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Comparative ethanol production by *Saccharomyces cerevisiae* and *Saccharomyces bayanus* using apple juice concentrate

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ABSTRACT

Fruit juice fermentation has been used historically as a conservation technique and to improve the preservation of bioactive components essential to human health. This research was investigated the potential fermentation of apple juice concentrate using *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The current investigation evaluated the yeast strains capacity to produce ethanol by comparing their ethanol concentrations and assessed the ability of yeast strains by evaluating their tolerance to sugar, ethanol, temperature, dry cell weight analysis. The ethanol tolerance of *S. cerevisiae* and *S. bayanus* strains was able to resist 12.5 % and 16.0 %, respectively, and both strains were a temperature of 25 °C. Furthermore, the cell dry weight of *S. cerevisiae* and *S. bayanus* describing cell growth increased with increasing fermentation time from 24 h to 96 h subsequently the cell has expressed a reducing growth phase. The highest production of ethanol after fermentation with the strain *S. cerevisiae* showed 11.01 g/L and *S. bayanus* produced ethanol 12.94 g/L at 25 °B more than *S. cerevisiae*. According to the current research, ethanol fermentation using different yeast strains (*S. cerevisiae* and *S. bayanus*) with apple juice concentrate lies in the potential to optimized fermentation performance, efficiency, and yield of ethanol. In contrast to *S. cerevisiae*, the strain *S. bayanus* was used as a potent starter culture to produce ethanol because it can tolerate high ethanol concentrations.

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Introduction

Global ethanol production in 2021 was estimated to be 100 billion liters of which 82 % was concentrated in Brazil (27 % mainly from sugarcane) and the United States (55 % primarily from corn) (Becerra-Pérez et al., 2023). Fruit based alcoholic beverages was created when their distinct aroma is infused into fermented liquor or distillate (Desai et al., 2015). These compounds were essential for scavenging free radicals. An enormous amount of apples was pressed each year to make cider and juice, producing pomace, a waste product made mostly of skin, pulp, and seeds. Pomace makes up 25 % - 30 % of the apple dry mass, depending on the variety (Grigoras et al., 2013). Global apple production reached 89 million tons in 2013, with the United States and China producing 43.7 million and 4.4 million tons, respectively, according to data from the Food

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and Agriculture Organization of the United Nations (FAOSTAT 2015). At 75 million tons produced in 2018–2019, it is the third most produced fruit globally, behind watermelon and bananas (Wani et al., 2022). India is the sixth-largest apple producer and consumer in the world. India's apple production is rising annually, particularly in Himachal Pradesh, despite the country's high alcohol consumption rates (Deodhar et al., 2006; Manrich, 2024). Since the establishment of apple juice concentrate production plants in Himachal Pradesh, there is far more apple juice concentrate available than can be used to prepare soft drinks. This has led to the investigation of the possibility of using the concentrate to make cider (Patil et al., 2020). Moreover, apple juice serves as the primary raw material for making the number of beverages. Depending on the circumstances, Cider, apple wine, calvados, and vinegar can all be made from fermenting apple juice. Some of the ingredients that give alcoholic beverages their flavor come from the raw material but that are changed in the presence of microorganisms during fermentation (Sattar et al., 2024). The potential use of cellulosic biomass as a feedstock for the synthesis of ethanol is very promising (Olughu et al., 2023). Fermentable polysaccharides can be found in cellulosic plant materials, particularly in non-food agricultural waste products like rice husk, bagasse, wheat straw, rice straw etc. (Goodman et al., 2020).

Today, the world produced an incredible amount of fermented alcohol more than 100 billion liters of fuel and beverage ethanol or bioethanol every year (Lee et al., 2017). The most prevalent type of fungus is yeast which is *Saccharomyces cerevisiae*. A key factor in the conversion of apple mixture to ethanol production is *Saccharomyces* (Walker & Stewart 2016). Yeast also produces a broad variety of odor compounds during fermentation, including esters, volatile acids, higher alcohols, carbonyl compounds, and many more (Guichard et al., 2017).

