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INFLUENCE OF PHYSICO-CHEMICAL FACTORS ON THE PROCESS OF *SACCHAROMYCES CEREVISIAE* MASS FORMATION DURING CULTIVATION ON MODIFIED NUTRIENT MOLASSES

The aim of the study was to investigate the effect of physico-chemical factors on the process of *Saccharomyces cerevisiae* mass formation during cultivation on modified nutrient molasses. The composition of molasses and the production of *Saccharomyces cerevisiae* mass during cultivation are influenced by genetic, environmental and technological factors. Therefore, molasses from different manufacturers vary in their biological, environmental, and technological characteristics. Molasses from three manufacturers (Aksu-kant, Koksu-Kant and Cheremnovsky Sugar Plant) was used in the research. Chemical studies showed that they had relatively similar indicators in terms of specific gravity (1.38-1.39 kg/m3) and the amount of fermentable sugars (44.36-46.23), and in terms of the mass fraction of sucrose, molasses from the manufacturer «Koksu-Kant» showed the highest values in terms of direct polarization and sucrose content (50.4%; 44.82%, respectively), whereas the lowest values were observed for Aksu-Kant molasses (45.98%; 42.57%, respectively). All three types of molasses met the quality and biosafety requirements. The cultivation temperature of 34 °C and the pH of the molasses-based nutrient medium of 5.07 are the most favorable conditions for growth of *Saccharomyces cerevisiae* in culture. The maximum biomass accumulation of *Saccharomyces cerevisiae* during cultivation on modified molasses was obtained with the addition of vitamin B6 (42.1 g/l).

Keywords: *Saccharomyces cerevisiae*, aerobic cultivation, modified nutrient molasses, yeast biomass.

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Модификацияланған қоректік мелассада өсіру кезінде *Saccharomyces cerevisiae* масса түзілу процесіне физика-химиялық факторлардың әсері

Зерттеудің мақсаты физика-химиялық факторлардың модификацияланған қоректік мелассада өсіру кезінде *Saccharomyces cerevisiae* масса түзілу процесіне әсерін зерттеу болды. Мелласса құрамы мен масса өндірісіне *Saccharomyces cerevisiae* өсіру кезінде генетикалық, экологиялық және технологиялық факторлар әсер етеді. Сондықтан әртүрлі өндірушілердің мелассасы биологиялық-экологиялық және технологиялық көрсеткіштер бойынша айырмашылықтарға ие. Зерттеулерде үш өндірушінің мелассасы қолданылды (“Ақсу-кант”,” Көксу-кант “ және “Черемновский қант зауыты”). Химиялық зерттеулер олардың меншікті салмағы (1,38-1,39 кг/м3) және ашытылған қанттардың қосындысы (44,36-46,23) бойынша салыстырмалы түрде ұқсас көрсеткіштерге ие екенін көрсетті, ал тікелей поляризация мен сахароза құрамындағы сахарозаның массалық үлесі бойынша “Кокс-кант” өндірушісінің мелассасы (тиісінше 50,4 %; 44,82 %), ал ең кішісі – “Ақсу-кант” мелассасы (тиісінше 45,98 %; 42,57%). Мелассаның барлық үш түрі сапа-биоқауіпсіздік талаптарына сәйкестігін көрсетті. Мәдениеттегі *Saccharomyces cerevisiae* дамуы үшін ең қолайлы-мелласса негізіндегі өсіру температурасы 34 °С және қоректік ортаның pH 5,07. Модификацияланған мелассада өсіру кезінде *Saccharomyces cerevisiae* массасының максималды жинақталуы B6 дәрумені (42,1 г/л) қосылған кезде алынды.

Түйін сөздер: *Saccharomyces cerevisiae*, аэробты өсіру, өзгертілген қоректік мелласса, ашытқы биомассасы.

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Влияние физико-химических факторов на процесс массообразования *Saccharomyces Cerevisiae* при культивировании на модифицированной питательной мелассе

Целью исследования было изучение влияния физико-химических факторов на процесс массообразования *Saccharomyces cerevisiae* при культивировании на модифицированной питательной мелассе. На состав мелассы и производство массы *Saccharomyces cerevisiae* при культивировании влияют генетические, экологические и технологические факторы. Поэтому меласса от разных производителей имеет различия по биолого-экологическим и технологическим показателям. В исследованиях использовалась меласса от трёх производителей («Аксу-кант», «Коксу-кант» и «Черемновский сахарный завод»). Химические исследования показали, что они имели относительно схожие показатели по удельному весу (1,38–1,39 кг/м^3) и сумме сбраживаемых сахаров (44,36–46,23), а по массовой доле сахарозы по прямой поляризации и содержанию сахарозы наибольшее значение имела меласса от производителя «Коксу-кант» (50,4 %; 44,82 % соответственно), а наименьшее – меласса «Аксу-кант» (45,98 %; 42,57 % соответственно). Все три вида мелассы показали соответствие по требованиям качества – биобезопасности. Наиболее благоприятным для развития *Saccharomyces cerevisiae* в культуре служат температура культивирования 34 °С и pH питательной среды на основе мелассы 5,07. Максимальное накопление массы *Saccharomyces cerevisiae* при культивировании на модифицированной мелассе было получено с добавлением витамина В6 (42,1 г/л).

Ключевые слова: *Saccharomyces cerevisiae*, аэробное культивирование, модифицированная питательная меласса, биомасса дрожжей.

