



UDC 663.5:661.7

DOI: 10.31548/animal.4.2025.59

Specific features of fermentation of higher gravity wort from starch-containing raw materials with a mixed culture of microorganisms

Oleksiy Oliynichuk

Postgraduate Student

Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine

02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine

<https://orcid.org/0009-0009-8014-1703>

Liubomyr Khomichak*

Doctor of Technical Sciences, Professor, Corresponding Member of NAAS

Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine

02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine

<https://orcid.org/0000-0001-9003-0315>

Olga Koval

PhD in Technical Sciences

Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine

02002, 4-A Ye. Sverstiuk Str., Kyiv, Ukraine

<https://orcid.org/0000-0003-1035-5895>

Abstract. The modern technological process of starch hydrolysis to fermentable sugars in the ethanol production from starch-containing raw materials is based on the use of amylolytic commercial enzyme preparations and the application of a low-temperature liquefaction stage for greater process efficiency, which significantly affects the final product's cost. Under such conditions, the search for alternative ways to reduce the costs of these technological process' components and, accordingly, the cost of the manufactured product is relevant. The purpose of the study was to investigate the use of a commercial mixture of microorganisms, namely alcohol yeast *Saccharomyces cerevisiae* and mould fungus *Rhizopus*, as components of the commercial product Angel Leaven for a single-stage process of starch hydrolysis and fermentation of higher gravity wort based on starch-containing raw materials. The methods and processes for preparing raw materials generally accepted in ethyl alcohol

Suggested Citation:

Oliynichuk, O., Khomichak, L., & Koval, O. (2025). Specific features of fermentation of higher gravity wort from starch-containing raw materials with a mixed culture of microorganisms. *Animal Science and Food Technology*, 16(4), 59-69. doi: 10.31548/animal.4.2025.59.

*Corresponding author



Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

production were used in the studies. The technological mode of single-stage starch hydrolysis and sugar fermentation in higher gravity wort by an association of mould fungus and alcohol yeast has been experimentally substantiated. It has been established that saccharification and starch fermentation by a mixed culture occurs in one stage and almost simultaneously at the temperature of 30-32°C, which creates prerequisites for energy saving and technological process simplification. The energy saving effect is enhanced by fermentation of higher gravity wort, obtained without the stage of low-temperature liquefaction, but with the stage of exposure with a proteolytic enzyme for 1 hour at the temperature of 50°C. This technological method is effective for a medium with a concentration of up to 306.6 g/dm³ of dry matter, which allows getting a wort with an ethanol content of up to 14.2% v/v. Increasing the concentration of the medium to 350 g/dm³ of dry matter does not allow getting the calculated ethanol content in mature wort in a single-stage process and requires further research to optimise the fermentation conditions. A set of technological solutions for optimising of starch hydrolysis and fermentation process with a mixed culture creates conditions for increasing the efficiency of raw materials and improving the energy efficiency of production process

Keywords: amylolytic enzymes; starch hydrolysis; mature mash; *Saccharomyces cerevisiae*; association of microorganisms

Introduction

Contemporary biotechnological methods for producing ethanol from starch-containing raw materials are based on the use of exo- and endoamylases for the starch hydrolysis to fermentable sugars. The use of enzyme preparations in practice helped to develop and introduce into production low-temperature methods of water-heat treatment of starch-containing raw materials, which allows saving heat resources, although even under such conditions, the costs of cooking make up to 10% of the costs of energy and fuel resources of production, according to G. Shmatkova & N. Hubenko (2013). However, according to V. Prybylskyi *et al.* (2024), the use of enzyme preparations (EP) for liquefaction and saccharification of starch entails certain financial costs, which can amount to up to 3% of the cost of produced ethanol. An alternative to the use of commercial enzyme preparations is the organisation of a single-stage technological process of hydrolysis and fermentation of starch under conditions of joint cultivation of amylolytic enzyme producers and *Saccharomycetes* yeast.

One variant for co-cultivation is to use a microbial complex, the predominant components

of which are mould fungi such as *Aspergillus* and *Rhizopus*. Such complexes are a traditional fermenting product in countries of Asia, produced based on raw materials rich in starch (wheat, soybeans, rice) inoculated with strains cultivated under controlled temperature and humidity. As mentioned by J. Zhang *et al.* (2023), they are mainly formed into spherical or square granules after drying and used as a starter for fermentation after grinding. The most common component of the mould starter of koji, particularly in Japan, is the fungus *Aspergillus oryzae*. *A. oryzae* is considered particularly suitable for fermentation given its ability to overexpress hydrolytic enzymes and degrade complex molecules such as polysaccharides and proteins (Allwood *et al.*, 2021; Daba *et al.*, 2021). It was also confirmed by N. Watarai *et al.* (2019) that of all the studies on microbial pairs for symbiotic cultivation, the most effective pair was *Aspergillus oryzae* and *Saccharomyces cerevisiae*.

