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# PROSPECTS FOR THE USE OF NEW YEAST STRAINS IN NON-ALCOHOLIC BEER PRODUCTION

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***Brewery production is currently one of the growing segments of the processing industry. Beer and beer drinks have long established themselves as competitive products in the beverage production market. Modern beer producers focus on creating traditional light and dark beers, birmix, and beer drinks with various fruit flavours. They also place particular importance on producing non-alcoholic beer. The increase in demand for non-alcoholic beer is due to the***

***partial transition to proper nutrition, the expansion of the assortment and the appearance of new flavours on the shelves. Non-alcoholic beer is a foamy beverage prepared according to classical beer production technology but with technological solutions for removing ethyl alcohol at the output. For the production of non-alcoholic beer, the permissible concentration of ethyl alcohol, which does not exceed 0.5%, is used. Vacuum distillation and membrane methods are used, and alcohol is removed by influencing the course of the technological process. One of these methods is the use of unique yeast strains in fermentation. The purpose of the presented research is to select new yeast strains for producing non-alcoholic beer. Four yeast strains were studied to determine their degree of digestion and influence on the profile of the finished beer. It was determined that yeast strain W 34/70 has the lowest degree of digestion. By technological and physicochemical parameters, the beer wort fermented by this yeast strain has suitable sensory parameters, which are not inferior to the production sample. The use of yeast strain W 34/70 also reduces the fermentation process and increases the shelf life of the finished beverage.***

**Keywords: beer production, non-alcoholic beer, yeast strain, ethyl alcohol, low digestion.**

## АЛКОГОЛЬСІЗ СЫРА ӨНДІРІСІНДЕ ЖАҢА АШЫТҚЫ ШТАМДАРЫН ҚОЛДАНУ ПЕРСПЕКТИВАЛАРЫ ҚОЛДАНУ ПЕРСПЕКТИВАЛАРЫ

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***Қазіргі уақытта өңдеу өнеркәсібінің дамып келе жатқан сегменттерінің бірі сыра қайнату болып табылады. Сыра мен сыра сусындары сусындар өндірісі нарығында бәсекеге қабілетті өнім ретінде ұзақ уақыт бойы өзін танытты. Заманауи сыра өндірісі сыраның дәстүрлі ашық және күңгірт сұрыптарын, сыра қоспаларын, түрлі жеміс-жидек хош иістендіргіштерін қосатын сыра сусындарын өндіруге негізделген, сонымен қатар алкогольсіз сыра өндірудің де маңызы ерекше. Алкогольсіз сыраға сұраныстың артуы дұрыс тамақтануға ішінара көшумен, ассортименттің кеңеюімен және сөрелерде жаңа дәмдік шешімдердің пайда болуымен байланысты. Алкогольсіз сыра-бұл сыра өндірісінің классикалық технологиясы бойынша дайындалған, бірақ шығу кезінде этил спиртін кетіруге арналған технологиялық шешімдерді қолданатын көбікті сусын. Алкогольсіз сыраны өндіру үшін этил спиртінің рұқсат етілген концентрациясы 0,5% - дан аспайтын, вакуумдық дистилляция және мембраналық әдістер қолданылады, сонымен қатар технологиялық процестің барысына әсер ету арқылы алкогольді алып тастайды. Осы әдістердің бірі ашыту кезінде ашытқының арнайы штамдарын қолдану болып табылады. Ұсынылған зерттеудің мақсаты – алкогольсіз сыра өндіру үшін ашытқылардың жаңа штаммдарын таңдау. Ашыту дәрежесін және дайын сыраның профиліне әсерін анықтау үшін ашытқылардың 4 штаммы зерттелді. W 34/70 ашытқы штаммының ашыту дәрежесі ең төмен екендігі және технологиялық және физика-химиялық көрсеткіштері бойынша осы ашытқы штаммымен ашытылған сыра суслосының жақсы сенсорлық қасиеттері бар, өнімділігі бойынша өндіріс үлгісінен кем түспейтіні анықталды. W 34/70 ашытқы штаммын қолдану да ашыту процесін қысқартады және дайын сусынның жарамдылық мерзімін арттырады.***

