

Extraction of resveratrol from the pomace of various grape varieties using an enzyme preparation

Abstract. *In the Turkestan region, there are factories for the production of wine materials and wine, the activity of which is associated with the formation of grape pomace, which can later be used as feed for cattle; making grape vinegar; in the production of alcohol; when preparing nutrient media for growing yeast. However, such use is not always advisable, given the unique biochemical composition of grape pomace. In addition, this product is not produced in Kazakhstan, and the cost of resveratrol powder produced in Russia is about 2,452.53 rubles per 50g. This article discusses methods for extracting resveratrol from different grape varieties using enzyme preparations.*

Resveratrol is a biologically active substance from the group of polyphenols, isolated from red grapes, grape pomace, and grape seeds, which have anticarcinogenic, antioxidant, and anti-inflammatory properties. Extraction experiments were carried out in three combinations using A. awamori F-RKM 0719 culture, Pectinol F-RKM 0719 enzyme preparation, and control. The analysis carried out has fully confirmed the effectiveness of using the enzyme preparation Pectinol F-RKM 0719. In all the experiments carried out, an increase in the amount of released resveratrol was observed almost twice as compared with the control experiments. Also, the use of the A. awamori F-RKM 0719 strain, the producer of the enzyme preparation Pectinol F-RKM 0719, showed a positive effect on the resveratrol yield. When using the Cabernet Sauvignon variety, the maximum amount of resveratrol which corresponds to the previously conducted experiments on the extraction of resveratrol from grape berries of various varieties.

Keywords: *grapes, grape pomace, resveratrol, polyphenols, enzyme preparation.*

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Introduction

Kazakhstan having huge land resources with a good biochemical soil composition and a favorable climate, especially in the south of the country, has every chance of becoming one of the leading countries in the world in the field of viticulture and winemaking. Grapes are used to obtaining wine, fresh and dried berries, jams, juices, etc. The traditionally recognized usefulness of grapes is confirmed by modern studies of the antioxidant, anti-inflammatory, and antitumor activity of the resveratrol contained in them. Positively affecting the cardiovascular system, resveratrol is present in various proportions in all of the above viticulture products. [1].

In the conditions of the south and southeastern zone of Kazakhstan, with an abundance of solar heat and the availability of irrigation water, vineyards allow getting a good harvest and can be highly profitable [2]. 70 percent of all Kazakhstani grapes are grown in South Kazakhstan [3], and in recent years there has been interesting from entrepreneurs in the field of wine production. So, in the Turkestan region, Saryagash district, LLP Chateau Silk Alley was recently opened, specializing in the first stage in the production of red wines, such as Cabernet Sauvignon, Cabernet Franc, Chardonnay, Muscat, Rkatseteli, Taifi, etc. season, its production base assumes a one-time supply of 3 million liters of wine material [4].

At the Issyk plant, located near Almaty, they began to use enzyme preparations for the enzymatic maceration of fruit and berry raw materials in the production of wine fifty years ago. This became a good basis for the development of winemaking [5]. Enzymes play a multifaceted role in the living cell. Using them in the implementation of processes associated with the destruction of the walls of plant cells allows for maximizing the extraction of the main ingredients of plant raw materials. Therefore, enzymes should be widely used in the food industry in the production of juices and wines.

Imported enzymes and enzyme preparations are widely used in Kazakhstan. Only the development of biotechnology, in particular the production of enzyme preparations, will make it possible to move the food industry to a new level without waste and low-waste technologies.

The most used industrial strains producing pectinases belong to the genus *Aspergillus*, synthesizing a wide range of hydrolytic enzymes, which provide effective destruction of polymers of plant raw materials in technologies for the production of juices fermented drinks based on juices [6].

The ingestion of polyphenols in the human body has a positive effect on the normalization of blood pressure. Grape polyphenols in a certain amount help to improve the metabolism of substances, especially glucose and lipids. However, it should be noted that the results of some studies show conflicting results. Resveratrol is a polyphenol found in red wine in an amount of 0.2-7 mg / l, depending on the grape variety from which it is produced.