The basic principles for improving of starch fermentation efficiency by direct conversion through the symbiotic cultivation of amylolytic and fermenting organisms were elucidated

by the co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* by I. Han & M. Steinberg (1987), which were further developed by many scientists, in particular A. Drosos *et al.* (2021). In South Korea, nuruk starter is widely used in the production of traditional rice wine. The microbes in the nuruk depend on the grain source and the fermentation environment, but the main of them essential for the fermentation are *Saccharomyces cerevisiae*, *Aspergillus* spp., *Lactobacillus* spp., *Rhizopus* spp., and *Penicillium* spp. The α -amylase, β -amylase, glucoamylase enzymes produced by *Aspergillus* spp. and *Rhizopus* spp. are responsible for the saccharification of starch, as noted in research by B. Wong *et al.* (2023). *Rhizopus* and *Mucor* fungi can be used as an alternative to *Aspergillus* in the production of fermented products, as stated in research by M. Kim & J. Seo (2021) and S. Heo *et al.* (2023).

The purpose of this study was to investigate the technological regimes of the single-stage process of saccharification and fermentation of starch in increased concentration wort with components of microorganisms' mixed culture in the commercial product Angel Leaven in comparison with the parameters of processes operating in the alcohol industry.

Materials and Methods

The experimental work was carried out from March to July 2025 in the Department of Technologies of Fermentation Products of Institute of Food Resources of National Academy of Agrarian Sciences of Ukraine. Physical and chemical analyses, including determination of alcohol content in wort and content of unfermented sugars, were carried out using the equipment of the institute's laboratory certified according to DSTU EN ISO/IEC 17025:2019 (2021). Grain waste from corn processing obtained from the State Enterprise Zarubinsky Distillery, Ternopil region, Ukraine, was used as a raw material for fermentation. The raw material was characterised by the following parameters: dry

matter content – $85.6 \pm 0.24\%$, starch content – $59.5 \pm 0.10\%$, moisture content – $14.4 \pm 0.1\%$. The commercial product Angel Leaven (Angel Yeast Co., Ltd, China), containing *Saccharomyces cerevisiae* alcohol yeast, *Rhizopus* mould fungi enriched with an additional enzyme complex, was used as a producer for single-stage starch hydrolysis and simultaneous fermentation into ethyl alcohol. The product dosage is $4\text{--}4.8 \text{ g/dm}^3$ in dry form. Before application, the product was activated by mixing with water in a ratio of 1:10 and exposure for 30 minutes at the temperature of $28\text{--}30^\circ\text{C}$.

The following enzyme preparations were used: thermostable α -amylase (Tegamyl BLHL, Tegaferm Holding GmbH, Austria), glucoamylase (TEGAMYL AG90L, Tegaferm Holding GmbH, Austria), protease (Tegalase AP75L, Tegaferm Holding GmbH, Austria). The dosage of α -amylase was based on the amylolytic activity of EP at the rate of 2 units of activity per 1 g of starch, the dosage of glucoamylase was based on the amylolytic activity of EP at the rate of 8 units of activity per 1 g of starch, protease was added according to the manufacturer's recommendation. The preparation Baktrilon (LLC TMA Tristan, Ukraine) was used as an antiseptic, in the dosage recommended by the manufacturer, for effective adherence of microbiological purity for use in alcohol production.

Grain waste from corn processing was ground in a laboratory mill to obtain a grinding of 99–100% of which passes through a sieve with the apertures of diameter 1 mm. The grinding was mixed with water in a ratio necessary to obtain a dry matter content in the medium at the level of $263.5\text{--}350 \text{ g/dm}^3$, which corresponds to the content of fermentable sugars and the estimated ethanol content in mature wort at the level of $183.2\text{--}243.3 \text{ g/dm}^3$ and $12 \pm 0.2\text{--}16.0 \pm 0.2\%$ v/v, respectively. The grinding weight was calculated according to the expected ethanol content in the mature wort, considering the starch content in the raw material, the regulated alcohol yield from a tonne of the raw material's

conditional starch, and the amount of wort required for the fermentation test. The grinding mixed with water was acidified to pH 5.0-5.1 to achieve the optimal value for the complex of amylo- and proteolytic enzymes action.

