

Non-conventional yeasts as a tool for the production of low-alcohol and alcohol free beer

Vladislava Todorova¹, Desislava Muhtarova¹, Polina Zapryanova^{1*}, Vesela Shopska¹, Georgi Kostov¹

¹ Department of Wine and beer technology, University of Food Technologies-Plovdiv, 26 Maritsa blvd, Plovdiv, Bulgaria

Abstract. The global trend for the production of beverages with reduced alcohol content is a result of both consumer desire for a healthy lifestyle and the producers' efforts to innovate their product portfolios. Although low alcohol and alcohol free beers are traditionally obtained by physical methods, non-conventional yeast strains that are unable to metabolize maltose has been studied extensively for the production of beer with reduced alcohol content. This paper investigated the possibilities of four non-conventional yeast species: *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, *Saccharomyces bayanus* (*S. oviformis*), and *Metschnikowia pulcherrima* for the production of low- and non-alcoholic beers at fermentation temperature of 15 °C. The strains displayed markedly different fermentation dynamics. *Z. bailii* produced the lowest ethanol concentrations and exhibited minimal sugar utilisation, making it suitable for alcohol-free brewing. *S. ludwigii* generated low ethanol and stable ester concentrations, while effectively reducing aldehydes. *M. pulcherrima* produced distinctive fruity esters and moderate ethanol levels. *S. oviformis* produced higher ethanol and citrus-like aromas, making it better suited for low-alcohol beer. Sensory analysis confirmed the chemical trends and highlighted the distinct aromatic signatures of each strain. The findings demonstrate that these yeast species can be selected to produce low- or alcohol-free beers with desirable flavour profiles.

1 Introduction

Global trends toward healthier living, increased awareness of alcohol-related health risks, and evolving consumer preferences have stimulated rapid growth in the market for low-alcohol and alcohol-free products. In Europe and many other regions, consumers increasingly seek beverages that provide the sensory and social experience of beer but with minimal or no alcohol. This shift is reflected in the development of advanced production methods and an expanding commercial portfolio of low- and no-alcohol beers [1, 2].

According to Regulation (EU) No 1169/2011 on the provision of food information to consumers, beverages with an alcohol-by-volume content above 1.2% are classified as "alcoholic beverages," while values below this threshold are not explicitly defined at the EU level. In most European countries, products containing alcohol up to 0.5% (v/v) are considered "non-alcoholic beverages" [3].

The production of low-alcohol and alcohol-free beers has been practiced for several decades and it includes two main methods: physical and biological. Physical methods generally involve two strategies capable of reducing ethanol levels: (i) thermal processes that evaporate ethanol, and (ii) alcohol removal using membrane technologies [4]. Although the physical methods are mainly used in brewing, beers produced often suffer from a loss of aromatic compounds (esters, aldehydes, and higher alcohols), and not merely ethanol

reduction. Due to these limitations, increasing attention has been directed toward biological methods, which include the use of non-conventional yeast strains, modification of mashing regimes and limitation of alcoholic fermentation [5,6,7]. Among these, a particularly promising strategy involves the application of non-conventional yeast strains, which are not capable of maltose utilization [8]. The metabolic characteristics of non-conventional yeasts — including their enzymatic systems, fermentation temperature preferences, and oxygen requirements — result in the formation of distinctive aromatic profiles in low-alcohol and alcohol-free beers. This highlights the need for detailed investigation of the volatile compounds produced and their contribution to the sensory properties of the beverage [9,10,11].

The aim of the present study was to evaluate the potential application of four yeast strain, namely *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, *Saccharomyces bayanus* (*S. oviformis*), and *Metschnikowia pulcherrima* in the production of low-alcohol and alcohol-free beers. Therefore, we investigated the primary and secondary metabolism of these non-conventional yeast strains during alcoholic fermentation at 15 °C and made sensory evaluation of the beers produced.

2 Materials and methods

* Corresponding author: p_zapryanova@uft-plovdiv.bg

2.1 Microorganisms

Three liquid cultures from the collection of the Department of Wine and Beer Technology were used, namely *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, and *Saccharomyces oviformis*, as well as one dry yeast strain of *Metschnikowia pulcherrima* (B-Nature) obtained from Bevision Ltd. The liquid cultures were pre-inoculated in sterile grape juice for 72 h at 28°C and the active dry yeast was rehydrated according to manufacturer's instruction.

