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RESEARCH PAPER

Combination of banana fruit (*Musa paradisiaca* L.) in the production of pineapple wine in Vietnam

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Abstract

Fruit wine products derived from Vietnamese fruit sources are increasingly gaining popularity and preference among consumers in the market. However, the assessment of influencing factors and the optimization of fruit combinations in fruit wine production remain underexplored. The objective of this study was to survey the main factors affecting the quality of banana-pineapple wine after fermentation using commercial yeast strains. From there, appropriate technological parameters were determined to create banana-pineapple wine products of high quality in terms of color, taste, and nutritional components. In this study, anaerobic fermentation utilizing the *Saccharomyces cerevisiae* yeast strain was employed to produce the wine product. At the same time, the resulting product had an average alcohol content of about 12 - 14% v/v. The fermentation process of banana-pineapple juice has been adjusted to mixing ratios of 5 - 10% and 15% v/v to create environmental conditions for yeast growth. In addition, pH was adjusted from 3.5 - 5.5, and total soluble solid from 20 - 28°Bx. After the fermentation process, the results showed that the fermentation solution with an initial mixing ratio of 15% v/v with an initial pH of 4.5 and a total soluble solid of 24°Bx would create the best quality fermented banana-pineapple wine product. The product had an ethanol content of 13.74% v/v. In green bananas, tannin precipitation occurs when combined with fruit protein, forming a precipitate. In wine production, after a fermentation period, this precipitate settles, contributing to a more precise final product and enhancing its quality. Therefore, an appropriate blending ratio is needed to achieve high-quality wine without compromising the yeast fermentation ability.

1. Introduction

Pineapple is a common fruit-bearing plant that serves as a rich source of various plant nutrients for treating and preventing several diseases such as cancer, coronary heart disease, obesity, elevated blood calcium, type two diabetes, hypertension, cataracts, colitis, dysmenorrhea, and indigestion (Zuraini *et al.*, 2021). In the market, pineapple wine products have been investigated and developed, as evidenced by research conducted by Idise (2012) and Qi *et al.* (2017), which includes findings on pineapple wine production and quality. However, research on the domestic production of pineapple wine remains limited. The first thing to consider in wine production is the aroma, color, quality, and clarity of the wine solution. Clarity significantly influences consumers' perception of wine products. Particularly, fruit wines often suffer from cloudiness due to proteins in fruit pulp, impacting consumer perception and preferences (Toy *et al.*, 2022). Incorporating specific components into wine could alter its clarity by creating adsorption complexes, enzymes, or ions with suspended particles, transforming them into larger molecules that could precipitate and settle out of the wine more easily and rapidly (Claus and Mojsov, 2018). Green bananas are a familiar tropical plant species with high tannins.

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Tannins precipitate with fruit pulp proteins, forming sediments in green bananas (Galani, 2019). In wine production, these sediments settled after a fermentation period, enhancing the wine's clarity and contributing to its overall quality. Additionally, tannins contribute bitterness to the wine, enhancing its flavor profile and sensory characteristics (Soares *et al.*, 2020). The use of banana peels in wine production has also bolstered the wine's antioxidant activity due to the peel's rich antioxidant content (Bratovcic *et al.*, 2021). Utilizing both bananas and green banana peels in wine production could benefit the banana industry economically and reduce annual waste management costs. Lin *et al*. (2018) investigated the influence of four commercial strains of *Saccharomyces cerevisiae* (D254, VIC, BV818, and CECA) on volatile compounds in pineapple juice fermentation (*Ananas comosus* L. Merr.). The study demonstrated that the different yeast strains formed distinct aromas in pineapple wine (Lin *et al.,* 2018). Furthermore, Dellacassa *et al*. (2017) utilized gas chromatography to analyze the aromatic compounds produced during pineapple wine fermentation using *S. cerevisiae* strains. The authors identified 61 volatile components, with 18 glycosidically bound aroma compounds (Dellacassa *et al.,* 2017).

This research aimed to investigate the key factors influencing the fermentation process and identify optimal parameters to achieve high fermentation efficiency while minimizing the formation of undesirable by-products. This study opened up a new direction for applying the characteristics of the tropical fruits of Vietnam. Additionally, the fermentation process to produce alcoholic beverages has contributed to diversifying and enhancing the value of fruit utilization in Vietnam.

2. Materials and methods

2.1 Preparation of samples, chemicals and reagents

Pineapple and green bananas used in the study were Queen pineapple varieties harvested directly from trees in Tien Giang province, Vietnam. In addition, the *S. cerevisiae* yeast used was Angel RV002 wine yeast (500 g package) provided by Nha Phuoc Thinh Company in 5 District, Ho Chi Minh City (Vietnam).

The chemicals used for the research included citric acid (purity 99%), sodium carbonate (Na_2CO_3, purity 99%), sodium metabisulfite ($Na_2S_2O_5$, purity 99%), hydrochloric acid (HCl), sodium hydroxide (NaOH), pectinase enzyme (Novozymes company, Denmark) and 3,5-dinitrosalicylic acid (DNS) reagent (purity 99%). All chemicals were supplied by Xilong in Shantou, China.

2.2 Banana-pineapple wine production

The pineapple was peeled, cleaned, and pressed to get the juice, which was then diluted with water at an additional water ratio of 50%. After that, the pineapple juice was mixed with the banana juice according to the additional banana juice ratio of 5%, 10%, and 15% (v/v), and the pH of the juice was adjusted at 3.5, 4.0, 4.5, 5.0, and 5.5 with citric acid and Na_2CO_3, adjusted the Brix to 20, 22, 24, 26, and 28°Bx with sugar. Next, the mixture was added with pectinase enzyme content at a ratio of 0.1% and $Na_2S_2O_5$ at 0.05 g/L, left at room temperature for a day. Finally, *Saccharomyces cerevisiae* yeast was added at 1 g/L, and fermentation was carried out for 7 consecutive days.