With the classic low-temperature liquefaction (variants 1-12, 3-12, 1-14, 1-16), recommended in production, mashing was carried out at a temperature of 88-90°C, after adding heat-stable α -amylase EP for three hours. Then the mixture was cooled to a temperature of 32-35°C, glucoamylase and protease EP, anti-septic were added, and inoculated with rehydrated Angel Leaven. The vessels with wort were kept for 96 hours at a temperature of $30 \pm 1^\circ\text{C}$.

In variant 3-12, no additional enzyme preparations were added. For a single-stage process of hydrolysis and fermentation of the formed sugars, a protease solution was added to the prepared wort to destroy protein-starch complexes and exposed in a thermostat at a temperature of $50 \pm 1^\circ\text{C}$ for 1 hour (variants 2-12, 2-14, 2-16). After exposing, the mixture was cooled to a temperature of 32-35°C, an antiseptic was added and inoculated with rehydrated Angel Leaven. In variant 3-14, exposing with protease was not performed. The duration of the single-stage process at a temperature of $30 \pm 1^\circ\text{C}$ was 96 hours. Differences in wort preparation by every variant are presented in Table 1.

Table 1. Research variants according to the processes of wort preparation and concentration

| Stage of the process of preparing the wort for fermentation | Medium concentration, g/dm ³ | | | | | | | |
|---------------------------------------------------------------------------|-----------------------------------------|------|------|------|-------------------------|------|------|------|
| | 263.5 g/dm ³ | | | | 350.0 g/dm ³ | | | |
| | Variant | | | | | | | |
| | 1-12 | 2-12 | 3-12 | 1-14 | 2-14 | 3-14 | 1-16 | 2-16 |
| Liquefaction with adding heat-stable α -amylase (88-90°C, 3 hours) | + | | | + | | | + | |
| Adding glucoamylase to fermentation ($30 \pm 1^\circ\text{C}$) | + | | | + | | | + | |
| Adding protease to fermentation ($30 \pm 1^\circ\text{C}$) | + | | | + | | | + | |
| Exposing with protease ($50 \pm 1^\circ\text{C}$, 1 hour) | | + | | | + | | | + |

Source: developed by the authors

The moisture content of the starch-containing raw materials was determined by the gravimetric method in accordance with DSTU 4864:2007 (2009), the dry matter content – by the calculation method, the starch content – by the fermentation test method in accordance with GSTU 46.045.2003 (2003). The fermentation activity of the microorganism association was determined by the gravimetric method, determining the amount of carbon dioxide that formed during the time the wort was in the thermostat. The samples were weighted after 24, 48, 72, 96 hours. The alcohol content in mature wort was determined by the areometric

method according to DSTU 7457:2013 (2014), the content of unfermented sugars – by the photoelectrocolorimetric method with anthrone reagent according to DSTU 4854:2007 (2009). The content of unfermented starch and dextrins was determined by the calculation method. In a series of studies, the samples were cultivated in 0.5 dm³ biological flasks equipped with air locks; each variant was cultivated in three replicates. The paper presents the average values of the research results. Comparison of means was performed using one method in SAS software. Statistical significance was determined at the level of $p < 0.05$.

Results and Discussion

An effective method for monitoring the enzymatic activity of microorganisms was the intensity of carbon dioxide emission during wort fermentation. The results for this indicator for all research variants were shown in Figure 1. It was found that with dry matter content in wort at the level of 263.5 g/dm³ (variants 1-12 – 3-12) intensity of CO₂ emission in the first day of fermentation was high and changed insignificantly depending on the variation of wort preparation, amounting to 57.87-64.77% of the total amount of carbon dioxide released, i.e., the consumption of sug-

ars during this period was about 60% of the input. As can be seen from Figure 1, CO₂ emission for these variants stopped after 72 hours from the start of fermentation, which allows confirming the completion of the sugar conversion process. Removal of the low-temperature liquefaction stage in case preprocessing by protease did not reduce the intensity of carbon dioxide emission during the fermentation process. Removing the stage of adding glucoamylase and proteolytic enzyme preparations after the liquefaction stage (variant 3-12) did not affect the intensity of carbon dioxide emission during the fermentation period.