Introduction

Relevance. Yeast production is a key segment of the food industry (production of food additives, bakery, and alcoholic beverages) as well as feed manufacturing. Therefore, optimizing the composition of molasses-based culture media is important for improving the efficiency of *S. cerevisiae* biomass production.

Sugar beet molasses, a by-product of sugar production, contains a total of 45–55% sucrose, glucose, fructose, raffinose, melibiose, and galactose (Kassa et al., 2024). In 2024, sugar beet yield in Kazakhstan reached 54 t/ha. A total of 549 thousand tons of sugar beet were produced from an area of 10 thousand hectares, of which 432 thousand tons were delivered for processing (Ministry of Agriculture of the Republic of Kazakhstan, 2024). Considering that 7 tons of sugar beet yield 0.25–0.35 tons of molasses (Schmid et al., 2019), or that 100 kg of processed plant raw material yield 3.5–4.5 kg of molasses (Ahmed, 2017), it can be concluded that in 2024 Kazakhstan's sugar factories produced between 15.1–19.4 thousand and 15.4–21.6 thousand tons of molasses. Because molasses can be stored at temperatures of up to 45°C, the use of molasses at yeast and feed plants as a nutrient medium for *Saccharomyces cerevisiae* biomass production is economically efficient.

Objective. To study the effect of physicochemical factors on the biomass accumulation process of *Saccharomyces cerevisiae* when cultivated on modified molasses-based nutrient media.

Tasks: To study the physicochemical and microbiological properties of molasses from three producers: «Aksu-Kant», «Koksu-Kant», and «Cheremnovsky Sugar Plant»; To study the effect of physicochemical factors on the growth characteristics of *Saccharomyces cerevisiae* during cultivation; To determine biomass accumulation of yeast when cultivated on modified molasses.

Scientific and practical significance. Studying the influence of physicochemical factors (temperature, pH) on the biomass accumulation process of *Saccharomyces cerevisiae* when cultivated on modified molasses-based nutrient media will make it possible to determine the optimal composition of the nutrient medium and the optimal cultivation regime, thereby improving the productivity of *Saccharomyces cerevisiae.*

Materials and methods

The materials for this study were *Saccharomyces cerevisiae* and molasses from three manufacturers: «Aksu-Kant», «Koksu-Kant», and «Cheremnovsky Sugar Plant».

Sampling of sugar beet molasses, determination of organoleptic characteristics, pH, and mass fraction of sucrose by direct polarization were carried out in accordance with GOST 30561-2017 (2017). The total fermentable sugars, sucrose content, and invert sugar content were determined according to the procedure described by Uprkhleb Minpishcheproma SSSR (1984). The specific gravity of molasses was determined according to GOST 18995.1-73 (1973).

The collected molasses samples were analyzed using microbiological methods to determine the presence of wild yeasts and molds (GOST 10444.12-2013, 2013), mesophilic aerobic and facultative-anaerobic microorganisms (CFU) (GOST 10444.15-94, 1994), and coliform bacteria (CB) (GOST 31747-2012, 2012) using the following agar media: Lysine, Sabouraud, Meat-Peptone Agar (MPA), and Endo agar, respectively. The content of nitrite-producing bacteria (NPB) in molasses was determined in accordance with GOST 32257-2013 (2013). Biomass accumulation by yeast during cultivation on modified molasses was carried out according to the procedure described by Uprkhleb Minpishcheproma SSSR (1984).

Results and discussion

Physicochemical and Microbiological Characteristics of Molasses

According to organoleptic parameters (appearance, color, odor, solubility in water), molasses from the three manufacturers – «Aksu-Kant», «Koksu-Kant» and «Cheremnovsky Sugar Plant» (Figure 1) – complied with the requirements of GOST 30561-2017 (thick and viscous, dark brown in color, with no foreign odor).

The results of physicochemical analyses of molasses are presented in Table 1.

As can be seen from Table 1, the physicochemical parameters of molasses from the three manufacturers are relatively similar in terms of specific gravity (1.38–1.39 kg/m³) and total fermentable sugars (44.36–46.23). However, the following differences were observed:

- pH: Molasses from «Aksu-Kant» exhibited a slightly acidic value (6.98), while molasses from «Koksu-Kant» and «Cheremnovsky Sugar Plant» showed alkaline values (8.13 and 7.65, respectively).

- mass fraction of sucrose (by direct polarization) and sucrose content: The highest values were found in molasses from «Koksu-Kant» (50.4% and 44.82%, respectively), whereas the lowest were observed in molasses from «Aksu-Kant» (45.98% and 42.57%, respectively).

- invert sugar: Molasses from «Aksu-Kant» contained higher levels of invert sugar (1.74%) compared to molasses from «Koksu-Kant» (0.32%) and «Cheremnovsky Sugar Plant» (0.25%).

Figure 1
Study of organoleptic characteristics of molasses from three manufacturers: «Aksu-Kant», «Koksu-Kant» and «Cheremnovsky Sugar Plant».



In addition to the above, it should be noted that variability among the three types of molasses was almost negligible (from 0 to 4.2%) for such parameters as specific gravity (0–0.59%), pH value (0–4.2%), mass fraction of sucrose (0.4–1.4%), total fermentable sugars (0.6–1.8%), and sucrose content (0.8–1.7%). For invert sugar content, the variability was low (9.4–24.9%).

