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STUDY ON THE PRODUCTION OF STRONG ALCOHOLIC BEVERAGE FROM JACKFRUIT FLESH AND SEEDS (*Artocarpus Heterophyllus*)

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Abstract – *Raw materials for strong alcoholic beverage fermentation are usually grains with high starch content such as regular rice, sticky rice, brown rice, corn, rice sprouts, and cassava. Nowadays, society is increasingly developing, human life is becoming enhanced. Therefore, people's demand for alcohol requires newness and strict quality. Vietnam is the land of many tropical fruits- a rich source of raw materials for researching and producing fruit-based strong alcoholic beverages. In this study, jackfruit (Artocarpus heterophyllus) flesh and boiled jackfruit seeds were used as raw materials to investigate the effects of factors including pretreatment of jackfruit seeds with enzymes, yeast/material ratio, initial dry matter concentration, pH, and fermentation time on alcoholic fermentation efficiency. This study forms the foundation for developing the technological process for producing jackfruit-based strong alcoholic beverages to introduce a new strong alcoholic beverage category for commercialization. Research results indicated that boiled jackfruit seeds treated with amylase enzyme exhibited soluble solids concentrations almost double that of samples without enzyme treatment. Accordingly, the added sugar content to the ingredient is reduced and fermentation time is shortened. Appropriate conditions for fermentation of jackfruit-based strong alcoholic beverage were yeast/raw material ratio of 1% (w/w), initial dry matter concentration of 24^{o}Bx, at pH 4.0, and fermentation time duration of 11 days.*

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I. INTRODUCTION

The tropical jackfruit tree (*Artocarpus heterophyllus*) is an evergreen tree commonly cultivated in tropical regions such as India, Bangladesh, Nepal, Sri Lanka, Cambodia, Vietnam, Thailand, Malaysia, Indonesia, and the Philippines. Jackfruit and its products are widely consumed globally. Small green jackfruits have a tough and spiky exterior, making them suitable for dishes such as soups, braised fish, and salads. The whole jackfruit bulbs contain flesh and seeds [1–3]. Once ripe, the flesh of the jackfruit becomes delectable and nutritious. In Vietnam and various Southeast Asian nations, ripe jackfruit is typically consumed fresh. While the jackfruit rags are commonly thrown away, they can also serve as pickled vegetables. Furthermore, jackfruit seeds are rich in essential nutrients such as carbohydrates, protein, vitamins, minerals, and antioxidants. The abundance of starch in jackfruit seeds makes them a common ingredient in certain dishes [4].

In Vietnam, strong alcoholic beverages have existed since ancient times. Strong alcoholic beverage in every region has different production methods. Each region has unique methods for producing strong alcoholic beverages. Various factors, including raw materials, yeast types, distillation techniques, and traditional knowledge, are crucial for crafting high-quality strong alcoholic beverage [5]. Vietnam boasts an array of tropical fruits like jackfruit, rambutan, mango, and pineapple, serving as abundant sources of raw materials for fruit-based strong alcoholic beverage production. Notably, jackfruit, with its nutrient-rich flesh, is derived from an easily cultivated tree. Jackfruit seeds can bring economic benefits yet be exploited effectively.

II. LITERATURE REVIEW

Previous studies have utilized jackfruit for strong alcoholic beverages and lactic fermentation [4, 6, 7]. Ho Thi Hao [4] carried out lactic acid fermentation using jackfruit seeds to take advantage of agricultural by-products. The lactic acid produced reached a content of 19.9 g/l, suitable for applications in food and various industries. In 2014, Phan Uyen Nguyen [6] studied the production of jackfruit-based strong alcoholic beverages through freeze distillation. Findings indicate that post-grinding, jackfruit fleshes are mixed with water in a 1:1 ratio, pH 4.5, dry matter concentration of 20^{o}Bx, yeast ratio of 0.2%, and then fermented to yield alcohol. For optimal sensory outcomes, the highest alcohol content achieved is 9.5%. In 2018, Tong Thi Anh Ngoc et al. [7] studied strong alcoholic beverage production from jackfruit fiber. The strong alcoholic beverage obtained retained the characteristic jackfruit aroma with a high concentration of 15%. While these studies focused on using jackfruit flesh and seeds for strong alcoholic beverage fermentation, none explored their potential as raw materials for high-concentration alcohol production. This study aims to innovate strong alcoholic beverage products and enhance economic benefits for jackfruit by experimenting with producing a high-concentration strong alcoholic beverage from whole jackfruit bulbs.

