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Supercritical Fluid Extraction in Resveratrol Isolation Technology

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Abstract

The article discusses the use of supercritical fluid extraction in the technology for the isolation of resveratrol, a phenolic compound found in *Vitis vinifera* L. A technology was developed for obtaining the sum of polyphenolic compounds with a quantitative content of resveratrol. As a raw material for the production of the substance, *Vitis* pomace was used after the production of wine and juice, which makes it possible to introduce complex processing of plant raw materials. For the first time, by the method of carbon dioxide extraction, the conditions for the isolation of resveratrol from *Vitis* pomace raw materials of the Kazakhstani varieties Saperavi and Cabernet were optimized. The influence of pressure (from 10 to 35 MPa), duration (from 60 to 180 min), temperature (from 50 to 70 °C) was studied when optimizing the extraction mode. The quantitative content of resveratrol in carbon dioxide extracts was determined by high-performance liquid chromatography(HPLC). The optimal parameters for the extraction of *Vitis vinifera* L. pomace (pressure, duration, temperature) were established, which provide a relatively high content of resveratrol in the extracts.

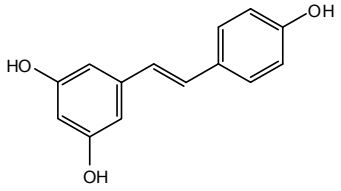
1. Introduction

Resveratrol (1) is a naturally occurring polyphenolic compound whose molecule has the structure of stilbene. Resveratrol (1) is isolated from more than 70 plant species, mainly found in all parts of *Vitis* and products of its processing, in berries, nuts, conifers [1].

Resveratrol (1) has antioxidant, anticarcinogenic, anti-inflammatory, hepatoprotective, neuroprotective, anti-aging and antidiabetic activities [2].

The chemical structure of resveratrol (3,5,4-trihydroxystilbene) was first described by Takaoka, who isolated it from the roots of *Veratrum grandiflorum* (Maxim. ex Baker) O. Loes. [3]. The basic structure of resveratrol consists of two phenolic rings linked together by a styrene double bond that forms 3,5,4-trihydroxystilbene. The double bond is responsible for the isometric cis- and trans-forms of resveratrol.

The peel of *Vitis* contains 50 to 100 μg of resveratrol (1) per gram of dry weight, seeds and stalks – about 6 μg/g [4].



(1)

Traditionally, resveratrol (1) is extracted from natural products using organic solvents [5]. However, these methods require a long extraction time and lead to low yields of the target component in the extract. Recently, supercritical fluid extraction, microwave and ultrasonic extraction have been used for the quantitative extraction of resveratrol, replacing classical methods such as Soxhlet extraction. The use of these extraction methods greatly simplifies the process of resveratrol production and reduces the consumption of solvents [6, 7].

Spanish researchers carried out work to increase the quantitative content of resveratrol in an extract from *Vitis* seeds, stems, peels and pomace of *Palomino fino*, using supercritical extraction with carbon dioxide with the addition of a co-solvent of ethyl alcohol [8].

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One of the ways to extract resveratrol (1) from *Polygonum cuspidatum* Siebold & Zucc is CO_2-extraction of raw materials with subsequent modification of the extract with absolute alcohol and 2-propanol [9].

In work [10], the ability of cyclodextrins to extract phenolic compounds from *Vitis* pomace was studied. The addition of cyclodextrins to the extractant has a positive effect on the quantitative yield of phenolic compounds.

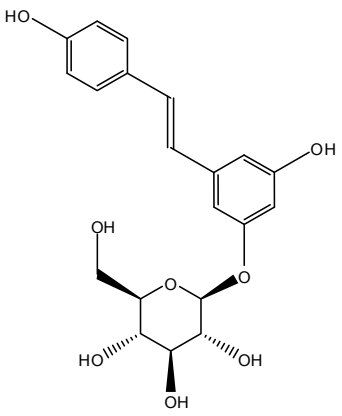
Extraction and microencapsulation of bioactive compounds from *Vitis vinifera* L. pomace have been optimized. The experimental results showed that an increase in temperature and an increase in extraction time lead to an increase in the yield, the total content of phenols, anthocyanin and resveratrol (1) [11].