Using enzymes, *Saccharomyces* members can transform sugar into carbon dioxide and alcohol, which is a remarkable ability. It is possible to produce industrial alcohols, beers, wines, baked goods, and distilled spirits by fermenting sugar using any strain of *Saccharomyces cerevisiae*. In an hour, a single yeast cell of this type can ferment almost its own weight of glucose, which is the most basic type of sugar (Britannica 2020).

Saccharomyces are the most efficient and safest microorganisms for converting sugars to ethanol, and they have long been employed in industry to convert agricultural goods containing glucose to ethanol. Invertase activity, sugar tolerance, and ethanol tolerance are a few crucial characteristics for usage in industrial ethanol production. Making alcoholic beverages from fermentable carbon sources using yeast is the most ancient and important application of biotechnology. Yeast is essential in the production of all alcoholic beverages. In order to maintain beverage sensory quality and maximize alcohol yield, it is crucial to choose the right yeast strains (Maicas, 2020).

Another yeast species, *Saccharomyces bayanus* belongs to the genus *Saccharomyces* is used to ferment wine, cider, and distilled drinks (Libkind et al., 2011). The high yield of hydrogen sulfide (H_2S), volatile acid, and foam produced by the EC-1118 strain makes it an excellent choice for fermentation. This organism has a high alcohol tolerance and ferments well over a broad temperature range of 10 °C to 30 °C. One of the most popular yeasts in use today is *S. bayanus* EC-1118. The intensity of flavor in wines fermented by *S. bayanus* is higher (Gamero et al., 2014). Comparative analysis of both the yeast strain *S. cerevisiae* and *S. bayanus* for ethanol production using apple juice concentrate and effect of hydroxymethylfurfural (HMF) on yeast growth was the main objective of the present study.

This study seeks to assess the performance of two yeast strains, namely *S. cerevisiae* and *S. bayanus*, in enhancing ethanol production.

Materials and Methods

Raw materials

The apple juice concentrate used in this study were obtained from fruit processing unit of HPMC (Himachal Pradesh Horticultural Produce Marketing and Processing Corporation LTD) India. The samples were transported in a packed container to the Amity Institute of Microbial Technology, Amity University, Rajasthan, Jaipur and maintained at 4 °C under cold chain storage until they were required for analysis.

Strain selection and maintenance

Two diverse species of *Saccharomyces* (*S. cerevisiae* and *S. bayanus* previously isolated) were used for ethanol fermentation process. All the strains were revived as per the media at 30 °C and instructions received from the MTCC, India. Yeast peptone dextrose (YPD) broth, which contained 10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose, was used to revive the yeast cultures for 24 h incubation period at 30 °C. The cultures were additionally kept on YPD agar plates, which contained 15 g/L agar, 20 g/L peptone, 20 g/L dextrose, and 10 g/L yeast extract. The cultures were further maintained using glycerol stock 50 % (v/v) at -80 °C and YPD agar slants at 5 °C till further use.

Ethanol tolerance

To study the ethanol tolerance, *S. cerevisiae* and *S. bayanus* were inoculated in the YPD broth media supplemented with different ethanol concentrations

ranging from 2.5 %, 5.0 %, 7.5 %, 10.0 %, 12.5 %, 15 %, 17.5 % and 20.0 % (v/v). The flasks were incubated for 24 h at 30 °C. After incubation all the flasks were observed and optical density was taken at 660_{nm} using spectrophotometer and the graph was plotted from the obtained data.

Temperature tolerance

To find out the temperature tolerance ability, YPD broth media were prepared and inoculated with different yeast strains. The inoculated flasks were incubated at different temperature conditions, *S. cerevisiae* incubated at three various temperature conditions (25 °C, 35 °C and 45 °C), and the another flasks with *S. bayanus* incubated at (25 °C, 35 °C and 45 °C). All the labeled flasks were incubated for 24 h.

Dry cell weight determination of the strain

The cell dry weight method was used to calculate the samples' cell biomass. Every sample of ethanol that was collected was centrifuged for 10 minutes at 4 °C and 4000 rpm, and the supernatant was disposed. The pellet spent an entire night for drying in the oven. Using an analytical balance, the dry weight of the biomass in the samples was determined.

