

Research on the Cocultured Fermentation of Soursop (*Annona muricata* L.) Fruit Juice by *Lactobacillus plantarum* and *Saccharomyces bayanus* Strain

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Abstract Soursop (*Annona muricata* L.) of *Annonaceae* is a great kind of nutritional and biological potential tropical plant. Fermented fruit products are currently a popular trend in consumption due to their various advantages. Nowadays, in addition to commonly used fermentation methods including alcoholic fermentation, acetic fermentation and lactic fermentation, multi-strain fermentation (or symbiosis) is increasingly applied in food production, especially the fruit juice sector. Through the process of altering the fermented conditions of this research, the appropriate process applied was a combination of 48-hour lactic fermentation with the addition of 10^7 CFU/mL *L. plantarum* bacterial strain at 30°C; followed by 24-hour combined alcoholic and lactic fermentation with the addition of 10^6 CFU/mL *S. bayanus* yeast strain at 15°C. The suitable conditions for fermentation of soursop fruit juice were established as the dilution ratio of soursop juice (SJ) and water (W) of 1:2; the total soluble solids (TSS) of 16°Brix; pH 5.0. Under these suitable fermentation parameters, the final product achieved total titratable acidity (TTA) of $0.488 \pm 0.026\%$, total sugar content of 14.881 ± 0.189 g/100mL, TSS of 15.37 ± 0.17 °Brix, pH index of 3.82 ± 0.04 , ethanol content of 0.42 ± 0.01 %v/v, vitamin C concentration of 29.26 ± 0.79 mg/100mL and total phenolic content of 95.35 ± 0.64 mg GAE /100mL. After assessment, the fermented soursop juice met the required microbiological quality standards. The sensory quality of the cocultured fermentation soursop juice was rated moderated like (7.41 ± 0.46 on 9-point hedonic scale).

Keywords: Soursop, multi-strain fermentation, fruit juice, *Lactobacillus plantarum*, *Saccharomyces bayanus*

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1. Introduction

Soursop is a tropical fruit tree native to Central America. Soursop belongs to the family *Annonaceae*, genus *Annona* [1] and is grown throughout the tropical and subtropical regions of the world, most commonly in the West Indies, North and South America, Africa, the Pacific Islands and Southeast Asia [2]. In Vietnam, soursop is substantially grown in the Southern regions with an average harvest yield of 15–17 tons per hectare [3] in which the Hau Giang soursop cooperative is typical of outstanding development recently.

Soursop is a climacteric fruit with promising nutritional valuation. Soursop is an exquisite source of carbohydrate (including sugar and fiber) [4], vital vitamins (including vitamin C, vitamin B1, vitamin B2) [5], along with a great amount of acid (including citric acid, malic acid, and isocitric acid) [6]. In addition, soursop has potentials in prevention and support of treating problems related to

cancers [7], cardiovascular diseases [8], mental disorders [9], etc. with the presence of biologically valuable compounds including alkaloids, phenolics, essential oils, and annonaceous acetogenin compounds.

Recently, consumer's awareness of healthy foods and eating habits has generated a massive market demand for functional foods with health benefits. Fermented foods, particularly non-dairy beverages, are gaining popularity and acceptance due to their functional benefits [10]. Fermented fruit juice is a product made by fermenting fruit juice that has been freshly extracted, with the fiber content removed [11]. Using optimized processes and technologies during or after fermentation can potentially extend the shelf life and improve the quality of fermented fruit drinks.

Currently, there are 4 fermentation methods commonly used to enhance the functional potential of fruit juice products. They are alcoholic fermentation, acetic fermentation, lactic fermentation and multi-strain fermentation [10]. Alcoholic fermentation is in charge of yeast strains [12]. Acetic fermentation is the fermentation

process of acetic acid bacteria (AAB) [13]. Lactic fermentation is the process of converting sugar substrates by homofermentative and heterofermentative lactic acid bacteria (LAB) strains [14]. The last fermentation method is multi-strain fermentation (symbiosis). Symbiosis, or the coexistence of various microorganisms, such as LAB and AAB or yeasts and molds, contributes to traditional fermentation [15]. Currently, the application of multi-strain fermentation is also increasingly popular in the beverage production, especially fruit juice.

