



Development and quality evaluation of pineapple (*Ananas comosus* L.) wine

S R Assumi^{1*}, Vanlalruati¹, H D Talang¹, M Bilashini Devi¹, S Hajong², H Kalita¹

¹ ICAR RC for NEH Region, Umiam, Meghalaya, India

² ICAR-NBPGR Regional Station, Shillong, Umiam, Meghalaya, India

Corresponding Author: S R Assumi

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Abstract

Pineapple (*Ananas comosus* L.) is a highly perishable tropical fruit that experiences considerable postharvest losses due to its high moisture content and rapid physiological deterioration. Fermentation into wine provides an effective value-addition strategy for extending shelf life and enhancing economic returns. The present study was conducted to evaluate the performance of three *Saccharomyces cerevisiae* strains, namely *S. cerevisiae* var. *ellipsoideus* ITCC 1030 (Y1), *S. cerevisiae* var. *ellipsoideus* UCD 595 (Y2) and *S. cerevisiae* strain Y11857 (Y3), for pineapple wine production. Pineapple juice was adjusted to 24°Brix, inoculated with 5% (v/v) yeast culture and fermented at 30±2°C for 72 h. Physicochemical changes, including total soluble solids (TSS), titratable acidity, pH, total carbohydrates, reducing sugars and alcohol content, were evaluated at 24h intervals. Fermentation resulted in a significant decline in TSS, carbohydrates and reducing sugars, accompanied by increased titratable acidity and alcohol content, while pH decreased progressively. Among the tested strains, Y2 exhibited the highest fermentative efficiency, reducing TSS from 22.59 to 8.31°Brix, total carbohydrates to 2.14%, and reducing sugars to 1.24% after 72 h. The same strain produced the highest alcohol content (7.81%) with a titratable acidity of 0.62% and pH of 3.40. Fermentation was essentially completed within 72h, as no appreciable increase in alcohol was observed thereafter. The results demonstrate that *S. cerevisiae* var. *ellipsoideus* UCD 595 is the most suitable yeast strain among those evaluated for efficient pineapple wine production, yielding superior fermentation performance and desirable physicochemical characteristics, thereby offering considerable potential for commercial-scale production and value addition of pineapple.

Keywords: Pineapple, yeast, fermentation, wine

Introduction

Pineapple (*Ananas comosus* L.) is one of the most economically important tropical fruits cultivated worldwide because of its pleasant flavour, aroma, nutritional value and consumer acceptability. However, its high moisture content (80-86%) and active postharvest metabolism make it highly susceptible to physiological deterioration and microbial spoilage immediately after harvest, resulting in considerable postharvest losses during transportation and storage. These losses reduce marketability and economic returns, particularly in tropical and subtropical regions where cold chain infrastructure is limited. Consequently, processing pineapple into value added products has become an effective strategy for reducing postharvest losses, extending shelf life and increasing profitability. Among the various processing alternatives, wine production represents an attractive option because it transforms surplus or lower grade fruits into a stable, high value fermented beverage while retaining desirable sensory and nutritional characteristics (Boondaeng *et al.*, 2022^[5]; He *et al.*, 2024).

Fruit wines have received increasing scientific and commercial attention owing to growing consumer interest in diversified alcoholic beverages with unique flavour profiles, natural antioxidants and functional properties. Compared with conventional grape wines, tropical fruit wines possess distinctive volatile compounds and bioactive constituents that contribute to their sensory appeal and nutritional value (Yuan *et al.*, 2024) ^[32]. Pineapple juice is particularly suitable for wine production because it contains adequate concentrations of fermentable sugars, balanced acidity, vitamins, minerals and nitrogenous compounds required for

yeast growth and alcoholic fermentation. Standard production of pineapple wine generally includes juice extraction, adjustment of soluble solids and acidity, inoculation with selected yeast cultures, controlled fermentation, clarification, maturation and bottling, with each stage influencing the physicochemical and sensory quality of the final product (Lin *et al.*, 2021) ^[14].