2.2 Wort preparation

Wort was prepared by mixing 4.5 kg Pilsner malt (Best maltz, Germany) with water in ratio 1:5 in a "Braumeister" brewing system. The mashing method is shown in Fig. 1 [12]. Lautering and boiling was conducted in the same Braumeister. Boiling lasted 60 minutes and 10 minutes after its start, bitter hop Magnum (α -bitter acids of 14.4%) was added to obtain wort with 90 mg/L α -bitter acids. After hot trub removal, wort was frozen at -18°C. The wort obtained was with an original extract of 11.5 °P and pH of 6.14.

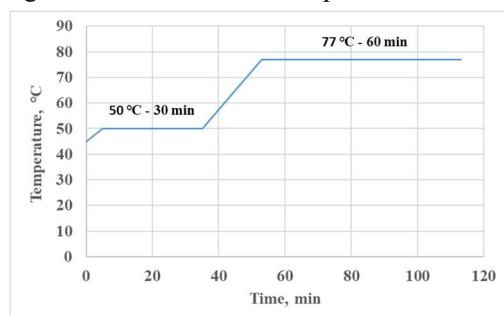


Fig. 1. Mashing method for the production of beer with reduced ethanol content.

2.3 Fermentation

Fermentation was carried out in 330 mL bottles equipped with airlock system. Each bottle was filled with 200 mL of wort and sterilized by means of a Koch steam sterilizer. After cooling, wort was inoculated with a yeast suspension of the corresponding strain, containing $5 \cdot 10^6$ cells/mL. Fermentation was carried out at a constant temperature of 15 ± 1 °C for 10 days. Fermentation was monitored daily by measuring the main beer parameters (extract, alcohol content, and pH). The concentration of secondary yeast metabolites (aldehydes, higher alcohols, and esters) in beer were also determined.

2.4 Analytical procedures

2.4.1 Beer main parameters

The main beer parameters (original and real extract, alcohol, and pH) were conducted according to the EBC methods of analysis [13]. Wort and beer extracts were measured using an Anton Paar DMA 35 density meter.

Alcohol content was also measured using the same density meter after simple distillation of the beer. The pH was determined by WTWInolab Ph7110.

2.4.2 Secondary metabolites

Secondary metabolites were determined after beer distillation. Higher alcohols were measured using the p-dimethylaminobenzaldehyde method according to AOAC [14]. Esters were determined after saponification with 0.1 N NaOH. [15]. Aldehydes were measured according to bisulphite method [15].

2.5 Sensory analysis

Sensory analysis was conducted as a descriptive screening test by a trained panel consisting of eight assessors (four males and four females, aged 39-63 years). All panelists had prior experience in beer sensory evaluation. Aroma and taste attributes were evaluated using a five-point intensity scale. A score of 1 corresponded to the poorest and unacceptable quality, whereas a score of 5 represented the best and most desirable quality. The evaluated aroma attributes were fruity, citrus, malty, sweet, and alcoholic, while taste attributes included malty, alcoholic, sour, bitter, astringent, and sweet. Individual scores for all quality parameters were expressed as arithmetic means of all evaluations [16].

2.6 Statistical analysis

Statistical comparison of the experimental results and the fitted models was performed using the algorithms implemented in the respective software packages. The kinetics of ethanol accumulation were described using the logistic growth equation, which exhibits the characteristic sigmoidal shape:

$$\text{Alc}(\tau) = \frac{\text{Alc}_{\max}}{1 + e^{-k(\tau - \tau_0)}} \quad (1)$$

where $\text{Alc}(\tau)$ is the alcohol concentration at time t (%), Alc_{\max} is the maximum alcohol concentration (%), k is the ethanol accumulation rate (h^{-1}), and τ_0 is the time at which 50% of Alc_{\max} is reached (h).

The model parameters were estimated by nonlinear regression using the least-squares method, implemented in Python (SciPy).

3 Results

3.1 Selection of non-conventional yeast for the production of beer with reduced ethanol content

In an initial series of studies, the brewing potential of more than 20 yeast strains, mainly used for wine production, were assessed by measuring: substrate consumption, ethanol production, concentrations of key metabolites (esters, aldehydes, and higher alcohols), and pH. The main criteria for the yeast selection was the alcohol content of the beer produced. Therefore, we

should select species that were not capable of maltose utilization. The second criteria for yeast selection was the amount of aldehydes produced because they are known to majorly contribute to characteristic malty and worty aroma of alcohol-free beers, especially those brewed by biological methods [17]. Furthermore, some aldehydes have extremely low sensory thresholds; for instance, trans-2-nonenal can be perceived at concentrations as low as 0.03 $\mu\text{g}/\text{dm}^3$ [18].

Therefore, we chose *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, *Saccharomyces oviformis* u *Metschnikowia pulcherrima*. The selected yeast strains produced aldehydes in the lowest concentration (8–14 mg/dm^3), which indicated that they may possess good reducing capabilities—an important factor in beer production.