2.3 Analysis methods

2.3.1 pH

The pH was determined using a WTW pH meter (pH3110-2AA112, Wissenschaftlich-Technische Werkstätten, Germany.

2.3.2 Total soluble solids content

The total soluble solids content was determined using an RHWN-25BrixATC refractometer (Atago Co., Ltd., Japan), with a measuring range of 0 - 32°Bx.

2.3.3 Reducing sugar content

The analysis of reducing sugar content was conducted according to the method described by Khatri and Chhetri (2020), using the DNS reagent solution.

2.3.4 Total sugar content

The analysis of total sugar contents was conducted similarly to the method described by Khatri and Chhetri (2020), using the DNS reagent solution.

2.3.5 Alcohol content

The volume of banana-pineapple wine sample (50 mL) was distilled. After distillation, the sample was allowed to cool to room temperature and adjusted to 50 mL. Finally, the alcohol content was measured using a DMA 35 Anton Paar device from Austria, with a density range of 0 to 3 g/cm³, temperature range of 0 to 40°C, and sample viscosity range of 0 to 1000 mPas.

2.3.6 Total acidity

The volume of banana-pineapple wine sample (10 mL) was titrated with 0.1 N NaOH and 1% phenolphthalein until a pink color persisted for 30 s.

2.3.7 Yeast density

The cell suspension was vigorously shaken, and a

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small drop was dispensed onto the surface of a counting chamber adjacent to the glass foil. The cell suspension entered the counting chamber via capillary action and moved to allow the suspension to fill the spaces (approximately 0.1 mm thick). Subsequently, the sample was observed under a microscope (B-352A Optika – Italy) with a 40× objective lens, and the number of cells within five representative large squares was counted.

2.4 Statistical analysis

Experimental data and images were processed using Microsoft Excel 2019 and the statistical analysis software JMP Pro 17 (SAS Institute Inc., USA).

3. Results and discussion

3.1 Effect of mixing ratio on the fermentation process

3.1.1 Acid content

The results of the investigation into the effect of mixing ratios on acid content during the fermentation process are depicted in Figure 1. The acid content decreased with the increased ratio of banana addition to the ingredient mixture. The results indicated that the acid content on the final day of fermentation was highest for the 5% mixing ratio, reaching 19.65 g/L, while for the 10% mixing ratio, the acid content was 13.23 g/L. Conversely, at the 15% mixing ratio, the acid content on the final day of fermentation was the lowest at 3.96 g/L. The decrease in acid content was due to the high acid content in pineapple, so the more pineapple in the mixture, the more acid content would increase (Adebanke Balogun *et al.*, 2017). The results of this study were consistent with the research report by Panyawoot *et al.* (2022).

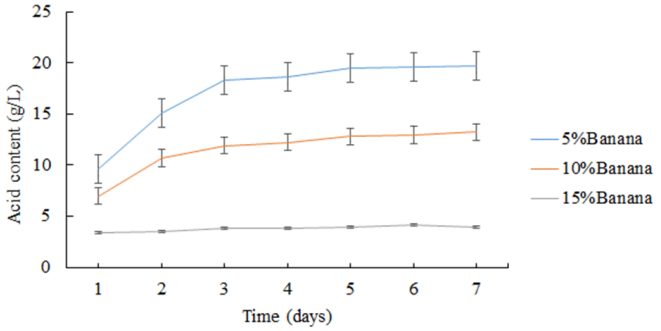

Figure 1. Effect of mixing ratio on acid content in banana-pineapple wine fermentation process.

3.1.2 Total soluble solid

The results of the investigation into the effect of mixing ratios on soluble solids content during the fermentation process are illustrated in Figure 2. The graph demonstrated that following the wine fermentation process, the soluble solids content gradually decreased on the final day of fermentation for the 5%, 10%, and 15% (v/v) mixing ratios, corresponding to soluble solids content values of 12.0°Bx, 10.5°Bx, and 7.0°Bx, respectively. Additionally, the highest soluble solids content was observed at the 5% mixing ratio, while the lowest was observed at the 15% mixing ratio.

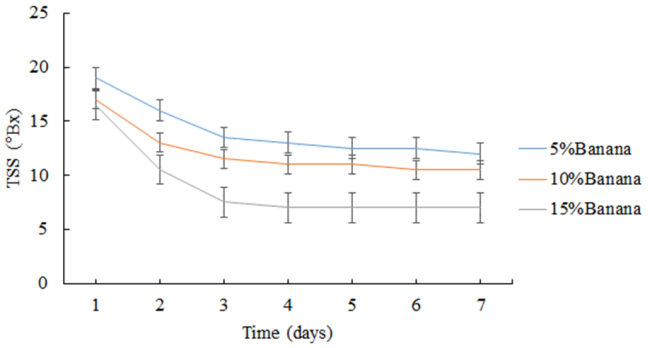

Figure 2. The effect of mixing ratio on total soluble solids in the fermentation process of banana-pineapple wine.

Based on the experimental results, the total soluble solids concentration decreased as the proportion of bananas increased. This concurred with the research conducted by Ravi *et al.* (2010), suggesting that the reduction in total soluble solids at different mixing ratios after the fermentation process might be attributed to changes in the fermentation broth's enzyme, chemical, biological, and physical properties.

3.1.3 Ethanol content

The results of the effect of mixing ratios on ethanol content during the fermentation process are presented in Figure 3. A lower proportion of banana supplementation corresponded to the mixture's higher pineapple juice content. The results of this study were consistent with the research report by Hien *et al.* (2022). At a 5% banana supplementation ratio, the ethanol content on the final day of fermentation was the lowest at 8.823%. For the 10% supplementation ratio, the ethanol content on the final day of fermentation was 11.007%. Lastly, with a 15% supplementation ratio, the ethanol content on the final day of fermentation was the highest at 13.560%. This suggested that lower pineapple content results in higher alcohol content of the product.

