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The fermentation conditions of low alcoholic three-leaved (*Cayratia trifolia* (L.) Domin) cider using *Saccharomyces cerevisiae* HG1.3

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ABSTRACT

Three-leaved *Cayratia*, a perennial climber, is widely distributed in India, Asia, Africa, and Australia. In Vietnam, it is found in the Mekong Delta with a specific aroma, dark purple berries, and contains an abundant source of bioactive compounds (yellow waxy oil, steroids/terpenoids, flavonoids, and tannins) in whole plants. Taking advantage of the raw source might enhance various fermented juice drinks and increase income for denizens. The initial parameters were pH 3, 4, and 5 with the total soluble solids (TSS) of 18, 20, 22, and 24 °Bx, respectively, to determine the appropriate *Cayratia* cider making condition. The yeast concentration (0.1, 0.15, 0.2, and 0.25% [w/v], respectively) and fermentation time (24, 48, and 72 h, respectively) at room temperature (28 ± 2°C) were also surveyed. Thereafter, the final product was analyzed for the sensory quality and microbiological and chemical criteria. The fresh *Cayratia* juice has a pH of 3.2 and 6 °Bx, and the juice recovery efficiency was 74%, equivalent to 0.74 L/kg of the fruit juice amount after squeezing. The low alcoholic juice has 4.62% v/v ethanol content which meets the Vietnam National Standard (TCVN 3215:79, and QCVN 6-2: 2010/BYT) after 72 h of fermentation time. The TSS of 22 °Bx, pH value of 4, and yeast concentration of 0.2% (w/v) are appropriate initial conditions for *Cayratia* juice with low ethanol content. *Cayratia* cider with 4.6% (v/v) ethanol content was produced by *Saccharomyces cerevisiae* HG 1.3 at room temperature 28 ± 2°C with initial TSS of 22 °Bx, pH 4, and inoculation yeast rate of 0.2% w/v. The quality analysis meets Vietnamese standards for food consumption.

1. INTRODUCTION

Cayratia trifolia (L.) Domin is an uncultivated glabrous tendril climber widely distributed in a bunch of bushes or glade areas of Asia such as Vietnam, Cambodia, Indonesia, India, China, Africa, and Australia. Numerous studies highlight the rich bioactive compounds in three-leaf *Cayratia*, contributing to antioxidant, anticancer, and anti-inflammatory properties [1,2]. In Vietnam, it is used as an ingredient in traditional cuisine, medicine, and winemaking particularly in Ca Mau and Kien Giang provinces. *Cayratia* wine was produced and commercialized following a traditional fermentation method for years which has the color as red-grape wine. In 2018, it was surveyed a completed winemaking process from raw material to final product using thermotolerant yeast sources isolated from three-leaf *Cayratia* berry [3-5].

The food fermentation industry is experiencing robust growth, marked by significant transformations driven by advancements in scientific technologies over the decades. Particularly, the exploration of yeast diversity underscores the crucial role of isolating novel strains for various applications in food fermentation, spanning wine, beer, pickles, bread, and cider. In this context, 20 yeast strains were isolated from six traditional marchas, microbial starters used in amylolytic fermentation, located in Sikkim, India. This initiative reflects the industry's commitment to harnessing microbial diversity for enhanced and diversified fermentation processes [6]; for instance, 21 *Saccharomyces* strains were found during cider production [7]; 2 thermotolerant yeasts were reported to have a high-temperature limit of 47°C among the total of 34 strains isolated from Indonesian rice wine [8]. In addition, yeasts were also isolated from different kinds of fruit such as watermelon [9], *Cayratia* berry [10], dragon fruit [11], Gemlik olives [12], and soursop [13,14].

The inclusion and diversification of yeast in food fermentation highlight the distinct capabilities of each strain in alcoholic beverage production under varied optimal conditions. For instance, *Saccharomyces cerevisiae* FBY015, sourced from soursop, efficiently yields 10% ethanol soursop wine within 12 days at room temperature [14]. In contrast, *S. cerevisiae* VK1 achieves natural fermentation of pineapple wine in just 5 days at room temperature [15]. The thermotolerant strain *S. cerevisiae* Y8 exhibits remarkable fermentability, producing

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7.45% (v/v) and 4.18% (v/v) ethanol content in pineapple wine at 37°C and 40°C within 5 days, respectively [16]. *Candida hellenica*, *Pichia anomala*, and *Candida pelliculosa* demonstrate resilience to high salt and low pH in brine natural fermentation media, facilitating olive fermentation within the temperature range of 15–28°C [3]. These findings underscore the tailored contributions of diverse yeast strains to alcoholic beverage production across different temperature and substrate conditions.