Table 1
Physicochemical characteristics of molasses

Physicochemical indicator	Statistical indicator	Sugar Plant		
		«Aksu-Kant»	«Koksu-Kant»	«Cheremnovsky Sugar Plant»
Specific gravity	$\bar{X} \pm m_{\bar{x}}$, kg/m^3	1,38	1,39 ± 0,004	1,39 ± 0,005
	C_v, %	0	0,47	0,59
pH value	$\bar{X} \pm m_{\bar{x}}$	6,98	8,13 ± 0,20	7,65 ± 0,10
	C_v, %	0	4,2	2,4
Mass fraction of sucrose (direct polarization)	$\bar{X} \pm m_{\bar{x}}$, %	45,98 ± 0,10	50,4 ± 0,3	49,48 ± 0,40
	C_v, %	0,4	1,2	1,4
Total fermentable sugars	$\bar{X} \pm m_{\bar{x}}$, %	44,36 ± 1,02	46,23 ± 0,22	45,70 ± 0,15
	C_v, %	1,8	0,8	0,6
Sucrose content	$\bar{X} \pm m_{\bar{x}}$, %	42,57 ± 0,36	44,82 ± 0,21	44,03 ± 0,44
	C_v, %	1,5	0,8	1,7
Invert sugar content	$\bar{X} \pm m_{\bar{x}}$, %	1,74 ± 0,08	0,32 ± 0,02	0,25 ± 0,04
	C_v, %	9,4	9,5	24,9

The composition of molasses is influenced by genetic factors (sugar beet variety), ecological and geographical factors (climatic, geographical, and soil conditions), and technological factors (production season, agronomic conditions of sugar beet cultivation, processing methods, and storage conditions of molasses). Therefore, molasses from different manufacturers differs in taste, color, viscosity, and nutrient composition.

Table 2
Microbiological characteristics of molasses

Microbial contamination, CFU	Sugar Plant		
	«Aksu-Kant»	«Koksu-Kant»	«Cheremnovsky Sugar Plant»
Mesophilic aerobic and facultative-anaerobic microorganisms	75	48	23
Molds / Yeasts	2/2	-	9/-
Wild yeasts	-	-	-
Coliforms	-	-	-
Nitrite-forming bacteria (NFB)	-	-	-

As can be seen, the parameters presented in Table 1 meet the requirements of the standards (GOST 10444.12-2013, 2013; GOST 30561-2017, 2017) applicable to molasses.

Microbiological control of molasses is necessary to ensure biosafety during yeast biomass production.

Molasses, as an object of control, is tested for the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), which allows detection of violations in molasses storage technology (Moiseyak et al., 2022); coliform bacteria (BGC), which serve as sanitary indicators of potential contamination (Martin et al., 2016)

and are capable of fermenting lactose with acid and gas formation at 32–35 °C (Davidson et al., 2004); their presence in raw material at levels of 1,000–5,000 cells/g can reduce yeast reproduction and weaken fermentation activity (Ponomareva, 2008); and mold fungi, whose presence indicates contamination and potential mycotoxin production (SanPiN 2.3.2.1078-01, 2004; TR TS 021/2011, 2011).

The presence of nitrites in molasses can be determined, according to GOST 32257-2013 (2013), by detecting nitrite-forming bacteria (NFB). It is known that nitrites are extremely toxic (Chamandoost et al., 2016). A concentration of 0.0005% in the medium inhibits normal yeast budding; 0.004% leads to a 40–50% reduction in yeast biomass accumulation; and 0.02% not only almost completely suppresses yeast growth and reproduction but also causes partial cell death (Eknikom et al., 2022; Saratovskiy GAU, 2016).

Microbiological studies showed, as presented in Table 2, that wild yeasts, coliform bacteria, and nitrite-forming bacteria were absent in all three types of molasses. Mold fungi were detected (no more than 10 colonies) in molasses from the «Aksu-Kant» and «Cheremnovsky Sugar Plant» producers, while in molasses from «Koksu-Kant» no mold fungi were found.

MAFAM contamination in the three types of molasses was determined at the following levels: 23 colonies («Cheremnovsky Sugar Plant»), 48 («Koksu-Kant»), and 75 («Aksu-Kant»).

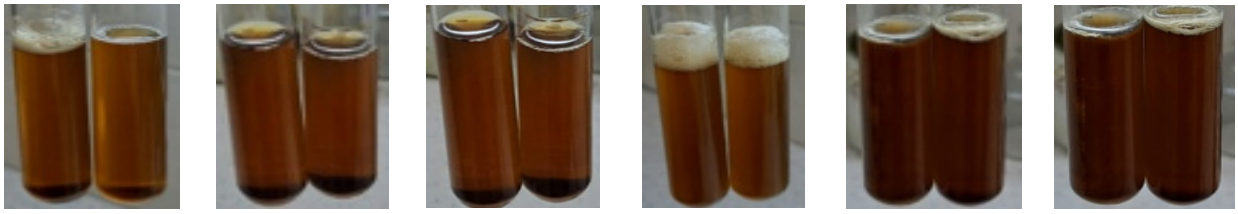
The obtained results indicate that all three types of molasses meet biosafety quality requirements (SanPiN 2.3.2.1078-01, 2004; TR TS 021/2011, 2011).

Influence of Physicochemical Factors on the Growth Characteristics of Saccharomyces cerevisiae During Cultivation on Sterile Molasses

Figures 2 and 3 present photographic images showing the results of 72-hour yeast cultivation depending on the medium's pH (5.07; 7.8) and temperature regime (34 °C, 37 °C).