3.2 Alcoholic fermentation

The ethanol-reduction strategy employed in this study combined two approaches: the use of wort with reduced maltose content and the application of low-temperature alcoholic fermentation [19]. These technological adjustments not only limit ethanol formation but also strongly influence the production of key yeast-derived flavour compounds. Although ethanol content is essential for beer classification, the concentration and balance of secondary metabolites such as higher alcohols, esters and aldehydes play a decisive role in defining the flavour quality of alcohol-free beer.

Higher alcohols play an important role in shaping the flavor profile of low-alcohol beer. If the higher alcohols are in low concentration, they contribute positively to fruity, floral and warming attributes, while high concentrations introduce harsh, solvent-like notes in beer. Esters in moderate quantities can add a pleasant, full-bodied character to beer aroma. When present in excess, they give beer aroma an overly fruity quality, which is considered undesirable by most consumers. Maintaining an appropriate ester-to-higher-alcohol ratio is particularly critical in low-alcohol beer, where the reduced ethanol content limits flavor fullness and requires careful optimization of fermentation conditions and yeast strain selection [20, 21]. Aldehydes are equally important, as they possess very low sensory thresholds and strongly influence the perception of freshness. Under-fermented worts and low-temperature fermentation conditions can result in insufficient reduction of wort-derived aldehydes, leading to undesirable green, worty or oxidized notes [17].

3.2.1 *Saccharomyces oviformis*

The results of fermentation with *S. oviformis* at 15 °C are presented in Fig. 2. The data show that the real extract decreased by approximately 2 °P within 168–240 hours, indicating partial fermentation of wort sugars. As a result, the alcohol content in the final beer was 1.6% v/v. The pH remains relatively stable, decreasing from an initial value of 4.64 to 4.31, suggesting limited organic acid formation. After 120 hours, a slight increase in pH is observed, most likely due to cell

lysis—an important factor to consider when conducting prolonged fermentations.

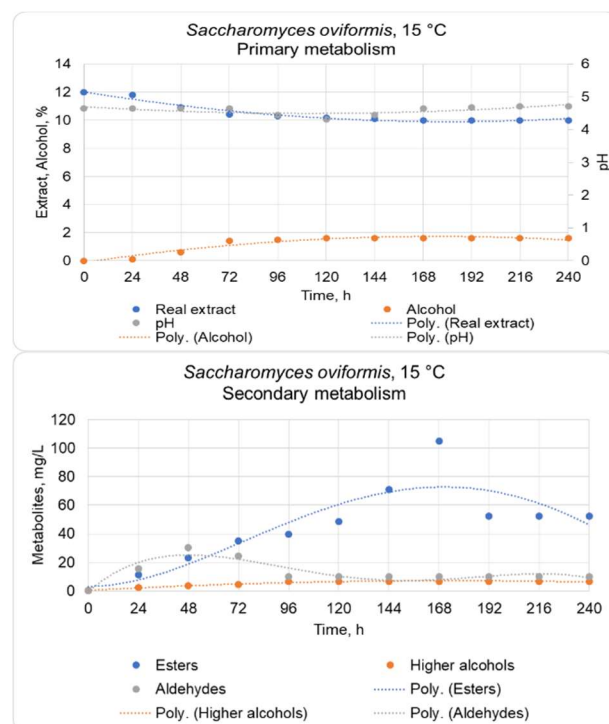


Fig. 2. Dynamic of beer fermentation with *Saccharomyces oviformis*.

The dynamics of ester formation showed that the strain produced substantial amounts of these aroma compounds. However, esters were synthesized only during the first 168 hours, after which a decrease was observed. This decline was attributed to the completion of alcoholic fermentation and the loss of esters due to carbonic acid stripping. Higher alcohols increased until 144 hours, reaching concentrations within an acceptable range between 120 and 144 hours. Aldehyde accumulation was intensive during the first 72 hours of fermentation, reaching a peak of 30.1 mg/L at 48 hours, followed by a gradual reduction. After 96 hours, the level stabilized at 9.75 mg/L , which indicated a reduction in green and harsh flavors.

The results of the fermentation process modelling using equation (1) are presented in Fig. 3 and Table 1.