**Негізгі сөздер: сыра өндірісі, алкогольсіз сыра, ашытқы штаммы, этил спирті, ашытудың төмен дәрежесі.**

## ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ НОВЫХ ШТАММОВ ДРОЖЖЕЙ В ПРОИЗВОДСТВЕ БЕЗАЛКОГОЛЬНОГО ПИВА

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***В настоящее время одним из развивающихся сегментов перерабатывающей отрасли является пивоваренное производство. Пиво и пивные напитики уже давно зарекомендовали себя как конкурентоспособная продукция на рынке производства напитков. Современное пивопроизводство основано на выпуске традиционных светлых и темных сортов пива, бирмиксов, пивных напитков с***

***добавлением разнообразных фруктовых вкусов, а также особое значение занимает производство безалкогольного пива. Повышение спроса на безалкогольное пиво связано с частичным переходом на правильное питание, расширением ассортимента и появленем на прилавках новых вкусовых решений. Безалкогольное пиво – это пенный напиток, приготовленный по классической технологии пивопроизводства, но с применением технологических решений по удалению этилового спирта на выходе. Для производства безалкогольного пива допустимая концентрация этилового спирта в котором не превышает 0,5%, применяют вакуумную дистилляцию и мембранные методы, также удаляют спирт с помощью влияния на ход технологического процесса. Одним из данных способов является применение специальных штаммов дрожжей при брожении. Целью представленного исследования является подбор новых штаммов дрожжей для производства безалкогольного пива. Исследовано 4 штамма дрожжей на определение степени сбраживания и влияния на профиль готового пива. Определено, что штамм дрожжей W 34/70 имеет наименьшую степень сбраживания и по технологическим и физико-химическим показателям. Пивное сусло, сброженное данным штаммом дрожжей, обладает хорошими сенсорными показателями, не уступающее по показателям производственному образцу. Применение штамма дрожжей W 34/70 также сокращает процесс брожения и увеличивает срок хранения готового напитка.***

**Ключевые слова: пивопроизводство, пиво безалкогольное, штамм дрожжей, этиловый спирт, низкая степень сбраживания.**

***Introduction***

Non-alcoholic beer is by far the most actively growing beer market segment. The growth of the non-alcoholic beer (NAB) market has increased significantly in recent years due to strict alcohol policies and consumers adopting healthier lifestyles [1, 2]. Non-alcoholic beer is a beverage similar in taste to traditional beer but containing a small amount of alcohol (0 to 0.5%). Due to the complete exclusion of alcohol, the production of non-alcoholic beer is a more complex process than the production of conventional beer [3].

One way to produce beer with lower alcohol content is to use a strain with the lowest fermentation activity. This results in a lower proportion of sugars used for ethanol formation. At the same time, it is necessary to consider the significant role of brewer's yeast's genetic properties in forming beer's sensory profile [4-6].

The type of fermentation divides beer into top (surface, warm) yeast fermentation and bottom (deep, cold) fermentation. Top fermentation takes place at temperatures of 14-25°C, sometimes higher, and at the final stage of fermentation, the yeast forms a "foam" on the beer's surface. Temperatures of 6-10°Coptimal for bottom fermentation, and the yeast settles at the bottom of the wort.

Brewing strains synthesize higher alcohols, organic acids, esters, sulfur compounds, and carbonyls, including critical organoleptic components like acetaldehyde, diacetyl, and pentanedione [7].

These compounds can give the beer an inappropriate flavour and non-specific odor at concentrations above the sensory threshold and a content that is too low. The technology and strain characteristics of yeast used for production determine the organoleptic profile of non-alcoholic beer [8].

In their research, T.I. Filimonova and O.A. Borisenko compared the quantitative ratio of using different yeast strains in cold fermentation. They concluded that most Russian breweries prefer yeast strains 308 and 34 [9].

In his study, S.G. Davydenko and D.V. Afonin compared yeast strains 129 and 776 used in cold fermentation. Yeast strain 129 showed better consumer properties than non-alcoholic beer [10].

Therefore, when creating a new technology of non-alcoholic beer, the organoleptic properties of which will be able to meet the consumer's requirements, it is necessary first to select yeast strains. For this purpose, four yeast strains were studied: W 34/70 (Finland), 34 (Belgium), 129 (France), and 308 (Russia). These strains differ significantly from each other, primarily in the rate of substrate utilization and biomass increase. Since ethanol metabolism has similar rates, its synthesis should occur in small amounts [11].