The patent [12] describes a method for obtaining a *Vitis* extract with increased content of resveratrol (1) by ultrasonic extraction at room temperature for 30–60 min, 80% ethanol.

A method for purifying resveratrol is proposed (1) by preliminary treating raw materials of *Vitis* leaves with liquid nitrogen for 20 min, followed by mechanical grinding of the leaves. Ultrasonic extraction of crushed *Vitis* leaves was carried out with ethanol in the ratio of raw materials and extractant 1:2, at a temperature of 55 °C, duration 40 min. The extract obtained is filtered, concentrated, and dried. Resveratrol (1) was purified by column chromatography using HPD450A macroporous resin sorbent, eluent 30% ethanol, at an elution flow rate of 1.0 ml/min [13].

The technology of Vitis processing waste extraction has been developed. The processed *Vitis* raw material is lyophilized using a LI05P lyophilizer, then the dried raw material is ground in a rotary mill. The dried and ground raw materials are extracted by supercritical fluid extraction at 150 bar, a temperature of 40–60 °C, a duration of 15 to 20 min, a CO_2 gas flow rate of 2 l/min, a co-solvents 7–10% ethanol. An extract saturated with resveratrol is obtained [14].

The method of isolating resveratrol from *Polygonum cuspidatum* is based on 95% ethanol extraction. The extract contains resveratrol (1), polydatin (2), emodin (3) and other components. Hydrolysis of polydatin (2) to resveratrol (1) was carried out by boiling in a water bath for 8 h at 75 °C, solution pH = 1. Due to acid hydrolysis of polydatin glycoside (2), the yield of resveratrol has increased by 4 times, and the content of resveratrol in the enriched fraction was 73.8% [15].



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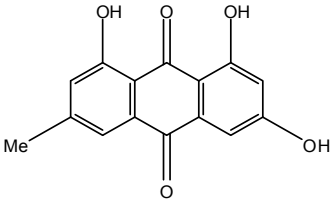

(3)

Liu W. et al. [16] studied the extraction of trans-resveratrol from Vitis rotundifolia pomace by alkaline extraction and foam fractionation. Optimum parameters: temperature 80 °C, sodium hydroxide concentration 12 mM and duration 86 min, then the extract is treated with fractionated foam at a temperature of 30 °C, the volumetric airflow rate of 150 ml/min, ionic strength of 0.3 M/kg. The yield of trans-resveratrol is 90.3%.

Trans-resveratrol has leached from *Vitis* rotundifolia pomace by alkaline extraction. Foam fractionation was used to recover trans-resveratrol from the leach solution, in which the trans-resveratrol concentration was 29.09 mg/l. To enrich the fractions containing trans-resveratrol, silicon dioxide nanoparticles were used and the degree of extraction of trans-resveratrol is 89.73±3.57% [17].

Afshin B. has developed ultrasonic extraction for the production of trans-resveratrol from *Vitis vinifera* L. wastes using a new combination of solvents and a response surface method – the Box-Behnken method. Ultrasonic solvent extraction was carried out using various ethanol: polyethylene glycol: water ratios, sonication times, and temperatures. The optimal extraction conditions were determined: duration – 19.4 min, temperature – 53.6 °C and the ratio of the mixture of extractants 48:32:20. The amount of trans-resveratrol is 862±4.67 mcg/g [18].

Efficient technology for the extraction of resveratrol from peanut sprouts by multistage count-

er-current extraction based on the method of alkaline extraction and acid precipitation was studied. Using one-factor experiments and a Box-Behnken design, the optimal extraction parameters were determined: extraction temperature 46.6 °C, calcium oxide to raw materials ratio 6:100, water to raw materials ratio 8.8: 1, and extraction duration 51.7 min. Resveratrol yield was 0.897±0.035% [19].

A regimen of supercritical liquid extraction for sequential fractionation of fatty acids and polyphenols from wine waste (two different *Vitis* varieties) was optimized. The effect of temperature, pressure and time of extraction with the use of cosolvents for the quantitative content of fatty acids and polyphenols were studied [20].

