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**SELECTION OF ACTIVE YEAST STRAINS  
FOR FERMENTED BEVERAGES FROM PLANT MATERIALS**

**Abstract.** Fresh juices obtained under sterile conditions, including pomegranate juice, cherries, cherries, red grapes, watermelon juice, beetroot juice, sugar cargo, as well as flushes from the surface of juice-containing berries growing in the Turkestan region were used as sources of yeast cultures. Of 180 isolated yeast species, the majority are *Saccharomyces* - 159, 71 pure cultures are the most typical for the region and suitable for fermentation. A subsequent study of the morphological characteristics of cells, physiological and biochemical properties, clarification of antagonistic activity, and resistance to antibiotics made it possible for further selection of strains. The most highly active and appropriate by technological parameters were selected: *Saccharomyces cerevisiae* AI-06 (from grapes), *Saccharomyces cerevisiae* GI -8 (from sugar sorghum juice) and *Saccharomyces cerevisiae*-Az-12 (from pomegranate juice). Thus, the analyzes showed the possibility of using plant materials not only as freshly squeezed juice of pomegranate, cherry, grape, watermelon juice, sugar cargo, but also as sources of active yeast.

**Key words:** yeast, *Saccharomyces cerevisiae*, pomegranate, fruit, berries, fermented juice.

**Introduction.** An analysis of the health status of the population in Kazakhstan shows that many residents of the country have certain health problems that depend on many factors, including their living conditions. At the same time, one of the serious factors is the environmental impact on public health. High levels of environmental pollution create stressful conditions for the human body. Fruits and vegetables are rich in vitamins, minerals, fiber, etc. They are not only beneficial for the body, but also able to remove toxins and various types of pollutants from the body [1-2]. The Turkestan region is the southernmost part of the country, with a long summer period and is rich in a number of medicinal, fodder, fruit and vegetable, ornamental plants characteristic of the Tien Shan floristic region. The climatic conditions of the Turkestan region allow growing a number of different fruits and vegetables [3]. Fruits containing various organic acids, sugar and other food sources of yeast and predominantly populated by yeast

The yeast strains associated with fruit surfaces are capable of converting large amounts of sugars into alcohol, and they can also tolerate a high concentration of alcohol. Although yeasts of different genera *Kloeckera*, *Hansensiaspora*, *Candida*, *Pichia* are involved in the process of transformation of sugars into acids and alcohols, in most cases, *Saccharomyces* species dominate the final stage of fermentation than any other types of yeast [4-8]. When cultivating yeast, like most living organisms, maintaining a certain temperature is of great importance. At the same time, the overwhelming majority of yeast species belong to the group of mesophilic microorganisms with temperature limits of growth ranging from 2–5 to 30–37° C and optimum at 26–28 °C. True yeast thermophiles are not known among the yeast, for growth of which temperatures are higher 50° – 60° C [5, 7].

**Aim of the study:** development of a fermented beverage based on selected yeasts.

**Materials and methods.** For extract the yeast cells we used the most acceptable methods, including washing with sterile water and scraping with a sterile scalpel. The sources of yeast cultures used washes from the surface of juice-bearing berries growing in the Turkestan region, as well as fresh juices obtained under sterile conditions, including pomegranate juice, cherry, red grapes, watermelon juice, table beet juice, sargo juice. The presence of yeast in them can be set directly under the microscope or after

concentrating on centrifuges at a frequency of 2000 rpm for 15–20 minutes. Samples of liquids were taken in sterile vessels [6, 13].

Wort agar was used as nutrient media; Sabur agar (glucose-peptone media), mycelium formation was investigated on corn-glucose agar medium. These media are used to fully account for and isolate most types of yeast. The most widely used full-fledged medium for growing yeast is also malt wort. It consists of glucose, fructose, sucrose, maltose, maltotriose and maltotetraose, as well as a small amount of pentoses - arabinose, xylose and ribose. Nitrogen components make up 6-7% dry matter (DM), among them ammonium nitrogen is 2.18-2.44 mg per 100 ml. In the wort there are amino acids, all the main B vitamins and minerals, the content of which depends on the water used. The wort is obtained from breweries. It is diluted with tap water to a concentration of 6-8% of DM. Wort agar can be made from dry malt extract. 20 g of the powder is dissolved in 400 ml of hot distilled water containing 12 g of agar, and sterilized at 121 °C for 15 minutes. After sowing, the plates are incubated 24 hours in the usual position so that the agar adsorbs the liquid, and then the Petri dishes are inverted to avoid dropping condensate from the lid to the surface [14-19].