***Materials and research methods***

The inoculum for the experimental part of the work was collected by growing a two-day yeast culture from 50 cm3 mashed wort agar followed by 500 $cm^3$ of wort. The yeast was then centrifuged and added to the wort at 18-20 million cells per 1 $cm^3$ with a dry matter mass fraction of 10.5% and an amine nitrogen concentration of 145 $mg/cm^3$. The medium's initial pH value was 5.2.

The fermentation process was carried out at 10±1°C and stopped when the final degree of fermentation was reached. The absence of changes in the C.P. content in the fermented wort evaluated it.

During fermentation, fermented wort samples were taken daily, and the amount of extract and yeast concentration was determined. At the end of the fermentation process, the amount of yeast and the concentration of diacetyl in beer, as the main indicator of secondary fermentation products, were determined using an HP-6890-Plas gas analyzer (Germany, 2010). The LPS method (USA, 2015) was used to evaluate the carbohydrate spectrum of beer (certificate of metrological certification of the method for determination of enzymatic carbohydrates № 56-09-03).

The exact degree of fermentation and mass fraction of ethanol was determined on an Anton Paar instrument (Austria, 2006). Beer color was analyzed at 440 nm, and isohumulone was analyzed at 275 nm.

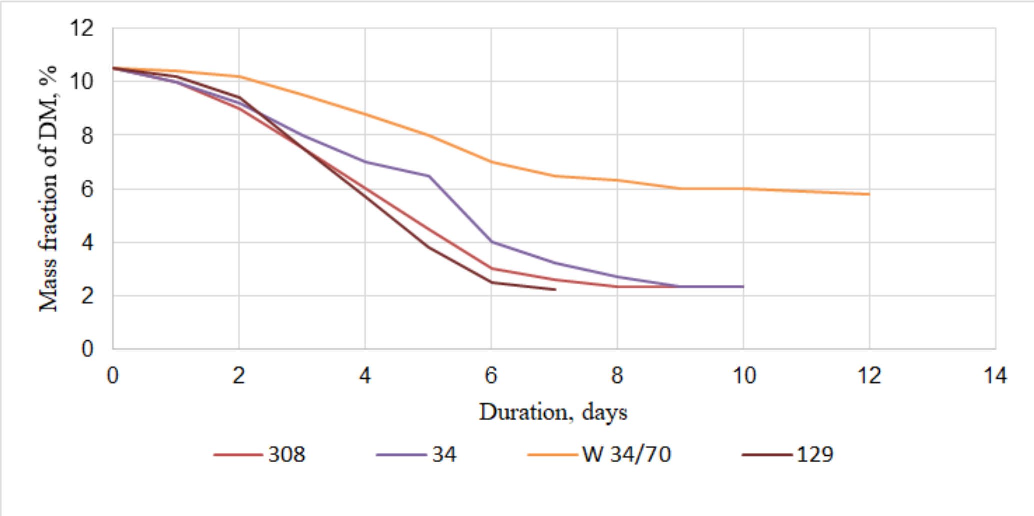


Figure 1. Kinetics of wort dry matters

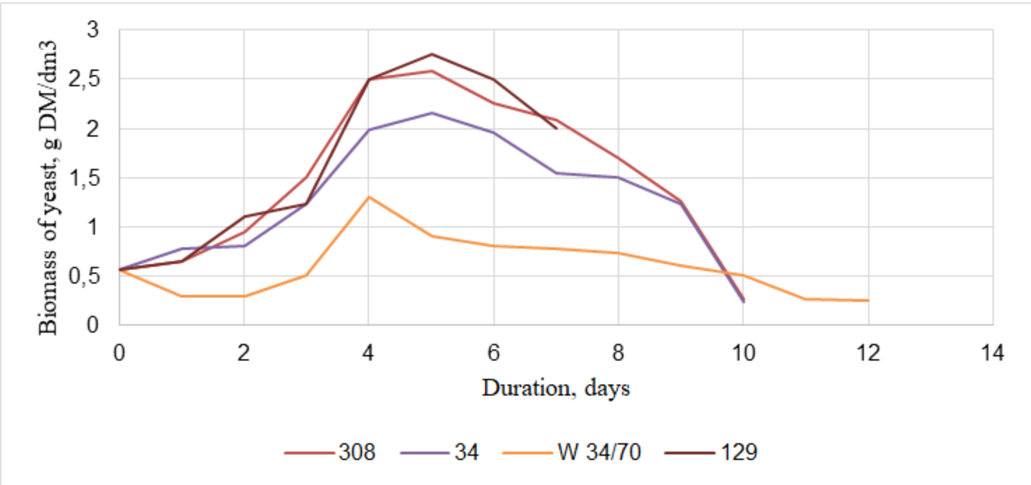


Figure 2. Concentration of wort yeast biomass

As shown in Figures 1 and 2, strain W 34/70 showed the lowest degree of wort fermentation, consuming only 45% of fermented carbohydrates after 12 days. This fact can be explained by the high flocculation capacity of yeast strain W 34/70, resulting in a biomass concentration of 2.5 g D.M/dm3 compared to 1.3 g of strain 34.