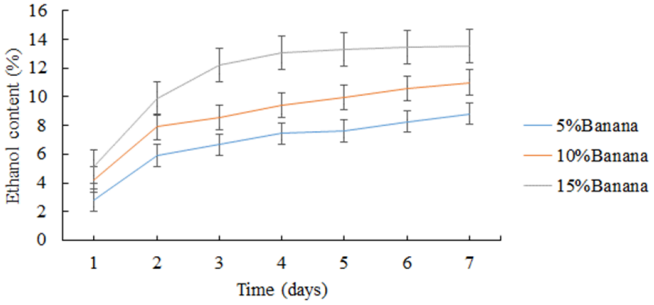

Figure 3. The effect of mixing ratio on ethanol content during the fermentation process of banana-pineapple wine.

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3.1.4 Reducing sugar content

The results of the investigation into the effect of mixing ratios on reducing sugar content are depicted in Figure 4. The graph showed a gradual decrease in reducing sugar content after fermentation. This reduction was attributed to yeast cells utilizing sugar for alcohol production (Joy Ajit *et al.*, 2018). Besides, the higher the banana addition ratio, the lower the sugar content. On the 7th day of the wine fermentation process, the highest reducing sugar content after fermentation was observed at 14.861 g/L for the 5% mixing ratio and 12.350 g/L for the 10% mixing ratio, while the lowest reducing sugar content after fermentation was recorded at 0.356 g/L for the 15% mixing ratio.

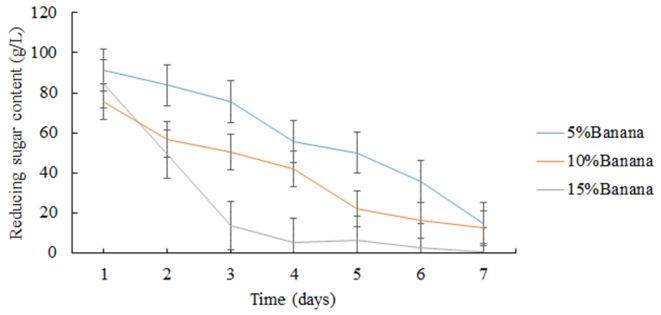

Figure 4. The effect of mixing ratio on reducing sugar content during the fermentation process of banana-pineapple wine.

The results indicated that higher pineapple content in the mixture led to higher sugar content. This was because pineapples contain 13 - 19% total solids, which include sucrose, glucose, and fructose as major components. Therefore, increasing pineapple supplementation resulted in a higher reducing sugar content (Adebanke Balogun *et al.*, 2017).

3.1.5 Total sugar content

Figure 5 presents the effect of the mixing ratio on the total sugar content during fermentation. The graph showed that total sugar content gradually decreased as a supplementary banana ratio increased. At a 5% supplementary banana ratio, the total sugar content peaked at 33.308 g/L, whereas at a 10% ratio, it decreased to 27.059 g/L. Finally, at a 15% ratio, the total sugar content dropped to its lowest point at 7.414 g/L.

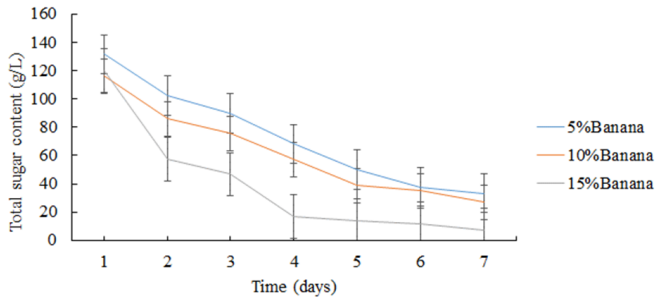

Figure 5. The effect of mixing ratio on total sugar content during the fermentation process of banana-pineapple wine.

The results of this study were consistent with the research report by Gao *et al.* (2016).

3.1.6 pH

The research findings on the effect of mixing ratio on pH during fermentation are presented in Figure 6. According to Figure 6, as the pineapple content increased, the pH of the sample decreased (Adebanke Balogun *et al.*, 2017). At a 5% ratio, the pH value after fermentation was 3.443, while for a 10% ratio, it was 3.647, and finally, for a 15% ratio, the pH value was 4.283. The variations in pH in wine could be attributed to microbial production of acids during fermentation. The highest pH value correlated with the microbial population metabolizing compounds in the environment. During fermentation, samples with higher pineapple supplementation contained higher acid levels, thus resulting in lower pH values (Joy Ajit *et al.*, 2018).

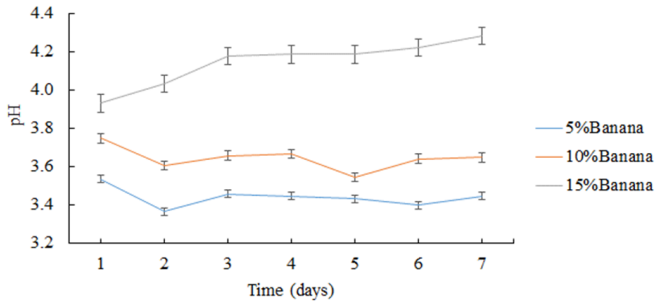

Figure 6. The effect of mixing ratio on pH during the fermentation process of banana-pineapple wine.

3.1.7 Yeast density

Based on the results in Figure 7, yeast cell density increased progressively from day 1 to day 4 of fermentation at a 15% dilution ratio (rising from 4.88×10^6 to 21.7×10^6), reaching its maximum biomass on the fourth day, which was also higher compared to the other ratios. The growth of yeast cells at a 5% dilution ratio was the lowest (increasing from 4.33×10^6 to 16.83×10^6). The results of this study were consistent with the research report by Ge *et al.* (2021).