In the production technology of table grape wines, the mass concentration of phenolic substances is not standardized [7]. At the same time, it is known that modern industrial technologies for the production of red wines make it possible to achieve a phenol content in the range of 1.5–5 g/dm³ [8]. Food concentrate of polyphenols "Enoant" prepared from a water-alcohol infusion of sweet pomace of Cabernet Sauvignon grapes with a total concentration of phenolic substances of 18-20 g/dm³ [9].

Among the many positive effects of resveratrol are the normalization and improvement of cellular metabolism, the regulation of fat and carbohydrate metabolism, strengthening of the vascular wall and reducing its permeability, improving blood rheological parameters, and anti-allergic, radioprotective, anti-inflammatory, anti-cancer and vasodilatory effects [10-13]. It should be noted that a significant amount of resveratrol remains in the grape pomace [12]. This healthy compound is present in the grapes, but a fraction is lost as waste after juice production and another fraction has limited bioavailability, reducing the effectiveness of these polyphenolic compounds [13].

Thus, a review of the literature shows the need to develop new efficient extraction methods to increase the bioavailability of polyphenols [11]. Resveratrol is found in red wine in an amount of 0.2-7 mg / l, depending on the grape variety from which it is produced. It should be noted that a significant amount of resveratrol remains in the grape pomace [12].

The purpose of the research is to determine the effectiveness of the use of enzymes to obtain a large amount of extractable resveratrol from the pomace of various grape varieties.

Materials and methods

A number of generally accepted methods were used in the studies: isolation of pure cultures of microorganisms-producers of pectinases from the environment; determination of the degree of pathogenicity and allergenicity of the strain by conventional methods (Birger MO, 1982) was carried out at the Kazakh Institute of Nutrition (LLP «Nutritest»); determination of the mass concentration of resveratrol by the method of high-performance liquid chromatography (HPLC), used for the analysis of polyphenols in plant raw materials [14]; determination of enzymatic activity was carried out according to the methods of the current standards (GOST R): GOST R 55298-2012 – Methods for determining pectolytic activity. The standard applies to enzyme preparations and enzyme-containing mixtures – sources of enzymes of the pectolytic complex used in the food industry and establishes methods for determining pectolytic activity; <https://docs.cntd.ru/document/1200100980>.

The determination of the total pectolytic activity was carried out using an LIR-2 interferometer (Russia), where, under the influence of enzymes of the pectolytic complex, the hydrolysis of pectin was not precipitated by zinc sulfate was determined. A unit of pectolytic activity was taken as the amount of enzyme that, at pH 5.0 and a temperature of 30°C, catalyzes 1 g of pectin in 1 min to products that are not precipitated by zinc sulfate.

GOST R 55293-2012 – Methods for determining cellulase activity. This standard applies to enzyme preparations and enzyme-containing mixtures of cellulolytic action used in the food industry and establishes a method for determining cellulase activity; <https://docs.cntd.ru/document/1200100977>.

Determination of cellulase activity is based on measuring the rate of formation of reducing sugars by the Somogyi-Nelson method during the hydrolysis of carboxymethyl cellulose. A 1% solution of sodium carboxymethyl cellulose in 0.1 M acetate buffer with pH 5 is used as a substrate for 5 minutes. The off-scale activity of cellulase activity is such an amount that in 1 minute at a temperature of 50 ° C and pH 5.0 hydrolyzes carboxymethyl cellulose with the formation of reducing sugars equivalent to 1 μmol of glucose.

GOST R – Methods for the determination of pectate and pectin lyase activity. This standard applies to enzyme preparations and enzyme-containing mixtures – sources of enzymes that destroy pectin substances by the transaminase mechanism of action, used in the food industry, and establishes methods for determining pectate and pectin-lyase activity;

The multienzyme complex was obtained by mixing enzyme preparations containing pectinase, cellulase, 3emicellulose, and β-glucanase, which were taken in optimal amounts determined experimentally. The enzyme preparation was mixed in a PM 100 ball mill (Retsch, Germany) for 10 min at 250 rpm at room temperature.