Ethanol fermentation

For conducting the experiment the fermentation media at three different TSS (Total soluble solid), 20 °Brix, 25 °Brix and 30 °Brix was prepared in erlenmeyer flasks separately with different volume of apple juice concentrate (AJC) and maintained the total volume with distilled water. The brix value was maintained by using Refractometer. The media was autoclaved for 15 minutes at 121 °C and 15 psi. Post sterilization 10 % (v/v) inoculum of both the strain *S. cerevisiae* and *S. bayanus* inoculate into the autoclaved medium and for ten to eleven days, fermentation was conducted in a shaker incubator with 30 °C and 120 rpm.

The samples were collected in cryo-vials for every 24 h of incubation for subsequent chromatographic analysis. After that selected a particular brix value at which the concentration of ethanol is higher for further fermentation at bioreactor scale.

The media was prepared at 25 °Brix for 30 L working volume and sterilized under 70 L pilot-scale bioreactor (FERMEX SOLUTIONS) for 20 min at 121 °C (Fig-1). Immediate after sterilization, the media was auto-cooled up to 30 °C with the help of chiller. Furthermore, the sterilized inoculum of *S. cerevisiae* (10% v/v) was added into the media and set all the parameters for the process of fermentation such as, pH- 4.5 to 5.5, temperature- 28 °C to 30 °C, agitation at 100 rpm and aeration was also required at 1 LPM for only initial 24 h. Since ethanol fermentation being an anaerobic process due to which aeration was stopped. Start the fermentation process for 10 to 11 days and samples were possessed after every 24 h for examination of alcohol content. Yield of alcohol was measured by HPLC (High performance liquid chromatography) technique. The whole process carried out with another strain of *Saccharomyces*, *S. bayanus* respectively.

Fig 1. Ethanol fermentation by *S. bayanus.*

Chromatographic analysis of ethanol

The Agilent Hi-Plex Ca (Duo) column with flow rate-0.60 mL/min (300×6.5 mm, 8 µm, Part No.: PL1F70-6850) was equipped with a Refractive Index Detector (RID) that operated at 55 °C and was kept at 80 °C to analyze the fermented ethanol (EtOH) using HPLC (Agilent Technologies, G2070-91126, 07/09). The mobile phase was HPLC-grade water, which was ultra-pure and had a low UV absorbance. The samples were processed immediately, and an HPLC column was filled with 20 µL of the sample. Prior to analysis, samples were filtered through 0.45 µm PES membrane filter after sediments were eliminated by centrifugation at 5000× g for 10 minutes.

HMF effect on yeast cell growth and ethanol fermentation

The study's methodology was applied to enhance *S. cerevisiae* and *S. bayanus* Hydroxymethylfurfural (HMF) resistance. YPD medium containing varying concentrations of HMF (10 mg/L to 60 mg/L) was used for the cultivations, which were incubated for 24 h. The estimation of yeast cells was done using a cell counting method or colony count after a 24 h period. For both yeast strains, the HMF effect on ethanol production was measured using the same methodology between the second to eleventh days of fermentation.

Results and Discussion

One of the most significant industrial cell for ethanol fermentation was yeast *S. cerevisiae* and *S. bayanus*, which can both assimilate glucose and make ethanol from apple juice concentrate. Different stresses were generally experienced by both yeast strains, which was exploited in the synthesis of bioethanol from concentrated apple juice fermentation. Yeast strains were subjected to several stressors that are common during fermentation settings in this investigation. A yellowish colony was observed after strains of *Saccharomyces* were cultivated on YPD agar plates. Both strains were tested for osmotic tolerance, ethanol tolerance, thermo-tolerance, dry cell biomass and HPLC analysis to produce ethanol by fermentation in order to identify the strain that has the potential to produce ethanol.