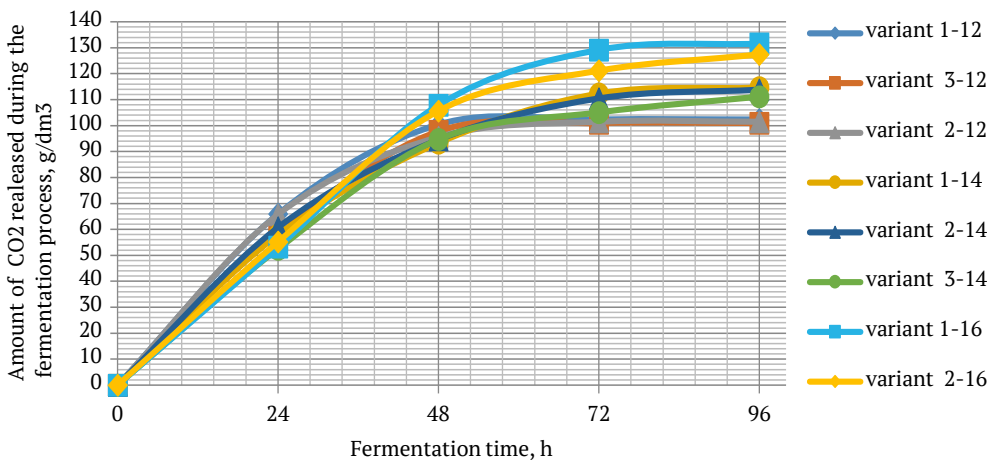


Figure 1. Intensity of carbon dioxide emission during the fermentation of wort based on starch-containing raw materials under various pre-processing conditions

Note: dry matter content in the wort – 263.5 g/dm³ (variants 1-12 – 3-12), 306.6 g/dm³ (variants 1-14 – 3-14) and 350 g/dm³ (variants 1-16 and 2-16)

Source: developed by the authors

In case of increasing the dry matter content to obtain the ethanol content in the mature wort at the level of 14.0-14.2% v/v, 3 variants for raw material pre-processing before fermentation were carried out: classic liquefaction, extraction of the liquefaction stage with the introduction of additional preprocessing with protease, and extraction of all preprocessing stages. According to the research results, it was found that the extraction of all pre-processing stages (variant

3-14) entails some decrease in the intensity of carbon dioxide formation both in the first day of fermentation and in the process as a whole. The duration of the fermentation process in a case an increase of the medium concentration rose for the Angel Leaven product both with classic liquefaction and under conditions of its use without high-temperature processing. Probably, further prolongation of the fermentation period in variant 3-14 can lead to an

increase in the alcohol content in the mature wort to the estimated one; however, excessive fermentation duration increases the risks of the medium contamination and, accordingly, deterioration of the final product quality. Fermentation of higher gravity wort with a dry matter content of 350 g/dm³ showed that the removal of the liquefaction phase from the technological process negatively affects the total amount of released carbon dioxide, reducing it by 3.2% compared to variant 1-16. However, in the first day, the amount of CO₂ was higher in variant 2-16, which can be explained by the lower rate of starch breakdown to fermentable sugars and, accordingly, a reduced negative impact on the yeast cell functionality.

Research by J. Li *et al.* (2021), using *Rhizopus nigricans* as an enzyme producer for the saccharification stage of starch-containing raw

materials followed by fermentation with *Saccharomyces cerevisiae* showed that the highest alcohol-forming capacity and conversion rate were with 16-23% of raw materials concentration. Based on the results of the intensity of CO₂ emission during the fermentation process, it can be argued that the starch conversion into fermentable sugars by a mixture of microorganisms in the product Angel Leaven at a concentration of 263.5 g/dm³ in a single-stage process of liquefaction and fermentation of wort from starch-containing raw materials was effective and, under certain conditions, did not lose efficiency when the concentration of dry substances rises to 306.6 g/dm³. For a more accurate understanding of the progress in the single-stage process in mature wort, the content of ethanol and residual fermentable sugars was determined; the research results are shown in Table 2.