Among fermentative microorganisms, *Saccharomyces cerevisiae* remains the preferred yeast for fruit wine production because of its high fermentation efficiency, ethanol tolerance, osmotolerance and ability to produce desirable volatile aroma compounds, esters and higher alcohols that define wine aroma and flavour. Recent advances in wine microbiology have also demonstrated that yeast selection plays a crucial role in determining fermentation kinetics, alcohol production, volatile metabolite composition, antioxidant capacity and sensory acceptability of fruit wines (Fazio *et al.*, 2023^[10]; Almeida dos Anjos *et al.*, 2024) ^[1]. Furthermore, fermentation performance is influenced by several interacting factors, including yeast strain, inoculum concentration, fermentation duration, sugar concentration, temperature, pH, dissolved oxygen and nutrient availability. Optimization of these parameters is therefore essential for ensuring complete fermentation, maximizing alcohol yield, minimizing microbial contamination and producing wines of consistent quality (Lin *et al.*, 2021; He *et al.*, 2024) ^[14, 30].

Following fermentation, ageing and storage are critical stages that continue to shape wine quality through complex biochemical and physicochemical reactions. During storage, changes occur in phenolic compounds, organic acids,

volatile aroma compounds and colour pigments, directly affecting the sensory characteristics and shelf stability of wine. Storage conditions, particularly temperature, oxygen exposure, light intensity, packaging material and storage duration, significantly influence these reactions. Appropriate ageing under controlled conditions promotes flavour integration, aroma development, and overall wine complexity, whereas unsuitable storage accelerates oxidation, colour deterioration, aroma loss and quality degradation (Jackson, 2020; Pretorius, 2020^[12, 18]; Waterhouse *et al.*, 2022).

Therefore, the present study was undertaken to investigate the feasibility of fermenting pineapple juice considering key fermentation parameters, particularly yeast strains and fermentation durations to develop pineapple wine, and to evaluate the physicochemical quality and sensory changes occurring during storage.

Materials and Methods

The present investigation was conducted in Postharvest laboratory, ICAR Research Complex for NEH Region, Umiam during 2022-23. Ripened pineapple fruits were washed, peeled, homogenized and strained through a sieve to extract the juice. Total soluble solids of pineapple juice was raised to 24°Brix with cane sugar, heat processed and stored at 2°C till further use. Three yeast cultures selected for this study viz. *S. cerevisiae* var. *ellipsoideus* ITCC 1030 (Y1), *S. cerevisiae* var. *ellipsoideus* UCD 595 (Y2) and *S. cerevisiae* strain Y 11857 (Y3) were sub-cultured and grown in MGYB agar slants (10ml). MGYB broth medium of Y1, Y2 and Y3 were prepared from these slants, these inoculated broth mediums were placed on a rotary shaker (100rpm, 30°C) for 48h. These broth were added (10% v/v) to pure pineapple juice (24°Brix) to prepare a starter culture (must) and incubated at 30±2°C for 48h. The inoculum of the must used was 10⁶ cfu ml⁻¹. In 250ml capacity erlenmeyer flasks, the respective juices (142.5ml) were inoculated with 5% (7.5ml) of activated broth yeast cultures of Y1, Y2, Y3 and the stoppered flasks were replaced with rubber seal and improvised S-shaped fermentation-lock. After 24h fermentation at room temperature (30±2°C), S-shaped fermentation-locks were blocked with water for release of carbon dioxide for allowing uniform growth of yeast. Samples were analyzed at intervals of 0, 24, 48 and 72h of fermentation.

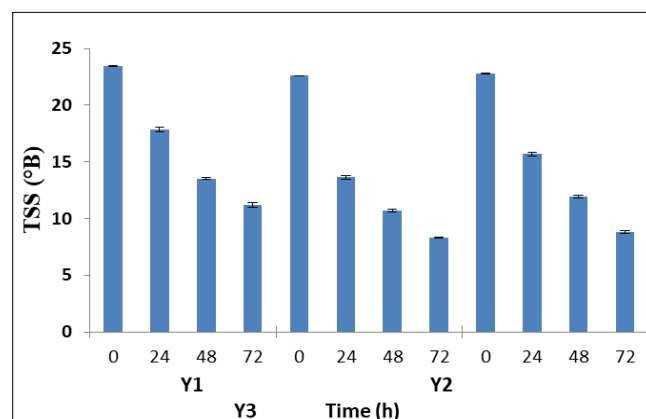
The samples were withdrawn by decanting them from the flask and used for quality analysis of TSS, titratable acidity, pH, total carbohydrates, reducing sugars and alcohol content. Total soluble solids (TSS) were measured using digital refractometer and expressed as degree brix (°B) at 20°C, pH of samples was determined directly by using a digital pH meter (µ pH system 361, Systronics) and titratable acidity was determined by titration of known weight of sample with 0.1N NaOH solution using a few drop of phenolphthalein as indicator (AOAC, 2000). Total carbohydrates was measured by Dubois's sulphuric acid method (Dubois *et al.*, 1956)^[9] which is a rapid and reproducible procedure for the determination of simple sugars and their derivatives which utilizes phenol as the specific organic colour developing agent, absorbance was read at 490nm and expressed as percentage (w/v). Reducing sugars was estimated by DNS (Dinitrosalicylic acid) method (Miller, 1959)^[15] wherein prepared samples were placed in hot water bath (80°C) for 20 minutes, cooled,