In addition to commonly used combined fermentation methods like kefir, kombucha, or mixing different LAB strains with dairy products [14], there is growing research interest in combining LAB bacteria with yeast for fermentation. Previous studies have shown that this combined fermentation offers many advantages. First, this symbiosis benefits LAB when yeasts act as the active organism, synthesizing missing elements such as vitamins, amino acids, and purines that are required for the growth of *Lactobacillus* [16,17]. Furthermore, specifically for Lactobacilli strains, which are catalase negative, the removal of oxygen by yeast is beneficial for the survival of LAB during growth and storage [18]. In addition, the combination of fermentation between the two strains increases the total phenolic content thereby increasing ABTS radical scavenging activity and ferric-reducing antioxidant power (FRAP) [18,19]. Some studies have demonstrated that this symbiotic fermentation inhibits low-density lipoprotein (LDL) oxidation [19] and induces beneficial growth of volatile components, especially esters, which are characteristic for moderate floral and fruity flavors [20]. Besides, early inoculation of yeasts and some LAB strains with antibacterial property such as *L. plantarum* can help protect fruit-based beverages from contamination by undesirable lactobacilli and cocci [21].

Many fruit juices have been successfully fermented using multi strains, resulting in final products with potential functional properties [22]. Specifically, the study of Q. Zhong et al. (2023) cocultured ferment mango juice product with LAB bacteria (*L. plantarum*, *L. casei*) and non-saccharomyces yeast (*Rhodotorula glutinis* or *Metschnikowia pulcherrima*) [23]. In addition, the study of L. Wanida et al. (2022) with a mixed fruit juice product of watermelon, pineapple and honey also chose a combined fermentation of LAB (*L. plantarum* or *L. salivarius*) followed by alcoholic fermentation by *S. boulardii* [24]. C. Gerardi et al. (2019) has produced *Prunus mahaleb* cherry juice from *S. cerevisiae* and *L. plantarum* strains with probiotic health-promoting properties [25]. With the combined fermentation of *Bacillus coagulans*, *L. plantarum*, and *S. cerevisiae* on blueberry juice, H. Zhong et al. (2021) showed the ability to significantly improve the antioxidant, antibacterial, and antidiabetic activities as an antihyperglycemic agent [26]. From the study of X. Jin et al. (2019) in mango slurry with the combination of *S. cerevisiae* DV 10 and *L. plantarum* symbiotically fermented for 24 hours to produce a juice product with all the good qualities mentioned above [19].

From the above studies, when cocultured LAB and yeast fermentation, the excellent choice for LAB is *L. plantarum* and yeast is *S. cerevisiae* due to the common application of the strain in the food industry. Our previously published studies on soursop fruit showed

similar results, selecting LAB strains and yeasts suitable for individual fermentation as *L. plantarum* [27] and *S. bayanus* [28], respectively.

Consequently, the aim of this work was to select suitable fermentation parameters corresponding to coculture of *L. plantarum* LB-1 and *S. bayanus* FD-3 strain by varying dilution ratio of soursop juice: water (SJ:W) concentration, total soluble solids (TSS), and fermentation time in order to develop fermented soursop juice which retains and enhances the nutritional and biologically active properties with good sensory value, from soursop harvested in Hau Giang (Vietnam).

2. Materials and Methods

2.1. Materials

Chemicals: Enzyme pectinase was obtained from Angel Yeast Co., Ltd (enzyme activity 60,000 U/g). Folin-Ciocalteu reagent (≥ 99.8), gallic acid (GA) ($\geq 99.9\%$), 3,5-Dinitrosalicylic acid ($\geq 98\%$) were supplied by Merck (Darmstadt, Germany). All other chemicals were analytical grade.

Starter culture: lactic acid bacteria LAB culture including *Lactobacillus plantarum* LB-1 (Chr. Hansen, Denmark) and yeast strain including *Saccharomyces bayanus* FD-3 (Fermentis, France). These strains were activated with pasteurization soursop fruit juice at a temperature of about 30-40°C for 15 minutes before conducting the experiment.