In the previous studies, 151 strains were found from *Cayratia* berries, in which, 57 thermotolerant yeasts were selected for winemaking at 37°C [5]. *Cayratia* wine was fermented using *S. cerevisiae* HG1.3 to produce an ethanol content of 9.9% v/v [3], and 12% v/v at 35°C in a 6-day incubation time [17]. Despite the growing global and Vietnamese preference for low-alcohol fruit drinks over traditional wines, it is necessary to develop the fermentability of *S. cerevisiae* HG1.3 for the production of low-alcoholic beverage products. *Cayratia*, a wild ingredient with a distinct purple color, is rich in polyphenols with antioxidative properties. Consequently, the authors aim to develop a fruit cider product with elevated polyphenol content and biological activity, diverging from traditional grape-based products. Using three-leaf *Cayratia* berries. Even wine production is better at low temperatures; this result reveals the prospect of wine production at high temperatures by *S. cerevisiae* HG1.3 is also suitable for the tropical countries like Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

Cayratia berries were purchased at local markets in An Giang, Kien Giang, Can Tho, Hau Giang provinces, Vietnam. The raw material was chosen as juicy, shiny, ripe, uncrushed ones, and immediately transferred to the laboratory. *S. cerevisiae* HG1.3 was isolated and stored at the microbial biotechnology laboratory, Institute of Food and Biotechnology, Can Tho University, Vietnam [13]. Sucrose (>99.7%) was produced by Bien Hoa sugar company, Dong Nai, Vietnam. Citric acid, calcium carbonate ($CaCO_3$), sodium bisulfite ($NaHSO_3$), ethanol, peptone, yeast extract, and D-glucose were purchased from Xilong, China. Folin-Ciocalteu's phenol reagent was produced by Sigma-Aldrich.

2.2. General Procedure of *Cayratia* Cider Fermentation

Shiny, juicy, and dark black ripe *Cayratia* berries were collected in the provinces of the Mekong Delta, in Vietnam. These fresh berries were washed under tap water, then, rinsed with distilled water and drained for an hour until the fruit's surface dried. Thereafter, 1000 g of *Cayratia* berry was pressed by a juicer and filtered through a filter cloth to collect 740 mL of juice. The juice was then diluted with water to a ratio of 1:1 (v/v) and adjusted to the appropriate pH value and TSS using 1 mol/L sodium carbonate and 0.5 mol/L citric acid. The adjusted *Cayratia* juice was fermented at room temperature (28 ± 2°C) using *S. cerevisiae* HG1.3 for 72 h after being sterilized with $NaHSO_3$ (140 mg/L for 2 h). The product was sterilized at 75°C in 15 min and allocated into 250 mL glass bottles for quality evaluation. The fermentation process is summarized in Figure 1. (Supplementary document, Figure S1).

S. cerevisiae HG1.3 was isolated from *Cayratia* berries and performed high fermentation ability [3,5,10]. A single colony was added to 100 mL YPD broth which had been sterilized at 121°C for 15 min, then was shaken at room temperature (28 ± 2°C) to obtain a rate of 10^8 cells/mL. Subsequently, the addition of 0.3% *S. cerevisiae* HG1.3 was inoculated to *Cayratia* juice for fermentation.

2.3. Effects of the pH Values and TSS on *Cayratia* Cider Production

Cayratia juice was diluted into water with a ratio of 1 to 1, adjusted to the total soluble solids (TSS) 18, 20, 22, and 24 Bx; pH 3, 4, and 5, respectively. To the juice mixture, 0.3% (w/v) *S. cerevisiae* HG1.3 was added to incubate at 28 ± 2°C (room temperature) for 72 h. After fermentation, the product was filtered, sterilized, and allocated into 250 mL glass bottles for further evaluation.