Figure 2

Yeast cultivation at 34 °C on the first (A1, A2; B1, B2), second (A3, A4; B3, B4), and third (A5, A6; B5, B6) day of subculturing at pH 5.07 (a: A1, A2, A3, A4, A5, A6) and pH 7.8 (b: B1, B2, B3, B4, B5, B6)

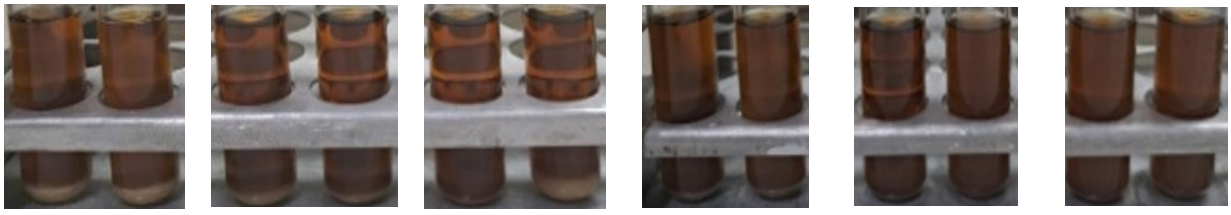


A1, A2 A3, A4 A5, A6 B1, B2 B3, B4 B5, B6

a) b)

Figure 3

Yeast cultivation at 37 °C on the first (C1, C2; D1, D2), second (C3, C4; D3, D4), and third (C5, C6; D5, D6) day of subculturing at pH 5.07 (a: D1, D2, D3, D4, D5, D6) and pH 7.8 (b: C1, C2, C3, C4, C5, C6)



D1, D2 D3, D4 D5, D6 C1, C2 C3, C4 C5, C6

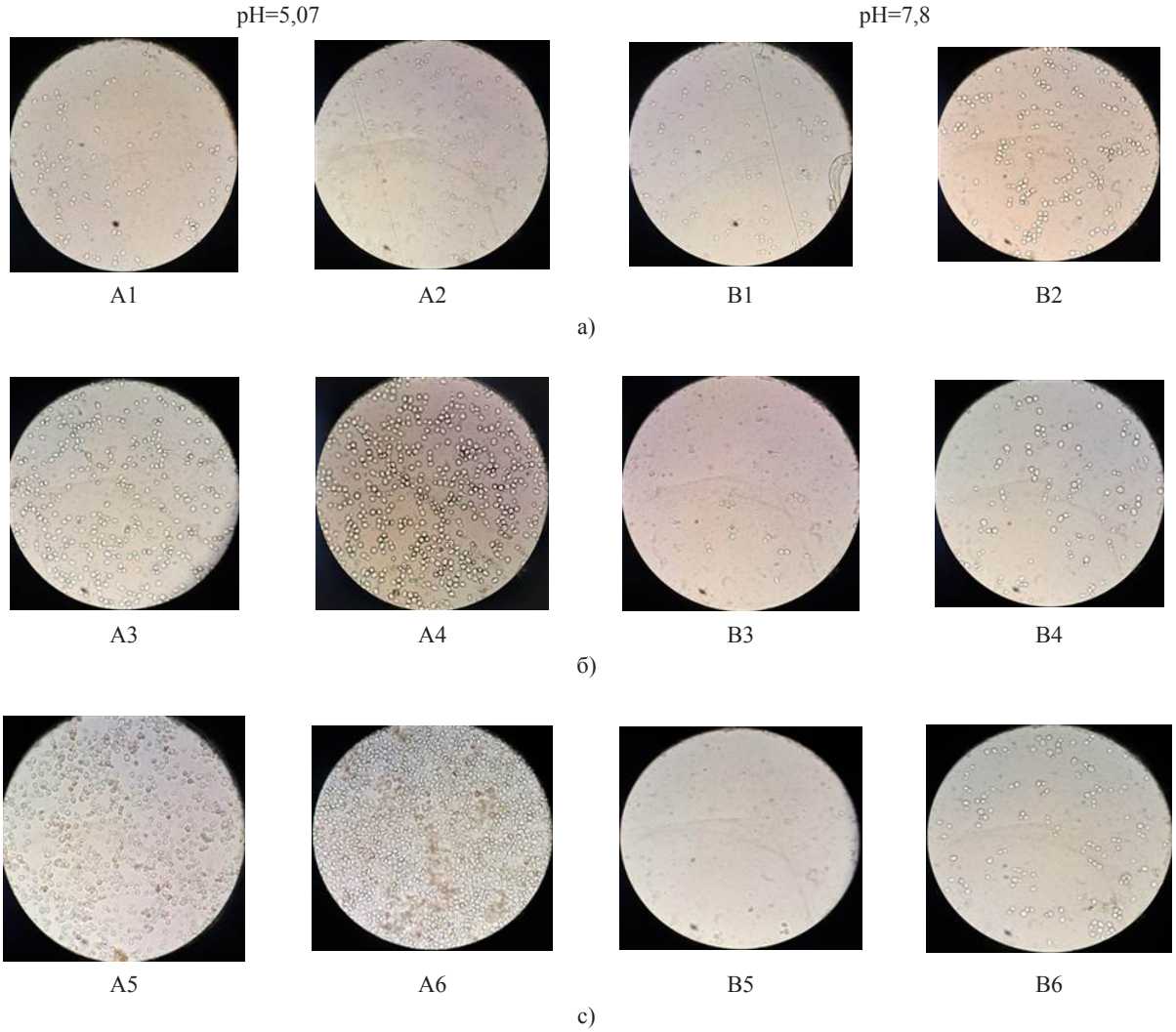
a) b)