Incubating yeast on dense media from the suspension being studied is made with a pipette, with 0.5 ml or one drop of the measured volume in each dish. A drop of the test suspension containing yeast cells is applied to the surface of the agarized solidified medium in a Petri dish. After that, a sterile glass spatula evenly distribute the drop to the surface. With the same spatula, you can still sow 2-3 dishes in case the first one is very dense growth of colonies [6, 9, 12].

The process of isolating a pure culture ends with the transfer from a separate, grown in isolation colony into a test tube. The isolated cultures were examined for cell homogeneity under a microscope, as well as the uniformity of the colonies on the plate during subsequent incubation [10-13].

Thus, the requirements that must be fulfilled in the research on determining the type of yeast are as follows: before determining each culture should be carefully checked for purity by microscopy and incubated on solid nutrient media; from each source culture, they prepare a so-called control culture by transferring it into a test tube with wort agar and retain its entire work period by definition. When describing morphological traits, standard media and cultivation methods are used, since these traits can vary significantly depending on the medium composition and growing conditions [12, 13].

**Research results.** Microorganisms isolated from various plant substrates were mostly separate budding cells, and yeasts were also found, forming pseudomycelium and individual species with a true well-formed mycelium. Among the studied representatives of the yeast were typical representatives of Oosporidium, also met Rhodoturola, capable of forming primitive pseudomycelium, colonies have a pronounced rather bright red or orange color; individual grown colonies in many respects belonged to Candida cells.

The results of the selection of pure cultures of *Saccharomyces* yeast are presented in table 1.

Of the 180 different types of isolated yeast, most belong to *Saccharomyces* 159 and only 21 cultures to Dipodascaceae, 71 pure cultures were isolated.

Table 1 – The results of the selection of pure cultures of yeast saccharomycetes

Research raw materials	The number of analyzed colonies on the dishes			Isolated pure cultures
	Total	Estimated <i>Saccharomyces</i> Yeast	Giving spores	
Pomegranate	31	24	16	16
Cherry	18	15	15	9
Grape	65	33	24	24
Watermelon	11	8	8	5
Beet	12	8	7	6
Sugar Cargo	43	32	21	11

Since the main goal of these studies is the development of a fermented drink, yeast capable of transforming sugars into biologically active substances useful for the body was selected from all yeast. Among the selected cultures, preference was given to representatives of the culture *Saccharomycets*, the family of *Saccharomycetaceae*. The sizes of the studied single cultures varied in width on average from 4.5  $\mu\text{m}$  to 9  $\mu\text{m}$  and in length up to 10  $\mu\text{m}$ . Form predominantly rounded, oval, elongated.

The most promising were *Saccharomyces cerevisiae* strains isolated from grapes, sugar sargo juice and pomegranate juice, which were obtained by multiple inoculation of individual yeast colonies on solid nutrient media. A further study of the morphological features of the cells, physiological and biochemical properties, clarification of the antagonistic activity, resistance to antibiotics made it possible for further selection. As a result, the strains identified as the following strains of the yeast *Saccharomyces cerevisiae* Al-06 (from grapes), *Saccharomyces cerevisiae* Gl-8 (from sugar sargo juice) and *Saccharomyces cerevisiae* Az-12 (from pomegranate juice), belong to the *Saccharomycecececeae* family species *Saccharomyces*.