2.4. Effects of Yeast Content and Fermentation Times on *Cayratia* Cider Production

The initial pH values and TSS of *Cayratia* juice were selected from the above experiment. *S. cerevisiae* HG1.3 was supplemented to the juice with a ratio of 0.1; 0.15; 0.2; and 0.25% (w/v) and fermented at 28 ± 2°C for 24, 48, and 72 h, respectively. The product was filtered, sterilized, and allocated into 250 mL glass bottles for the next experiments.

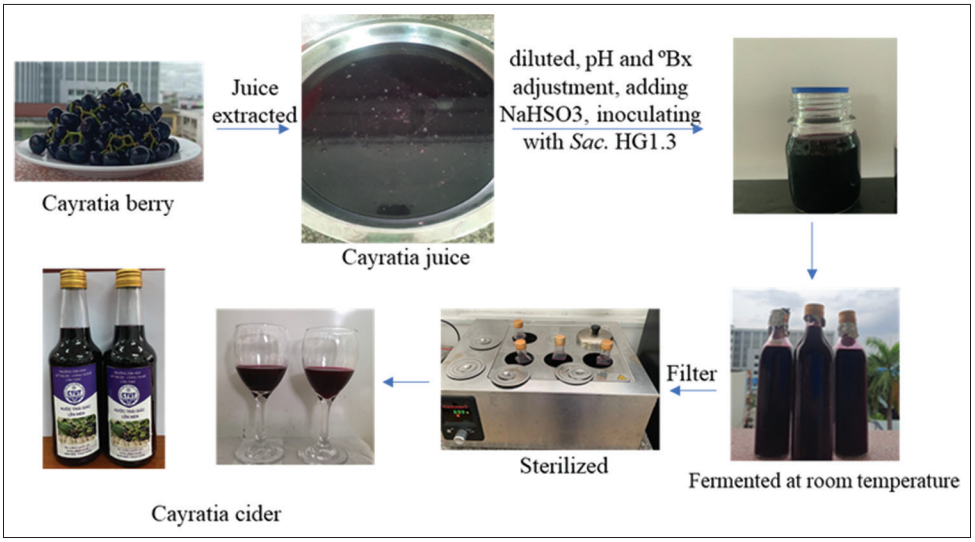

Figure 1: The procedure of *Cayratia* cider fermentation.

2.5. Physicochemical Analyses

Dark black ripe *Cayratia* berries were removed stalk and washed under tap water, thereafter, the juice was collected using a juicer (Philips HR1811, Netherlands). The pH value was measured using a digital pH meter (Hanna, America), and the (TSS, °Bx) were measured using a manual Atago refractometer (0–33 °Bx, France). Ethanol content (% v/v) at 20°C was determined using the distillation method.

The total phenolic content was determined by the Folin-Ciolcateau method as follows: 1.25 mL folin solution (10% v/v) was added to 0.5 mL methanol-caryatid juice solution (60 µg/µL) and incubated for 5 min. Then, the mixture samples were reacted with 1 mL Na_2CO_3 (2% w/v) for 45 min in the dark. The optical density was measured at 760 nm [4].

2.6. Microbiological and Sensory Analyses

The microbiological parameters were conducted by the National Agro-Forestry Fisheries Quality Assurance Department – Branch 6 (Cantho City, Vietnam). *Cayratia* cider was analyzed for microbiological and chemical quality parameters: total microaerophile (CFU/mL), total yeast and mold (CFU/mL), *Clostridium perfringens* (CFU/mL), Coliforms (CFU/mL), and *E. coli* CFU/mL) according to International Organization for Standardization [18]; methanol and ethanol content according to Association of Official Agricultural Chemists [19]. Sensory evaluation of wine in the criteria of clarity and color, aroma, taste, and overall confidence was done according to Vietnam National Standard 3217:79 [20].