Ethanol tolerance

Since ethanol inhibits the growth of yeast cells, yeast strains can tolerate high ethanol concentrations was better suited to produce high ethanol titers. The ability of yeast strains to resist ethanol is demonstrated by their capacity to produce ethanol at high levels (Da silva et al., 2022). The absorbance value at 660_{nm} of yeast cells after exposure to ethanol at different concentrations (2.5 %, 5.0 %, 7.5 %, 10.0 %, 12.5 %, 15.0 %, 17.5 %, and 20.0 %) was used to compare the tolerance of ethanol of *S. cerevisiae* and *S. bayanus* strain in figure (2). When the ethanol concentration increased from 2.5 % to 12.5 % in the growth medium the growth of *S. cerevisiae* strain increased but beyond that a sharp decline in the growth of strain was observed when the ethanol concentration was increased from 15.0 % to 20.0 %. Strain *S. bayanus* showed increased ethanol tolerance from 2.5 % to 16.0 % after that slow and steady decline phase observed with increased ethanol concentration from 17.0 % to 20.0 %.

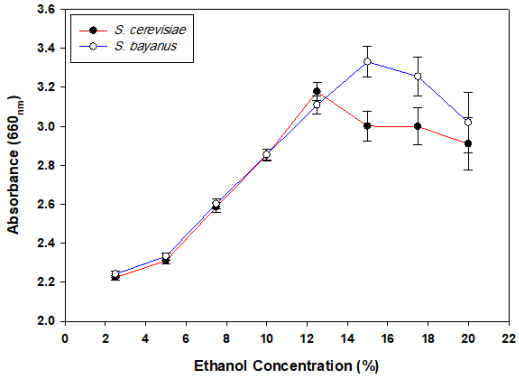

Fig 2. Ethanol tolerance evaluation of *S. cerevisiae* and *S. bayanus*

Few researchers concluded that the most crucial factor is ethanol tolerance because it is hard to prevent the rising ethanol concentration in the medium throughout fermentation. Using four strains isolated from fermenting cashew apple juice, demonstrated strain tolerance by showing significant growth in a medium containing 9 % (v/v) ethanol (Osho, 2005). According to another study the majority of yeast isolates could tolerate ethanol concentrations of up to 10 % (v/v) (Techaprin et al., 2017). Another study determined that the highest percentage of ethanol (v/v) that prevented *S. cerevisiae* and *Pichia kudriavzevii* from growing was 5 % and 10 %, respectively. The growth of yeast isolates was totally inhibited at a 10 % ethanol concentration (SR et al., 2024). Additionally, the modified *S. cerevisiae* strain showed an 80.2 % increase in ethanol tolerance, reaching 30 g/L, while maintaining excellent activity at 50 g/L (Zhang et al., 2023).

Temperature tolerance

Temperature tolerance ability of *S. cerevisiae* and *S. bayanus* at three different temperature situations 25 °C, 35 °C and 45 °C was compared based on the absorbance value at 660_{nm} after incubation at 24 h (Fig. 3). The absorbance value of *S. cerevisiae* was decreased at temperature 35 °C and 45 °C as compared to 25 °C. *S. bayanus* witnessed a slow and steady decline as the temperature expanded from 35 °C to 45 °C. Conventional yeast *S. cerevisiae* is used for industrial fermentation, which is normally conducted at low temperatures of 25 °C – 30 °C. On the other hand, high-temperature fermentation has grown a lot of attention and offers benefit since it produces ethanol at a temperature that lowers cooling costs and allows for more efficient fermentation (Saini et al., 2017). In a different study, thermo-tolerant yeast strains were examined in order to enhance ethanol production at higher temperatures. *P. kudriavzevii* NUPHS33 and NUPHS34, two yeast strains, have demonstrated remarkable resistance to ethanol, withstanding concentrations as high as 15 % (v/v). Furthermore, these strains demonstrated the ability to flourish at 45 °C temperatures (Pongcharoen et al., 2021). According to a different study, denaturation of ribosomes and enzymes, as well as issues with membrane fluidity, may be caused indirectly by high temperatures. The maximum specific growth rate and maximum specific ethanol production rate with varying initial glucose concentrations were measured between 30 °C and 45 °C (Abdul Salam et al., 2024). At 42 °C, other researchers showed noticeably greater rates of cell viability and growth (Zhang et al., 2023).

Dry cell weight determination

Cell concentration is measured during fermentation by analyzing the dry mass of the cells. The cell dry weight can be used to measure the growth of microorganisms.