The amount of accumulated yeast in strain W 34/70 was at the same level as the other strains D.M/dm3 (Figure 3). The high flocculation ability of the cells of strain W 34/70 and their low fermentation activity may be of interest in producing non-alcoholic or low-alcoholic beer.

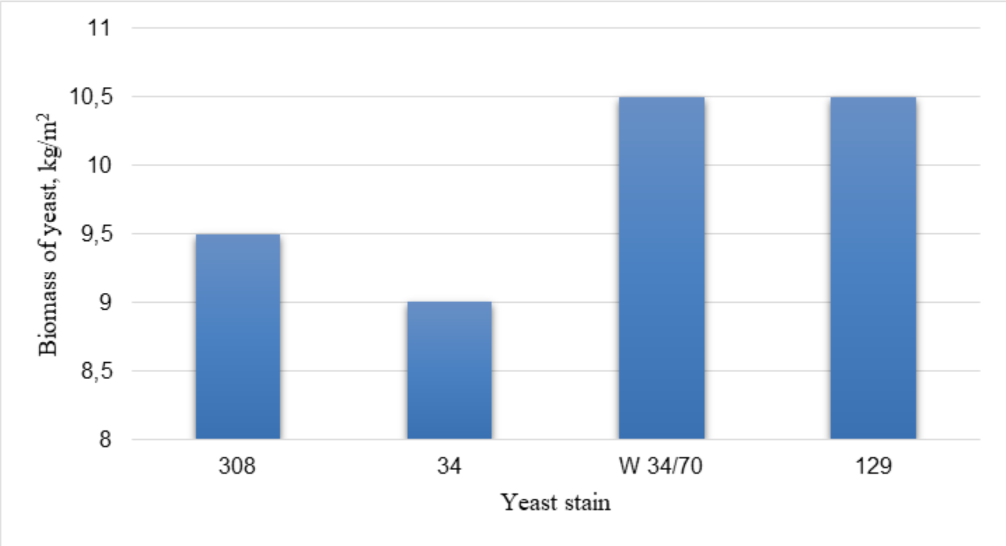


Figure 3. Accumulation of yeast biomass at the end of fermentation

In addition to fermentation activity, one of the essential fermentation characteristics is beer yeasts, which contain acids, higher alcohols, esters, carbonyls, and sulphur compounds. Although the sensory level is shallow, beer yeasts determine the organoleptic profile of beer, carbonyls, and fatty acids and the stability of beer flavour during storage.

Therefore, secondary product synthesis was performed in addition to evaluating indicators characterizing yeast enzymatic activity, and strains were compared for their ability to synthesize secondary metabolic products.

***Results and discussion***

One of the main carbonyls, namely diacetyl (2,3-butanedione), is crucial in the choice of fermentation process regime. The intermediate metabolite in the biosynthesis of diacetyl valine is formed from α-acetolactate. The chemical conversion of α-acetolactate to diacetyl is an oxidative decarboxylation reaction, the rate of which depends on yeast genetic characteristics, fermentation temperature and pH value. The odour of diacetyl, comparable to that of fat and burnt sugar, appeared at concentrations above 0.05 mg/dm³. The concentration of diacetyl in open-lager beers must not exceed 0.05 mg/dm³, while 0.20 mg/dm³ is allowed in quality fermented beer.