Results and Discussion

To select plant raw materials as an industrial source of biologically active substances, one should be guided by the following criteria: a relatively high content of resveratrol in the raw material, a stable raw material base, indicators of the quality of the isolated resveratrol in accordance with the requirements of the food and medical industry. Resveratrol is a natural phytoalexin secreted by some plants, including grapes, as a defense against parasites such as bacteria and fungi, with most of it, concentrated in grape skins. Studies carried out on model objects have proven the therapeutic effect of resveratrol, but its effect is limited by its low bioavailability. It was found that the highest mass concentration of phenolic and coloring substances was achieved with a sugar content of grapes of 180-250 g / dm³ during the entire ripening period.

To isolate resveratrol from grape pomace, pre-crushed, extraction was carried out using an aqueous solution of alcohol - 70% as one of the optimal extractants. To maintain the stability of polyphenols in a concentrated extract of grape pomace, a method was developed that involves the distillation of ethanol under vacuum from an equilibrium extract to a strength of 3% by volume. Then, after evaporation, hot water was added, and after the removal of non-polar carotenoid compounds, fats, and other lipophilic substances, resveratrol was extracted from the aqueous medium with aglycones.

Further, the identification of resveratrol from Cabernet Sauvignon grapes was carried out. Detection of resveratrol in the HPLC study was performed at a wavelength of 307 nm. Resveratrol retention time 8.3 min. Extraction experiments were carried out in three combinations using *A. awamori* F-RKM 0719 culture, Pectinol F-RKM 0719 enzyme preparation, and a control using only chemicals. The most promising for the isolation of resveratrol is Cabernet Sauvignon because the highest mass concentration of phenolic and coloring substances is found in berries from the selected variety (Figure 1-3).

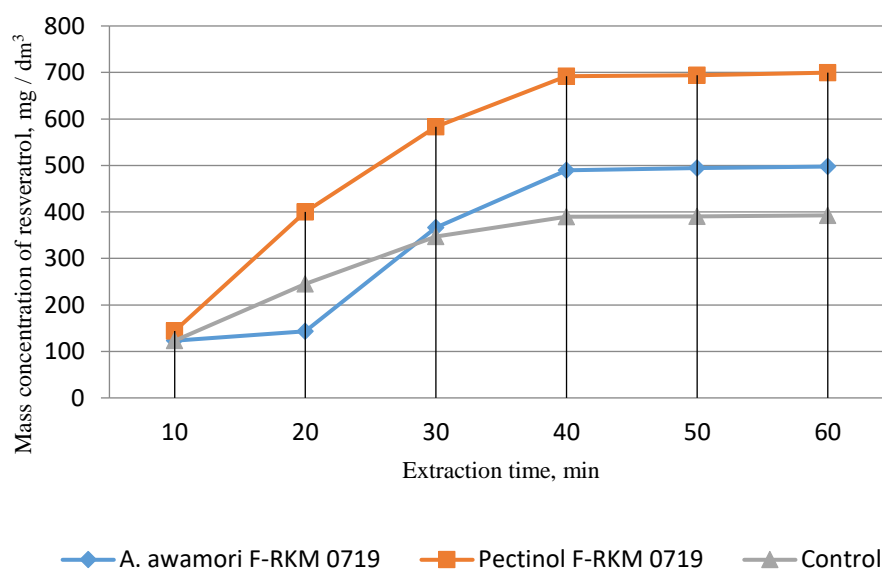


Figure 1. Effect of the enzyme preparation Pectinol F-RKM 0719, produced by the A. awamori F-RKM 0719 strain on the extraction of resveratrol from the pomace of Cabernet Sauvignon grapes

Enzyme preparation Pectinol F-RKM 0719, isolated from A. awamori F-RKM 0719, purification using the sorption method and ion analysis with a high degree of color purification - 99%, with a specific activity of 525.0 units / ml of protein, with a high the yield of enzymes by pectinase activity - 10.8 U / ml and polygalacturonase activity - 6.8 U / ml.