III. RESEARCH METHODS

A. Ingredient

Thai jackfruit (*Artocarpus heterophyllus*) was purchased at Hang Bong market, Phu Loi Ward, Thu Dau Mot City, Binh Duong Province, Vietnam. Vietnamese yeast is Mai Linh compressed yeast, produced at Ngoc Kha strong alcoholic beverage yeast processing facility, Tan Ngai Ward, Vinh Long City (Vinh Long Province, Vietnam). Belarusian yeast was purchased from Drozhzhevoy Kombinat Co., Ltd (Minsk, Belarus).

Liquid amylase BAA-400 was purchased from Biozym Co., Ltd (Ho Chi Minh City, Vietnam). This product includes two strains of enzymes α-amylase and α-amylase produced from the Bacillus subtilis strain with optimum temperature: 60 to 90^{o}C, optimal pH: 5.0–7.0, activity: 4,000 U/ml. Liquid Gluco–Amylase GA-260 was purchased from Angel Yeast Co., Ltd (Hubei, China). This product is a strain of Gluco–Amylase produced from Aspergillus Nigeria with an optimum temperature of 55–62^{o}C, optimal pH of 3–5, and activity of 260,000 U/g. Refined sugar was purchased from Bien Hoa Sugar Joint Stock Company (Dong Nai Province, Vietnam). Natri Cacbonat (Na_2CO_3) and Citric acid were purchased from Tianhe Chemical Co., Ltd (Shanghai, China).

B. Methods

The experiments were performed with the following constant parameters: 300 g of fresh jackfruit flesh and 100 g of boiled jackfruit seeds were chopped and mixed with water at a ratio of 1:1 (w/v). Sugar was added to the mixture, pH was adjusted by citric acid, and yeast was added at appropriate values for every specific experiment.

C. Investigation of the effect of yeast/ingredient ratio on the obtained alcohol concentration

Yeast was added at different ratios of 0.75%, 1.0%, 1.25%, and 1.5% (w/w). The ingredients were afterward fermented statically for seven days at room temperature. The fermented solution was distilled to determine the obtained alcohol concentration and the amount of residual sugar remaining in the fermented solution.

D. Investigation of the effect of initial soluble dry matter concentration on the obtained alcohol concentration

The experiment was conducted similarly to the prior experiment mentioned in Section IIIC. In which, the yeast ratio was the result of the previous experiment, the initial soluble dry matter concentration was adjusted with sugar to achieve concentrations of 12^oBx, 16^oBx, 20^oBx, 24^oBx, 28^oBx. The fermented solution is determined for alcohol concentration and residual sugar content.

E. Investigation of the effect of pH on the obtained alcohol concentration

The initial pH of the fermented solution was adjusted with citric acid to achieve pH 3.5; 4.0; 4.5; 5.0 and 5.5. Other parameters were the results of previous experiments (Section IIIC, IIID). The fermented solution was determined for alcohol concentration and residual sugar content.

F. Investigation of the effect of time on the obtained alcohol concentration

Fermentation time was varied from 5 days, 7 days, 9 days, 11 days, 13 days. Other parameters were the results of previous experiments (Section IIIC, IIID, and IIIE). The fermented solution was determined for alcohol concentration and residual sugar content.

G. Investigation of the influence of yeast strains on the obtained wine concentration

In this experiment, the fermentation efficiency of two Vietnamese and Belarusian yeast strains was investigated. Other parameters were the results of previous experiments (Section IIIC, IIID, IIIE, and IIIF). The fermented solution was determined for the obtained alcohol concentration.

H. Investigation of the effect of pretreatment on the efficiency of alcoholic fermentation

The raw materials were combined with two types of amylase preparations, BAA-400 and GA-260, at a ratio of 1% of the total mass of the original mixture of raw materials and water. The mixture was then incubated at 60^oC for an hour. After the incubation process, the fermentation liquid was tested for soluble solids content using a brix meter. Refined sugar was added to the liquid to reach a dry matter of 24^oBx. Vietnamese yeast was then added to the samples, and the fermentation process was carried out with the same parameters as the previous experiments (Section IIIC, IIID, IIIE, IIIF, and IIIG). The alcohol concentration obtained after the fermentation process was determined.

