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II. TECHNOLOGIES

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RESEARCH OF TECHNOLOGICALLY VALUABLE BAKER'S YEAST STRAINS
OF ARMENIA

*The study is devoted to the study of the biological characteristics of strains of baking yeast *Saccharomyces cerevisiae* of Armenia, as well as the effect of long-term maintenance (1996-2021) in laboratory conditions on their enzymatic activity.*

Key words: *Saccharomyces cerevisiae* - lifting force - α -glucosidase activity - osmosis resistance - generative activity.

The basis for the intensification of the fermentation process and maturation of the dough in baking is the biological characteristics of the yeast strains used [2, 13]. Previous studies at Scientific Production Center in the bakery industry provide strong evidence for the importance of yeast α -glucosidase activity in bakery [10]. Yeast with high maltase (α -glucosidase) activity at a much lower dosage accelerates the process of dough making and contributes to the production of bakery products with quality indicators [6, 12, 16].

The production culture medium in which yeast is grown contains a number of substances that determine its osmotic pressure. It is necessary to take into account the peculiarity of yeast to reduce its enzymatic activity in the presence of substances that increase osmotic pressure, which is reflected in their osmosensitivity index [6]. The value of this indicator in the characterization of industrial strains of baker's yeast has especially increased due to the fact that the main direction of technological progress in yeast production is the use of accelerated methods of growing yeast in a highly concentrated molasses medium (at DF = 6-8). At the same time, the growth rate of *Saccharomyces* increases, and therefore the yield of yeast increases [10]. However, in a highly concentrated molasses solution, the effect of harmful substances of the raw material on yeast cells is more pronounced, namely, the ability of *Saccharomyces* to reproduce during transfers decreases faster, which does not allow the use of long-term cultivation technologies with numerous production stages [4, 17].

The aim of the work was to find active cultures of *Saccharomyces cerevisiae* of Armenia, to study their technologically valuable biological features, the subsequent selection of baker's yeast strains in the conditions of yeast and bakery production, as well as to study the effect of long-term maintenance (1996 - 2021) in laboratory conditions on their enzymatic activity.

The objects of the study were 25 yeast cultures isolated from samples of local bread sourdoughs of spontaneous fermentation (tthmor) and dry baker's yeast produced in the Netherlands, France, Turkey, as well as those obtained as a result of selection under production conditions of the Abovyan baker's yeast plant. The selection of strains was carried out according to the gas-forming ability of yeast in Dunbar tubes.

The selected cultures were identified based on the study of a complex of cultural-morphological and physiological-biochemical properties [1, 14, 15].

The lift of *S. cerevisiae* yeast was determined by the accelerated pop-up ball method using 85% milled wheat flour. Maltase and zymase activities were determined by the time of release of 10 ml of CO₂ during the fermentation of 20 ml of a 5% solution of maltose or sucrose with yeast, taken in an amount of 2.5% by volume of the medium, in a microgasometer of the Yeletsky system [3].

The optimal fermentation temperature was determined by the amount of released CO₂ at different temperatures by the gravimetric method in flasks with Meisel sulfuric acid locks. Osmosensitivity was studied according to the White method modified by us, by the difference in time between the lifting power of yeast in a dough without salt and in a dough with an increased salt concentration (up to 4%, instead of 3.35% according to White [7]).

The generative activity of yeast was assessed by determining the relative number of small cells in uterine yeast by counting them under a microscope [3].

Determination of yeast resistance to molasses was carried out in a molasses solution with a content of 10% DM instead of 5% accepted by the method, since modern technology for obtaining baker's yeast biomass is focused on the use of accelerated methods of growing yeast in a highly concentrated molasses medium. Determination of the stability of the finished yeast product (moisture content 75%) during storage was carried out according to the time of softening of the yeast in a thermostat. Yeast is considered resistant if it retains a consistency of 100 hours or more at 35°C [3, 7].

The evaluation of the technological properties of strain S-1 in baking was carried out by baking prototypes of bread [8, 9, 11].