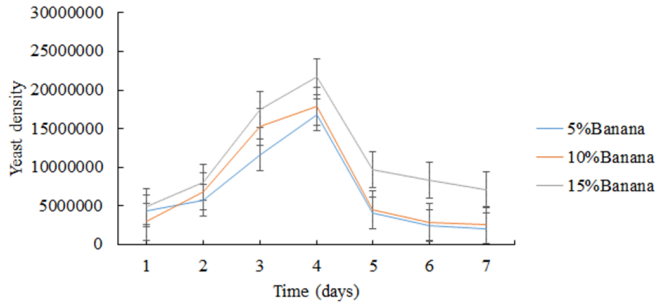

Figure 7. Depicts the effect of the mixing ratio on yeast cell density during fermentation.

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3.2 Effect of initial pH on the fermentation process

3.2.1 Acid content

The effect of initial pH on acid content during fermentation is presented in Figure 8. The results indicated a high initial pH sample would produce low acid content after fermentation. The highest acid content was achieved at 7.23 g/L with a pH of 3.5. The lowest acid content was 3.87 g/L and 3.75 g/L, respectively, corresponding to pH values of 5 and 5.5 (the difference is not statistically significant). Additionally, the acid content of samples with different initial pH values gradually increased over the 7-day fermentation period.

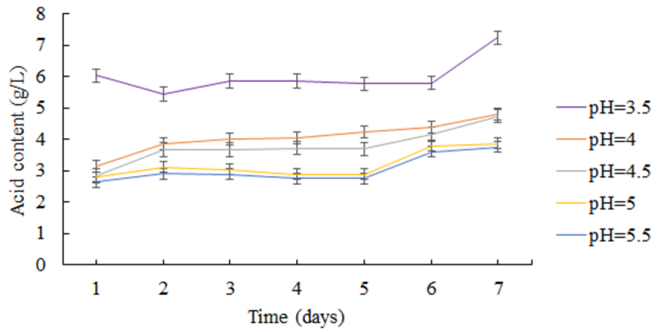

Figure 8. The effect of initial pH on acid content during the fermentation process of wine.

The increase in acid content was attributed to the conversion of sugar into ethanol and partly into various organic acids (such as acetic, succinic, and malic) during fermentation. Even after fermentation, the acid formation process continued for some time due to the presence of enzymes in the environment (Mendes Ferreira and Mendes-Faia, 2020). Furthermore, low pH promoted the formation of yeast-derived acids. A low initial pH extends the lag phase of yeast, affecting biomass accumulation, altering the rate of total sugar consumption, and increasing the final content of acetic acid and glycerol (Dennis-Eboh *et al.*, 2023). On the contrary, excessively high pH levels may hinder fermentation and lead to low acid content (Owusu *et al.*, 2012).

3.2.2 Total soluble solid

Figure 9 presents the effect of initial pH on total soluble solids after wine fermentation. After 2 days of fermentation, the concentration of total soluble solids decreased rapidly. At pH 3.5, the total soluble solids decreased from 18°Bx to 12°Bx. At pH 4, pH 5, and pH 5.5, they decreased from 16.5°Bx to 10.5°Bx; at pH 4.5, they decreased from 16°Bx to 11.5°Bx. On the final fermentation day, the total soluble solids' concentration decreased to its lowest value of 7°Bx, with the sample having a pH of 4.5. Additionally, the total soluble solids concentration at pH 3.5 and 4 was 8°Bx (not statistically significant), and at pH 5 and 5.5, it was 7.5°Bx. Therefore, excessively low or high initial pH values could alter the protein structure of many enzymes involved in fermentation and reduce the enzyme's activation ability (Chilakamarry *et al.*, 2022). Hence, samples at initial pH = 3.5, 4, 5, and 5.5 resulted in high levels of total soluble solids.

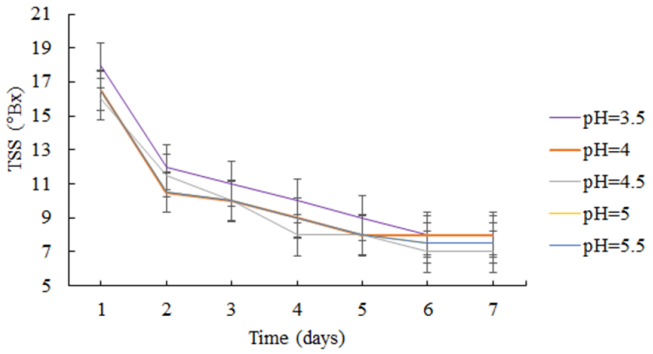

Figure 9. The effect of initial pH on total soluble solids during the fermentation process of wine.

3.2.3 Ethanol content

The effect of initial pH on ethanol content is depicted in Figure 10. The results revealed a gradual increase in ethanol content over the 7-day fermentation period across all samples. Specifically, ethanol content increased rapidly from day 1 to day 3, after which the fermentation process slowed down. On the final day, the sample with an initial pH of 4.5 exhibited the highest ethanol content (13.723%), significantly different from the other samples. According to the report by Patil *et al.* (2021), the optimal pH for the wine fermentation process was 4.5 - 5.0. However, pH 5.0 might not be conducive to yeast growth, reducing fermentation efficiency and resulting ethanol content (Patil *et al.*, 2021). A higher pH led to increased glycerol and organic acid formation, which limited ethanol production; hence, ethanol content decreased with increasing pH values. This was also reported in the study by Chidi *et al.* (2018). Moreover, for samples at a low pH (pH = 4.0), although the low pH inhibited contaminants, fermentation at such pH was not favorable for yeast activity. Thus, yeast activity was partly inhibited from the early stages of fermentation, resulting in less vigorous growth in subsequent stages

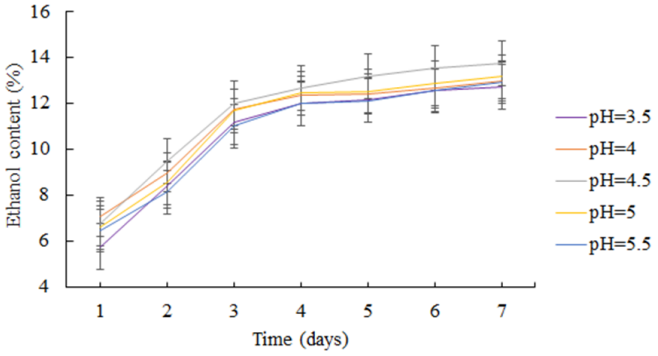

Figure 10. The effect of initial pH on ethanol content during the fermentation process of wine.