Raw material: Soursop fruits (*Annona muricata*) was purchased from Thuan Hoa soursop cooperative, Hau Giang province, Vietnam, transferred to the laboratory within 48 hours with the affinity in terms of maturity, weight and without any defects or crushes. The process of pretreatment soursop to receive soursop juice is according to the process in our previous research [26].

2.2. Processing of Fermented Fruit Juice

Fermented soursop fruit juice was firstly produced by the combination of lactic acid fermentation by *Lactobacillus plantarum* LB-1 culture and the alcoholic fermentation by *Saccharomyces bayanus* FD-3. The fermentation process is carried out as follows. Primarily, soursop extracted juice was adjusted to achieve 16°Brix with dilution ratio of soursop juice and water (SJ:W) ratio of 1:2 and pH 5.0, followed by pasteurization at 65°C for 15 minutes. The fixed *L. plantarum* concentration of 10^7 CFU/ml was added after activation. Lactic acid fermentation was conducted at 30°C for 48 hours [27]. Subsequently, the fixed *S. bayanus* concentration of 10^6 CFU/ml was added after activation and the fermentation were conducted at 15°C [28]. The processing of fermented juice is concluded by centrifugation at 4000 rpm for 30 min to separate the yeast residue and pasteurization at 65°C for 15 minutes. The product is stored at 4°C to keep in the best condition.

The suitable fermentation parameters are evaluated by One Factor at a Time (OFAT) method [29] in which one tested factor in each experiment is changed while all the other factors are kept constant. The factors investigated in this

experiment included fermentation time (M1), the dilution ratio of SJ: W (M2) and TSS content (M3) (Table 1).

Table 1. Variables of the fermentation parameters

Parameters	Value		
Alcoholic fermentation time (hours) (M1)	24	32	40
Dilution ratio of soursop juice:water (M2)	1:1	1:1.5	1:2
Total soluble solids (°Bx) (M3)	14	16	18

The fermentation process is similarly presented as above with the change of the one tested factor. Fermentation progress was monitored by measuring total soluble solid (TSS), reducing sugar content, total titratable acidity (TTA), pH index every 8 hours. After fermentation time, the ethanol content and sensory evaluation of the product was also performed concurrently. Each experiment was repeated three times.

2.3. Chemical Composition Analysis

2.3.1. Total Titratable Acidity, pH and Total Soluble Solids

The technique outlined by AOAC (2010) was used to determine the titratable acidity [30]. The process is as follows: mix 5 mL of sample with 20 mL of distilled water. Three to four drops of phenolphthalein addition were utilized as the indication. Each 25 mL solution was titrated with 0.1N NaOH solution until the color became pink and stayed for 30 seconds.

The pH value was employed by pH meter (Thermo Scientific STARA1117) and total soluble solids was measured by the portable refractometer.

2.3.2. Reducing Sugars

By using Miller's technique, the reducing sugars were measured [31]. The solution 3 mL of DNS and 0.5 mL of juice sample (diluted with distilled water), is boiled for five minutes at 100°C, then cooled for ten minutes in an ice water bath. Using an Apel PD-3000UV UV-vis spectrophotometer, the solution's absorbance was measured at 540 nm. In order to create the standard curve, glucose (0.1–1 g/L) was assessed.

The total sugars were determined by the same Miller's method excepting the juice sample was hydrolyzed by HCl 2% (at 100°C for 45 minutes) and neutralized with 10% NaOH before starting the procedure.

2.3.3. Total Phenolic Content

With a slight modification, the Folin-Ciocalteu method [32], as reported by Obanda et al. (1997), was used to determine the total phenolic content. A precise transfer of 0.2 mL of diluted soursop juice was made to a test tube holding 1 mL of Folin-Ciocalteu's phenol reagent. After vortexing and incubating for three minutes, 0.8 mL of 7.5% (w/v) Na₂CO₃ was added, and the mixture was allowed to react for an hour at room temperature in the dark. At 765 nm, the solution's absorbance was measured by a spectrophotometer. Gallic acid was used in a

concentration range of 25 mg/L to 125 mg/L to create the standard curve.