The optimal values of pressure during extraction, duration of extraction, extraction temperature, and solid-to-solvent ratio were 1.02 MPa, 24.89 min, 152.32 °C and 1:15 g/ml, respectively, the yield of resveratrol reaches 6.90 μg/g in the extract [21].

Pascual-Marty studied the supercritical fluid extraction of resveratrol from the grape peel of *Vitis vinifera* L. Extraction parameters such as pressure, modifier concentration (ethanol) and extraction time are optimized. Final extraction conditions: 40 °C, 150 bar, 7.5% ethanol and extraction time 15 min. Extraction and accuracy were calculated (coefficient of variation from 0.2 to 1.0%) [22].

In a study [23], resveratrol was analyzed in *Vitis vinifera* cv. Pinot Noir by HPLC with a combination of fluorometric and photodiode detection, the sample was extracted in methanol: water (8:2), and the extract was purified. The content of resveratrol in grapes was (102±25) mg/l.

Based on the collected material for the quantitative extraction of resveratrol (1), ultrasonic, alkaline and supercritical fluid extraction using solvents were used. Supercritical extraction is one of the promising and effective methods for extracting resveratrol (1) from plant raw materials and the development of a technology for the production of a substance from *Vitis pomace* using this method is an urgent task.

2. Materials and methods

The objects of the study were samples of red *Vitis* varieties of Kazakhstan selection Saperavi and Cabernet Sauvignon, which are dried waste after *Vitis* processing (pomace, seeds, twigs).

Physicochemical and biochemical parameters were determined: titratable acidity according to GOST 54134-99 (Fruit and vegetable juices. Method for determination of titratable acidity), dry substances according to GOST R 51433-99 (Fruit and vegetable juices. Method for determination of soluble solids content with refractometer), determination of pH according to GOST 26188-84 (Fruit and vegetable products, canned meat and meat-vegetable mixtures. Method for determination of pH), determination of vitamin "C" according to GOST 24556-89 (Products of fruits and vegetables processing. Methods for determination of vitamin C), determination of sugar according to GOST 8756.13-87 (Fruit and vegetable products. Methods for determination of sugars).

Solvent carbon dioxide CO_2 (Mr 44.2). A colorless gas, odorless and tasteless. The volume fraction of carbon dioxide is 99, 98%. Miscible in all proportions with oxygen. GOST-8050-76 food-grade (Carbon dioxide, gaseous and liquid. Technical conditions). Technological processes for obtaining CO_2-extracts from *Vitis* pomace of Saperavi and Cabernet Sauvignon varieties were carried out on a USFE-2/5 unit (Russia).

The quantitative content of resveratrol in CO_2 extracts of *Vitis* pomace was determined using a Hewlett Packard Agilent 1100 Series liquid chromatograph in isocratic mode. An analytical column 4.6×150 mm filled with a Zorbax SB-C18 sorbent (5 μm) was used as a stationary phase; mobile phase: methanol – 5% acetic acid (40:60) (0.5 ml/min). UV detection at 307 nm.

3. Results and discussions

The study of the physicochemical and biochemical parameters of the Saperavi and Cabernet varieties showed that in the Saperavi grapes soluble solids were 23.6%, pH – 4.4, the mass fraction of vitamin "C" – 6.85 mg/%; total sugar – 22%; titratable acidity 7.79 g/dm^3, and in Cabernet grapes – soluble solids amounted to 22.49%, pH – 4.4, mass fraction of vitamin "C" – 7.29 mg/%; total sugar – 21%; titratable acidity 7.61 g/dm^3.

A comparative study of physicochemical and biochemical parameters in whole grapes and *Vitis* pomace of Saperavi and Cabernet varieties was carried out. The results are shown in Table 1.

As can be seen from Table 1, the chemical composition of the area-specific *Vitis* varieties and waste of their processing showed that the data for the two varieties do not differ significantly and correspond to the average indicators for red *Vitis* varieties growing on the territory of the Almaty region.