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(Seo *et al.*, 2020).

3.2.4 Reducing sugar content

The effect of initial pH on reducing sugar content is shown in Figure 11. Figure 11 shows that reducing sugar content decreased gradually over the 7-day fermentation period. The sample with an initial pH of 4.5 had the lowest reducing sugar content at 5.211 g/L, while the remaining samples with pH values of 3.5, 4, 5, and 5.5 had reducing sugar contents of 15.287 g/L, 7.960 g/L, 9.319 g/L, and 9.897 g/L, respectively. At low pH levels such as 3.5 or 4, unfavorable conditions for vigorous yeast activity, fermentation efficiency decreased, resulting in higher residual sugar content in the wine after fermentation. Similarly to the analysis of ethanol content, the sample with pH = 5.0 was not conducive to yeast growth, leading to decreased fermentation efficiency and lower ethanol content in the wine, hence, higher residual sugar content (Bagheri *et al.*, 2020).

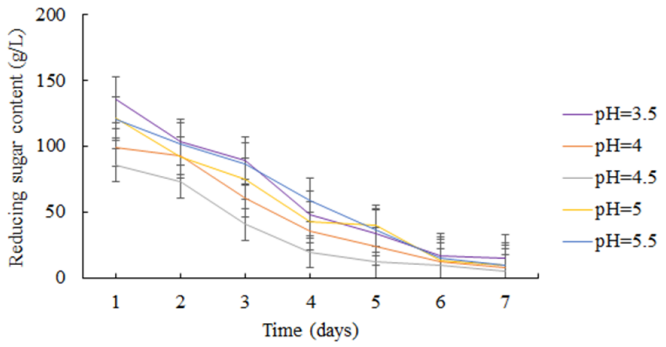

Figure 11. The effect of initial pH on reducing sugar content during the fermentation process of wine.

3.2.5 Total sugar content

The results of the investigation into the effect of initial pH on total sugar content are presented in Figure 12. At pH 4.5, the sample with the lowest total sugar content on the 7th day of fermentation was 8.722 g/L, significantly different from the other samples. For samples with initial pH values of 3.5, 4, 5, and 5.5, the total sugar contents obtained were 18.358 g/L, 12.413 g/L, 12.950 g/L, and 16.956 g/L, respectively. Similar to the analysis of reducing sugar content, higher residual sugar content occurred at lower pH values. This is because excessively low pH inhibits favorable conditions for yeast growth, leading to suppressed yeast activity (Saranraj *et al.*, 2017). As pH increased, the sugar content decreased because yeast fermentation was more efficient at pH values above 4, facilitating better fermentation and conversion of reducing sugars into alcohol, resulting in reduced sugar content.

However, samples with initial pH values such as pH 5 and 5.5 might not be conducive to yeast growth, thereby reducing fermentation efficiency and resulting in lower ethanol content after fermentation and, hence, higher residual sugar content (Bagheri *et al.*, 2020). Additionally, when the environmental pH was not suitable for yeast activity, yeast had to consume additional nutrients to adjust the pH of the environment.

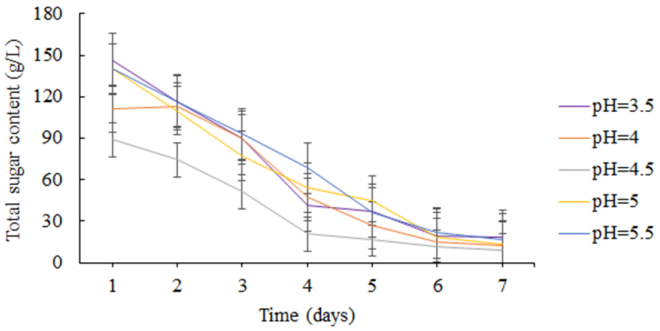

Figure 12. The effect of initial pH on total sugar content during the fermentation process of wine.

3.2.6 pH

The effect of initial pH on pH value during the fermentation process is depicted in Figure 13. After 7 days of fermentation, the samples with initial pH values of 3.5 and 4 had final pH values of 3.705 and 3.918, respectively. For an initial pH of 4.5, the sample had a final pH value of 4.093. Meanwhile, for initial pH values of 5 and 5.5, the final pH values at the end of fermentation reached their highest values at 4.265 and 4.178, respectively.

Based on Figure 13, the low initial pH of the solution led to low pH during fermentation. For initial pH values of 5 and 5.5, the initial pH of the solution was too high, resulting in a high final pH. This condition facilitated rapid bacterial growth, leading to spoiled fermentation and poor-quality wine with an undesirable color. Therefore, the fermentation process needed to be controlled at an appropriate pH (De Melo Pereira *et al.*, 2015).

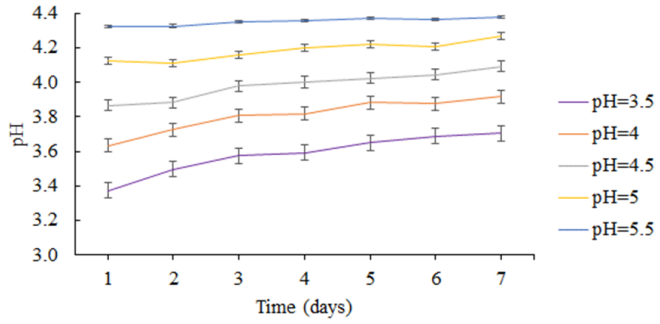

Figure 13. The effect of the initial pH of the sample on pH during the fermentation process of wine.