2.3.4. Vitamin C

The iodine titration method [33] was used to determine the content of vitamin C. Starch indicator solution was prepared by dissolving 1 g of starch with 200 mL of boiling water then left for cool. Iodine solution 0.01N was prepared by mixing 5.00g of KI and 0.268g of KIO₃ in 200mL of distilled water then adding 30mL of 3M H₂SO₄ and bringing the total volume to 500mL. Using three to four drops of 1% starch indicator solution, the iodine solution was titrated against 5 milliliters of 1% ascorbic acid standard solution. The same procedure was used using a 5 mL juice sample. The following formula was used to calculate the ascorbic acid content in the samples:

$$\text{Ascorbic acid} = \frac{V1}{V2} \times 1000 (\text{mg} / 100\text{mL})$$

where V1 is titre (mL) from the titration of the sample solution

V2 is that of standard ascorbic acid solution.

2.3.5. Alcohol Concentration

By using distillation, alcoholic volatile components in the samples were isolated. Next, the amount of ethanol was measured with an ebulliometer (Dujardin-Salleron, France) [34]. The foundation of an ebulliometer is the idea that an alcoholic combination's boiling point is lower than that of water because of the alcohol concentration in the mixture. After distillation, 50 mL of the sample was added to the ebulliometer chamber and heated to a continuous boil. The ethanol content is obtained by comparing the boiling point of distilled water and sample by the device's included ebulliometer disk.

2.4. Microbiological Analysis

2.4.1. Viability of Lactic Acid Bacteria *L. plantarum* in Fermented Juice Product

Serial dilutions were accomplished by homogenizing a precisely 1 mL sample of fermented soursop juice in a sterilized peptone solution. Using the spread plate method, 0.1 mL of diluted probiotic soursop juice was plated onto MRS agar and incubated anaerobically for 48 hours at 37°C [35]. Distinct colonies were counted after the incubation period, multiplied by the reciprocal of the dilution factor, and represented as colony forming units per milliliter of juice (CFU/mL). Every microbiological analysis was carried out duplicate.

2.4.2. Total Bacteria Count of Fermented Fruit Juice Product

Fermented fruit juice samples were diluted decimally, and 0.1 mL of each sample was plated in duplicate on plate count agar (PCA) media to calculate the total bacterial count [36]. Plates were incubated at 30-32°C for 48 hours. Plates with 20-200 colonies were counted, and the results were reported as CFU/mL.

2.4.3. Microbiological Quality: Coliform, Yeast and Mold Counts of Fermented Product

On MacConkey Agar, the coliform count was determined, and the medium was incubated for 24 hours at 37°C [37]. *Coliform* bacteria are often represented by dark red colonies. The pour plate count method was used to count the yeast and mold in the fermented probiotic soursop juice. This was calculated on potato dextrose agar (PDA) media with 0.01% chloramphenicol added (to restrict bacteria development) at 30°C for 48 hours [35].

2.4. Sensory Evaluation

The sensory analysis was conducted with untrained panelists. Fifty individuals, ages 18 to 28, participated in the panel to rate the fermented soursop fruit juice. Samples of 10 milliliters were placed in clear 25 milliliter-glasses. Before serving, the samples were cooled to 4°C in the refrigerator. The evaluation sessions took place at room temperature (30 to 32°C) between 9 and 10 a.m. The samples were assessed on a nine-category hedonic scale to determine their overall acceptance [38]. Extreme dislike = 1; very much dislike = 2; moderately dislike = 3; slightly dislike = 4; neither like nor dislike = 5; slightly like = 6; moderately like = 7; very much like = 8; and extremely like = 9.

2.5. Statistical Analysis

The experiment results from triplicate assay were expressed mean $\pm$ SD. One-way ANOVA with a level of significance at 5% performed by MS Excel was used in statistical analysis.