I. Evaluation of the quality of strong jackfruit-based alcoholic beverage

The jackfruit-based strong alcoholic beverage was obtained by processing raw materials under fermentation conditions obtained from the results of the above experiments. The quality of the strong alcoholic beverage product was determined by the following factors: Ethanol concentration, aldehyde content, ester content, acetic acid content, and fufurol content.

The quality of jackfruit-based strong alcoholic beverage products was assessed using the hedonic sensory scoring test outlined in TCVN 3217-79 [8].

J. Analytical method

Determination of residual sugar content after fermentation

A refractometer was used to determine residual sugar content.

Determination of alcohol level

The mixture after fermentation was filtered to remove the residue of the raw materials and collect the solution. The solution was distilled until the temperature of the fermentation solution reached 95^oC. The process ends at this point. The alcohol obtained after distillation was then determined for alcohol concentration using an alcohol meter.

Determination of furfural in white wine

Furfural in white wine was determined according to AOAC 960.16 [9].

Determination of acid and ester content in alcohol

Acid and ester content in alcohol was determined according to AOAC 972.07 [10].

Determination of aldehyde content

Aldehyde content was determined according to TCVN 8009:2009 [11].

K. Data analyzation

Each treatment was repeated three times. The results are shown as mean and standard error.

The experimental data underwent Anova analysis using Statgraphics Centurion XV statistical software, with a 95% confidence level.

IV. RESULTS AND DISCUSSION

A. Effect of yeast/ingredient ratio on the obtained alcohol concentration

In the fermentation process, the yeast ratio was crucial, impacting fermentation speed and alcohol production [12]. A low yeast ratio prolonged fermentation and reduced alcohol yield. Conversely, an excessive ratio led to nutrient scarcity, hindering yeast growth and causing cell death, resulting in an off-flavor product. Based on the data presented in Figure 1, an increase in yeast ratio from 0.75% to 1% (w/w) led to a 12.3% rise in alcohol concentration. Conversely, a rise from 1.25% to 1.5% (w/w) showed no change in alcohol content. This highlights the insufficiency of a 0.75% yeast amount for fermentation, resulting in low alcohol yield and high residual sugar. Consequently, extending the fermentation duration is essential [13]. Beyond a 1% (w/w) yeast ratio, there is no notable difference in alcohol production compared to the 1% (w/w) ratio, indicating that the optimal yeast ratio for jackfruit wine fermentation is 1% (w/w).

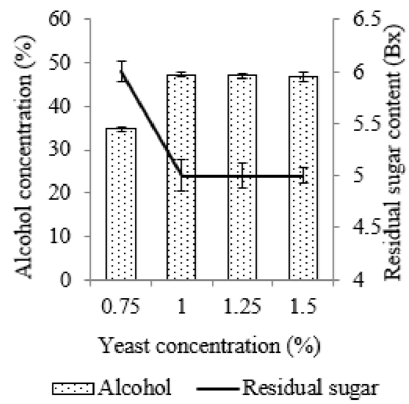

Fig. 1: Effect of yeast/raw material ratio on alcohol concentration

B. Effect of initial dry matter concentration on the obtained wine concentration

The initial dry matter concentration of raw materials significantly influenced yeast growth and fermentation rate. The concentration of dry matter, mainly sugar, also affected the fermentation ability of yeast when too high or too low [14–16]. It is necessary to choose an appropriate initial dry matter concentration to maximize alcohol production by yeast.

According to the results in Figure 2, the initial dry matter concentration changed from 12^oBx to 24^oBx, and the alcohol concentration increased from 47.6% to 66.3%. When the dry matter concentration reached 28^oBx, the alcohol concentration plateaus. At a dry matter concentration of 12^oBx, the residual sugar concentration was at least 8% with alcohol content also at a minimum of 47.6%. This indicates that yeast initially utilizes nutrients in the environment to boost biomass, leading to a substantial decrease in nutrients [17]. After a few days of fermentation, the nutrient source in the environment was depleted, leading to the gradual death of the yeast. The initial dry matter concentration of 12^oBx was insufficient for the fermentation process.

Dry matter concentrations of 16^oBx and 20^oBx promoted yeast growth, yet optimal fermentation results were not attained. Elevating the concentration to 24^oBx and 28^oBx yielded a maximum alcohol concentration of 66.3%. This implies that the environment has established favorable fermentation conditions, supplying sufficient nutrients for yeast growth. The ethanol concentration in the 24^oBx and 28^oBx samples did not differ. However, the 28^oBx sample had a higher residual sugar content compared to the 24^oBx sample due to the higher initial dry matter concentration in the 28^oBx sample. Hence, the suitable initial dry matter concentration value for jackfruit-based strong alcoholic beverage fermentation is 24^oBx.