diluted with distilled water, read at 540nm absorbance against reagent blank and expressed as percentage (w/v). Alcohol content was determined by spectrophotometric method (Caputi *et al.*, 1968)^[7] and concentration of ethanol expressed as percentage (w/v), here the distillate samples was diluted with distilled water and dichromate reagent, heated at 60°C for 20 minutes in hot water bath. Mean comparison among cultures and treatments were performed using the Tukey's Honest Significant Difference (HSD) test, a difference was considered statistically significant when *p*-value was less than 0.05 (*p*<0.05). All analysis was performed with SAS 9.3 (TS1MO) software package developed by SAS Institute (2000).

Results and Discussions

Total soluble solids (TSS)

Changes in TSS during fermentation of pineapple juice inoculated with three yeast strains (Y1, Y2 and Y3) are presented in Figure 1. After 24h of fermentation onwards, there was a fast reduction in TSS and the differences in their reduction were a manifestation of different yeast strains used wherein the order of fermentability in decreasing manner was Y2>Y3>Y1. Y2 strain showed highest fermentability with respect to maximum reduction in TSS (22.59 to 8.31°B) and Y1 demonstrated the lowest (23.43 to 11.20°B). The initial higher decrease in TSS during fermentation is attributed to the higher fermentability of musts because of more availability of sugar and less alcohol in the medium. In general, reduction in TSS was a function of time and was evidently due to the fermentation of sugar by the yeast, this is typical fermentation behaviour of any alcoholic fermentation of fruit juice into wine. The decrease in TSS content of pineapple wine indicates the utilization of the sugar present in the must during fermentation where soluble solids decline rapidly during the first 48-72h before reaching a stable phase as sugar becomes limiting and ethanol accumulation inhibits yeast metabolism (Almeida *et al.*, 2024)^[1]. Similar reduction in TSS of banana, jamun and guava must have been reported by Brathwaite and Badrie (2001), Sonar *et al.* (2004) and Sevda and Rodriguez (2011). Pawar *et al.* (2011)^[6, 17, 19, 24] reported that TSS content of must decreased at faster rate till middle of fermentation and later on the rate of fermentation declined but continued at a much slower rate till the end of fermentation.



Y1- *Saccharomyces cerevisiae* var. *ellipsoideus* 1030; Y2- *S. cerevisiae* var. *ellipsoideus* 595; Y3- *S. cerevisiae* 11857

Fig 1: Effect of yeast strains and fermentation time on total soluble solids (°Brix) of pineapple wine.

Titrateable acidity (TA) & pH

The changes in TA during fermentation show (Table 1) that there was a general increase in TA of the wines of different yeast strains and after 24h of fermentation, TA increased significantly irrespective of the yeast strains used. The highest TA (0.62%) was observed in case of yeast strain Y2 after 72h of fermentation followed by Y3 (0.59%). Acidity is an important factor, since it contributes both directly and indirectly to the quality of wines (Clarke and Bakker, 2004)^[8] and TA of wines increased as fermentation progressed due to the presence of organic acids formed as a by-product. The increment of TA during fermentation is attributed to the production of different organic acids as observed in kiwi wine (Towantakavanit *et al.*, 2011)^[26] and fermenting

medium of sugar syrup from cassava flour (Ocloo and Ayernor, 2008)^[16]. This rise in acidity corresponds to the fall in reducing sugars content and increase in alcohol concentration, and the increase in acidity may be due to the increased alcohol production from the high initial sugar concentration (Attri, 2009)^[4]. The increase in TA observed during fermentation is consistent with recent reports demonstrating the formation of succinic, acetic and other organic acids as secondary metabolites of yeast metabolism and these acids contribute to microbial stability, freshness and overall sensory quality of fruit wines (Fazio *et al.*, 2023^[10]; Waterhouse *et al.*, 2022). Acid inhibits the development of spoilage bacteria and encourages the growth and activity of wines.