Biological species of the genus *Saccharomyces* are a good model for studying the fundamental biological processes: speciation and adaptability of organisms to the environment. Currently, the genus *Saccharomyces* is clearly defined and includes, in addition to *S. cerevisiae*, the species *S. arboricolus*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii*, *S. mikatae* and *S. Paradoxus* [5]. The cultural gene pool of *Saccharomyces* yeast is represented by *S. cerevisiae* and *S. bayanus* species. Different strains of yeast used in the production of various drinks allow you to get drinks unique in taste and aroma [14-18].

Yeast culture *Saccharomyces cerevisiae* Al-06 (from grapes) grows in 1.5% milk at a temperature of 30 °C, fermentation of milk does not occur, gas ( $\text{CO}_2$ ) is formed during the fermentation of juices. When they grow in a solid medium, form beige colonies in a round shape 1.5-2.0 mm in diameter. The culture has a characteristic smell of yeast. The strain does not form pigments diffusing into the medium (figure 1).



Figure 1 – Culture of the yeast *Saccharomyces cerevisiae* Al-06 (from grapes)

The average cell size is  $6.5 \times 7.2 \mu\text{m}$ . The shape of the cells is oval and rounded. Reproduces by budding.

On aqueous agar containing sodium acetate cells form asci with spherical spores with smooth shells, 1 to 4 in cell. Colonies are large, smooth, and convex, with smooth edges on the malt wort agar.

Physiological and biochemical properties. Many simple compounds, such as glucose, fructose, galactose, sucrose, glycerin, can be used as a carbon source. As a result of the fermentation of sugars,  $\text{CO}_2$  and ethanol are formed.

Features of growth: Temperature optimum is  $26 \pm 1^\circ\text{C}$ . Cells grow in the range of 4 °C to 40 °C. The optimum pH of the medium is 4.5-5.5. Keeps viability in the pH range from 2.0 to 10. It grows when the content of bile in the medium is up to 2.5%.

The cultural and morphological properties of *Saccharomyces cerevisiae* Gl-8 yeast strain (from sugar sargo juice) are the following: On the surface of a solid agar medium, round convex, light cream-colored opaque colonies with a smooth edge, 3-3.5 mm in size, smooth surface, glitters, the consistency is soft, buttery (figure 2).



Figure 2 – Yeast culture of *Saccharomyces cerevisiae* Gl-8 (from sugar sargo juice)

The average cell size is  $5.0 \times 8.7 \mu\text{m}$ . The shape of the cells is oval and rounded. Reproduces by budding. On agar medium containing sodium acetate, the cells form aski with spores of spherical shape with smooth shells, 1-4 askies in cell.

Physiological and biochemical properties. Ferments: glucose, sucrose, maltose, galactose, 1/3 raffinose. Does not ferment: lactose and simple dextrans.

Features of growth: Temperature optimum is  $26 \pm 1^\circ\text{C}$ . Cells grow in the range of 4 to  $40^\circ\text{C}$ . The optimum pH of the medium is 3.5-5.5. Keeps viability in the pH range from 2.0 to 10. It grows when the content of bile in the medium is up to 2.5%.

Cultural and morphological properties of *Saccharomyces cerevisiae* Az-12 (from pomegranate juice): on the malt wort-agar colonies are small, smooth, convex, with plain edges (figure 3). The average cell size is  $5.0 \times 6.4$  microns. The shape of the cells is mostly rounded. Reproduces by budding. The yeast does not form a yeast spore.

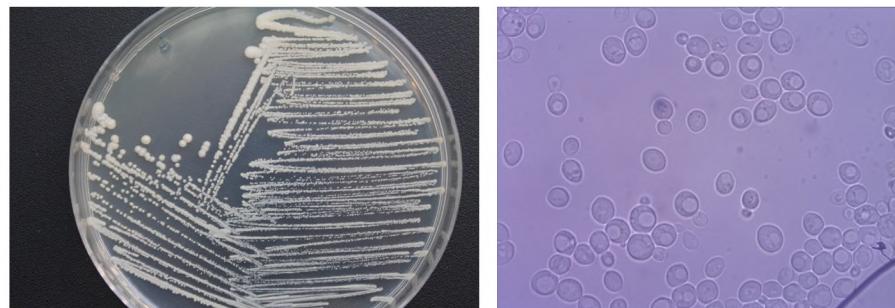


Figure 3 – Culture of *Saccharomyces cerevisiae* Az-12 yeast (from pomegranate juice)

Physiological and biochemical properties. Ferments: glucose, fructose, sucrose, maltose, maltotriose, does not use galactose, consumes pentose in a small amount - arabinose, xylose and ribose, can use many simple glycerol compounds as a carbon source, as a result of fermentation of sugars it forms  $\text{CO}_2$  and ethyl alcohol.