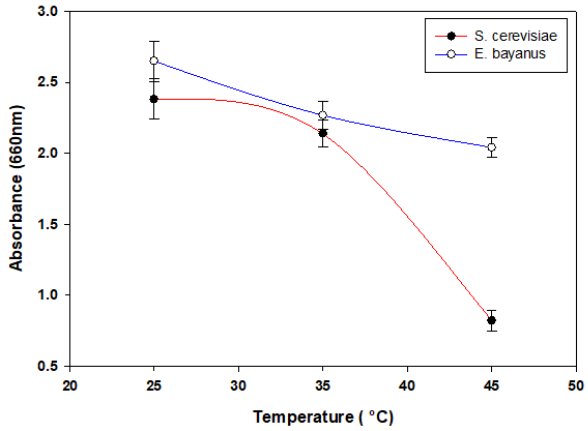

Fig 3. Temperature tolerance of *S. cerevisiae* and *S. bayanus.*

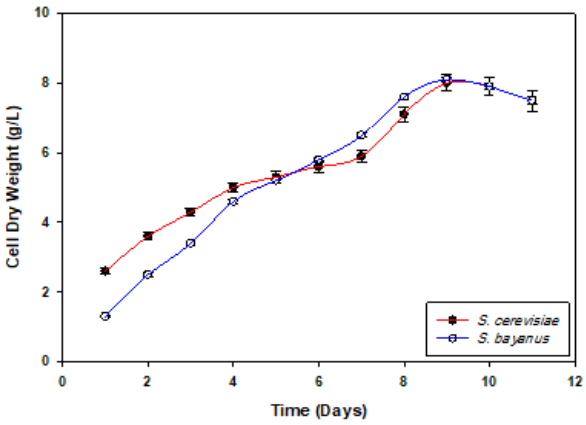

Fig 4. Cell dry weight of yeast strain *S. cerevisiae* and *S. bayanus*

Figure 4 displays data from measurements of *S. cerevisiae* and *S. bayanus* cell dry weight during the ethanol fermentation process. The cell concentration of both the yeast strain increased with increasing fermentation time from 24 h to 96 h. After that, the concentration was decreased and the cell has expressed a slowing growth phase or death phase. Because the yeast is still adjusting to the fermentation medium at this early stage, it is not producing its maximum number of cells, which is why cell dry weight is still comparatively low after 24 h of fermentation. Because the cells can divide and reproduce more quickly because they have advanced through the adaptation phase, the cell dry weight produced at 48 h exceeded the 24 h fermentation time. The cells dry weight at 72 h and 96 h of fermentation increased slightly over the 48 h fermentation period, which was not a very noticeable change. This showed that the cell enters the stationary phase at 72 h and 96 h. This is the stage when microorganisms reach their maximum state of growth and the ratio of living to dead microorganisms is relatively balanced due to the small amount of nutrition. The cell has gone through a reducing growth phase or death phase if the dry weight of the cell decreases after the 120 h of fermentation (Ahmad et al., 2019). According to other study the strain *S. cerevisiae* BLR and CFB strains had a 60 h lag phase during cell growth. The lag phase in BLR did not decrease when it reached the value of 16 h, while in CFB it did so in 23 h. The cells in both situations took 44–48 h to reach the stationary phase (Mavrommati et al., 2024).

Parameters of fermented ethanol

The highest ethanol concentration of apple juice concentrate, fermented by the yeast *S. cerevisiae* at the 10th day of fermentation period and different TSS conditions was 10.06 g/L at 20 °Brix, 11.01 g/L at 25 °Brix and 10.94 g/L at 30 °Brix. On the other hand, the highest ethanol concentration for the same media fermented by *S. bayanus* yeast was equal to 10.25 g/L at 20 °Brix, 12.94 g/L at 25 °Brix, and 11.18 g/L at 30 °Brix. The results showed that using the *S. bayanus* strain instead of the *S. cerevisiae*, enhanced the yield of ethanol production by about 2-4%.