On the other hand, when the pH dropped to around 4.2, bacterial inhibition occurred. In contrast to the sample at pH 5, the sample at pH 4.5 lowered the pH to as low as 4.1 after a period of fermentation. This low pH

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value had the capability to inhibit most bacteria, thus halting the acid-producing fermentation process (Henick-Kling, 1993).

3.2.7 Yeast density

The results of the survey on the effect of initial pH on yeast density during the fermentation process are shown in Figure 14. During the fermentation process, pH had a significant effect on the growth and development of yeast. The optimal pH for yeast development was 4.0 - 5.0 (Olee *et al.*, 2022). Therefore, yeast cell growth was partially inhibited at pH levels of 5.0 and 5.5, with maximum biomass levels at these pH values being 15.60×10^6 and 14.33×10^6, respectively. At a pH of 4.5, the maximum biomass value was 22.77×10^6 because the pH of the sample fell within the optimal range for the fermentation process.

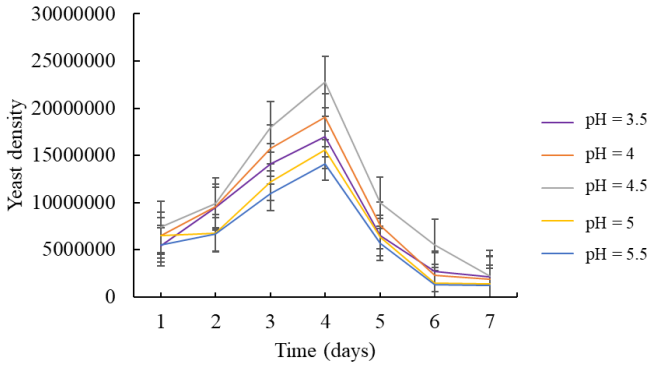

Figure 14. The effect of initial pH on yeast density during wine fermentation process.

In practice, however, the pH of the fermentation medium was often adjusted to a lower range of about 3.5 - 4.5 to limit bacterial fermentation. Nevertheless, at a low pH (pH = 3.5), fermentation efficiency was weak, and the yeast cell density in the fermentation medium was low because the low pH inhibited yeast growth. At this pH level, the maximum yeast biomass reached 16.97×10^6.

3.3 Effect of initial total soluble solid concentration on the fermentation process

3.3.1 Acid content

The effect of initial total soluble solids on acid content is presented in Figure 15. The results showed a strong acid generation in the early stages of the fermentation process. This was attributed to the effective fermentation process at this stage, where a significant amount of CO_2 dissolves in water while also producing some organic acids (such as acetic, lactic, citric, pyruvic, and succinic acids) in the solution. The predominant acid in wine was acetic acid, primarily generated through the oxidation-reduction reaction between two acetic aldehydes or produced by acetic bacteria activity in the fermentation solution, utilizing sugar and generating acetic acid (Joshi *et al.*, 2021). Furthermore, after fermentation of samples with initial total soluble solids of 28°Bx, the acid content was the lowest (6.54 g/L), while the highest acid content was 19.20 g/L for samples with 20°Bx. As the initial total soluble solids increased from 20°Bx to 28°Bx, the acid content decreased gradually. This demonstrates decreased acid content with increasing initial total soluble solids (Nguyen *et al.*, 2016).

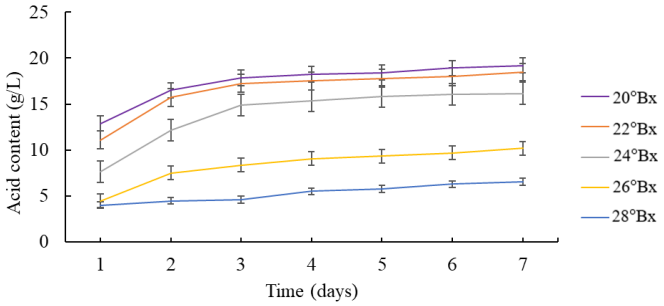

Figure 15. The effect of initial total soluble solids on acid content during wine fermentation process.

If the initial Brix values were low, such as 20°Bx and 22°Bx, it would result in high acid content, namely 19.2 g/L and 18.48 g/L, respectively. The reason was the low alcohol production, insufficient to inhibit other harmful bacteria and wild yeast, leading to higher acid content (Taherzadeh and Karimi, 2011). Additionally, pH decreased sharply on the first day and slowed down from day 2 to day 4 due to the formation of by-products of the fermentation process. Therefore, the acid content on the first day of total soluble solids content of 26°Bx and 28° Bx yielded no significant difference in acid content.

3.3.2 Total soluble solids

The effect of the initial Brix on the total soluble solid after fermentation is presented in Figure 16. The results showed that the lowest post-fermentation soluble solids content for samples with an initial Brix value of 20°Bx and 22°Bx was 7.5°Bx (no statistically significant difference). Additionally, these samples exhibited a faster solid reduction rate than the other samples. This was because the growth rate of yeast was less affected by the

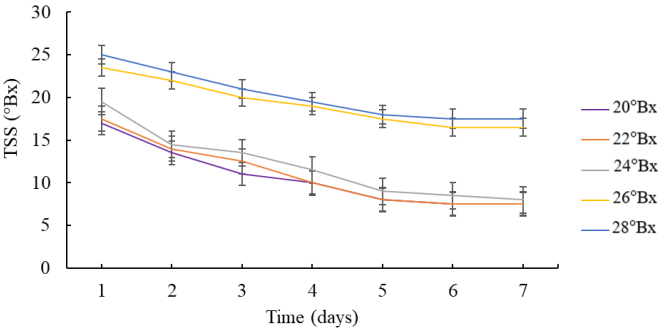

Figure 16. The effect of initial Brix on the total soluble solid during wine fermentation.

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soluble solids content.