Features of growth: The temperature optimum is  $37 \pm 1^\circ\text{C}$ . Cells grow in the range of 5 to  $45^\circ\text{C}$ . The optimum pH of the medium is 3.5-5.5. Keeps viability in the pH range from 1.2 to 10. It grows when the content of bile in the medium is up to 3.0%.

In relation to oxygen, all of the studied strains are optional.

Antibiotic resistance: *Saccharomyces cerevisiae* Al-06 strains are resistant to gentamicin, cefazolin, amoxiclav, tetracycline, norfloxacin, vancomycin, erythromycin, ciprofloxacin, cefuroxime, amphotericin. Show moderate antagonistic properties in relation to *E. coli*.

The strain *Saccharomyces cerevisiae* Gl-8 is resistant to gentamicin, oxacillin, amoxiclav, tetracycline, norfloxacin, vancomycin, erythromycin, ciprofloxacin, metronidazole, ketonazole, amphotericin. It shows pronounced antagonistic properties in relation to *E. coli* и *Staphylococcus aureus*.

*Saccharomyces cerevisiae* Az-12 is resistant to gentamicin, oxacillin, cefazolin, amoxiclav, tetracycline, norfloxacin, vancomycin, erythromycin, cefotaxime, ciprofloxacin, cefuroxime, metronidazole, ketonazole, amphotericin. They exhibit pronounced antagonistic properties against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The most acceptable cultures were selected in accordance with their relatively fast ability to ferment fruit juices, where the leading factor was high organoleptic characteristics, natural fruit smell, without the appearance of turbidity or large sediment and a pleasant slightly sour taste (таблица 2).

Tasting evaluation of the finished product was carried out on a ten-point scale, where the leading indicators were: color, transparency, smell (aroma), taste, each of the above indicators was given the maximum and minimum rating, which was summarized and determined as the final one. The tasting was attended by students, teachers and technologists of food enterprises.

In the southern region of Kazakhstan, a large number of fruits, berries and vegetables are grown, but due to the lack of effective technologies for processing vegetable raw materials and recipes for obtaining fermented beverages, it is impossible to expand the range of fermented fruit drinks intended for the prevention of various common diseases.

The development of fermented beverages based on the juice of fruit and berry raw materials will allow to replenish the range of products for therapeutic and preventive purposes by enriching the final product with a number of functional ingredients, and as a result will give an overall improvement in the health of the population [19-20].

Table 2 – Chemical indicators and tasting evaluation of mixed fruit juices, fermented by experienced yeast

Fruit Juice	Kind of Yeast	Volume fraction of ethyl alcohol, %	Mass concentration of sugars, g/dm <sup>3</sup>	Mass concentration of titratable acids, g/dm <sup>3</sup>	Mass concentration of volatile acids, g/dm <sup>3</sup>	Tasting evaluation
Watermelon juice	<i>Saccharomyces cerevisiae</i> Al-06	1,5	4,2	5,01	0,45	6
	<i>Saccharomyces cerevisiae</i> Gl-8	2,3	3,1	5,64	0,51	7
	<i>Saccharomyces cerevisiae</i> Az- 12	1,6	3,9	5,21	0,54	8
Pomegranate juice	<i>Saccharomyces cerevisiae</i> Al-06	2,6	5,8	6,93	0,72	8
	<i>Saccharomyces cerevisiae</i> Gl-8	3,7	4,1	7,82	0,75	8
	<i>Saccharomyces cerevisiae</i> -Az- 12	2,8	5,3	6,15	0,78	9
Mixed juice (watermelon-pomegranate)	<i>Saccharomyces cerevisiae</i> Al-06	2,3	4,9	5,92	0,55	8
	<i>Saccharomyces cerevisiae</i> Gl-8	3,1	2,7	8,43	0,59	9
	<i>Saccharomyces cerevisiae</i> -Az- 12	1,8	4,5	5,15	0,64	10