According to another record, the amount of ethanol produced during the orange juice fermentation process by *S. cerevisiae* UFLA CA1774 was 5.831% (Santos et al., 2013), which is less than the results we found and displayed in the graph form figure 5 (a, b). Zhang and their colleague acquired final ethanol concentration near to 6.5%, which is also below to our results (Zhang et al., 2023). The evaluation between concentrations of produced ethanol from both the strain *S. cerevisiae* and *S. bayanus* at different TSS also showed in the graph figure 6 (a-c).

According to the present results, ethanol production at 20 °Brix by *S. cerevisiae* was higher from the *S. bayanus,* rather than, the concentration of ethanol was higher at 25 °Brix and 30 °Brix by *S. bayanus* than the value we obtained from the strain *S. cerevisiae*. Therefore, compared to strain *S. cerevisiae*, strain *S. bayanus* is able to produce a higher concentration of ethanol. Our results for the apple juice were higher, reported on the fermentation of existing juices and showed that final ethanol concentrations varying from 34.6 g/L to 44.7 g/L depending on the yeast strain used (Kelanne et al., 2020). Another results showed that the strawberry juice contained 41.87 g/l of total sugar. At 6 g/l (strawberry juice per hour), 35 g/l (raspberry juice), and 7 g/l (per liter of strawberry juice), respectively, the final ethanol percentage, ethanol yield, and maximum productivity were recorded (Robati et al., 2023).

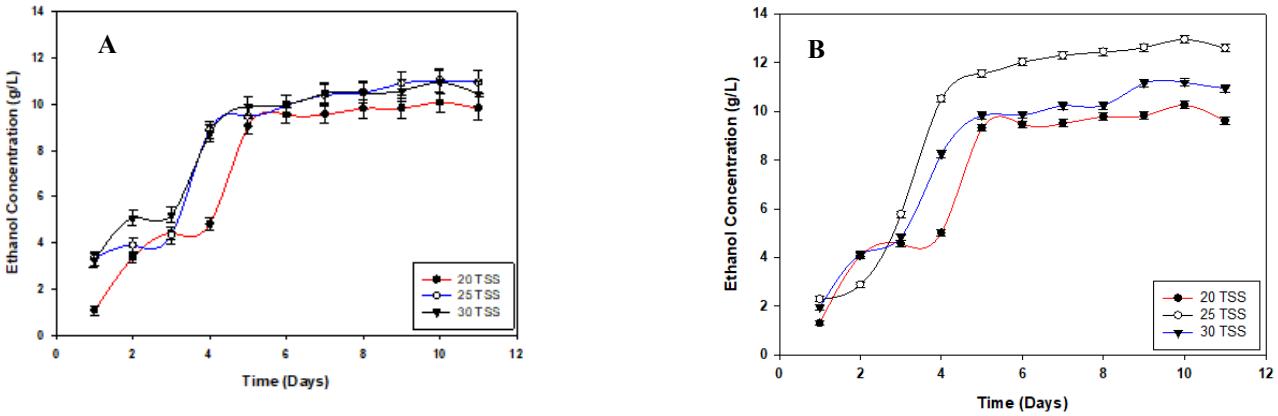

Fig 5. Ethanol production at different total suspended solids (TSS) concentrations by (a) *Saccharomyces cerevisiae* and (b) *Saccharomyces bayanus*.