For samples with initial Brix values of 26 and 28, the post-fermentation soluble solids content is 16.5°Bx and 17.5°Bx, respectively. A slower reduction in soluble solid content was due to the inhibitory effect of high soluble solid content on yeast growth, resulting in slower nutrient utilization (Udeagha *et al.*, 2020).

The sample with an initial Brix value of 24 had a lower remaining soluble solids content than the sample with a Brix of 28, resulting in a lower osmotic pressure of the sample with Brix value of 24 environment on yeast cells than the sample with Brix value of 28. This led to a significant reduction in total soluble solid content. The sample with a Brix value of 28 and a higher nutrient content exerted a higher osmotic pressure on yeast cells, thus slowing down yeast activity. High osmotic pressure inactivated important enzymes responsible for DNA replication, inhibiting yeast activity due to low intracellular pH (Lin *et al.*, 2022). Besides, when the nutrient concentration reached a certain threshold, water activity decreased, causing cell shrinkage, which affected the growth and development of yeast, resulting in a slower reduction in soluble solids content compared to the sample with an initial total soluble solids concentration of 24°Bx.

The optimal environment for wine fermentation had a sugar concentration ranging from 18 to 25%. When the sugar concentration exceeded 25%, the fermentation process slowed down, and fermentation might halt altogether (Huynh *et al.*, 2018). Therefore, a high sugar concentration led to an excess of nutrients, which inhibited the growth and metabolism of yeast, resulting in inefficient fermentation and slower nutrient utilization by the yeast. Samples with total soluble solids of 20, 22, and 24°Bx provided favorable conditions for yeast growth, leading to faster consumption of sugars by yeast.

3.3.3 Ethanol content

The effect of the initial Brix degree in the samples on ethanol content during fermentation has been presented in Figure 17. The results showed that samples with initial Brix value of 24, 26, and 28 had ethanol concentrations after 7 days of fermentation of 13.170%, 13.103%, and 13.157%, respectively (with no statistically significant differences).

The increase in sugar concentration led to increased alcohol content in the product. According to the report by Aßkamp *et al.* (2019), an amount of sugar was used for biomass growth, while the rest was converted into ethyl alcohol and other by-products such as glycerol and pyruvate (Aßkamp *et al.*, 2019). When fermented under the same pH and yeast ratio conditions, ethanol content significantly increased when the initial fermentation medium had a high soluble solids concentration. As the sugar content increased, the alcohol content produced gradually increased, increasing the pressure on the yeast cell membranes and limiting yeast growth and alcohol fermentation (García-Ríos and Guillamón, 2019). When the sugar concentration became excessively high, osmotic stress occurred, inhibiting yeast activity and reducing fermentation capacity (Lin *et al.*, 2022).

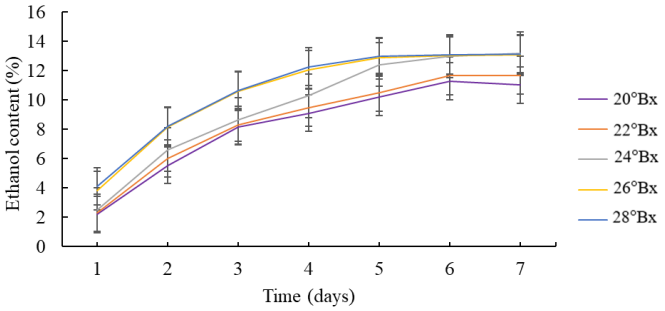

Figure 17. The effect of total soluble solids on ethanol content during wine fermentation process.

For samples with initial Brix values of 20 and 22, the ethanol content was 11.02% and 11.70%, respectively. When the supplemented sugar concentration was low, the ethanol content was also low because less sugar was available for the ethanol conversion process (as yeast utilizes it for biomass growth), resulting in lower ethanol production.

3.3.4 Reducing sugar content

Figure 18 shows the results of the influence of initial Brix on reducing sugar content during fermentation. The results indicated that at initial Brix values of 26 and 28, the reducing sugar content obtained after fermentation was 30.461 g/L and 30.731 g/L, respectively. After fermentation, the high remaining sugar content indicated an excess in terms of organic matter, or it could also be that the initial concentration of solids was too high, partially inhibiting yeast growth and metabolic activity, leading to ineffective fermentation (Walker and Basso, 2020). At initial soluble solids concentrations of 20, 22, and 24°Bx, the reducing sugar content after fermentation was 10.283 g/L, 10.900 g/L, and 12.904 g/L,

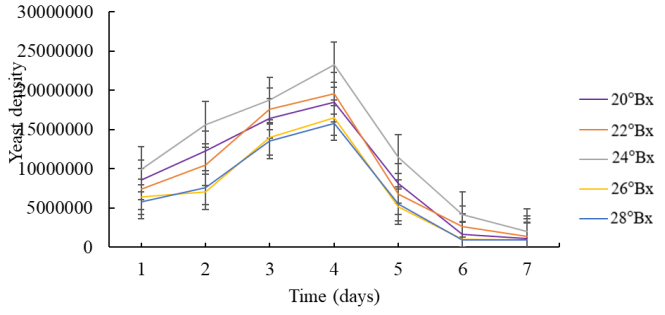

Figure 18. The effect of initial total soluble solids on reducing sugar content during wine fermentation.

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respectively. With low initial sugar concentrations, yeast might be deprived of nutrients, leading to poor fermentation efficiency (Labuschagne and Divol, 2021). Towards the end of the fermentation process, the reduction in sugar content and Brix was minimal due to nutrient depletion in the environment; *Saccharomyces cerevisiae* cells typically aged, ceasing the conversion of sugar into alcohol and dying rapidly.