Thus, performed analyzes show the possibility of using plant materials not only as freshly squeezed juice of pomegranate, cherry, grapes, watermelon juice, sugar sargo juice, but also as sources of active yeast. Of the yeast isolated from plant substrates, the most acceptable from a technological point of view, as well as those with pronounced antagonistic abilities in relation pathogens are *Saccharomyces cerevisiae* Az- 12 и *Saccharomyces cerevisiae* Gl-8

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**Conclusion.** As a result of the selecting work, *Saccharomyces cerevisiae* strains isolated from grapes, sugar sargo juice and pomegranate juice were chosen and obtained by multiple passages of individual yeast colonies on solid nutrient media and identified as *Saccharomyces cerevisiae* Al-06, *Saccharomyces cerevisiae* Gl-8 и *Saccharomyces cerevisiae* Az- 12.

The most promising were *Saccharomyces cerevisiae* Gul-8 and *Saccharomyces cerevisiae* Az-12 with the ability to ferment fruit juices relatively quickly, and the leading factor was high product quality: organoleptic characteristics, natural fruit odor, no turbidity, and a pleasant slightly sweet, slightly sour taste.

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## **СЕЛЕКЦИЯ АКТИВНЫХ ШТАММОВ ДРОЖЖЕЙ ДЛЯ ФЕРМЕНТАТИРОВАННЫХ НАПИТКОВ ИЗ РАСТИТЕЛЬНОГО СЫРЬЯ**

**Аннотация.** Анализ состояния здоровья населения в Казахстане показывает, что проблемы со здоровьем в той или иной мере имеют многие жители страны, которые зависят от многих факторов, включая и условия их проживания. Фрукты и овощи, обладая высоким содержанием витаминов, минералов, клетчатки и т.д., не

только благоприятно воздействуют на организм в целом, но и способны выводить токсины и различные виды поллютантов из организма. Фрукты, содержащие различные органические кислоты, сахар и другие источники питания дрожжей, преимущественно населены дрожжами.

В качестве источников дрожжевых культур использовали свежие соки, полученные в стерильных условиях, в том числе сок граната, вишни, черешни, красного винограда, арбузный сок, сок столовой свеклы, сок сахарного сарго, а также смывы с поверхности сокосодержащих ягод, произрастающих в Туркестанской области. Так как основной целью данной работы было разработка ферментированного напитка, из выделенных культур микроорганизмов селективно отбирали дрожжи, способные трансформировать сахара в биологически активные вещества, полезные для организма.

Среди выделенных культур предпочтение отдавали представителям культуры класса сахаромицетов семейства *Saccharomycetaceae*. Размеры исследуемых одиночных культур варьировали по ширине в среднем от 4,5 мкм до 9 мкм и по длине до 10 мкм. Формы преимущественно округлые, овальные, удлиненные. Из 180 выделенных видов дрожжей большинство относится к *Saccharomyces* – 159, как наиболее типичные для данного региона и приемлемые для ферментации выделена 71 чистая культура. Последующее изучение морфологических особенностей клеток, физиологических и биохимических свойств, прояснения антагонистической активности, устойчивости к антибиотикам дали возможность для дальнейшей селекции штаммов. Наиболее высокоактивные и соответствующие по технологическим параметрам были отобраны: *Saccharomyces cerevisiae* Al-06 (из винограда), *Saccharomyces cerevisiae* Gl-8 (из сока сахарного сарго) и *Saccharomyces cerevisiae*-Az-12 (из гранатового сока).

Таким образом, проведенные анализы показали возможность использования растительного сырья не только в качестве свеже выжатых соков граната, вишни, винограда, арбузного сока, сока сахарного сарго, но и в качестве источников активных дрожжей.