Yeast was cultivated at 34 °C (Figure 2) and 37 °C (Figure 3) in molasses-based nutrient media at pH 5.07 and 7.8. As observed in the photographic images in Figure 2, foam formation was more pronounced during the first (A1, B1, B2) and second days (B1, B2) of cultivation, indicating high yeast activity at these stages. In contrast, under the 37 °C regime (Figure 3), foam formation was minimal. With each subsequent subculturing step, the foam volume gradually decreased across all media in series A, B, C, and D. This decline is associated with nutrient depletion during cultivation, accumulation of metabolic by-products, and reduced yeast cell activity. In experiments from series A and D (pH 5.07), sediment formation was observed in the tubes due to acidification of the medium by the addition of ammonium and orthophosphoric acids. These additions disrupted the ionic balance, contributing to cell precipitation. In contrast, no sediment formation was observed in experiments from series B and C (pH 7.8). These results suggest that foam formation is influenced not only by cultivation temperature and medium acidity but also by the degree of substrate consumption.

Photographic images in Figures 4 (34 °C) and 5 (37 °C), as well as data presented in Tables 3 (34 °C) and 4 (37 °C), provide further evidence of the growth characteristics of yeast in the investigated nutrient media.

Figure 4

Yeast growth at pH 5.0 (experiment group A) and pH 7.8 (experiment group B) on the first (a), second (b), and third (c) day of molasses subculturing



As shown in the photographic images in Figures 4 and 5, the surface of the solid nutrient medium during cultivation was more actively colonized by yeast at a temperature of 34 °C and pH 5.07 (experiment series A) compared to pH 7.8 (series B). A similar result was observed for yeast cultivated at 37 °C in experiment series D (pH 5.07), in comparison with series C (pH 7.8).

Overall, yeast cells were able to adapt to the tested temperature values (34 °C, 37 °C) and pH levels (5.07, 7.8); however, the most favorable conditions for their development were 34 °C and pH 5.07.

When comparing the temperature regimes (34 °C vs. 37 °C) and experiment series A and D at pH 5.07, a more pronounced and intensive yeast cell growth was observed under the conditions of 34 °C and pH 5.07 (series A).

In the study by Wasungu and Simard (1982), the continuous growth of baker's yeast was examined under temperatures ranging from 15 °C to 40 °C and pH values from 2.5 to 6.0 under steady-state conditions in 1% ethanol. The results showed that 30 °C and pH 4.5 were optimal, as they yielded the highest growth compared to other experimental conditions.

Yalcin and Ozbas (2008) demonstrated that high specific growth rates and dry cell mass of baker's yeast were achieved at 30 °C and pH 4.0.

In our study, under locally defined conditions, the temperature of 34 °C and pH 5.07 were found to be optimal for increasing the specific growth rate of baker's yeast.

Figure 5

Yeast growth at pH 5.0 (experiment group D) and pH 7.8 (experiment group C) on the first (a), second (b), and third (c) day of molasses subculturing at 37 °C