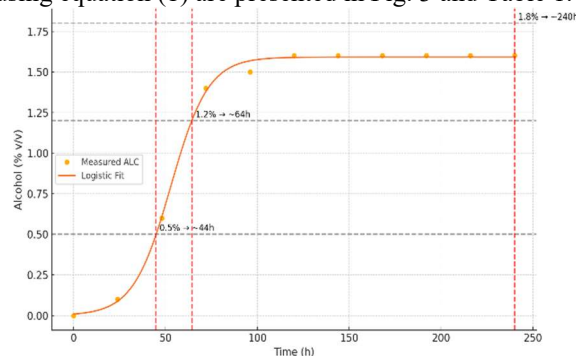


Fig. 3. Modelling of beer fermentation with *Saccharomyces oviformis* with logistic curve.

The model outputs (Table 1) can be used to determine the appropriate time for stopping alcoholic fermentation in order to obtain non-alcoholic or low-

alcoholic beer. In the case of the fermentation process with *Saccharomyces oviformis* at 15 °C, fermentation should be stopped at approximately 45 h to obtain alcohol-free beer and at around 64 h to produce low-alcohol beer. The model data further indicated that the ethanol concentration did not exceed 1.6%, which confirmed the suitability of this strain for the production of low-alcohol beer.

Table 1. Logistic curve parameters for fermentation description.

Alc _{max} , %	k, h ⁻¹	τ ₀ , h
<i>Saccharomyces oviformis</i> 15 °C		
1.59	0.098	53
<i>Zygosaccharomyces bailii</i> 15 °C		
0.70	0.034	73
<i>Saccharomycodes ludwigii</i> 15 °C		
1.21	0.051	67
<i>Metschnikowia pulcherrima</i> 15 °C		
1.22	0.018	147

3.2.2 *Zygosaccharomyces bailii*

The results for beer fermentation with *Zygosaccharomyces bailii* are shown in Fig. 4 and Fig.5. The data showed that the real extract decreased from 11.5 °P to 10.8 °P, indicating poor utilization of wort sugars. Alcohol increased slowly to 0.7% v/v and remains stable after 168 hours, which was below the regulation limit for “low-alcohol beer.” The results demonstrated that *Z. bailii* produced alcohol at low concentration at 15 °C, likely due to temperature stress or low activity of key enzymes. According to the model, the fermentation process could be stopped at around 70 hours.

The pH values remained stable (4.59–4.64) throughout the entire period, indicating moderate organic acid synthesis without intensive acidification.

The peak of ester content was measured (approximately 105 mg/L) at 168 hours. Afterwards the esters concentration decreased significantly, again due to carbonic acids esters stripping. This suggests that identifying an optimal fermentation-stop point is essential for preserving high ester concentrations. The accumulation of higher alcohols was limited during the first 168 hours, reaching its maximum at the end of fermentation (14.24 mg/L). This dynamic resulted in an unbalanced flavor–aroma profile, indicating the need to optimize the fermentation process. Aldehyde synthesis

was moderate, with a maximum reached between 96 and 120 hours, followed by slight reduction and stabilization around typical beer values (~15 mg/L).

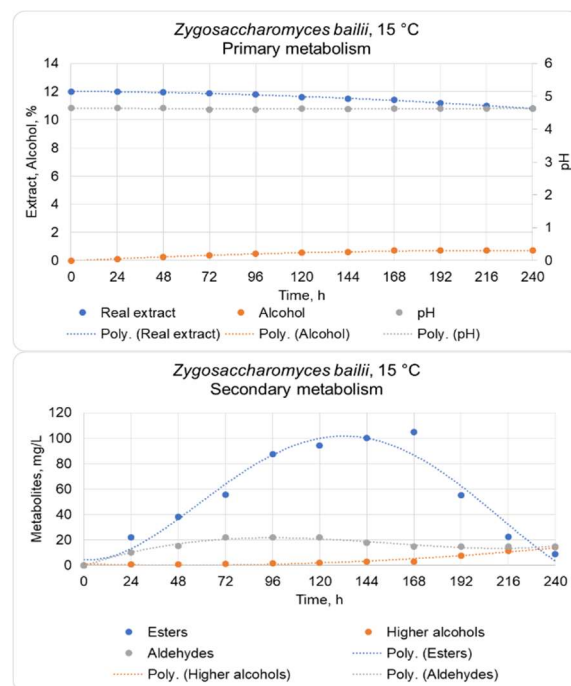


Fig. 4. Dynamic of beer fermentation with *Zygosaccharomyces bailii*.

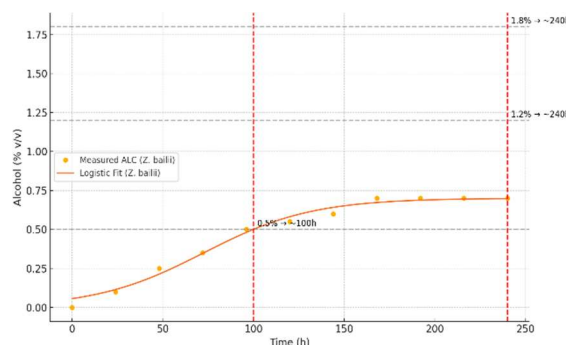


Fig. 5. Modelling of beer fermentation with *Zygosaccharomyces bailii* with logistic curve.