2.7. Statistical Analysis

All the experiments were performed in triplicate. The data were analyzed using Statgraphics Centurion version XIX-X64 (Statpoint Technologies Inc., USA) and Excel 2016 software (Microsoft Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characteristics of the Juice

Due to the appropriate taste, flavor, availability, high sugar, and water content, and overall chemical composition, numerous fruits are cultivated and selected for winemaking throughout the world. Cider, non-grapefruit wine, is fermented from temperate fruits or tropical fruits such as apples, berries, cherries, kiwifruit, plums, peaches, strawberries, mango, jackfruit, banana cashew apple, pineapple, watermelon, and wild apricot. Physicochemical analysis of fruit juice is an initial important step during the cider fermentation process to evaluate fruit characteristics. As of the results of physicochemical analyses [Table 1], *Cayratia* berry had a low content of TSS and pH, dragon fruits 11.8 °Bx [21], however, high concentration of total phenolic compounds.

Table 1: The pH value, TSS, and total phenolic content of *Cayratia* juice.

Criteria	Mean±SE
pH	3.2
Soluble solid content (°Bx)	6.0
Cayratia juice volume (L/kg)	0.74±0.05
Total phenolic content (mgGAE/g)	18.89±0.04
Anthocyanin content	0.62±0.02

SE: Standard error, TSS: Total soluble solids.

3.2. TSS Content and pH Value of *Cayratia* Cider Production

In this study, the pH value and TSS of *Cayratia* juice were determined at 3.2 and 6 °Bx, respectively; however, a TSS of 20 °Bx and pH below 4 is recommended for cider production [22]. It must be adjusted, therefore, an optimal pH and TSS for yeast's activity during the fermentation process to produce a good quality *Cayratia* cider. A high concentration of polyphenol *Cayratia* wine, 11.68–12.0% v/v ethanol, was fermented by *S. cerevisiae* HG1.3 at conditions (35°C, pH 4.5, 20 °Bx, 10^5 cells/mL, 6 days) [5,17]. On the other hand, cider is one of the low alcoholic drinks (0.5–6.5% v/v alcohol) [23]. According to Huan, the optimal conditions of fermented dragon fruit juice were selected as 18 °Bx, 2% yeast inoculation rate, and 44 h incubation time to produce 3.54% v/v alcohol dragon fruit drink with remaining sugar content of 14.6 °Bx [21]. Similarly, *S. cerevisiae* produced 5.2% v/v alcoholic *Docynia indica* juice at 18 °Bx of initial TSS [24]. Based on these findings, pH of 3, 4, 5 and TSS of 18, 20, 22, and 24 °Bx were selected to optimize the *Cayratia* cider fermentation process, the effect of factors on ethanol content is presented in Table 2. The lowest ethanol content was 3% v/v produced in treatment 4 (pH 3, 20 °Bx) and 10 (pH 3, 24 °Bx), whereas the highest ethanol content was 5% v/v created in treatment 6 (pH 5; 20 °Bx) and 8 (pH 4; 22 °Bx). Meanwhile, the ethanol content in treatments 2, 5, 7, and 9 is not significantly different at the 95% confidence level ($P < 0.05$).

Yeast growth is affected by osmotic stress due to the high sugar concentration, as well as low pH during winemaking. The pH value and TSS decreased after fermentation, the highest pH and TSS were 4.12 and 15.67 °Bx, and the lowest pH and TSS were 2.00 and 11.30 °Bx, respectively. The logarithmic concentration term, pH, displays hydrogen ion activity which plays an important role in winemaking. Its involvements range from physicochemical and biological concerns to sensory properties and potential defects. Yeast could grow in an environment of pH 2.0–8.0 but optimized in pH 4.0–6.0, depending on temperature, the presence of oxygen, culture, and the strain of yeast [25]. A low initial pH prolongs the yeast lag phase which leads to an effect on accumulating mass loss, changes in total sugar consumption rate, increase of final acetic acid and glycerol content, and decrease of final ethanol [26]. This could be seen that when the initial pH is decreased from 4 to 3, the ethanol content was significantly reduced by about 1% v/v. In addition, the results on the influence of initial pH showed a significant difference in sensory quality at the 5% level. A pH value in the range of 4 resulted in a better sensory quality of the product in terms of color (86%), aroma (84%), and taste (82%). At pH values of 3 and 5, the resulting products showed no significant sensory differences and were even quite similar in terms of aroma and taste [Figure 2a, Supplementary information, Table S1].