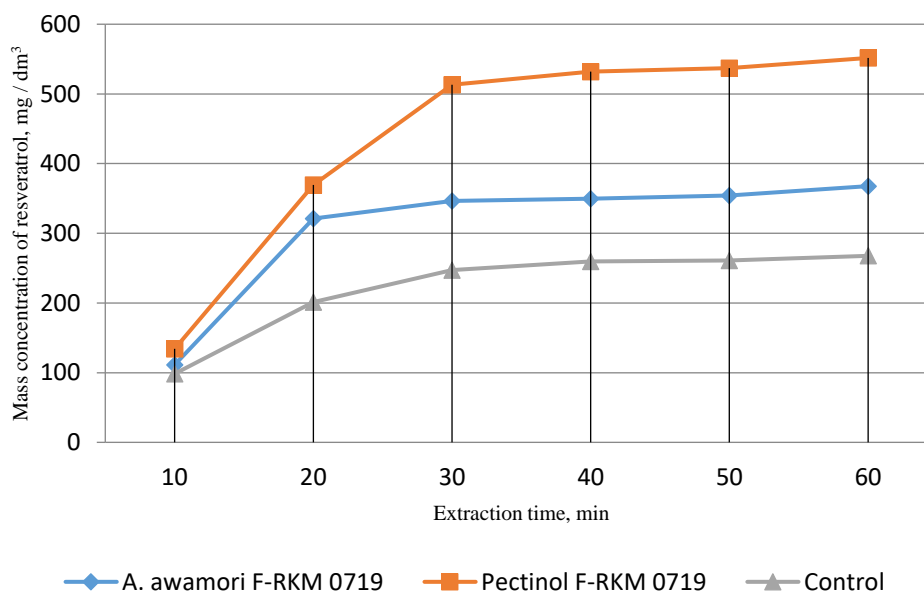


Figure 2. Influence of the enzyme preparation Pectinol F-RKM 0719 produced by the A. awamori F-RKM 0719 strain on the extraction of resveratrol from the pomace of Saperavi grapes

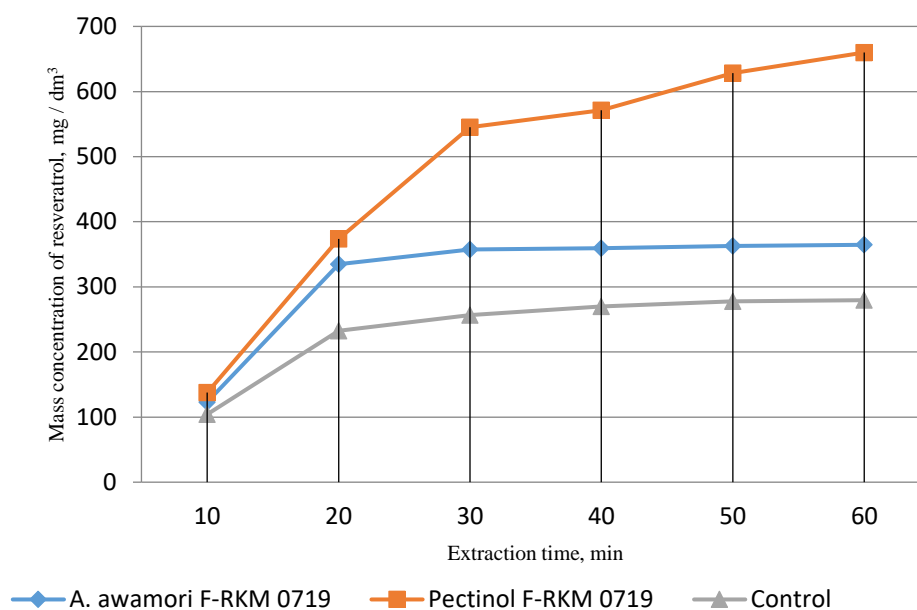


Figure 3. Influence of the enzyme preparation Pectinol F-RKM 0719, produced by the A. awamori F-RKM 0719 strain on the extraction of resveratrol from grapes pomace of the Matrasa variety

The A. awamori F-RKM 0719 strain was deposited in the Republican State Enterprise on the right of economic management "Republican Collection of Microorganisms" of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan. The enzymatic activity of the new strain was: pectinase - 1.65 U / ml, polygalacturonase - 0.97 U / ml, pectinesterase - 0.52 U / ml, pectin-lyase - 1.30 U / ml, β -glucanase - 1.22 U / ml. / ml, cellulolytic - 0.90 units / ml [15].