3.2.3 *Saccharomycodes ludwigii*

The results of the fermentation with *Saccharomycodes ludwigii* are presented in Fig. 6 and Fig. 7, as well as in Table 1.

The data show that the real extract decreased from 11.5 °P to 10.5 °P, indicating limited sugar consumption. It is well known that this species does not metabolize maltose from wort, and therefore a low alcohol content was expected. Alcohol increased slowly to 1.2–1.3% v/v and remained constant after 168 hours, which was below the threshold for “low-alcohol beer.” Based on the model predictions, alcoholic fermentation could be stopped within 60 hours to obtain alcohol-free beer, and up to 164 hours for low-alcohol beer. The pH decreased from an initial value of 6.06 to 4.6 within the first 72–120 hours, after which it didn’t change. This reduction was typical during beer fermentation and pH

values around 4.6 affected positively microbiological stability and beer sensory profile.

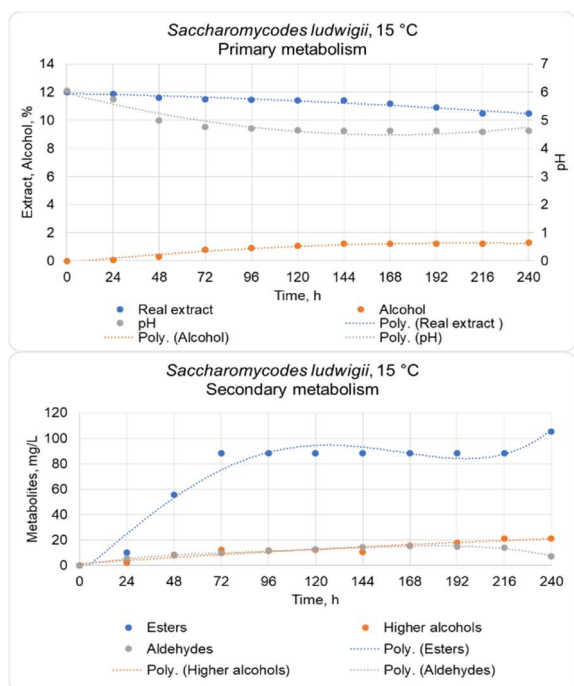


Fig. 6. Beer fermentation with *Saccharomyces ludwigii*.

The esters concentration increased during first 72 hours and this concentration remained consistent until the end of fermentation. The constant ester concentration ensured a pleasant fruity aroma that compensated for the low alcohol content. The formation of higher alcohols was moderate, reaching 21.29 mg/L after 120 hours.

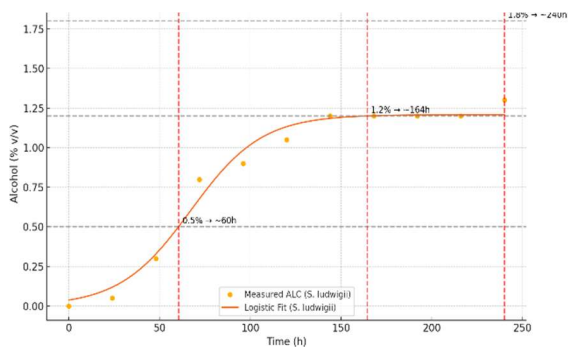


Fig. 7. Modelling of beer fermentation with *Saccharomyces ludwigii* with logistic curve

Aldehydes increased from 0 to a peak of 15 mg/L at 168 hours, after which they decrease to 7.3 mg/L by the end of fermentation. This late-stage reduction indicates an ability to reduce aldehydes, which at higher concentrations impart a “raw” or “green” flavour.

3.2.4 *Metschnikowia pulcherrima*

The results of the fermentation with *Metschnikowia pulcherrima* at 15 °C are presented in Fig. 8, Fig. 9, and Table 1. The data show that the real extract decreased from 12 °P to 10.3 °P, again indicating limited sugar consumption. After 240 hours, the ethanol concentration

in beer was 1.1% v/v. However, the results in Table 1 showed that for the production of alcohol-free beer fermentation could be stopped at around 120 hours. The pH decreased gradually throughout the fermentation, reaching 4.8–4.9 by the end of the fermentation.