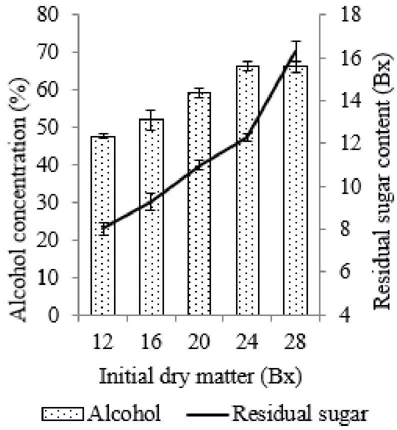

Fig. 2: Effect of initial dry matter concentration on the alcoholic fermentation process

C. Effect of pH on obtained alcohol concentration

The pH level of the environment significantly affects yeast growth as it heavily relies on the environmental pH. The optimal pH range for yeast growth is between 3.5 and 6. Nonetheless, besides beneficial yeast, various contaminants can thrive, impacting both the fermentation process and the final fermented goods. Hence, maintaining an appropriate pH during fermentation is crucial to inhibit the proliferation of undesirable microorganisms. The results in

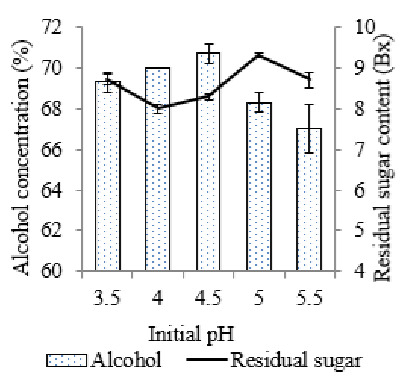

Fig. 3: Effect of initial pH value on alcoholic fermentation process

Figure 3 demonstrate that experiments with pH values ranging from 3.5 to 4.5 exhibited a rise in alcohol concentration from 69.3% to 70.7%. This indicates that pH levels within this range foster favorable conditions for yeast growth. A pH of 3.5 results in the fermentation medium having a greater concentration of H+ ions compared to mediums with pH levels of 4.0 and 4.5.

The results in Figure 3 demonstrate that experiments with pH values ranging from 3.5 to 4.5 exhibited a rise in alcohol concentration from 69.3% to 70.7%. This indicates that pH levels within this range foster favorable conditions for yeast growth. A pH of 3.5 results in the fermentation medium having a greater concentration of H+ ions compared to mediums with pH levels of 4.0 and 4.5. The presence of H+ ions in the environment has an impact on the yeast cell wall's charge state, which can affect the level of osmosis through the cell membrane. As a result, it can reduce the yeast's ability to ferment [18]. A pH of 4–4.5 yielded the highest alcohol concentration and minimal residual sugar, indicating yeast thrived under these conditions. When the pH was raised to 5.0 using a 10% Na_2CO_3 solution, the alcohol concentration in wine tended to decrease to only 68.3%. In this environment, both yeast and bacteria grow within the same pH range, which can lead to competition between the two microorganisms [18]. Despite the high density of yeast, there can still be competition from bacteria which can result in lower fermentation efficiency. However, the yeast can still overpower the bacteria due to their higher density. When the pH reached 5.5, the fermentation conditions became unfavorable for yeast growth. Bacteria thrived, suppressing yeast growth and leading to rapid yeast cell death. Consequently, the alcohol concentration after fermentation was reduced to 67%. Thus, pH values of 4.0 and 4.5 yielded similar alcohol concentrations. Statistically, no difference exists in alcohol content at pH 4.0 and 4.5. Opting for pH 4.0 aids the fermentation process due to the initial material's proximity to this value.