Table 2. Physical and chemical characteristics of mature wort

| Indicators | Estimated ethanol content in mature wort, % v/v | | | | | | | |
|-----------------------------------------------------------------------------|-------------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | 12.0-12.2 | | 14.0-14.2 | | | 16.0-16.2 | | |
| | Variants | | | | | | | |
| | 1-12 | 2-12 | 3-12 | 1-14 | 2-14 | 3-14 | 1-16 | 2-16 |
| Ethanol content in mature wort, % v/v | 12.18±0.04 | 12.17±0.03 | 12.18±0.03 | 14.17±0.03 | 14.17±0.03 | 13.77±0.10 | 16.18±0.03 | 15.78±0.03 |
| Content of total unfermented carbohydrates, g/100 cm ³ | 0.304±0.030 | 0.295±0.007 | 0.341±0.003 | 0.262±0.007 | 0.534±0.007 | 0.872±0.005 | 0.345±0.005 | 0.875±0.004 |
| Content of water-soluble unfermented carbohydrates, g/100 cm ³ | 0.285±0.035 | 0.202±0.004 | 0.302±0.003 | 0.243±0.003 | 0.525±0.005 | 0.817±0.003 | 0.283±0.003 | 0.820±0.005 |
| Content of unfermented starch, g/100 cm ³ | 0.017±0.004 | 0.082±0.005 | 0.035±0.002 | 0.017±0.004 | 0.008±0.003 | 0.049±0.003 | 0.056±0.003 | 0.014±0.001 |
| Content of alcohol-soluble unfermented carbohydrates, g/100 cm ³ | 0.057±0.006 | 0.071±0.003 | 0.112±0.003 | 0.021±0.004 | 0.040±0.002 | 0.042±0.002 | 0.068±0.002 | 0.137±0.006 |
| Content of dextrins, g/100 cm ³ | 0.205±0.027 | 0.118±0.004 | 0.171±0.004 | 0.200±0.005 | 0.437±0.004 | 0.698±0.002 | 0.193±0.002 | 0.614±0.004 |
| Yield of ethanol from a ton of conditional starch, dm ³ | 664.8 | 664.3 | 664.8 | 664.9 | 664.9 | 646.2 | 665.0 | 648.6 |

Source: developed by the authors

According to the data of Table 2, it was established that with a dry matter content in the wort from starch-containing raw materials at the level of 263.5 g/dm³, the commercial product Angel Leaven allowed obtaining an alcohol yield from the mash at the level of 664.3-664.8 dm³ from a tonne of conventional starch, regardless of the raw material preprocessing method used in the study. Accordingly, processing of raw materials at such medium concentration allows the costs reducing of using additional enzyme preparations and heat treatment of the wort without deteriorating of the process quality. The content of undissolved starch fluctuated slightly depending on the preprocessing variant from 0.017 to 0.082 g/100 cm³, however, remaining within the indicator typical for high-quality liquefaction process. The content of total unfermented carbohydrates, which characterises the overall efficiency of raw material fermentation, for variants 1-12 – 3-12 was within 0.293-0.341 g/100 cm³, which was 1.6-1.9% of the starch content introduced into fermentation. Accordingly, this indicator did not go beyond the standards established for Ukrainian production. The low content of alcohol-soluble carbohydrates in mature mash – no more than 0.112 g/100 cm³ – characterised the high efficiency of fermentation of the medium by yeast cells. The low content of dextrans – no more than 0.205 g/100 cm³ – is an indicator that the amount of enzymes with glucoamylase activity is sufficient for effective saccharification of the wort. The findings correlate with the data from C. Zhao *et al.* (2023) on the effect of *Rhizopus* in the composition of the microorganism mixture on the amount of reducing sugars and the alcohol content in the final product.

Increasing the dry matter content in the wort to 306.6 g/dm³ when using Angel Leaven in the process with standard raw material preprocessing allowed achieving the estimated alcohol content in the wort, which helped to obtain a regulated yield from a tonne of conditional starch of raw materials. Removing the

stage of separate liquefaction and adding amylolytic enzymes with the replacement of processing with protease did not have a negative effect upon the course of fermentation and the alcohol content in the mature wort. However, the content of the spectrum of unfermented sugars was higher than the control variant 1-14, which, together with achieving the normative alcohol yield, may indicate that the gradual release of sugars during the destruction of starch by the fungal component enzymes reduced the stress effect of excess nutrients on yeast cells and, probably, reduced the amount of osmoprotectors formed by yeast (Saito & Posas, 2012; Auesukaree, 2017). However, according to P. Puligundla *et al.* (2011), long-term exposure to both higher gravity wort and increasing ethanol stress prevented yeast from efficiently metabolising residual sugars.

The variant in which protease was not added and liquefaction was not applied was characterised by a decrease in the ethanol content in the mature wort to 3% compared to the variant with classical processing. Accordingly, the ethanol yield from a tonne of conditional starch decreased by 18.7 dm³, and, therefore, the use of Angel Leaven on an industrial scale for wort with a concentration of 306.6 g/dm³ and higher without pre-processing is impractical. When the concentration of the wort increased to 350 g/dm³, 2 processing variants were compared: classical (variant 1-16) and the exposure with protease (variant 2-16). It was found that with such a concentration of dry substances in the medium, the extraction of the stage of the temperature treatment from the process negatively affected the course of fermentation, reducing the alcohol content in the mature wort by 2.5% compared to the classical technology, respectively, the ethanol yield from a tonne of conditional starch also did not meet the normative one.