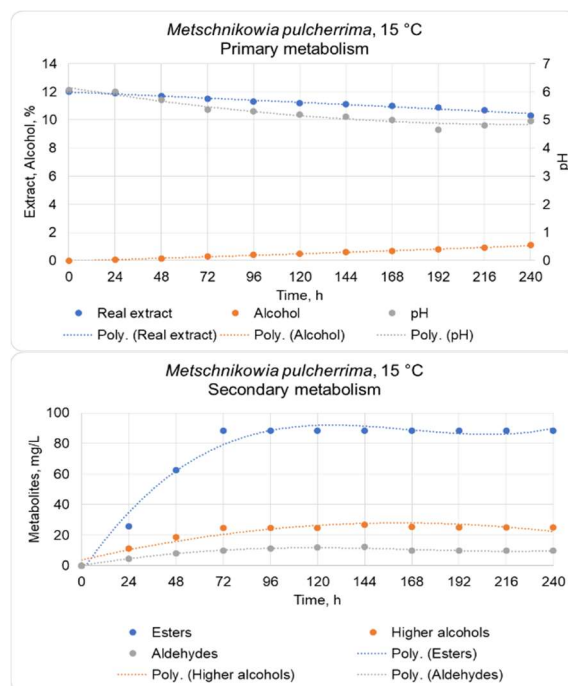


Fig. 8. Beer fermentation with *Metschnikowia pulcherrima*.

Ester concentration increased rapidly during the first 72 hours and then remained steady at around 90 mg/L. A slight increase till 105 mg/L at 240 hours was observed. It may be due to more active ester formation as yeast growth slowed. This pattern is beneficial, as the aromatic esters add pleasant fruity and floral notes that enhance the flavour profile. Higher alcohols increased rapidly during fermentation reaching 26.8 mg/L at 144 h. After that, they remained stable. The strain was characterized by limited aldehydes synthesis because in the final beverage aldehydes were below 10 mg/L.

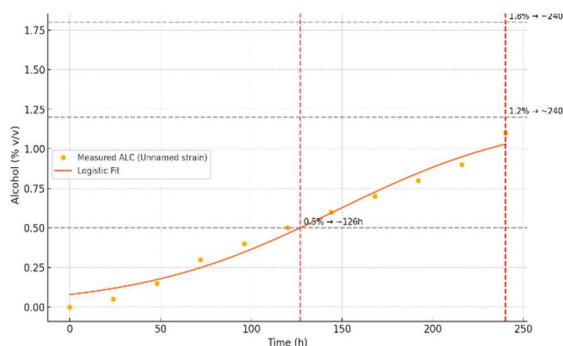


Fig. 9. Modelling of beer fermentation with *Metschnikowia pulcherrima* with logistic curve

3.3 Sensory evaluation

The beverages produced with the non-conventional yeast strains showed no major differences in their

aromatic scores, though their flavour profiles varied, as illustrated in Figures 10–13.

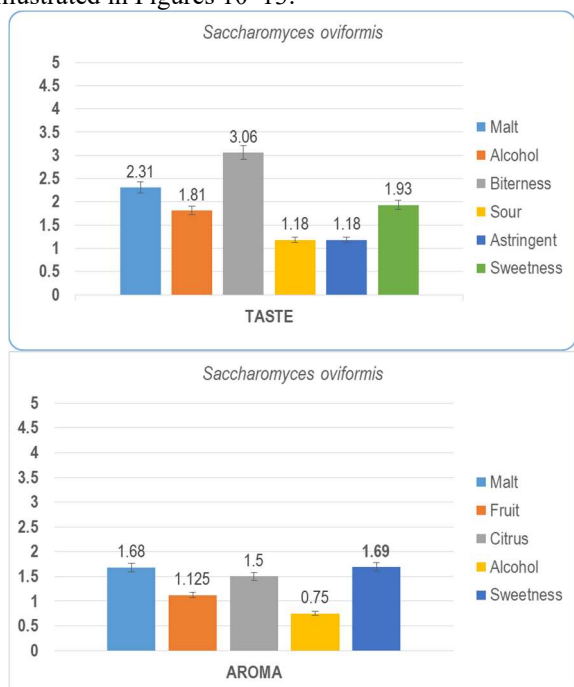


Fig. 10. Sensory evaluation of beer produced with *Saccharomyces oviformis*

Among the samples, only the beer fermented with *S. oviformis* differed aromatically, displaying the most pronounced citrus notes. However, it also received the highest rating for perceived alcohol, which is less desirable for low-alcohol and alcohol-free products.