D. Effect of fermentation time on obtained alcohol concentration

Fermentation time is a crucial factor in wine fermentation [19]. If the fermentation process is too short, it can lead to high residual sugar levels and low alcohol content in the wine; conversely, if the process is too long, undesirable by-products may impact the overall quality of the wine. Simultaneously, alcohol oxidation can lead to the formation of acetic acid, decreasing alcohol levels and resulting in wine spoilage [18]. Typically, to ascertain the conclusion of the fermentation process, the fermentation liquid ceasing to bubble was observed. This indicates that CO_2 production in the wine has ceased, signifying that the yeast is no longer converting sugar into alcohol either due to nutrient depletion in the fermentation environment or the alcohol concentration reaching a point where it inhibits yeast activity. According to Figure 4, the alcohol concentration increases gradually as the fermentation time extends, but only up to a certain limit. After five days of fermentation, the alcohol concentration reached 66.5%, but the remaining sugar was still at a significant level of 9.7%. This observation suggests that five days are not sufficient for the yeast to grow, and hence the produced alcohol concentration is low.

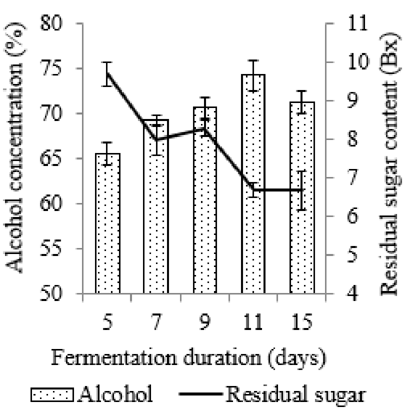

Fig. 4: Effect of time on the wine fermentation process

During the fermentation process, the alcohol concentration increased significantly from day 7 to day 9, going from 69.3% to 70.7%. During this period, the yeast fermented efficiently but incompletely, leaving a substantial amount of residual sugar. By day 11, a noticeable change was observed, with the highest alcohol concentration reaching 74.3% and a residual sugar content of at least 6.7%. On day 13, the alcohol concentration dropped to 71.3%, with the residual sugar level remaining almost the same as on day 11. This indicates a halt in yeast activity due to the depletion of nutrients.

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E. Effect of yeast strains on the efficiency of the wine fermentation process

In this study, the optimal parameters from prior experiments were set, and examined the fermentation efficiency of two yeast strains, one Vietnamese and one Belarusian. According to the results in Figure 5, the alcohol concentration obtained by the normal yeast strain is 6.6% higher than that of the Belarusian yeast. In Vietnamese commercial yeast, aside from the main *Saccharomyces cerevisiae* strain responsible for fermenting alcohol, there are also various other yeast and mold strains. These additional microorganisms support the smooth hydrolysis of starch into sugar, making the subsequent alcohol fermentation process more effective [21]. On the other hand, the Belerus yeast strain only consists of pure *Saccharomyces cerevisiae* strain. As a result, the process of hydrolyzing starch from jackfruit seeds was not as thorough, leading to a less effective fermentation process compared to the Vietnamese strain. Yeast first converts starch into glucose before fermenting it into alcohol. This initial starch hydrolysis by yeast can be time-consuming, potentially hindering effective fermentation. To address this, raw materials are pre-processed to enhance the hydrolysis of starch in jackfruit seeds when yeast is added. Consequently, the Vietnamese yeast strain is better suited for strong alcoholic beverage fermentation from jackfruit.

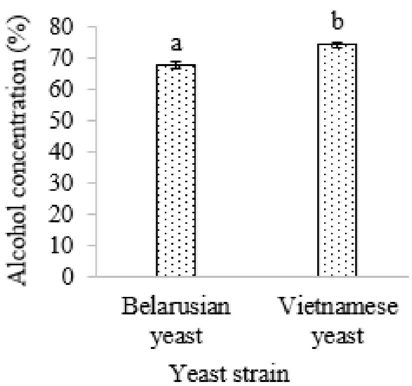

Fig. 5: Effect of yeast strain on wine fermentation process

F. Effect of pretreatment on the efficiency of alcoholic fermentation

In this study, the ingredients used comprised jackfruit flesh and seeds. Starch constitutes 77.76% of jackfruit seed weight [20]. Yeast metabolizes starch into glucose before fermenting it into alcohol. Consequently, yeast requires time to hydrolyze starch, resulting in prolonged fermentation and potentially incomplete hydrolysis. Therefore, the raw materials are processed before adding yeast to hydrolyze the starch in jackfruit seeds more effectively.