Table 1
Physicochemical and biochemical parameters of seeds, stalks and peels of *Vitis*

Name	Dry substances, %	pH	Mass fraction of vitamin "C", mg/%	Total sugar, %	Titratable acidity, %
Vitis Saperavi					
Seeds	8.71	6.89	1.55	4.45	0.64
Seeds	8.22	6.74	-	2.72	0.64
Peel	16.51	5.51	2.09	20.81	0.73
Vitis Cabernet Sauvignon					
Seeds	8.59	6.87	1.54	4.40	0.68
Stalk	8.29	6.70	-	2.66	0.58
Peel	16.47	5.57	2.5	20.64	0.67
Vitis Saperavi after processing					
Seeds	8.76	6.86	1.52	4.41	0.62
Stalk	8.20	6.75	-	2.72	0.58
Peel	16.50	5.50	2.16	20.89	0.72
Vitis Cabernet Sauvignon after processing					
Seeds	8.62	6.88	1.55	4.36	0.67
Stalk	8.31	6.71	-	2.68	0.55
Peel	16.51	5.53	2.54	20.69	0.69

The next stage of research was the selection of the optimal conditions for the carbon dioxide extraction of *Vitis* pomace for the quantitative extraction of resveratrol (1).

Taking into account the physicochemical properties of resveratrol, supercritical carbon dioxide extraction of *Vitis* pomace was carried out, aimed at the quantitative extraction of resveratrol from raw materials.

For supercritical carbon dioxide extraction of *Vitis* pomace with the regulation of technological parameters: pressure, temperature, duration of the raw material extraction process.

Analysis of the dependence of resveratrol yield on pressure (Fig. 1). The quantitative content of resveratrol in CO_2-extracts was determined by HPLC. As can be seen from the results obtained, an increase in pressure from 10 to 20 MPa during extraction leads to an increase in the yield of carbon dioxide extract and the content of resveratrol in it. A further increase in pressure from 20 to 35 MPa during extraction does not significantly affect the yield of CO_2-extract but leads to a decrease in the content of resveratrol in it.

In the course of the experiment, it was established that the quantitative extraction of resveratrol from *Vitis* pomace is observed at a pressure of 20 MPa, while the yield of CO_2-extract is 18 g with a resveratrol content of 0.025%.

The next step in selecting the optimal mode of CO_2 extraction for *Vitis* pomace was to determine the effect of the duration of the extraction process on the yield of resveratrol.

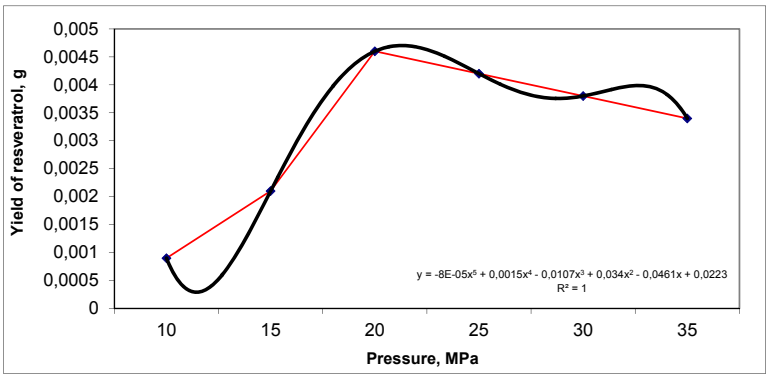

Fig. 1. Quantitative content of resveratrol in carbon dioxide extracts depending on the pressure.

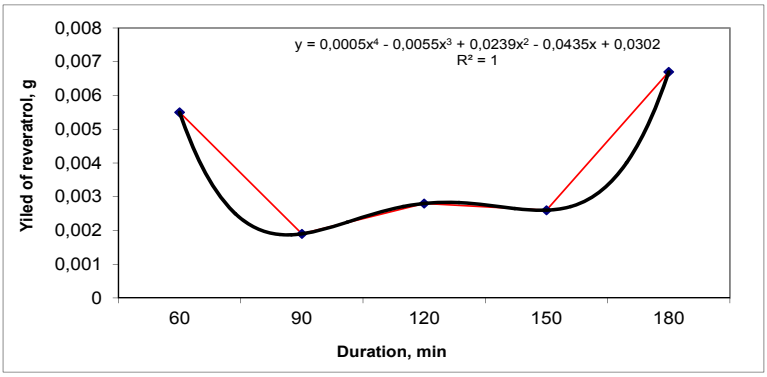

Fig. 2. Quantitative content of resveratrol in carbon dioxide extracts depending on the duration of extraction.