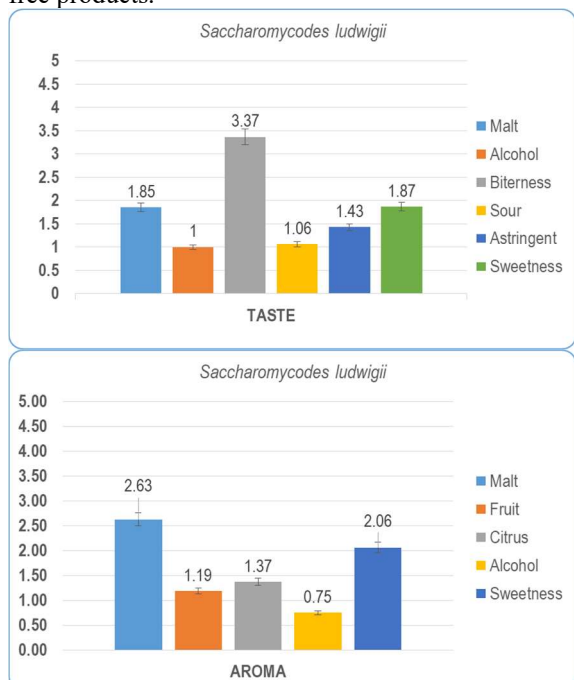


Fig. 11. Sensory evaluation of beer produced with *Saccharomyces ludwigii*

Flavour-wise, the samples diverged more clearly. Malt character was rated differently across all beverages, with the *Zygosaccharomyces bailii* sample receiving the highest malt score. This sample also showed the lowest ratings for bitterness, astringency,

and alcohol perception. Sweetness and acidity did not differ substantially among the beers.

In summary, all tested strains are suitable candidates for low-alcohol beer production, although the resulting beverages will exhibit distinct aromatic and flavour characteristics

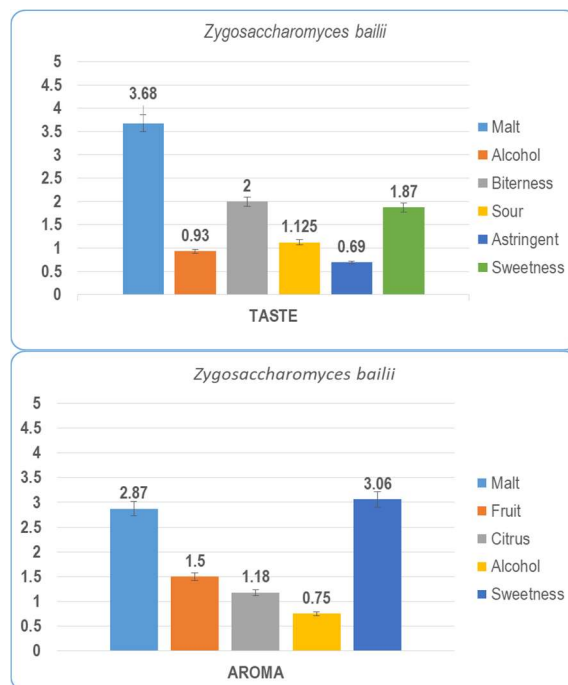


Fig. 12. Sensory evaluation of beer produced with *Zygosaccharomyces bailii*

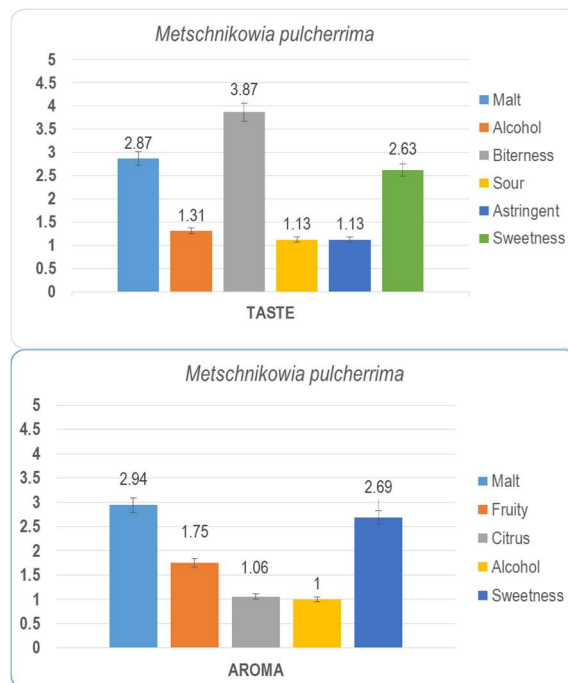


Fig. 13. Sensory evaluation of beer produced with *Metschnikowia pulcherrima*

4 Discussion

The four non-conventional yeast strains examined in this study exhibited markedly different fermentation behaviours, reflecting their variable abilities to

metabolise wort carbohydrates and produce aroma-active compounds at low temperature. Because these strains are either maltose-negative or possess limited maltose transport capacity, ethanol formation was significantly lower than in conventional brewing yeasts.