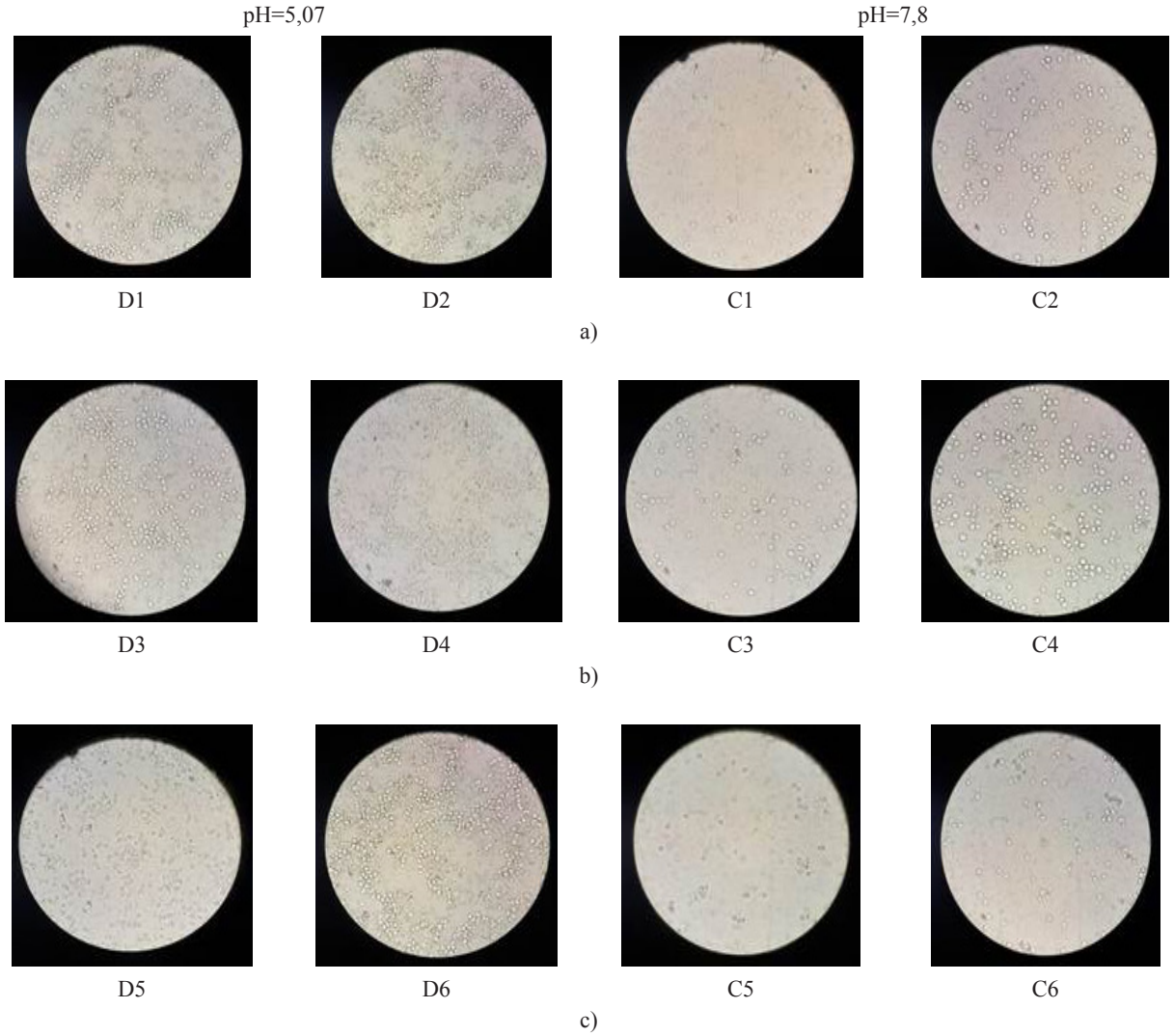


Table 3
Growth characteristics of Saccharomyces cerevisiae on different subculturing days at 34 °C

Group	pH	Subculturing Day					
		First		Second		Third	
		Subgroup	Cell morphology description	Subgroup	Cell morphology description	Subgroup	Cell morphology description
A	5,07	A1	Small, round, uniform	A3	Small, round, uniform	A5	Many well-developed budding cells. Some dead cells observed
		A2	Small, round, uniform. Some large cells observed	A4	Large, uniform	A6	Many well-developed budding cells. Some dead cells observed
B	7,8	B1	Round, uniform	B3	Small and large cells	B5	Underdeveloped with small buds
		B2	Large budding cells	B4	Many single large budding cells	B6	Small

Table 4
Growth characteristics of Saccharomyces cerevisiae on different subculturing days at 37 °C

Group	pH	Subculturing Day					
		First		Second		Third	
		Subgroup	Cell morphology description	Subgroup	Cell morphology description	Subgroup	Cell morphology description
D	5,07	D1	Predominantly small, round cells; few cells with small buds	D3	Cells are round, oval, medium-sized. Some large oval cells observed, most with clearly visible buds	D5	Many oval, medium- and large-sized cells. Budding is actively expressed, buds are larger. Some dead cells observed
		D2		D4		D6	
C	7,8	C1	Small and round cells; budding weakly expressed	C3	Small and large budding cells	C5	Small cells; some dead cells present
		C2		C4		C6	

At the next stage of the research, as shown in Tables 3 and 4, the growth characteristics of *Saccharomyces cerevisiae* on different days of cultivation at different temperatures (34 °C, 37 °C) and pH (5.07; 7.8) were studied:

1) at the initial stage of growth (after 24 hours of cultivation), when adaptation to the environment occurs, the studied yeast cells at a temperature of:

- 34 °C were small and rounded (pH 5.07) and large budding (pH 7.8),
- 37 °C were small and rounded (pH 7.8), but there were also cells with small buds (pH 5.07);

2) during the active reproduction phase (after 48 hours of cultivation), the yeast cells studied at a temperature of

- 34 °C was small (A3) at pH 5.07, but large homogeneous (A4) cells were also found, and at pH 7.8, small and large with particulate inclusions (B3) and single large cells (B4),
- 37 °C, both small and large cells with particulate inclusions were found, while there were many single large cells in the field of view (pH 7.8: D3, D4), as well as numerous round and oval cells of medium to large size with clearly visible buds (pH 5.07: C3, C4);

3) at the peak of the lag phase of growth, the yeast under study showed active division at temperatures of

- 34 °C at pH 5.07, many swollen, developed cells and dead cells (A5, A6) were found, and at pH

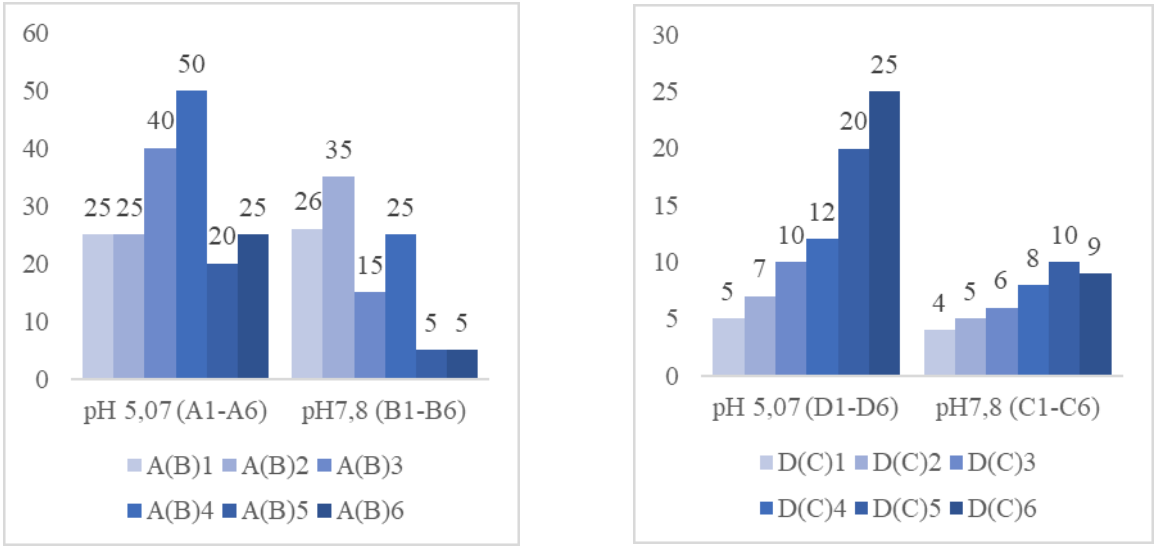
7.8, underdeveloped with small buds (B5) and small (B6);