A detailed analysis of the spectrum of unfermented sugars in the mature wort showed that the liquefaction process in both variants

occurs within the normal range, as evidenced by the low content of undissolved starch. However, the total content of unfermented sugars in variant 2-16 was significantly higher, 0.835 g/100 cm³ versus 0.345 g/100 cm³ in the variant with classic liquefaction. In addition, the content of alcohol-soluble carbohydrates in both variants was low, so it means that the yeast cells were able to consume the sugars available to them to the maximum. High content of dextrans in variants 2-16 can indicate the lack of glycoamylase enzymes. It is obvious that some enzymes present in the commercial product, and those created by *Rhizopus*, were not sufficient for dextrans to be converted into sugars available to yeast with a required speed. Probably, a high starting concentration of the medium, an increase in the amount of acids as a fermentation byproducts and an increase in the amount of ethanol in wort negatively affected the growth and cultivation of *Rhizopus* as enzyme producers, which, accordingly, delayed the overall process of converting sugars into the final product. These data need to be confirmed, since studies by E. Uyar *et al.* (2010) in this area were focused on the accumulation of trehalose under the influence of individual stress factors and did not consider the specifics of the use of the producer in the fermentation process of starch-containing raw materials.

One of the options of the problem solution may be to increase the fermentation time, which would allow the glucoamylase enzymes to complete the transformation process. However, such process duration is not advisable when using the technology in the production process. Moreover, one of the variants for adjusting the fermentation efficiency may be to increase the amount of Angel Leaven inoculum, since it is known that increasing the amount of *Rhizopus* inoculum can have a positive effect on the amount of alcohol in the mature wort, as mentioned by A. Büyükkileci *et al.* (2006). Considering the above-mentioned results of research using the commercial product Angel

Leaven as a producer of a mixed culture of microorganisms during wort fermentation at a concentration that ensures the alcohol content in the mature wort not exceeding 14% vol., the effectiveness of replacing the stage of low-temperature wort liquefaction by an exposure with a proteolytic enzyme for 1 hour at a temperature of 50°C was confirmed.

Conclusions

Studies have shown that the efficiency of single-stage fermentation of wort based on starch-containing raw materials with a mixed culture of alcohol yeast *Saccharomyces cerevisiae* and mould fungus *Rhizopus*, enriched with additional amylolytic enzymes contained in the Angel Leaven product complex, depends on the concentration of the medium. With an increase in the initial concentration of the medium, the duration of the process of converting starch into the final product can be extended to 96 hours. On the condition that the calculated alcohol content in the mature wort is obtained up to 14.2% vol., the Angel Leaven product can effectively ferment the wort without the low-temperature liquefaction stage; however, it requires a stage of exposing with a proteolytic enzyme for 1 hour at a temperature of 50°C. The content of total unfermented sugars is higher in comparison with the current technology (0.534 ± 0.007 versus 0.262 ± 0.007 g/100 cm³, respectively) but does not exceed the regulated values. The content of alcohol-soluble carbohydrates is low regardless of the characteristics of the process – no more than 0.137 ± 0.006 g/100 cm³, which indicates the efficiency of the process of assimilation of the obtained fermentable sugars by yeast cells. Increasing the concentration of the medium to 350 g/dm³ of dry matter does not allow obtaining the calculated alcohol content in the mature wort in a single-stage process and requires further research on optimising fermentation conditions, in particular, the efficiency of increasing the amount of the initial mass of producer. The obtained results provide a basis

for developing a regime of single-stage starch hydrolysis and fermentation of the obtained sugars in larger-scale, in particular plant, conditions. The implementation of the proposed technological regime will help not only to reduce the costs of commercial enzyme preparations, but also to reduce the energy intensity of the liquefaction process.

None.

None.

None.