At present, the addition of sugar to fermenting and the finished wine is permitted in the wine industry. Sugar is generally used in the form of concentrates, dry dextrose, sucrose, or syrups of the latter two. However, the sugar concentration can inhibit yeast growth and decrease the maximum population, which affects ethanol production. Herein, the remaining sugar resulted similarly in the final products fermented at the same initial TSS but with different pH values. It also showed that, however, the higher Bx level before fermentation was, the more Bx level after fermentation was obtained. In detail, around initial 11 °Bx of remaining sugar was found in the final products started with TSS 18 °Bx, similarly to other ones, fermentation environments of 20, 22, and 24 °Bx resulted in 13, 14, and 15 °Bx of fermented products, respectively. Nevertheless, ethanol content was reduced in high sugar

Table 2: Effects of pH and TSS on ethanol content.

Treatment	Factors		Results after fermentation		
	pH value	TSS (°Bx)	pH value	TSS (°Bx)	Ethanol (% v/v) ở 20°C
1	3	18	2.00	11.30	3.16^{c}
2	4	18	2.49	11.30	4.66^{ab}
3	5	18	4.05	11.70	3.66^{bc}
4	3	20	2.09	13.67	3.00^{c}
5	4	20	2.84	13.33	4.66^{ab}
6	5	20	3.88	13.67	5.00^{a}
7	3	22	2.07	14.33	4.66^{ab}
8	4	22	3.13	14.67	5.00^{a}
9	5	22	4.04	14.33	4.66^{ab}
10	3	24	2.15	15.33	3.00^{c}
11	4	24	3.18	15.33	4.00^{abc}
12	5	24	4.12	15.67	4.33^{ab}
	P=0.0000	*P*=0.0001			*P*=0.0036

The values in this table were the average values of triplication. The average values in a group with the same letter were not significantly different at the 95% confidence level ($P<0.05$), TSS: Total soluble solids.

concentration, and no significant change (5% v/v ethanol of fermented product) when the initial TSS increased from 20 to 22. Yeasts use glucose and fructose as carbon and energy sources to grow; therefore, it would be in nutrient storage to increase biomass if the initial TSS was too low. In contrast, the addition of too much sugar would hinder yeast activity due to increased osmotic pressure, unbalancing the physiological state and metabolism of yeast, and leading to a reduction of ethanol content [27].

Furthermore, the effect of initial sugar concentration on color and clarity, aroma, and taste was significantly different at the 5% statistical level ($P < 0.05$). Particularly, at 22 °Bx, the sensory quality of the product was the highest of others, approximately 88% with 4.3 points [Figure 2a and b]. The 18 °Bx treatment had the lowest sensory evaluation than that of, followed 20 and 24 °Bx treatments, respectively [Supplementary information, Table S2]. Consequently, pH 4 and TSS 22 °Bx were selected for further experiments to optimize *Cayratia* cider fermentation.

3.3. Yeast Inoculation Level and Fermentation Time of *Cayratia* Cider Production

Low alcoholic beverages could be produced by many modifications involving one or more fermentation methods, shorter processing times, and lower temperatures to reduce the final ethanol concentration [23]. In this study, low alcoholic *Cayratia* cider fermentation was optimized with yeast inoculation levels (0.1, 0.15, 0.2, and 0.25% w/v) at 28 ± 2°C in 24, 48, and 72 h, respectively. The results are illustrated in Table 3 with a 95% confidence level, ($P < 0.05$).

During fermentation, the yeast uses sugar for growth, which is converted into ethanol and carbon dioxide in the fruit juice when the process is fulfilled. According to the results, Bx level and pH value reduced after 24–72 h of incubation time, the more fermentation time is prolonged, the more ethanol is produced. The lowest ethanol content is 0% v/v produced at a fermented environment of 0.1% and 0.25% w/v in 24 h. On the contrary, the ethanol content fermented for