Table 1: Effect of yeast strains and fermentation time on titrateable acidity (%) of pineapple wine.

Time (Hours)	0	24	48	72
Yeast strains				
Y1	0.38 ± 0.01 ^{aC}	0.48 ± 0.01 ^{aB}	0.55 ± 0.01 ^{aBA}	0.58 ± 0.02 ^{bA}
Y2	0.39 ± 0.01 ^{aD}	0.49 ± 0.01 ^{aC}	0.57 ± 0.01 ^{aB}	0.62 ± 0.01 ^{aA}
Y3	0.38 ± 0.01 ^{aD}	0.51 ± 0.02 ^{aC}	0.55 ± 0.01 ^{aB}	0.59 ± 0.01 ^{bA}

According to HSD Tukey's test, values with different lowercase superscripts in the same column are significantly different between the yeast strains ($p < 0.05$); values with different uppercase superscripts in the same row are significantly different between fermentation time ($p < 0.05$); each value is the mean ± SD ($n = 3$).

The increase in TA was concomitant with a decrease in pH ranging from 3.83 to 3.39 (Table 2) and with the progress in fermentation time pH decreased. The decrease in pH with increase in acidity of wine observed may be due to dissociation of parental acids and formation of hydrogen ions. The decrease in pH with increase in acidity was also reported by in grape and sapota wines (Pawar *et al.*, 2011)

^[17] fermentations. Soni *et al.* (2009)^[25] reported that the pH of amla wine revealed a gradual fall with the progress in fermentation, showing a final value of 3.4 at the end of fermentation (6d). The pH of the wine depends on composition of the must, amount of organic acids and sugars present in the wine. The gradual decline in pH during alcoholic fermentation is widely recognized as a consequence of increasing organic acid concentration and yeast metabolism. Similar observations have been reported for pineapple, mango and mixed tropical fruit wines, where decreasing pH contributes to microbiological stability and improves storage quality (He *et al.*, 2024; Shen *et al.*, 2024)^[20].

Table 2: Effect of yeast strains and fermentation time on pH of pineapple wine.

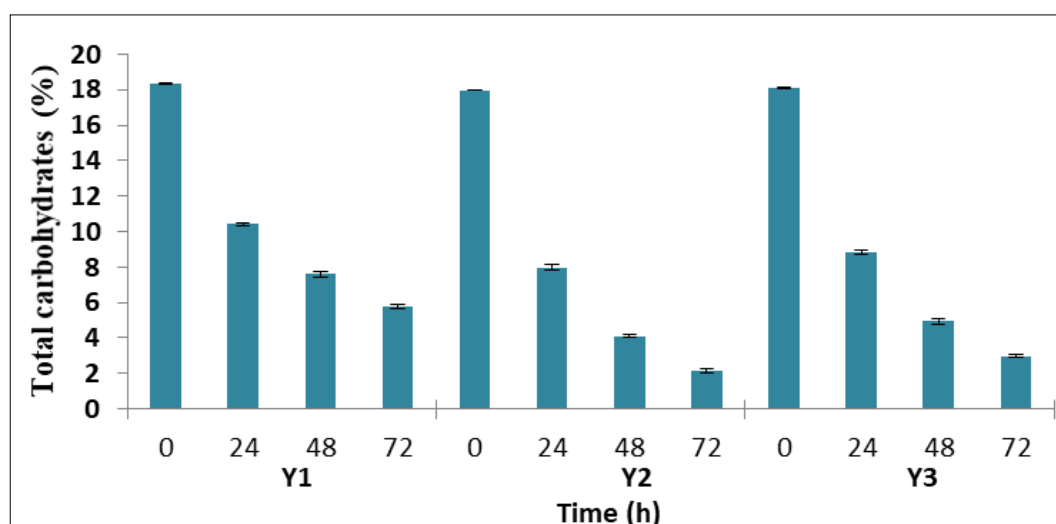
Time (Hours)	0	24	48	72
Yeast strains				
Y1	3.83 ± 0.01 ^{aA}	3.61 ± 0.02 ^{aB}	3.50 ± 0.02 ^{aC}	3.39 ± 0.02 ^{aC}
Y2	3.81 ± 0.04 ^{aA}	3.56 ± 0.10 ^{aB}	3.43 ± 0.09 ^{aB}	3.40 ± 0.06 ^{aB}
Y3	3.82 ± 0.03 ^{aA}	3.58 ± 0.02 ^{aB}	3.44 ± 0.04 ^{aCB}	3.41 ± 0.03 ^{aC}

According to HSD Tukey's test, values with different lowercase superscripts in the same column are significantly different between the yeast strains ($p < 0.05$); values with different uppercase superscripts in the same row are significantly different between fermentation time ($p < 0.05$); each value is the mean ± SD ($n = 3$).