Acknowledgements

Funding

Conflict of Interest

References

- [1] Allwood, J.G., Wakeling, L.T., & Bean, D.C. (2021). Fermentation and the microbial community of Japanese koji and miso: A review. *Journal of Food Science*, 86(6), 2194-2207. doi: [10.1111/1750-3841.15773](https://doi.org/10.1111/1750-3841.15773).
- [2] Auesukaree, C. (2017). Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *Journal of Bioscience and Bioengineering*, 124(2), 133-142. doi: [10.1016/j.jbiosc.2017.03.009](https://doi.org/10.1016/j.jbiosc.2017.03.009).
- [3] Büyükkileci, A.O., Hamamcı, H., & Yucel, M. (2006). Lactate and ethanol productions by *Rhizopus oryzae* ATCC 9363 and activities of related pyruvate branch point enzymes. *Journal of Bioscience and Bioengineering*, 102(5), 464-466. doi: [10.1263/jbb.102.464](https://doi.org/10.1263/jbb.102.464).
- [4] Daba, G.M., Mostafa, F.A., & Elkhateeb, W.A. (2021). The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool. *Bioresources and Bioprocessing*, 8, article number 52. doi: [10.1186/s40643-021-00408-z](https://doi.org/10.1186/s40643-021-00408-z).
- [5] Drosos, A., Boura, K., Dima, A., Soupioni, M., Nigam, P.S., Kanellaki, M., & Koutinas, A.A. (2021). A cell-factory model of *Saccharomyces cerevisiae* based on bacterial cellulose without GMO for consolidated bioprocessing of starch. *Food and Bioprocesses Processing*, 128, 202-214. doi: [10.1016/j.fbp.2021.05.006](https://doi.org/10.1016/j.fbp.2021.05.006).
- [6] DSTU 4854:2007. (2009). *Starch-containing raw materials for alcohol production. Determination of mass concentration of fermentable carbohydrates by photoelectrocolorimetric method*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=85091.
- [7] DSTU 4864:2007. (2009). *Starch-containing raw materials for alcohol production. Methods for determining moisture content*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=85099.
- [8] DSTU 7457:2013. (2014). *Aqueous-alcoholic solutions. Methods for determining the content of ethyl alcohol*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=61147.
- [9] DSTU EN ISO/IEC 17025:2019. (2021). *General requirements for the competence of testing and calibration laboratories*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=88724.
- [10] GSTU 46.045.2003. (2003). *Grain. Methods for determining conditional starch content*. Retrieved from <https://zakon.rada.gov.ua/rada/show/v0250555-03#Text>.
- [11] Han, I.Y., & Steinberg, M.P. (1987). Amylolysis of raw corn by *Aspergillus niger* for simultaneous ethanol fermentation. *Biotechnology and Bioengineering*, 30(2), 225-232. doi: [10.1002/bit.260300212](https://doi.org/10.1002/bit.260300212).
- [12] Heo, S., Park, J., Lee, K.G., Lee, J.H., & Jeong, D.W. (2023). Quality characteristics of soybean fermented by *Mucor*, *Rhizopus*, and *Aspergillus* from *meju*. *Heliyon*, 9(3), article number e14092. doi: [10.1016/j.heliyon.2023.e14092](https://doi.org/10.1016/j.heliyon.2023.e14092).

- [13] Kim, M., & Seo, J.A. (2021). Fermentation profiling of rice wine produced by *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter. *Applied Biological Chemistry*, 64(1), article number 25. doi: [10.1186/s13765-020-00582-2](https://doi.org/10.1186/s13765-020-00582-2).
- [14] Li, J., Tang, X., Qian, H., Yang, Y., Zhu, X., Wu, Q., Mu, Y., & Huang, Z. (2021). Analysis of saccharification products of high-concentration glutinous rice fermentation by *Rhizopus nigricans* Q3 and alcoholic fermentation of *Saccharomyces cerevisiae* GY-1. *ACS Omega*, 6(12), 8038-8044. doi: [10.1021/acsomega.0c05452](https://doi.org/10.1021/acsomega.0c05452).
- [15] Prybylskiy, V.L., Kuts, A.M., Boiarchuk, Ya.A., & Dulka, O.S. (2024). The impact of enzyme preparations and growth activators on yeast in the technology of alcohol fermentation. *Journal of Chemistry and Technologies*, 32(2), 333-342. doi: [10.15421/jchemtech.v32i2.299359](https://doi.org/10.15421/jchemtech.v32i2.299359).
- [16] Puligundla, P., Smogrovicova, D., Obulam, V.S.R., & Ko, S. (2011). Very high gravity (VHG) ethanolic brewing and fermentation: A research update. *Journal of Industrial Microbiology and Biotechnology*, 38(9), 1133-1144. doi: [10.1007/s10295-011-0999-3](https://doi.org/10.1007/s10295-011-0999-3).
- [17] Saito, H., & Posas, F. (2012). Response to hyperosmotic stress. *Genetics*, 192(2), 289-318. doi: [10.1534/genetics.112.140863](https://doi.org/10.1534/genetics.112.140863).
- [18] Shmatkova, G.K., & Hubenko, N.Yu. (2013). [Formation of the cost price of ethanol production in the context of innovative development](#). *Scientific Works of the National University of Food Technologies*, 48, 169-174.
- [19] Uyar, E.O., Hamamci, H., & Türkel, S. (2010). Effect of different stresses on trehalose levels in *Rhizopus oryzae*. *Journal of Basic Microbiology*, 50(4), 368-372. doi: [10.1002/jobm.200900339](https://doi.org/10.1002/jobm.200900339).
- [20] Watarai, N., Yamamoto, N., Sawada, K., & Yamada, T. (2019). Evolution of *Aspergillus oryzae* before and after domestication inferred by large-scale comparative genomic analysis. *DNA Research*, 26(6), 465-472. doi: [10.1093/dnares/dsz024](https://doi.org/10.1093/dnares/dsz024).
- [21] Wong, B., Muchangi, K., Quach, E., Chen, T., Owens, A., Otter, D., Phillips, M., & Kam, R. (2023). Characterisation of Korean rice wine (*makgeolli*) prepared by different processing methods. *Current Research in Food Science*, 6, article number 100420. doi: [10.1016/j.crfs.2022.100420](https://doi.org/10.1016/j.crfs.2022.100420).
- [22] Zhang, J., Liu, K., Duan, X., Wang, X., Ge, W., & Jin, W. (2023). Restoration of Choujiu Koji and evaluation of its brewing performance. *LWT*, 183, article number 114933. doi: [10.1016/j.lwt.2023.114933](https://doi.org/10.1016/j.lwt.2023.114933).
- [23] Zhao, C., Su, W., Mu, Y., Luo, L., Zhao, M., Qiu, S., Su, G., & Jiang, L. (2023). Effects of Jiuqu inoculating *Rhizopus oryzae* Q303 and *Saccharomyces cerevisiae* on chemical components and microbiota during black glutinous rice wine fermentation. *International Journal of Food Microbiology*, 385, article number 110012. doi: [10.1016/j.ijfoodmicro.2022.110012](https://doi.org/10.1016/j.ijfoodmicro.2022.110012).