3. Results and Discussion

3.1. The Influence of Different Alcoholic Fermentation Time on Product Quality During Cocultured Fermentation

The fermentation process integrating lactic acid bacteria and yeast strains is initiated by a 48-hour lactic fermentation by *L. plantarum* strain followed by a cocultured alcoholic fermentation with the yeast addition of *S. bayanus*. This 48-hour lacto-fermentation period was selected based on the results of the study by Nguyen Thi Hanh et al. (2024) [27] with a similar research subject, soursop juice, when fermented with a single strain of *L. plantarum*. Meanwhile, the suitable time for consecutive alcoholic fermentation following the initial 48-hours lactic fermentation process has not been investigated. Therefore, the sequential cocultured alcoholic fermentation periods including 24h, 32h and 40h will be studied in this experiment to select the suitable total fermentation time (72, 80 or 88 hours) to produce the multi-strain fermented soursop juice product.

The evaluation of the influence of fermentation time on the quality of soursop juice products when symbiosis fermenting with *L. plantarum* and *S. bayanus* strains is presented in Figures 1 at the points 0h, 8h, 16h, 24h, 32h and 40h after adding the yeast strain to start the combined alcoholic fermentation process.

The pretreatment soursop juice had the following quality characteristics: total soluble solid 16°Brix, pH 5.0,

total sugar content 15.985 g/100mL and titratable acidity value of 0.110%. After 48 hours of lactic fermentation with *L. plantarum* strain, these nutritional indicators changed to: total dry matter content 15.8 °Brix, pH 3.93, total sugar content 15.380 g/100mL and titratable acidity value of 0.403%.