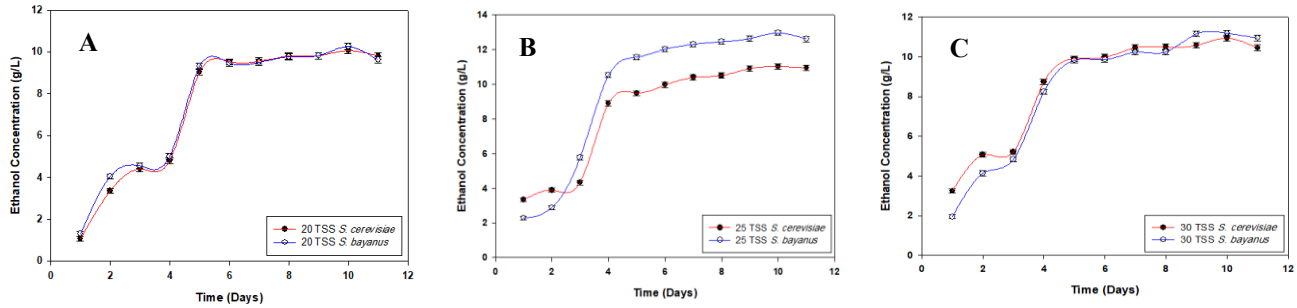

Fig 6. Evaluation of ethanol production by *Saccharomyces cerevisiae* and *Saccharomyces bayanus* at different °Brix levels: (a) 20 °Brix, (b) 25 °Brix, and (c) 30 °Brix.

In the case of formation of other by-products such as, acetaldehyde, methanol and glycerol, it was observed that as compared to ethanol production, the amount of acetaldehyde that forms at 20 °Brix is extremely low, slightly higher at 25 °Brix and 30 °Brix, but still much less than that of ethanol produced by *S. bayanus*. The stability of the product, toxicology, and aroma balance are all impacted by acetaldehyde production (Dzialo et al., 2017).

Low concentrations of acetaldehyde are known as a typical aromatic compound and help to create a pleasant, fruity aroma (Zea et al., 2015). With the strain *S. cerevisiae*, acetaldehyde formation is somewhat higher at 20 °Brix and then decreases at 25 °Brix and 30 °Brix with increasing TSS, but it is still far less than that of ethanol. The concentration of methanol during ethanol production by *S. cerevisiae* was increased with TSS level as compared to the strain *S. bayanus* but also less than the concentration of ethanol. The crucial role that, glycerol play as a compatible solute in hyperosmotic stress is another factor. The yeast strain produces more glycerol and stores it inside its cells in response to rise in external osmolarity, which balances the osmotic pressure (Singh et. al., 2020). Similar to the acetaldehyde, more glycerol is formed at 20 °Brix with *S. cerevisiae* than at 25 °Brix and 30 °Brix. With the *S. bayanus* strain, the glycerol percentage increased with increasing TSS level, but it was less than the ethanol percentage from both strains. The findings showed that other by-products were produced at a higher rate during the ethanol production process when strain *S. cerevisiae* was present compared to strain *S. bayanus*; however, the other compounds formed less than ethanol with both strains of yeast.

Effect of HMF on yeast cell growth and ethanol concentration

A colony counting unit was used to assess how HMF affected the growth of yeast cells. Yeast cultures were treated with varying concentrations of HMF (10 mg/L to 60 mg/L). As the HMF concentration increased, the number of colonies decreased for both strains, but *S. bayanus* was capable of resisting HMF concentrations than *S. cerevisiae*, as shown in figure 7(a). The HMF

concentrations used in the experiments ranged from 10 mg/L to 60 mg/L. At the conclusion, the amount of ethanol in the fermentation broth was measured. The production of ethanol declined as the concentration of HMF increased, as indicated by figure (7 b). With *S. cerevisiae*, an average ethanol production of 10 g/L was obtained at an HMF concentration of 50 g/L. Conversely, a mean ethanol concentration of 12 g/L with *S. bayanus* was obtained at the highest HMF concentration (60 mg/L).