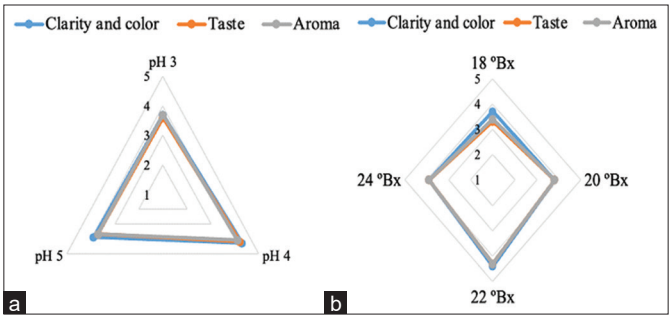

Figure 2: The effect of pH and total soluble solids on *Cayratia* cider sensory charts. (a) pH effect on clarity and color, taste, and aroma of *Cayratia* cider. (b) pH effect on clarity and color, taste, and aroma of *Cayratia* cider. (Sensory attributes including clarity and color, taste, and aroma were evaluated using a 5-point Hedonic scale (where 1 = dislike extremely, dislike = 2, neither like nor dislike = 3, like = 4, and 5 = like extremely) following Vietnam National Standard TCVN 3215-79 by 50 panelists selected from professors, graduate and undergraduate students, staff, and faculty of several related departments in Can Tho University of Technology.

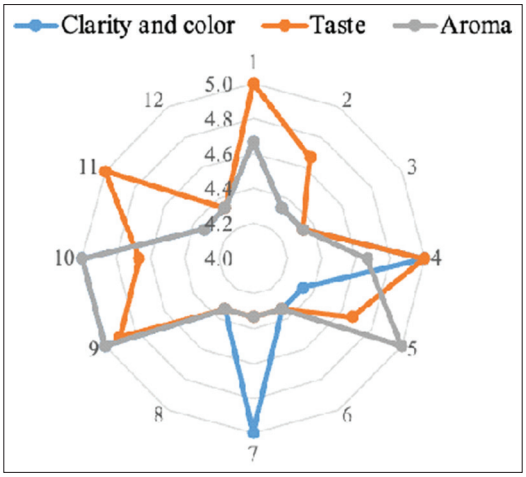

Figure 3: The effect of yeast inoculation level and fermentation time on *Cayratia* cider sensory chart. (Sensory attributes including clarity and color, taste, and aroma were evaluated using a 5-point Hedonic scale (where 1 = dislike extremely, dislike = 2, neither like nor dislike = 3, like = 4, and 5 = like extremely) following TCVN 3215-79 Vietnamese standard by 50 panelists selected from professors, graduate and undergraduate students, staff, and faculty of several related departments in Can Tho University of Technology).

72 h was higher than that of, and highest at 0.2% and 0.25% w/v yeast concentration but not significantly different at 95% confidence (4.6 and 4.5% v/v ethanol, respectively).

Moreover, sensory quality evaluation achieved the highest clarity and color, taste, and aroma value with points of 5.0, 4.9, and 5.0, respectively, fermented for 72 h at 28 ± 2°C with 0.2% inoculation yeast level [Figure 3]. *Cayratia* cider sensory quality affected by yeast ratio and fermentation time was evaluated by 50 members. At the yeast ratio of 0.2%, it is appreciated with the highest score in terms of clarity and color, 86% (4.3/5), aroma 86% (4.3/5), and taste 86% (4.3/5) [Supplementary information, Table S3]. For the fermentation time effect, all 50 members agreed that the 72-h treatment had good sensory quality with 84% clarity and color (4.2/5), 82% aroma (4.1/5), and 82% taste (4.1/5) [Supplementary information, Table S4]. These

Table 3: Effects of yeast inoculation levels and time fermentation on ethanol content.

Treatment	Factors		Results after fermentation		
	Yeast inoculation (% w/v)	Time (h)	pH value	TSS (°Bx)	Ethanol (% v/v) at 20°C
1	0.10	24	3.80	21.7	0.0^{e}
2	0.10	48	3.13	15.0	1.3^{d}
3	0.10	72	2.96	15.0	2.6^{b}
4	0.15	24	3.90	21.3	0.0^{e}
5	0.15	48	3.34	14.7	2.0^{c}
6	0.15	72	3.09	15.0	3.0^{b}
7	0.20	24	3.94	21.7	0.0^{e}
8	0.20	48	3.31	14.3	1.6^{cd}
9	0.20	72	3.04	14.7	4.6^{a}
10	0.25	24	3.97	21.3	0.0^{e}
11	0.25	48	3.51	14.7	2.0^{c}
12	0.25	72	3.27	14.7	4.5^{a}
	P=0.0001	P=0.0000			P=0.0001

The values in this table were the average values of triplication. The average values in a group with the same letter were not significantly different at the 95% confidence level (P<0.05), TSS: Total soluble solids.