Особливості зброджування сусла підвищеної концентрації з крохмалевмісної сировини змішаною культурою мікроорганізмів

Олексій Олійнічук

Аспірант

Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
<https://orcid.org/0009-0009-8014-1703>

Любомир Хомічак

Доктор технічних наук, професор, член-кореспондент НААН

Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
<https://orcid.org/0000-0001-9003-0315>

Ольга Коваль

Кандидат технічних наук

Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Є. Сверстюка, 4-А, м. Київ, Україна
<https://orcid.org/0000-0003-1035-5895>

Анотація. Сучасний технологічний процес гідролізу крохмалю до зброджуваних цукрів у виробництві етанолу з крохмалевмісної сировини базується на використанні комерційних ферментних препаратів амілолітичної дії та застосуванні стадії низькотемпературного розрідження для більшої ефективності процесу, що суттєво впливає на собівартість готового продукту. За таких умов пошук альтернативних шляхів зменшення витрат на дані компоненти технологічного процесу і, відповідно, собівартості виробленої продукції є актуальним. Метою роботи було дослідження використання комерційної суміші мікроорганізмів, а саме спиртових дріжджів *Saccharomyces cerevisiae* та плісеневого грибу *Rhizopus* як складових комерційного продукту «Angel Leaven» для одностадійного процесу гідролізу крохмалю та зброджування сусла підвищеної концентрації на основі крохмалевмісної сировини на етанол. В дослідженнях використовували методи та процеси підготовки сировини, що є загальноприйнятими у виробництві спирту етилового. Експериментально обґрунтовано технологічний режим одностадійного гідролізу крохмалю та зброджування цукрів в суслі підвищеної концентрації асоціацією плісеневого гриба та спиртових дріжджів. Встановлено, що оцукрювання та зброджування крохмалю змішаною культурою відбувається в одну стадію і практично одночасно за температури 30-32 °С, що створює передумови для енергозбереження та спрощення перебігу технологічного процесу. Ефект енергозбереження посилюється за рахунок зброджування сусла підвищеної концентрації, отриманого без стадії низькотемпературного розрідження, однак зі стадією витримки з протеолітичним ферментом впродовж 1 години за температури 50 °С. Даний технологічний прийом був ефективним для середовища з концентрацією до 306,6 г/дм³ сухих речовин, що дозволило отримати бражку зі вмістом спирту до 14,2 % об. Підвищення концентрації середовища до 350 г/дм³ сухих речовин не дозволяє отримати розрахунковий вміст спирту в зрілій бражці за одностадійного процесу і потребує подальших досліджень щодо оптимізації умов зброджування. Сукупність технологічних рішень оптимізації процесу гідролізу і зброджування крохмалю змішаною культурою створює умови для підвищення ефективності використання сировини та зростання енергоефективності виробничого процесу

Ключові слова: амілолітичні ферменти; гідроліз крохмалю; зріла бражка; *Saccharomyces cerevisiae*; асоціація мікроорганізмів