- 37 °C oval, medium and large cells were found, while budding was actively expressed and the buds were large, swollen and dead (pH 5.07: D5, D6), small and dead (pH 7.8: C5, C6).

As a result of the analysis of bioassays, it was revealed that the yeast reaches optimal growth on the 3rd day of re-cultivation at pH 5.07. Yeast growth at pH 7.8 on the first day of subculturing was abundant, but with each subsequent subculturing, yeast reproduction became slow and low yeast growth was observed, and there were more dead cells. There were fewer budding cells under two pH regimes (5.07; 7.8) by the third subculturing.

Figure 6

Budding rate of Saccharomyces cerevisiae on different subculturing days at temperatures of 34 °C (a) and 37 °C (b), and at pH 5.07 and 7.8



a) b)

The x-axis represents experimental groups at pH 5.07 and 7.8 at 34 °C (a) and 37 °C (b); subgroups 1–2, 3–4, and 5–6 correspond to the first, second, and third days of subculturing, respectively. The y-axis represents the proportion of budding cells (% of the total cell population).

According to the study by Meledina and Davydenko (2015), the obtained yeast cells met the morphological requirements–oval and round cell shapes with clear and smooth contours–as well as the expected budding rate. In the active growth phase (A4, B4), the proportion of budding cells was no less than 40–50% of the total population (Figure 6). A low percentage of budding cells in the active growth phase (below 40%) may indicate reduced metabolic activity or unfavorable cultivation conditions. As shown in Figure 6 (b), the budding rate of yeast cultivated at 37 °C was lower compared to that of yeast grown at 34 °C.

In terms of organoleptic properties, the yeast produced during cultivation met the requirements of GOST 171-81 (1981): they exhibited a uniform light creamy color, had a dense and friable texture, and possessed a characteristic yeast-specific aroma and taste.

Biomass Accumulation by Yeast During Cultivation on Modified Molasses

Yeast biomass accumulation during cultivation on modified molasses was assessed by filtering the cells.

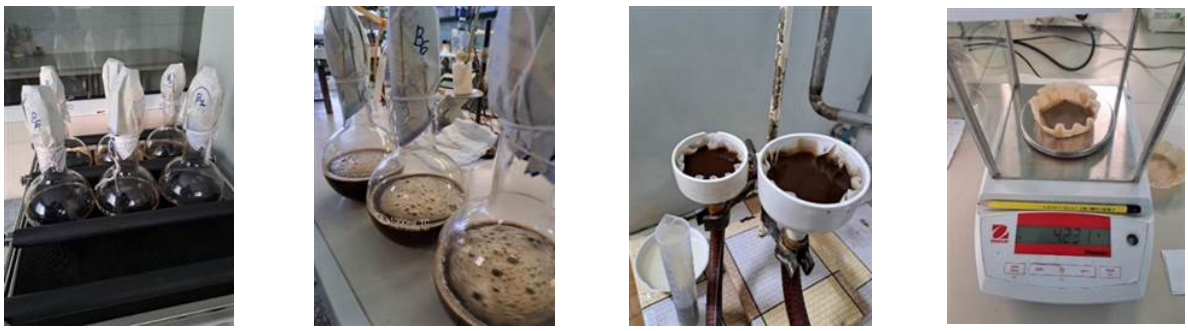
The modified sterile molasses, diluted with distilled water to a volume of 3 L, had a density of 17°Bx and contained the following components: 27 g NaCl, 0.25 g $MgSO_4$, 0.25 g Zn, and 0.0025 g $C_4H_6O_4$. Depending on the vitamin added, two experimental variants were used: a medium supplemented with vitamin B1 and a medium supplement-

ed with vitamin B6. The control medium consisted of sterile molasses without any additives.

The prepared media were inoculated with yeast and cultivated on a shaker for 24 hours (Figure 7, a).

After 24 hours of cultivation, the yeast biomass concentration was determined using a Büchner funnel (Figure 7, b), assessing the effect of vitamin supplementation on biomass accumulation in the medium. Biomass collection on the Büchner funnel took 15–20 minutes, after which the collected mass was weighed using analytical scales (Table 5).

Figure 7
Cultivation of Saccharomyces cerevisiae on modified molasses



a) Yeast cultivation

b) Determination of yeast biomass accumulation

The growth of *Saccharomyces cerevisiae* in molasses-based media at pH = 5.07–without vitamins (a), with the addition of vitamin B1 (b), and with vitamin B6 (c)–is shown in Figure 8.