Получение ферментированных напитков на основе соков плодово-ягодного сырья позволяет пополнить ассортимент продуктов лечебно-профилактического назначения за счет обогащения конечного продукта рядом функциональных ингредиентов, и как следствие дает общее улучшение здоровья у населения.

**Ключевые слова:** дрожжи, *Saccharomyces cerevisiae*, гранат, фрукты, ягоды, ферментированный сок.

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## **ӨСІМДІК ШИКІЗАТЫНАН ФЕРМЕНТЕЛГЕН СУСЫНҒА АРНАЛҒАН АШЫТҚЫНЫҢ БЕЛСЕНДІ ШТАММ СЕЛЕКЦИЯСЫ**

**Аннотация.** Қазақстандағы халықтың денсаулық жағдайын талдау денсаулық мәселелері қандай да бір дәрежеде елдің көптеген тұрғындарында кездесетін көрсетеді ері бұл олардың өмір сүру жағдайын қоса алғанда, көптеген факторларға байланысты. Жемістер мен көкөністер витаминдердің, минералдардың, клетчаткалардың жоғары құрамына ие бола отырып, жалпы ағзаға жағымды эсер етіп қана қоймай, сонымен қатар уыт пен түрлі поллютанттарды ағзадан шығаруға қабілетті. Құрамында түрлі органикалық қышқылдар, қант және ашытқы қоректендірудің басқа да көзі кездесетін жемістер негізінен ашытқылармен орналасқан.

Ашытқы дақылдарының көзі ретінде стерильді жағдайда алынған жаңа шырын, соның ішінде анар шие, тәтті шие, қызыл жұзім, қарбыз, асхана қызылшасы, қант сорғосының шырыны, сондай-ақ Түркістан облысында өссетін құрамында шырыны бар жидектер бетінен шайындылар пайдаланылды. Осы жұмыстың негізгі мақсаты ферменттелген сусынды әзірлейтіндіктен, микроорганизмдердің бөлінген дақылдарынан қантты биологиялық белсенді заттарға ағзаға пайдалы түрлендіруге қабілетті ашытқы іріктеліп алынды.

Бөлінген дақылдар арасында сахаромицет класының өкілдеріне, *Saccharomycetaceae* тұқымдасына артықшылық берілді. Зерттелетін бір дақыл мөлшері ені бойынша орташа 4,5 мкм-ден 9 мкм-ге дейін және ұзындығы бойынша 10 мкм-ге дейін өзгеріп отырады. Нысаны негізінен дөңгелек, сопақ, ұзартылған. 180 бөлінген ашытқы түрінің көпшілігі *Saccharomyces*-159-ға жатады, осы аймақ үшін ең типтік және ферментация үшін қолайлы 71 таза дақыл бөлінді. Клеткалардың морфологиялық ерекшеліктерін, физиологиялық және биохимиялық қасиеттерін, антагонистік белсенділікті, антибиотиктерге төзімділікті кейіннен зерттеу штаммдарды одан ері селекциялауға мүмкіндік берді. Ең жоғары белсенді және технологиялық параметрлер бойынша сәйкес келетін: *Saccharomyces cerevisiae* Al-06 (жұзімнен), *Saccharomyces cerevisiae* Gl-8 (қант сарго шырынынан) және *Saccharomyces cerevisiae*-az - 12 (анар шырынынан) ірікеп алынды.

Осылайша жүргізілген талдаулар өсімдік шикізатын анар, шие, жұзім, қарбыз, қант сорғосының шырыны ретінде ғана емес, сонымен кatar белсенді ашытқы көзі ретінде пайдалану мүмкіндігін көрсетті.

Жеміс-жидек шикізатының шырыны негізінде ферменттеген сусын алу емдеу-профилактикалық мақсаттағы өнімдер ассортиментін бірқатар функционалдық ингредиенттердің соңғы өнімін байыту есебінен толықтыруға және осының салдарынан халықтың денсаулығын жалпы жақсартуға мүмкіндік береді.

**Түйін сөздер:** ашытқы, *Saccharomyces cerevisiae*, анар, жеміс, жидек, ферментирленген шырын.

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