The medium for maintaining yeast cultures is wort agar 10% dry matter (DM). The frequency of reseeded is once a year.

Studied cultures: *Saccharomyces cerevisiae*: 8 strains (numbers 100, 101, 105, 107, 110, 115, 123 and 131) were isolated from samples of spontaneous fermentation bread starters (ttkhmor) from the villages of Goris and Sisian regions, Syunik Marz; 6 strains (numbers 60, 63, 67, 71, 79 and 82) were isolated from samples of spontaneous fermentation bread starters (tthmor) from the villages of Sevan and Gavar regions, Gegharkunik marz; 2 strains (numbers 90 and 99) were isolated from samples of spontaneous fermentation bread starters (tthmor) from the villages of Spitak district, Lori marz; 4 strains (numbers S-1, S-3, S-4 and S-5) were obtained as a result of breeding under the production conditions of the Abovyan baker's yeast plant and 5 strains were obtained by isolating pure cultures from dry yeast produced in France (2 strains, numbers F -1, F-2), the Netherlands (1 strain, number H-1) and Turkey (2 strains, numbers T-1, T-2). All studied strains are presented under their laboratory numbers.

Determination of the gas-forming ability of yeast in malt wort with a concentration of 10% DM in Dunbar tubes made it possible to select the 25 most powerful gas-forming agents from 58 cultures, with the release of 7–9 ml of CO₂ in 24 h. Based on taxonomic studies, the selected strains were assigned to the species *Saccharomyces cerevisiae*. Microscopy showed that the largest sizes (8-9 x 11-13 microns), in comparison with other strains, are the cells of strain S-3.

The main criteria for further study of yeast strains were lifting force, osmosensitivity, zymase and maltase activities. Of the 25 strains, strains S-1, S-3, S-4, H-1, F-2 and T-1 had a high lifting force from 6 min in strain S-1 to 8-9 min in other strains. The same strains are the most promising in terms of other biochemical characteristics, showing high zymase (21-25 min) and maltase (25-30 min) activities.

The study of the influence of temperature on the strength of yeast fermentation showed that the strains differ in optimal fermentation temperatures (28-34°). The most heat-resistant in comparison with other studied strains is strain 100 with a maximum temperature of 43°.

To characterize baker's yeast strains as a production race, their resistance to molasses is of no small importance [3]. As a result of the study, only breeding strains withstood 6 or more passages in a molasses solution containing 10% dry matter, which corresponds to 100% resistance of strains to molasses. Strains S-1 and S-4 withstood the greatest number of passages of 12.

However, yeasts that are resistant to the high osmotic pressures created by sugars in the medium are not always as resistant to the pressures created by salts. In this regard, the effect of an increased content of sodium chloride (up to 4%) on the lifting power of yeast was studied. The dependence of yeast fermentation activity on NaCl concentration in the medium was confirmed. Nevertheless, all 25 cultures tested in terms of osmosensitivity from 0 to 10 min meet the requirements for active strains of baker's yeast [10]. The lifting force of strains H-1 and F-2 was not affected by an increase in the concentration of sodium chloride.

A study of yeast sensitivity to NaCl showed that the degree of sensitivity depends to a large extent both on the strain and on the type of fermented sugar. It was found that sodium chloride suppresses the fermentation of maltose to the greatest extent and, to a lesser extent, of sucrose. The study of the effect of NaCl on the maltase activity of 25 yeast cultures showed that 6 strains are the least sensitive to salt. Table 1 shows that sodium chloride at a concentration of 1.5% does not affect the rate of maltose fermentation by these strains.

Based on the research, a complex of technologically valuable properties of yeast cultures in the laboratory and the results obtained, strain S-1 was selected for testing under production conditions at the Abovyan Baker's Yeast Plant.