3.3.5 Total sugar content

Figure 19 shows the influence of initial soluble solids concentration on the total sugar content during fermentation. The results presented that the remaining total sugar content after fermentation in samples with initial total soluble solids of 26 and 28°Bx was relatively high, at 39.194 g/L and 39.509 g/L, respectively. In samples with initial Brix values of 20 and 22, the remaining sugar content was low, at 8.816 g/L and 13.985 g/L, respectively. Meanwhile, the sample of 24° Bx yielded a final total sugar content of 23.593 g/L. From the results in Figure 19, the higher Brix in the fermentation solution results in higher residual sugar content.

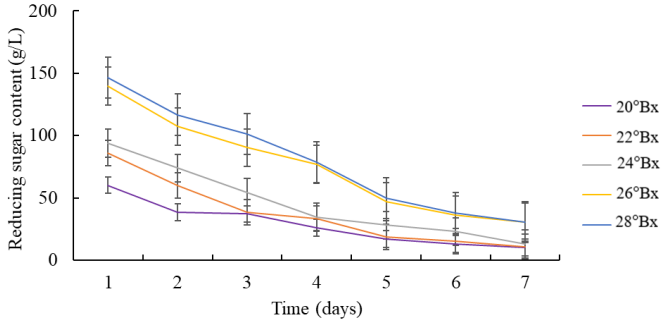

Figure 19. The effect of initial total soluble solids on total sugar content during wine fermentation.

3.3.6 pH

The effect of initial Brix values on pH during fermentation is depicted in Figure 20. For samples with an initial Brix value of 28, the pH produced after fermentation was the highest, reaching 3.570. Conversely, for samples with an initial soluble solids concentration of 20°Bx, the pH after fermentation was the lowest, at 2.803. For samples with 22, 24, and 26°Bx, the pH after fermentation was 2.873, 3.303, and 3.357, respectively.

Based on the results in Figure 20, higher initial total soluble solids led to higher pH values. Specifically, pH decreased sharply on the first day and decreased more slowly from day 2 to day 4 due to the formation of by-products during fermentation. The reduction in pH helped maintain the acidity of the wine to inhibit the growth of undesirable microorganisms during the fermentation process (Morata *et al.*, 2020).

For samples with soluble solids concentrations of 20 - 22°Bx, the pH value obtained was low. It is explained that at these soluble solids concentrations, the rate of solid reduction was faster compared to other samples in the initial days due to the lesser influence of solid content on yeast growth, producing more organic acids. Therefore, the pH of samples with soluble solids concentrations of 20 - 22°Bx was lower than that of the other samples.

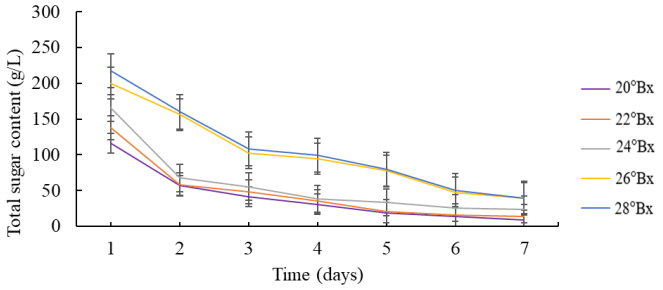

Figure 20. The effect of initial total soluble solids on pH during wine fermentation process.

3.3.7 Yeast density

The results of the study on the effect of total initial soluble solids on yeast density during fermentation are presented in Figure 21. For samples with high initial soluble solids concentrations, such as 26°Bx and 28°Bx, the osmotic pressure in the fermentation solution was higher compared to other samples, resulting in the inhibition of yeast growth and creating favorable conditions for the growth of harmful microorganisms (Walker and Basso, 2020). Therefore, the biomass increase of these two samples was insignificant, reaching values of 16.53×10^6 and 15.83×10^6, respectively.

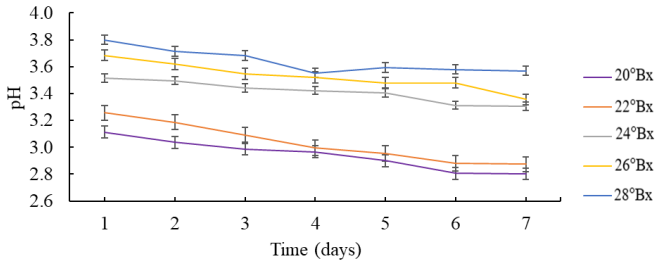

Figure 21. The effect of initial total soluble solids on yeast density during wine fermentation.

Conversely, samples with lower Brix values, such as 20, 22, and 24°Bx, were less affected, leading to higher yeast biomass production. However, due to the rapid increase in cell numbers, they would utilize a large number of soluble solids for growth, resulting in an unbalanced flavor profile of the product. The maximum biomass production of these samples was 18.47×10^6, 19.60×10^6, and 23.30×10^6, respectively. Among them, the sample with a soluble solids concentration of 24°Bx

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had the highest biomass production.

4. Conclusion

Using the commercial yeast strain *S. cerevisiae* RV0002 in this study, the optimal blending ratio for the fermentation process was determined to be 15% (v/v). As the proportion of banana supplementation increased (to 15%), the concentration of soluble solids decreased compared to other ratios. Correspondingly, a higher proportion of pineapple juice in the mixture (5% banana ratio) led to a lower ethanol content after fermentation, resulting in alcohol levels not meeting the standards for alcohol content by volume in QCVN 6-3:2010/BYT. The optimal pH for facilitating the fermentation process was found to be pH = 4.5. The pH of the fermentation solution was typically adjusted using $NaHCO_3$, $CaCO_3$, tartaric acid, or citric acid. Low pH levels could alter the protein structure of various enzymes directly involved in the fermentation process, reducing their activation potential. The optimal soluble solids content was 24°Bx, which maximized fermentation efficiency. Successful production of wine products from Queen pineapple and ripe banana (*Musa paradisaca* L.) varieties represented a potential solution for diversifying products and reducing post-harvest losses of pineapple and banana crops today.

Conflict of interest

The authors declare there is no conflict of interest.

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