The results shown in Figure 6 indicate that the dry matter concentration in samples pretreated with the enzyme systems α and β amylase (BBA-400) and with α, β, γ amylase (BBA-400 and GA-260) was much higher than in raw materials not treated with enzymes. Materials treated with the enzyme system BBA-400 and GA-260 exhibited the highest dry matter concentration, almost double that of untreated samples. The sample treated with enzyme (BBA-400) also showed a 64% increase compared to the control sample. This is attributed to the amylase enzyme system, the primary component of the BBA-400 enzyme system, which catalyzes the conversion of starch into dextrins and disaccharides, consequently enhancing the dry matter content. Furthermore, enzyme C contains glucoamylase, the primary component responsible for converting disaccharides into simple sugars. Consequently, the dry matter concentration in the sample BBA-400 and GA-260 is higher than in the sample BBA-400. Therefore, the pretreatment process is considered to notably boost the dry matter content of the raw materials prior to the fermentation stage, thereby decreasing the necessitate for additional sugar in the initial ingredients. The finding is similar to a previous study on the impact of α-amylase and glucoamylase preparations on rice wine fermentation [22]. In that study, the combination of α-amylase and glucoamylase produced an alcohol concentration 9.8% higher than using only α-amylase, and 42.5% higher than samples without enzyme supplementation. Therefore, the optimal pretreatment method for jackfruit flesh and seeds is utilizing a blend of three enzyme preparations (BBA-400 and GA-260). This approach results in achieving the highest dry matter content in the raw materials, lowering the added sugar content while maintaining a high fermentation efficiency.

G. Evaluation of the quality of strong jackfruit-based alcoholic beverage

A quality test on fermented wine products was conducted under optimal conditions based on previous experiments. Based on the analysis re-

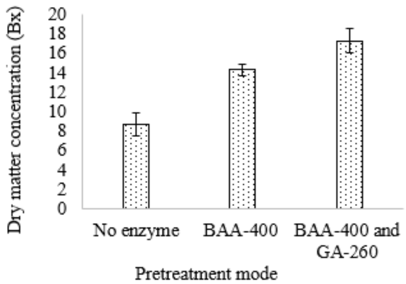

Fig. 6: Effect of pretreatment on dry matter content in raw materials

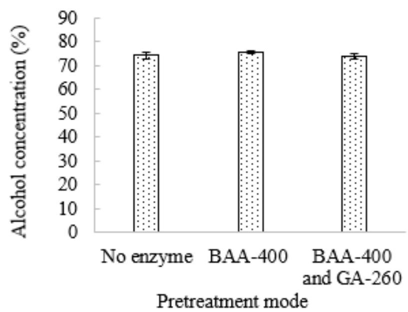

Fig. 7: Results of pretreatment effect on fermentation efficiency

sults presented in Table 1, all the indicators complied with the requirements specified in TCVN 7043:2013 [23]. Furthermore, according to the scoring test's sensory evaluation results (Table 2), the finished jackfruit wine scored 16.1 points, which corresponds to a good wine on the wine quality assessment scale [8]. Therefore, the wine produced from jackfruit flesh and seeds is safe, well-rated by consumers, and widely accepted for consumption.

Table 1: Results of analyzing the quality of obtained jackfruit wine

Criteria	Jackfruit-based strong alcoholic beverage
1. Ethanol content, % volume at 20°C	40
2. Aldehyde content, calculated as acetaldehyde, mg/l 100o ethanol	4.5
3. Ester content, calculated as ethyl acetate, mg/l ethanol 100%	11
4. Acid content, calculated as acetic acid, mg/l ethanol 100%	15
5. Furfurol content	Not detected

Table 2: Scoring test sensory evaluation results according to TCVN 3217-79 [8]

Sensory attributes	Average score	Coefficient	Exchange score
Clarity and color	3.86	0.8	3.1
Odor	4	1.2	4.8
Flavor	4.1	2.0	8.2
Total			16.1

V. CONCLUSION AND RECOMMENDATIONS

Jackfruit is a kind of fruit highly cherished by the Vietnamese. Its flesh has significant nutritional value, notably the enduring aroma. Despite being rich in starch, jackfruit seeds are frequently discarded unused. Jackfruit wine blends the sweet aroma of fruit flesh with jackfruit seeds as a fermentation medium. In this study, optimal fermentation conditions are using a yeast ratio of 1% (w/w), starting with a dry matter concentration of 24^{o}Bx, maintaining a pH level of 4.0 during the fermentation process and an 11-day fermentation period. Jackfruit seeds were processed with amylase enzymes before fermentation reducing the need for additional sugar. The quality of the jackfruit wine adapts to the required standards and is highly appreciated by tasters.

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