When developing combined methods for obtaining an enzyme preparation, the efficiency of using methods of purification from interfering impurities was taken into account. The method should also ensure the maximum thermal and pH stability of the enzymes. After pre-treatment of the cultural biomass, active enzymes were extracted, and then they were purified and concentrated. The cleaning process includes the following steps: treatment with microporous coals; treatment with anion exchange resins; isolation and activation of pectinases with nanosized Si-hydroxyapatite; concentration and desalting by ultrafiltration and freeze drying [15].

The analysis carried out fully confirmed the effectiveness of the use of the enzyme preparation Pectinol F-RKM 0719. In all the experiments carried out, an increase in the amount of resveratrol released was observed almost twice as compared to the control experiments. Also, the use of the A. awamori F-RKM 0719 strain, the producer of the enzyme preparation Pectinol F-RKM 0719, showed a positive effect on the resveratrol yield. When using the Cabernet Sauvignon variety, the resveratrol yield is maximum, which corresponds to the previously conducted experiments on the extraction of resveratrol from grape berries of various varieties.

The temperature of the extraction process has a significant effect on the amount of resveratrol released due to changes in the structure of the protein complex of the multienzyme component, and the efficiency of the exchange of individual components with their contents and a set of phenomena that change the microenvironment of the main enzyme - Pectinol F-RKM 0719 by other drugs.

Experiments on the extraction of resveratrol showed an increase in the isolated extract with an increase in temperature from 20 to 35 ° C, a further increase in temperature had a positive effect only in control experiments without using the A. awamori F-RKM 0719 strain and the Pectinol F-RKM 0719 enzyme preparation. The maximum extraction of resveratrol was in experiments with grape pomace of Cabernet Sauvignon. The mass concentration of resveratrol is approximately 700 mg/ dm³. The optimum extraction temperature was chosen at 35 ° C, with a duration of 30 minutes.

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Conclusion

In South Kazakhstan, 70 percent of all Kazakhstani grapes are grown, and the area of grapes is increasing every year. Grapes are valuable raw materials for obtaining grape juice, wine, grape oil, and raisins. Grape pomace is a by-product of grape processing and winemaking. By their chemical composition, secondary products of grape processing are valuable raw material for obtaining a variety of new products.

The use of enzyme preparations will increase the amount of extractable resveratrol from grape pomace. Its industrial production will displace the imported product. Its use in the food industry will positively affect the health of the population. Extraction and continued use of resveratrol are medically recommended. Resveratrol has a positive effect on the body's metabolism, in particular on the normalization of blood pressure and blood glucose levels, as well as improving lipid metabolism. However, some studies have shown conflicting results, which may be related to significant restrictions on their bioavailability. Consequently, more extensive and in-depth scientific research is needed to more accurately determine the role of polyphenols, methods of extraction, and enhancement of their bioavailability.

The experiments showed the effectiveness of using the *A. awamori* F-RKM 0719 strain, the producer of the enzyme preparation Pectinol F-RKM 0719, and the multienzyme complex of the enzyme preparation Pectinol F-RKM 071, while the optimum extraction temperature was chosen at 35 ° C, with a duration of 30 minutes. Maximum extraction of resveratrol was in experiments with the pomace of Cabernet Sauvignon, the mass concentration of resveratrol approximately 700 mg / dm³.