Table 4: Quality evaluation of *Cayratia* cider.

Criteria	Vietnam National Standard QCVN 6-3:2010/BYT	Method	Result
Total microaerophile (CFU/mL)	1,000	ISO 4833-1:2013	<1
Total yeast and mold (CFU/mL)	100	ISO 215227-2:2008	<1
Coliforms (CFU/mL)	Abs	ISO 4832:2006	0
E. coli (CFU/mL)	Abs	ISO 16649-2:2001	0
Clostridium perfringens (CFU/mL)	Abs	ISO 7937:2004	0
Methanol (%)	Abs	Ref.AOAC 972.11 (LOQ=0.025%)	nd
Ethanol (% v/v)		Ref.AOAC 972.11 (LOQ=0.025%)	4.62

These parameters were analyzed by National Agro-Forestry Fisheries Quality Assurance Department - Branch 6, Vietnam. Wherein, abs: absence; nd: not detected.

Figure 4: *Cayratia* cider.

results are found consistent with the studies on the cider made from King orange (*Citrus nobilis* L. Osbeck) and soursop fruit (*Annona muricata L.*) [28,29].

3.4. Microbiological and Chemical Evaluation of *Cayratia* Cider

Food safety is always an important factor in the food industry. The quality of the product on microbiological and chemical criteria was conducted by the National Agro-Forestry Fisheries Quality Assurance Department - Branch 6, Vietnam. The results are shown in Table 4, followed by Vietnam National Standard QCVN 6-3:2010/BYT. The final product is presented as in Figure 4 and analysis results are shown in Table 4. The cider produced meets the Vietnamese standard requirements with 4.62% (v/v) ethanol content, and the absence of methanol, Coliforms, *E. coli,* and *C. perfringens,* while the total microaerophile, yeast, and mold of *Cayratia* cider were acceptable based on the requirements of microbiological safety standards [Table 4].

4. CONCLUSION

Fermented *Cayratia* cider successfully produced 4.6% (v/v) ethanol content by *S. cerevicsiae* HG1.3 at room temperature 28 ± 2°C with initial TSS of 22 °Bx, pH 4, and inoculation yeast rate of 0.2% w/v. In general, *Cayratia* cider has a purple color, clear, and favorable taste

and aroma which meets the Vietnamese standards for sensory quality as well as microbiological and chemical quality. Therefore, *Cayratia* cider will be a potential new product in the fermented food industry in Mekong Delta in particular, and Vietnam in general.

5. AUTHOR CONTRIBUTIONS

Concept and Design: Doan T.K. Tien, Nguyen H. Thanh, Le T.B. Son, and Nguyen N. Thanh. **Data Acquisition:** Nguyen H. Thanh, Le T.B. Son, and Nguyen N. Thanh. **Data Analysis/Interpretation:** Huynh T.N. Mi, Nguyen H. Thanh, Le T.B. Son, Nguyen N. Thanh, and Huynh X. Phong. **Drafting Manuscript:** Tran T.M. Thu and Huynh T.N. Mi. **Critical Revision of Manuscript:** Doan T.K. Tien, Tran T.M. Thu, and Huynh X. Phong. **Statistical Analysis:** Huynh T.N. Mi, Nguyen H. Thanh, Le T.B. Son. **Admin, Technical or Material Support:** Tran T.M. Thu. **Supervision:** Doan T.K. Tien and Huynh X. Phong. **Final Approval:** Doan T.K. Tien and Huynh X. Phong.

6. FUNDING

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7. CONFLICTS OF INTEREST

Doan T.K. Tien, Tran T.M. Thu, Huynh T.N. Mi, Nguyen H. Thanh, Le T.B. Son, Nguyen N. Thanh, and Huynh X. Phong declare that they have no conflicts of interest.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the relevent data is available with the authors and can be accessed on request.

10. SUPPLEMENTARY MATERIAL:

The supplementary material can be accessed at the journal's website. Link Here [https://jabonline.in/admin/php/uploadss/1206_pdf.pdf].

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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