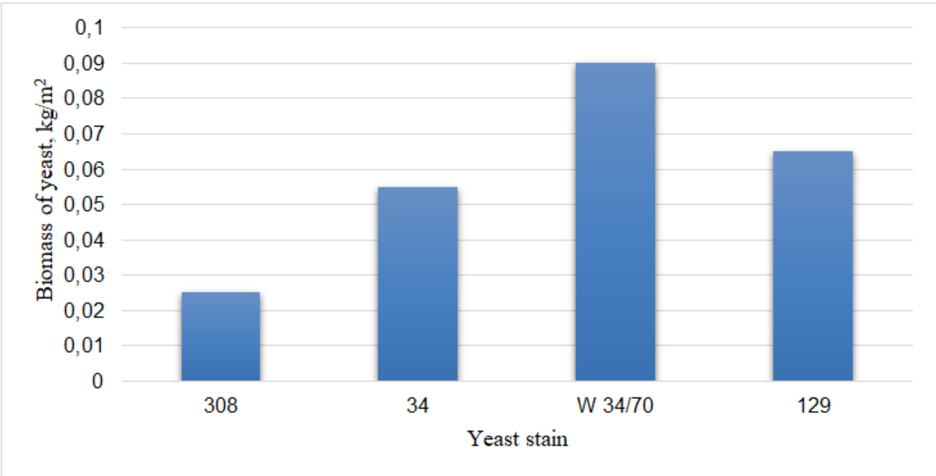


Figure 4. Concentration of diacetyl in young beer

According to the studies (Figure 3), yeast strain W 34/70 (0.09 mg/dm3) showed the maximum diacetyl in beer. Meanwhile, it is known that diacetyl reduction to acetoin occurs on the yeast cell surface [12].

Thus, yeast strain W 34/70 is particularly interesting for producing non-alcoholic beer due to its low fermentation activity. However, the low concentration of organoleptic components in the fermented wort may result in the absence of beer's characteristic flavour and aroma. Therefore, the following experiments used this yeast strain to produce non-alcoholic beer. Nonalcoholic beer "Baltika O" was used as a control to produce yeast of lower fermentation and remove ethyl alcohol from beer with a mass fraction of 12% by dialysis [13].

The experiments were conducted at the brewery of the "Educational and Scientific Centre of Fermentation Products Production" of the Almaty Technological University.

The hoped wort with a mass fraction of 5.5% CB was cooled to 9°C and pumped into a cone-bottom fermentation tank (CCT). Yeast was dosed at 3 million cells per 1 $cm^3$ of wort per wort stream. The fermented wort was stirred once daily for 30 minutes by carbon dioxide bubbling. After reaching an ethyl alcohol concentration of 0.45-0.5 vol.%, the beer was filtered and saturated with carbon dioxide.

During this process, the maximum number of cells was recorded on the fifth day of fermentation (6 million/cm3), but this does not mean that the yeast biomass growth factor is 2. Each day, the yeast settled into the cone of the apparatus before the bubble was removed from the CKT, which caused cell autolysis and prevented the accumulation of unpleasant aromas in the beer.

On the fifth day of fermentation, the temperature of the fermented beer decreased to 3°C for 24 h, and the pressure above the liquid layer increased to 1 bar when the mass fraction of CP reached 4.9%. The development of the young beer lasted for five days.

The table presents the analyses of non-alcoholic beer produced by yeast strain W 34/70 and “Baltika 0” non-alcoholic beer.

Table 1. Analyses of the results of experimental non-alcoholic beer and Baltika 0 beer produced by yeast strain W 34/70

| Indicator | Beer | |
|---|---|---|
| | experimental | Baltika 0 |
| Real extract, % | 4,48 | 7,5 |
| Apparent extract, % | 4,22 | 2,8 |
| Ethyl alcohol content, wt. % | 0,38 | 0,32 |
| pH | 4,5 | 4,2 |
| Titratable acidity, NaOH/100 $cm^3$ | 1,9 | 2,1 |
| Isohumulone, bitterness units (EBC) | 17,6 | 17,0 |
| Colour, colour units | 0,6 | 0,5 |
| Oligosaccharides, $g/dm^3$ | 23,0 | 42,5 |
| Including: | | |
| Trisaccharidter | 5,4 | 5,2 |
| Maltose | 7,5 | 18,8 |
| Glucose | 0,5 | 8,0 |
| Fructose | 0,3 | 0,1 |
| Dimethyl sulphide, $\mu g/dm^3$ | 14 | 4 |
| Diacetyl, $\mu g/dm^3$ | 35 | 20 |
| Pentanedione, $\mu g/dm^3$ | 28 | 18 |

Based on the data analysis in the table, it can be concluded that the control beer sample significantly differs from the experimental sample in terms of the mass fraction of the residual extract and its carbohydrate content.

