33.
40. Sukmanov, V. Establishing the equipment-methodical support for determining the properties of extracts of grape pomace extracts produced in the subcreative water environment [Text] / V. Sukmanov, A. Ukrainets, V. Zavialov, A. Marynin // EUREKA: Life Sciences. – 2017. – Issue 5. – P. 18–25. doi: 10.21303/2504-5695.2017.00434
41. Sukmanov, V. O. Apparaturnoe oformlenie processa ehkstragirovaniya biologicheski aktivnyh veshchestv iz vyzhimok vinograda v srede subkriticheskoy vody [Text] / V. O. Sukmanov, Yu. M. Petrova, I. O. Lagovskiy // Aktualni problemy ta perspektyvy rozvytku kharchovykh vyrobnytstv, hotelno-restorannoho ta turystychnoho biznesu. – Poltava: PUET, 2015. – P. 276–277.
42. Alexandrov, A. Management system IAPWS-IF97 for calculating of thermodynamic properties of water and steam for industrial calculations. Additional equations [Text] / A. Alexandrov // Fittings. – 1998. – Issue 10. – P. 64–72.
43. Veshnyakov, V. A. Cravnenie metodov opredeleniya reduciruyushchih veshchestv: metod Bertrana, ehbuliostaticheskiy i fotometricheskiy metody [Text] / V. A. Veshnyakov, Yu. G. Habarov, N. D. Kamakina // Himiya rastitel'nogo syr'ya. – 2008. – Issue 4. – P. 47–50.
44. Ryzhova, G. L. Poluchenie suhogo ehkstrakta iz plodov ryabiny sibirskoy i izuchenie ego himicheskogo sostava [Text] / G. L. Ryzhova, S. A. Matasova, S. G. Bashurov // Himiya rastitel'nogo syr'ya. – 1997. – Issue 2. – P. 37–41.