The uterine yeast of strain S-1 is characterized by a relatively low content of 10-15% of small cells (with an allowable rate of up to 25%), which indicates a high generative activity of the strain. Strain S-1, due to its high osmosis resistance, showed an increased specific growth rate at the BIN stage (0.289 h⁻¹ instead of 0.249 h⁻¹) in the process of growing yeast in a molasses solution with a concentration of 15% DM in comparison with strain Odessa-14 according to the technological regulations of the plant, and also a higher yield of biomass (yeast yield is 9% higher than in the variant with strain O-14). Pressed yeast obtained using the S-1 strain is distinguished by a high dry matter content of 30.3%.

Table 1.

Effect of sodium chloride on the lifting force and maltase activity of the most osmosis-tolerant yeast cultures ($n= 5$; $P<0.05$)

Strains	Maltase activity, min		Dough rising, min		Osmosensitivity, min
	без NaCl	NaCl, 1,5%	без NaCl	NaCl, 4%	
S-1	25,00±0,44	25,00±0,63	6,00±0,44	7,00±0,31	1
S-3	27,00±0,63	27,00±0,77	6,50±0,31	7,50±0,31	1
F-2	29,00±0,89	29,00±0,44	9,00±0,44	9,00±0,31	0
H-1	30,00±0,89	30,00±0,63	10,00±0,31	10,00±0,31	0
S-4	25,00±0,31	25,00±0,44	7,00±0,31	8,00±0,44	1
T-1	28,00±0,77	28,00±0,89	8,00±0,63	10,00±0,44	2

The ability of the S-1 strain to develop in an environment with high osmotic pressure makes it possible, when cultivating at production stages in a molasses medium with an increased contamination by foreign microflora, to obtain high-quality finished products with a lifting force of 6-7 minutes. This is fully consistent with the literature data that the increased osmotic pressure of the medium is a factor that determines its protective properties: wild yeast fungi in a highly concentrated molasses medium reproduce much more slowly than Saccharomycetes due to the fact that the intracellular osmotic pressure of the latter is 2 times higher [3].

The final assessment of the technological properties of strain S-1 was carried out by baking prototype bread samples [8, 11].

Table 2 shows that as a result of the use of a new yeast culture, the fermentation activity of the dough improves (20.1 ml of CO₂ in the S-1 strain versus 9.0 in the control). Experimental samples of bread differed in the best porosity of the crumb. With the same weight as the control sample, the volume yield of the prototype bread was 28% higher, and the porosity was 7%. In addition, the use of yeast strain S-1 in baking contributes to the intensification of the dough process due to the high maltase activity of the culture: the maturation time of the dough is reduced by 30 minutes, proofing by 15 minutes.

The ability of yeast strain S-1 to intensify the process of dough making and thereby improve the quality of bread was a prerequisite for studying the possibility of reducing the consumption of yeast for dough kneading. In these experiments, when preparing the dough in a non-dough way, the consumption of yeast was reduced by 30, 50 and 70% of the norm provided for in the recipe. The control dough was prepared with the addition of 1% commercial pressed yeast by weight of flour.

The study of the dynamics of gas formation showed that at all selected dosages of yeast, the total amount of released CO₂ in the experimental test exceeded the control by 3.1-15.7%. At the same time, in terms of physicochemical parameters, the prototype bread samples in all variants with reduced dosages of yeast had a volume yield of bread by 7.4-16.8%, and porosity by 2.3-5.1% more.

Table 2.

Qualitative indicators of yeast, dough and bread obtained using pressed yeast of the experimental strain ($n= 5$; $P < 0.05$)

Indicators	Pressed yeast, 75% moisture	
	Commercial yeast (control)	S-1 (experience)
Yeast		
Dough rising, min	15,00 ±1,78	5,00 ±0,89
Dough		
Fermentation activity, ml CO ₂ /20 g	9,00 ±1,41	20,10 ±1,26
Number of yeast cells, mln/g	120,00±2,28	175,00±1,41
Fermentation duration, min	60	30
Proofing time, min	40	25
Bread		
Weight of 1 loaf, g	700	700
Bread volume, ml	450,00±4,04	576,00±2,82
Porosity, %	70,0±2,0	75,00±1,78
Moisture, %	43,6	43,5
Acidity, °H	3,0	3,0

The high baking qualities of the S-1 strain make it possible to use pressed yeast with a moisture content of 75% in the amount of 0.3-0.4% by weight of flour in dough making, ensuring a normal technological mode of dough making and obtaining high-quality finished products.