The amount of vicinal diketones (diacetyl and pentamidine) and dimethyl sulfide (DMS) in the experimental sample is higher than in the control but does not exceed the detection limit (40 μg/L) in both samples [14,15].

Physicochemical parameters cannot give a complete picture of beer, which contains more than 800 components, each of which determines the organoleptic properties of the individual and overall product. Therefore, the tasting was conducted with the participation of qualified specialists. Specialists-tasters of the brewing company "Carlsberg Kazakhstan" LLP took part in the tasting.

To analyze the beverage's taste and aroma, we conducted a descriptive test to characterize its organoleptic properties. After the tasting, we documented the protocol and processed the data from each taster using a specific computer program. The results are presented in profilograms (Figures 5, 6).

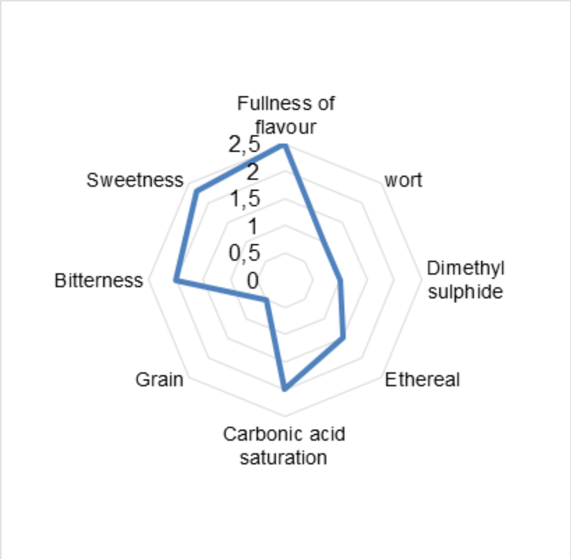


Figure 5. Tasting evaluation of “Baltika 0” beer

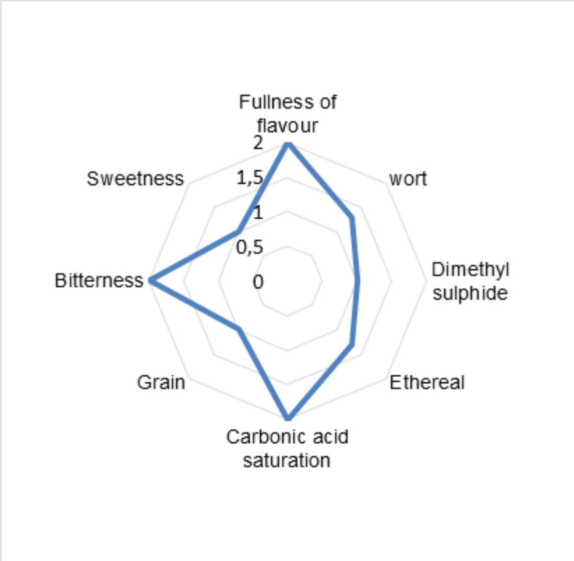


Figure 6. Tasting evaluation of beer produced by yeast strain W 34/70

The profilograms show that sweetness and fullness of flavour increased in the control samples compared to the experimental variants due to higher values of fermentable carbohydrate concentration. The main disadvantage of the experimental non-alcoholic beer is the presence of wort and grain aromas, which are practically imperceptible in the control. It is not due to the peculiarities of the yeast strain. However, it is caused by the fermentation process technology, which is interrupted when a specific ethanol concentration is reached.

***Conclusion***

Thus, non-alcoholic beer obtained using yeast strain W 34/70 corresponds to non-alcoholic beer produced by the best domestic samples in terms of its physicochemical and organoleptic properties. In addition, when using strain W 34/70, it is easier to control the fermentation process until the desired ethyl alcohol concentration, which is in the range of 0.3-0.5 vol.%, unlike strains with high fermentation activity.

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