Carbohydrates (CHO)

The results of the effect of three yeast strains on total CHO content during fermentation of pineapple juice are presented in Figure 2. Decrease in total CHO during fermentation could be due to the availability of nutrients, favourable acidity, pH and ability of the yeast to consume sugars which is converted in to alcohol. At 24h of fermentation, there was a significant ($p < 0.05$) difference in the utilization of CHO which is a reflection of difference in the fermentability of yeast strains, subsequently, after 24h there was a clear declining trend. At 72h, minimum amount of CHO was recorded in Y2 (1.82%),

Y3 (2.35%) and Y1 (4.97%) and wherein Y2 exhibited highest fermentability as reflected by the lowest CHO at the end of fermentation period. CHO utilization at the end of fermentation ranged from 65.72 to 89%. Similar observations were reported in mahua wine (Singh *et al.*, 2013)^[22] and in litchi wine production (Singh and Kaur, 2009)^[21] where total sugar decreased from 85.25 to 3.5% (w/v) after five days fermentation. Singh *et al.* (2009)^[21] reported utilization of the total carbohydrates in 24h after the start of fermentation and this decrease was significant with increase in alcohol content. The progressive reduction in total carbohydrates reflects efficient utilization of fermentable substrates by yeast for biomass formation and ethanol production. Comparable reductions have recently been reported during fermentation of tropical fruit wines prepared using *Saccharomyces cerevisiae*, with carbohydrate depletion directly associated with increasing alcohol concentration (Yuan *et al.*, 2024; Tan *et al.*, 2024)^[27, 32].



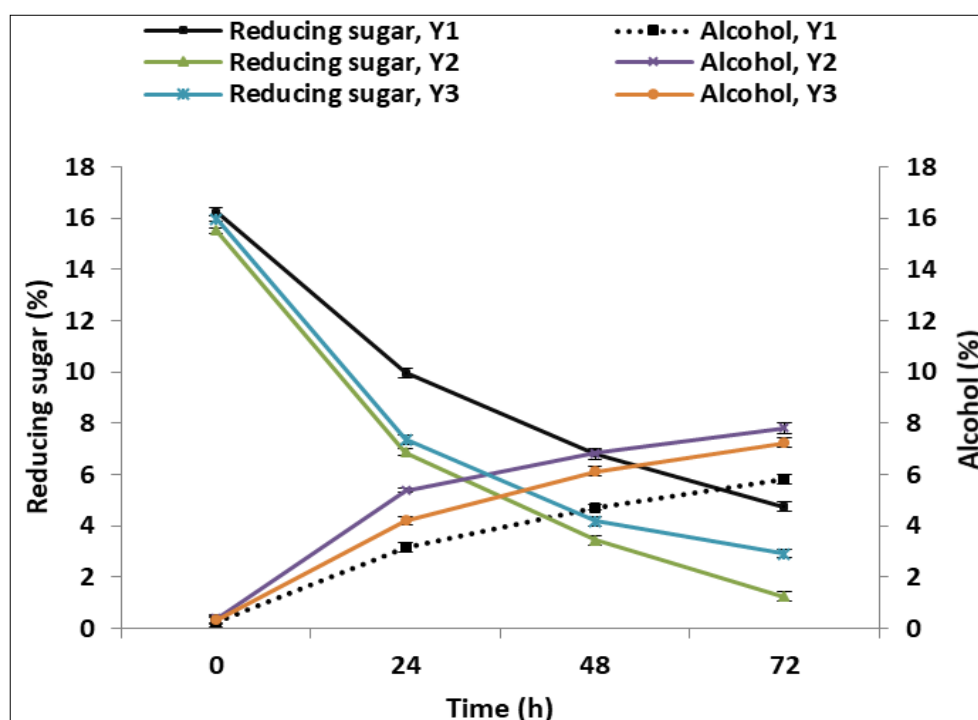
Y1- *Saccharomyces cerevisiae* var. *ellipsoideus* 1030; Y2- *S. cerevisiae* var. *ellipsoideus* 595; Y3- *S. cerevisiae* 11857

Fig 2: Effect of yeast strains and fermentation time on total carbohydrates (%) of pineapple wine.