To select a technologically justified duration of the process for the isolation of resveratrol from *Vitis* raw materials, a series of extraction was carried out. The duration of the extraction was increased by 30 min (Fig. 2).

As can be seen from Fig. 2, the extraction of raw materials of *Vitis* pomace, depending on the duration of the process, the most optimal extraction time is 180 min, which helps to increase the yield of the extract and extract the content of resveratrol in it up to 0.029%.

To determine the effect of temperature on the quantitative yield of the extract and the target compound of resveratrol, studies were carried out on the selection of temperature regimes (Fig. 3).

In the course of the experiment, it was revealed that an increase in the extraction temperature from 50 to 60 °C provides an increase in the yield of CO_2-extract and the quantitative content of resveratrol in it. An increase in the extraction temperature from 65 to 70 °C leads to a slight increase in the yield of CO_2-extract, but at the same time, the content of resveratrol in it significantly decreases.

Thus, as a result of the experimental work, the optimal parameters of the extraction process for *Vitis* pomace were determined, using supercritical carbon dioxide extraction, which ensures the quantitative extraction of resveratrol from plant raw materials (Table 2).

Table 2

Parameters of the optimal mode of CO_2-extraction of *Vitis* pomace, providing quantitative recovery of resveratrol

Parameters	Extract of *Vitis* pomace
Pressure, MPa	20
Extraction time, h	180
Extraction temperature, °C	60

Based on the analysis of experimental data, it was established that *Vitis* pomace is a promising raw material for obtaining biologically active extracts, which predetermines the feasibility of developing a technology for their production.

As a result of the experimental work, the optimal parameters of the extraction mode for *Vitis* pomace using CO_2-gas in the supercritical state were determined: pressure 20 MPa, temperature 60 °C, extraction duration – 180 min. Based on the studies carried out, a technology for obtaining resveratrol with the use of CO_2 extraction of *Vitis* pomace was developed, parameters were determined that provide a quantitative yield of resveratrol.

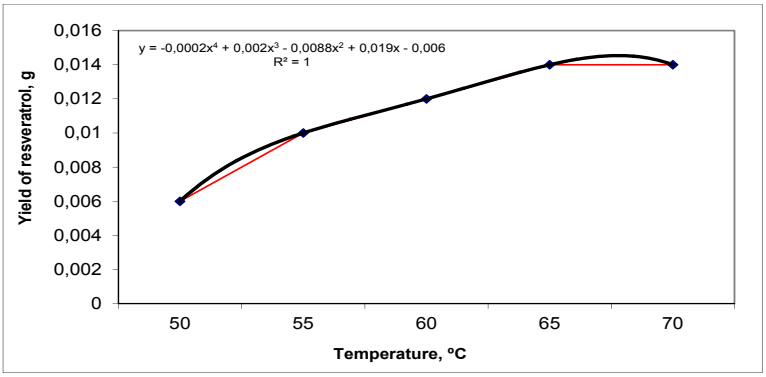

Fig. 3. The quantitative content of resveratrol in carbon dioxide extracts of *Vitis*, depending on the extraction temperature.

4. Conclusions

The raw material of *Vitis* pomace is a potential source of resveratrol – stilbene, which has a pronounced antioxidant effect, which also has a beneficial effect on biomarkers of diabetes, cardiovascular diseases.

According to the results of a comparative study of the physicochemical and biochemical indicators of whole grapes of Saperavi and Cabernet varieties and pomace after processing, it was established that the data for the two varieties do not differ significantly and correspond to the average indicators for red grapes.

Technology has been developed for the production of carbon dioxide extract from *Vitis* pomace using supercritical fluid extraction to ensure the quantitative yield of the target component of resveratrol. The optimal modes of extraction of *Vitis vinifera* L. pomace raw material have been determined, so the quantitative content of resveratrol is achieved at a pressure of 200 bar, an extraction duration of 3 h and a temperature of 60 °C. A regulation for the production of a substance from *Vitis* pomace with a quantitative extract yield of more than 77% is proposed.

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