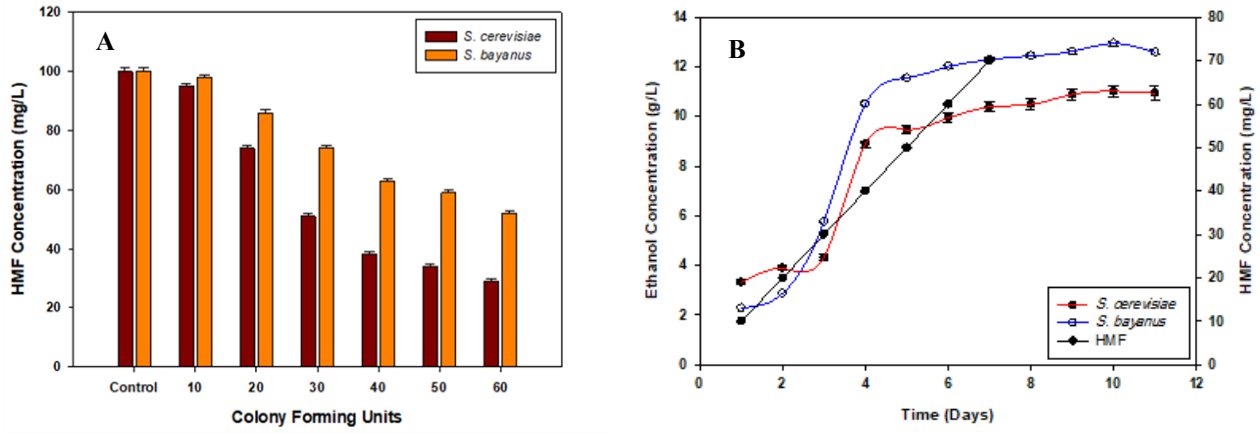

Fig 7. Effect of hydroxymethylfurfural (HMF) on (a) yeast cell growth and (b) ethanol production.

HMF is an inhibitor of furan derivatives that affects the yield of ethanol by blocking the activity of enzymes like glycolysis, alcohol dehydrogenase, and aldehyde dehydrogenase. HMF is the most notable effect on organisms is its interference with microbial growth, which is why it's regarded as the most powerful inhibitor of bioethanol production (Gencturk & Ulgen 2022). Previous research indicates that the addition of 0.3 g/L furfural did not affect yeast cells that grew at comparable specific growth rates (0.4/h), biomass concentrations (≈4 g/L), and biomass yields (0.080 g/g). Growing the cells in the presence of 4 and 4.5 g/L of furfural caused the specific growth rate to gradually decline above 0.3 g/L, reaching the lowest level of 53 % of the control specific rate. As there was a maximum reduction of 25 % for the assays with more than 4 g/L of furfural, the presence of furfural had less of an impact on the yeast biomass concentration and yield than it did on the specific growth rate (Lopes et al., 2017). Furan aldehydes were found to significantly reduce the intracellular concentrations of redox co-factors and the catabolic and anabolic reduction charges of *S. cerevisiae* strain VTT C-10883 when compared to cultivations without inhibitors. This was observed in a different study using furfural and HMF. When furfural and HMF were present and inhibitors were added, the intracellular ATP concentration decreased, but this only slightly affected the energy charge, which decreased from 0.87(±0.002) to 0.85(±0.004) relative to the control (Ask et al., 2013). The 5-hydroxymethylfurfural (HMF) concentration tolerance of immobilized *S. cerevisiae* during ethanol production in glucose-based media was also investigated, and ethanol formation, biomass growth, and substrate consumption were modeled. Findings indicated that in 6 g/L of HMF production, the maximum ethanol yield was computed to be 43.867 % (32.07 g/L). Furthermore, the system operating with 10 g/L of HMF produced the lowest yield and production values, which came out to be 32.14 % and 3.993 g/L. The production of ethanol and the consumption of sugar were less impacted by HMF than the growth of biomass (Erkan et al., 2022).

Conclusion

This study revealed that compared to *S. cerevisiae* and *S. bayanus,* the strain *S. bayanus* exhibited enhanced fermentation capabilities. The higher alcohol content was the outcome of a faster reduction in sugars. The use of the *S. bayanus* strain rather than the *S. cerevisiae* strain resulted in an increase in the ethanol concentration up to 16 %, which is approximately 4% higher than *S. cerevisiae*. The yeast *S. cerevisiae* produced 13.96 % of ethanol on fermentation of concentrated apple juice. The concentration of acetaldehyde, methanol and glycerol was lower than ethanol with both the strain. Temperature tolerance ability of both strains was consistent and increased with temperature 25 °C and then decreased with 35 °C to 45 °C. From an industrial perspective, the use of apple juice concentrate in the process of turning sugar into ethanol is beneficial, and the findings offer insightful

information about the potential uses of *S. cerevisiae* and *S. bayanus* in the future for the synthesis of acetic acid with high ethanol productivities.

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Conflicts of Interest
We all declare no conflict of interest among all the authors and co-authors.

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