At the last stage of research, the effect of long-term maintenance and storage of *S. cerevisiae* strains in laboratory conditions on their enzymatic activity was studied. This aspect of the study is important, since long-term maintenance of pure yeast cultures is necessary both in production and during storage in collections. It is also important that the production races of yeast, with the chosen method of storage, do not lose their productive properties [1].

As a way to maintain yeast cultures, the method of periodic transfers on wort agar with 10% DM was used, once a year. Data on the lifting power and maltase activity of the most active yeast strains, carried out with an interval of 25 years, are presented in Table 3.

Table 3.

Effect of long-term maintenance of the most active yeast strains on their enzymatic activity ($n= 5$; $P < 0.05$)

Strains	Maltase activity, min		Dough rising, min	
	1996	2021	1996	2021
S-1	25	25,00±0,89	6	7,50±0,77

Strains	Maltase activity, min		Dough rising, min	
	1996	2021	1996	2021
S-3	27	28,00±1,0	6,5	9,00±0,44
F-2	29	29,00±0,31	9	10,00±0,31
H-1	30	30,00±0,44	10	11,00±0,44
S-4	25	26,00±1,0	7	9,00±0,63
T-1	28	29,00±0,89	8	10,00±0,63

Table 3 shows that when using this maintenance method for 25 years, *S. cerevisiae* strains almost completely retained their maltase activity. The slight decrease in yeast lift scores may be due to the difference in quality between the 85% milled wheat flour used in this analysis in 1996 and 2021.

As a result of the research and selection, a strain of *S. cerevisiae* S-1 was obtained, resistant to increased osmotic pressure of the medium and harmful impurities of molasses, the use of which in yeast and bakery production allows:

- to cultivate yeast of 12 or more production stages in a highly concentrated molasses medium (at the dilution ratio of molasses DF-6-8);
- to carry out the process of growing yeast at the production stages without sterilizing the molasses medium and at the same time to obtain high-quality commercial yeast with a high yield;
- reduce the use of pressed yeast with a moisture content of 75% in dough preparation by 2-2.5 times from the standard dosage and obtain high quality finished products.

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ՀԱՅԱՍՏԱՆԻ ՀԱՅԱԹԻՄԱՆ ԽՄՈՐԻՉԻ ՏԵԽՆՈԼՈԳԻԱԿԱՆ ԱՐԺԵՔԱՎՈՐ ՇՏԱՄՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆ

Ուսումնասիրությունը նվիրված է հացաթխման խմորիչի՝ հացաթխման շաքարասունկի շրամների կենսաբանական բնութագրերին, ինչպես նաև լաբորատոր պայմաններում երկարապրև պահպանման (1996-2021 թթ.) ազդեցությանը դրանց ֆերմենտային ակտիվության վրա:

Առանցքային բառեր. *հացաթխման շաքարասունկ, վերամբարձ ուժ, մալրազային ակտիվություն, օսմոտիկ կայունություն, գեներալիզ ակտիվություն:*

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ИССЛЕДОВАНИЕ ТЕХНОЛОГИЧЕСКИ ЦЕННЫХ ШТАММОВ ХЛЕБОПЕКАРНЫХ ДРОЖЖЕЙ АРМЕНИИ

*Исследование посвящено изучению биологических особенностей штаммов хлебопекарных дрожжей *Saccharomyces cerevisiae* Армении, а также влияния длительного поддержания (1996 – 2021гг.) в лабораторных условиях на их ферментативную активность.*

Ключевые слова: *Saccharomyces cerevisiae, подъемная сила, α-глюкозидазная активность, осмотическая, генеративная активность.*

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