The results for *Saccharomyces ludwigii* fermentation confirmed prior data showing that *S. ludwigii* was especially effective in suppressing ethanol formation under typical wort fermentation conditions. For instance, a study on non-alcoholic beer production with *S. ludwigii* reported ethanol concentrations approximately six times lower than those obtained with conventional brewer's yeast under comparable conditions [23]. Similarly, in a comparative study including *S. ludwigii* and another non-conventional yeast, the final beers exhibited substantially reduced ethanol—highlighting the strain's suitability for low-alcohol beer when fermentation is arrested [24].

Although in the scientific literature there is no data for the production of low-alcohol beer with *Zygoaccharomyces bailii*, the lowest ethanol produced (~0.7% v/v) further illustrate that certain non-*Saccharomyces* yeasts may be better suited for truly “alcohol-free” beers, provided fermentation is terminated before residual sugars degrade or off-flavours develop.

Although the alcohol content of beer, produced with *Metschnikowia pulcherrima* was higher than that reported in [16] this yeast strain can be applied successfully for the production of low-alcohol beer.

The only yeast strain that was not suitable for the production of low-alcohol beer at these fermentation conditions was *Saccharomyces oviformis*. However, our investigations with this yeast strain will continue by changing wort extract or fermentation temperature.

A critical challenge in low- or no-alcohol brewing is preserving desirable aroma and masking or avoiding off-flavours. Our findings that *M. pulcherrima* produced stable, fruity esters throughout fermentation are well supported by the literature: a recent study evaluating *M. pulcherrima* strains isolated from vineyards underscored their strong potential as starter cultures for craft beer, due to their enzymatic activities and low off-flavour generation [25]. Moreover, the metabolic modelling study of non-*Saccharomyces* yeasts suggests that *M. pulcherrima* tends toward respiration rather than full alcoholic fermentation, which leads to lower ethanol but also modifies by-product distribution, favouring aroma compounds over ethanol production [26].

In contrast, *S. ludwigii* in our work maintained stable ester concentrations while keeping aldehydes low — a combination highly desirable for low-alcohol beer. This consistency aligns with what others have reported: non-*Saccharomyces* and maltose-negative yeasts can generate acceptable flavour compounds even under restricted fermentation conditions, making them viable alternatives to dealcoholisation [16].

For *Z. bailii*, our observation of late ester peak followed by decline and slight aldehyde accumulation under long fermentation mirrors the caution expressed in prior studies: some non-conventional yeasts may require tight fermentation control to avoid flavour deterioration [27].

The use of non-*Saccharomyces* yeasts — particularly those that lean on respiration or limited sugar metabolism — may help breweries avoid the drawbacks of physical dealcoholization (loss of volatiles, need for aroma recovery). As shown in studies exploring non-conventional yeasts for low-alcohol beers, such biological methods can yield beers in the 0.5–1.05% v/v range while achieving favourable sensory ratings. Nonetheless, our findings aligned with previous reports indicating that extended fermentation elevated the risk of generating unwanted metabolites—such as carbonyls, diminished ester levels, and other off-flavours—emphasizing the importance of strict process control and cessation at the optimal point.

5 Conclusion

This study demonstrated that non-conventional yeast strains represented a promising biological alternative for producing low-alcohol and alcohol-free beers, offering clear advantages over physical dealcoholization in terms of aroma retention and flavour quality. Three of the four strains evaluated showed fermentation behaviours consistent with findings from other authors, confirming that maltose-negative or weakly fermentative yeasts can effectively limit ethanol formation while still generating desirable aroma compounds. Among them, *Saccharomyces ludwigii* proved the most reliable for producing clean and well-balanced low-alcohol beers due to its stable ester formation and efficient reduction of aldehydes, while *Zygosaccharomyces bailii* showed potential for alcohol-free products when fermentation is carefully controlled. *Metschnikowia pulcherrima* produced richer aromatic profiles, aligning with previous reports describing the ability of certain non-*Saccharomyces* strains to enhance fruity esters and flavour complexity. Only *Saccharomyces oviformis* was not suitable for the production of beer with reduced ethanol content at the studied conditions. Therefore, future investigations will be needed for proving its application in beer production. However, the study supported the growing consensus that non-conventional yeasts provide a versatile toolbox for developing high-quality, flavourful reduced-alcohol beers through controlled biological fermentation.

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