Based on the results presented in Figure 8 and Table 5, the following conclusions can be drawn:

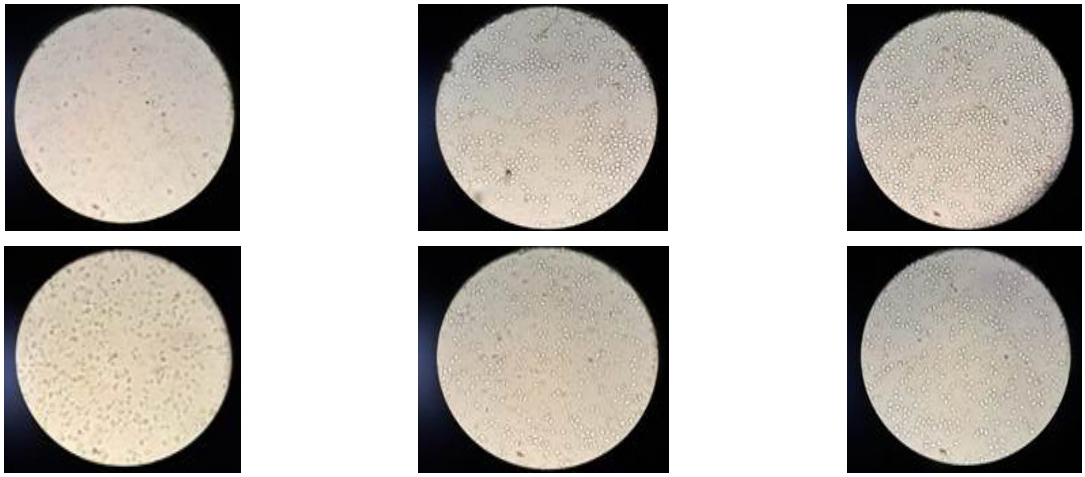
1) Modified media supplemented with vitamins yielded a higher biomass compared to the control group: 37.8 and 42.1 g/L versus 30.7 g/L, respectively;

2) Vitamin B6 resulted in the highest yeast biomass production (42.1 ± 5.4 g/L), compared to vitamin B1 (37.8 ± 3.2 g/L);

3) In media without additives, yeast cells appeared smaller, whereas in vitamin-enriched media, the cells exhibited larger morphological forms.

As shown, the best results were achieved with yeast cultivated in the modified medium supplemented with vitamin B6 (pyridoxine).

Figure 8
Growth of Saccharomyces cerevisiae in molasses-based media at pH = 5.07 without additives (a) and with the addition of vitamins B1 (b) and B6 (c)



a) b) c)

Table 5
Biomass formation of Saccharomyces cerevisiae during cultivation on modified molasses

Modified medium	Indicator	
	Statistical parameter	Experimentally obtained value
Control	$\bar{X} \pm m_{\bar{X}}$, g/l	30,7 ± 1,9
	C_v, %	10
With vitamin B1 (thiamine)	$\bar{X} \pm m_{\bar{X}}$, g/l	37,8 ± 3,2
	C_v, %	10
With vitamin B6 (pyridoxine)	$\bar{X} \pm m_{\bar{X}}$, g/l	42,1 ± 5,4
	C_v, %	20

Thus, aerobic cultivation on a modified nutrient medium based on beet molasses enables a high biomass yield of *Saccharomyces cerevisiae*. Under aerobic conditions, the biomass yield per cell can reach up to 0.5 g^{-1}. With an initial inoculation of 20 g/L, the final yeast concentration may reach 55–60 g/L (George et al., 1998; Martin & Chan, 2024).

In the study by Nasr and Zaky (2011), biomass production during cultivation in a medium with pH 5.0 on a shaker at 30 °C and 200 rpm for 3 days demonstrated that an initial yeast concentration of 10^3–10^4 CFU/mL was the most optimal for achieving higher biomass yield. In another study (Jamir et al., 2021), it was found that further optimization of medium components significantly enhanced biomass production of *Saccharomyces cerevisiae.*

Conclusion

The research was aimed at studying the influence of physico-chemical factors on the process of *Saccharomyces cerevisiae* mass formation during cultivation on modified nutrient molasses. Analysis of the physicochemical properties of molasses from three manufacturers – Aksu-Kant, Koksu-Kant, and Cheremnovsky Sugar Plant – showed relatively similar specific gravity (1.38–1.39 kg/m^3) and total fermentable sugar content (44.36–46.23%). Koksu-Kant molasses showed the highest mass fraction of sucrose determined by direct polarization (50.4%) and the highest sucrose content (44.82%), whereas the lowest values were observed for Aksu-Kant molasses (45.98% and 42.57%, respectively). The invert sugar content in Aksu-kant molasses had higher values (1.74%) compared to molasses from the manufacturers Koksu-kant (0.32%) and Cheremnov Sugar Factory (0.25%).

Microbiological studies have shown the absence of wild yeast, BGCP and nitrite-forming bacteria in any of the three molasses samples. The content of mold fungi was recorded (no more than 10 colonies) for molasses from the manufacturers Aksu-kant and Cheremnovsky Sugar Plant, and no mold fungi were found in the molasses of Koksu-kant. The CMFAM contamination for the three molasses samples was determined at the level of 23 (Cheremnov Sugar Factory), 48 (Koksu-kant) and 75 (Aksu-kant) colonies. The results obtained indicate that all three types of molasses comply with biosafety quality requirements. The study of the influence of physico-chemical factors (temperature, pH) on the growth characteristics of *Saccharomyces cerevisiae* during their cultivation showed that 34 °C and pH 5.07 are the most favorable for their development. Studying the process of accumulation of biomass by yeast during their cultivation on modified molasses, it was shown that the highest biomass yield was obtained with the addition of vitamin B6 (pyridoxine): 42.1 g /l compared with vitamin B1 (37.8 g/l) and control (30.7 g/l).

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