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Әр түрлі жүзім сорттарынан ферментті препараттың көмегімен ресвератролды алу

Аңдатпа. Оңтүстік Қазақстанда барлық қазақстандық жүзімнің 70 пайызы өсіріледі және жыл сайын жүзім алқабы артуда. Түркістан облысында шарап материалдары мен шарап өндіретін зауыттар жұмыс істейді, олардың қызметі жүзім сығындыларының түзілуімен байланысты, оларды кейіннен ірі қара малға азық ретінде пайдалануға; жүзім сірке суын дайындауға; спирт өндіруге; ашытқы өсіру үшін қоректік ортаны дайындау кезінде қолдануға болады. Бірақ, жүзім сығындыларының ерекше биохимиялық құрамын ескеріп, мұндай пайдалану әрдайым ұсынылмайды. Мақалада ферментті препараттарды қолданып, жүзімнің әртүрлі сорттарынан ресвератролды алу әдістері қарастырылады.

Ресвератрол-бұл қызыл жүзімнен, жүзім сығындыларынан және жүзім сүйектерінен бөлінген, антиканцерогендік, антитотықтырғыш және қабынуға қарсы қасиеттері бар полифенолдар тобындағы биологиялық белсенді заттар. Бөліп алу тәжірибелері А. awamori F-RKM 0719 мәдениетін, пектинол F-RKM 0719 ферменттік препаратын және бақылауды қолданып, үш комбинацияда жүргізілді. Жүргізілген талдаулар Пектинол F-RKM 0719 ферменттік препаратын пайдалану тиімділігін толық анықтады. Барлық жүргізілген тәжірибелерде бақылау тәжірибелерімен салыстырғанда бөлінген ресвератрол мөлшерінің екі есе өсуі байқалады. Пектинол F-RKM 0719 ферменттік препаратын өндіруші А. awamori F-RKM 0719 штаммын қолдану ресвератролдың шығымына оң әсерін көрсетті. Каберне Совиньон түрін қолданған кезінде ресвератролдың шығымы өте көп болады, бұл әртүрлі сорттардың жүзім жидектерінен ресвератролды алу бойынша алдын-ала жүргізілген тәжірибелерге сәйкес келеді.

Түйін сөздер: жүзім, жүзім помасы, ресвератрол, полифенолдар, ферментті препарат.

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Извлечение ресвератрола из разных сортов винограда с помощью ферментного препарата

Аннотация. В Южном Казахстане выращивается 70 процентов всего казахстанского винограда, и с каждым годом площади винограда увеличиваются. В Туркестанской области действуют заводы по производству виноматериала и вина, деятельность которых сопряжена с образованием виноградных выжимок, которые в дальнейшем могут быть использованы в качестве корма для крупного рогатого скота; приготовления виноградного уксуса; в производстве спирта; при подготовке питательных сред для выращивания дрожжей. Однако такое использование не всегда целесообразно, с учетом уникального биохимического состава виноградных выжимок. В данной статье рассматриваются методы извлечения ресвератрола из разных сортов винограда с помощью ферментных препаратов.

Ресвератрол - это биологически активное вещество из группы полифенолов, выделенное из винограда красных сортов, виноградных выжимок и виноградных косточек, обладающее антиканцерогенными, антиокислительными и противовоспалительными свойствами. Опыты по извлечению проводили в трех комбинациях: с использованием культуры *A. awamori* F-RKM 0719, ферментного препарата *Пектинол* F-RKM 0719 и проведение контрольного опыта. Проведенные анализы полностью подтвердили эффективность использования ферментного препарата *Пектинол* F-RKM 0719. Во всех проведенных экспериментах наблюдается увеличение количества выделенного ресвератрола почти в два раза по сравнению с контрольными опытами. Также положительное воздействие на выход ресвератрола оказало использование штамма *A. awamori* F-RKM 0719, продуцента ферментного препарата *Пектинол* F-RKM 0719. При использовании сорта Каберне Совиньон выход ресвератрола максимальный, что соответствует предварительно проведенным опытам по извлечению ресвератрола из виноградных ягод различных сортов.

Ключевые слова: виноград, выжимки винограда, ресвератрол, полифенолы, ферментный препарат.

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