Reducing sugars (RS)

RS is most important sugar for fermentation as it is easy to metabolize by yeast. There was a significant difference ($p < 0.05$) with respect to utilization of sugars among the yeast strains and between time period during fermentation although the dynamics of utilization, reflected in a sharp declining trend, was similar irrespective of the yeast strains (Fig. 3). The initial RS content in different strains ranged from 15.51 to 16.24%, arising due to variation in RS content coming from musts of different strains used for inoculation and at 24h, a steep decrease in RS content was observed which ranged from 6.87 to 9.96%. This decline was attributed to utilization by the yeast for the production of alcohol. Subsequently, after 48 and 72h a clear declining trend was observed, maximum amount of residual reducing sugar was recorded in wine prepared from Y1 (4.74%). With

respect to fermentability of the yeast strains, strain Y2 registered the lowest RS value depicting as the best strain with respect to sugar utilization and subsequent alcohol production during the fermentation process. In all the cases, after the third day of fermentation process almost all of residual sugars were fermented. Similar fermentation behaviour has been documented in recent studies on pineapple and other tropical fruit wines where glucose and fructose are preferentially metabolized during the exponential growth phase of *Saccharomyces cerevisiae* (Lin *et al.*, 2021; Wang *et al.*, 2025). Kawa-Rygielska *et al.* (2013) [13, 14, 29] who worked on sugar beet for ethanol production reported increase in glucose and fructose content immediately after 5h of fermentation. Rapid depletion of reducing sugars during the early stages of fermentation indicates active glycolysis and efficient sugar uptake by yeast cells.



Y1- *Saccharomyces cerevisiae* var. *ellipsoideus* 1030; Y2- *S. cerevisiae* var. *ellipsoideus* 595; Y3- *S. cerevisiae* 11857

Fig 3: Effect of yeast strains and fermentation time on utilization of reducing sugars (%) and production of alcohol (%) in pineapple wine.

Alcohol content

With increase in fermentation time, concentration of alcohol increased, irrespective of the yeast strains and this trend continued up to 72h. At the end of fermentation, alcohol production with respect to the yeast strains was in the order Y2>Y3>Y1 (Fig. 3), Y2 fermented the substrate faster and gave a considerable amount of alcohol (7.81%) with 5% (v/v) inoculum rate followed by Y3 (7.23%) and Y1 (5.80%). The result is in conformity with their respective mean fall of TSS, total carbohydrates and utilization of reducing sugars (Figs. 1, 2 and 3). It was also found that concentration of alcohol between 72 and 96h (data not shown) of fermentation in all the yeast strains did not differ, which suggests that fermentation up to 72h was optimum. This difference among the yeast strains with respect to the alcohol yield might be due to the variation in performance of the yeast to utilize the fermentable sugars affecting the fermentability, hence the varied alcohol production (Amerine *et al.*, 1980). Yadav *et al.* (2012)^[2, 31] observed that mahua wine fermented at 16°C had higher content of alcohol (9.9%) after 15 days of fermentation. The continuous increase in ethanol concentration during fermentation corresponded closely with reductions in TSS, CHO and RS, confirming efficient conversion of fermentable sugars into ethanol. The superior alcohol yield obtained with strain Y2 indicates greater fermentative efficiency and ethanol tolerance than the other strains. Similar findings have recently been reported for pineapple and other tropical fruit wines, where yeast strain selection significantly influenced ethanol yield, fermentation kinetics, volatile aroma formation and overall sensory quality (Almeida *et al.*, 2024; Wang *et al.*, 2024)^[1, 28].

Conclusion

Amongst the three yeast strains screened for alcoholic fermentation of pineapple wine, Y2 i.e., *S. cerevisiae* var. *ellipsoideus* UCD 595 showed the higher rate of sugar consumption accompanied with maximum production of alcohol (8.65%). The process showed a maximum utilization of reducing sugars (97%) and total carbohydrates (89%) with 5% v/v inoculum level after 72h of fermentation. This application of different yeast led to exploration of alcohol production and reduction of fermentation time, and this best performing strain can therefore be selected for further scaling up studies.

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