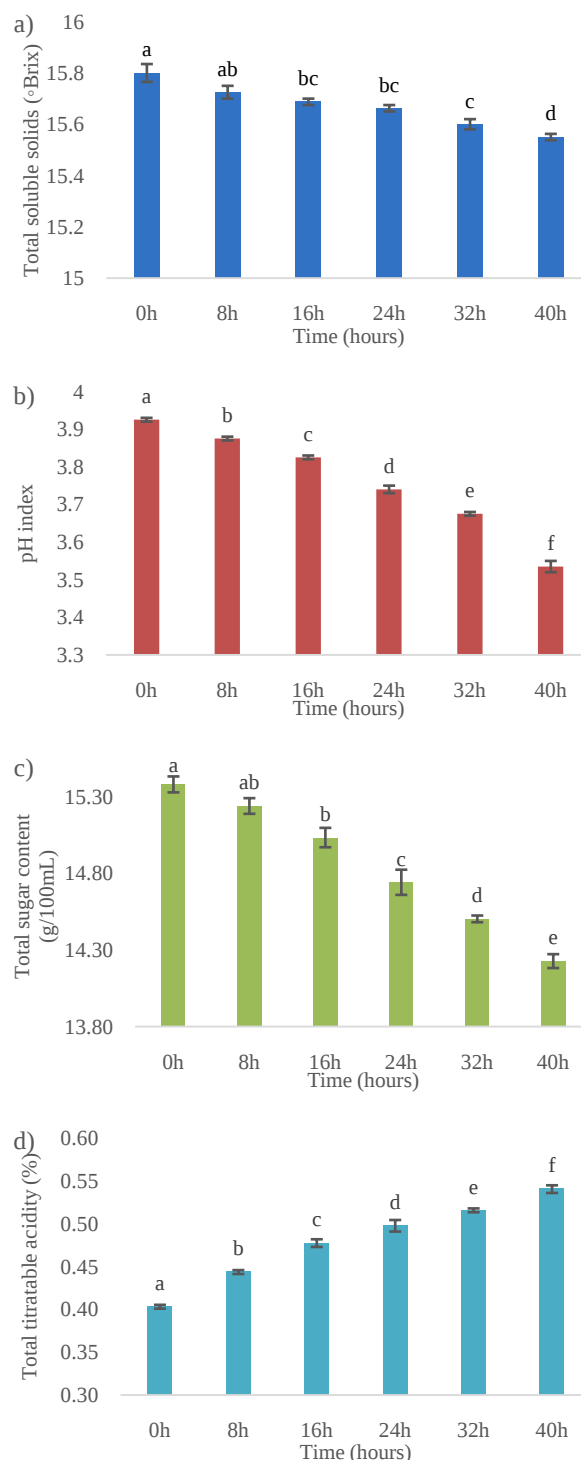


Figure 1. Changes in some physico-chemical quality parameters of multi-strain fermented soursop juice while apply different alcoholic fermentation time. (a) Total soluble solid (°Brix), (b) pH, (c) Total sugar content (g/100mL), (d) Total titratable acidity (%)

During the fermentation process, yeast and LAB bacteria use soluble solids, mainly sugar, as a source of nutrients to grow and metabolize into ethanol, organic acids, CO₂ and other by-products [39]. Longer

fermentation times generally result in more complete fermentation, in which more sugars are converted into fermentation products, thereby significantly reducing the sugar and TSS, as can be seen in Figure 1(a) and (c). A 72-hour fermentation (48 hours of lactic fermentation combined with 24 hours of alcoholic fermentation) reduced the sugar content of the product by 0.34 °Brix and 1.243 g/100mL, while an 80-hour fermentation (48 hours of lactic fermentation combined with 32 hours of alcoholic fermentation) reduced the total sugar content by 0.40 °Brix, 1.482 g/100mL, and an 80-hour fermentation (48 hours of lactic fermentation combined with 40 hours of alcoholic fermentation) reduced the total sugar content by 0.49 °Brix, 1.757 g/100mL. The significant reduction in total sugar content as well as TSS with prolonged fermentation time was directly proportional to the efficiency of fermentation and metabolic activity of yeast and LAB bacteria [40].

Prolonged fermentation time also changes other nutritional indicators of the product such as pH value and total acid content. From Figure 1(b) and (d), we can see that the fermentation process of 72 hours, 80 hours and 88 hours increases the total acid content (TTA) calculated as lactic acid from 0.110 ± 0.002 to 0.498 ± 0.007 , 0.516 ± 0.002 and 0.540 ± 0.005 , respectively; reduces the product pH from 5.04 ± 0.00 to 3.74 ± 0.01 , 3.68 ± 0.01 and 3.54 ± 0.02 . Longer fermentation times resulted in changes in TTA and pH due to the production of various organic acids. *L. plantarum* bacteria produced mainly lactic acid while *S. bayanus* yeast produced some by-products such as acetic acid and lactic acid. Furthermore, yeast metabolized the principle by-product, CO₂, which interacted with water in the fermentation medium to generate carbonic acid (H₂CO₃), which eventually broke down into hydrogen ions (H⁺) and bicarbonate ions (HCO₃⁻), lowering the medium's pH [11]. The longer the fermentation time, the more sugar substrates in the juice product were converted into organic acids, increasing TTA and decreasing product pH. However, excessive fermentation times can lead to excessive acid production or changes in pH, affecting the sensory quality of the product. Typically, for juice products, TTA can be maintained between 0.4% and 1.0%, depending on the natural acidity of the fruit and the desired flavor.

Table 2. Ethanol content and sensory value of multi-strain fermented soursop juice apply different alcoholic fermentation time

Parameters	Cocultured alcoholic fermentation time		
	24h	32h	40h
Ethanol content (% v/v)	0.42 ± 0.01^a	0.56 ± 0.01^b	0.76 ± 0.02^c
Sensory overall acceptability	7.17 ± 0.64^A	6.35 ± 0.44^B	5.95 ± 0.56^C

Soursop juice products when fermented for different lengths of time give statistically significant differences in ethanol content. The product of 24-hour cocultured alcoholic fermentation has an ethanol content of 0.42 ± 0.01 %v/v, 32-hour is 0.56 ± 0.01 %v/v and 40-hour is 0.76 ± 0.02 %v/v. The length of fermentation time is directly proportional to the growth of ethanol content produced by *S. bayanus*. The significant increase in ethanol content also has a great impact on the sensory quality of the output product.

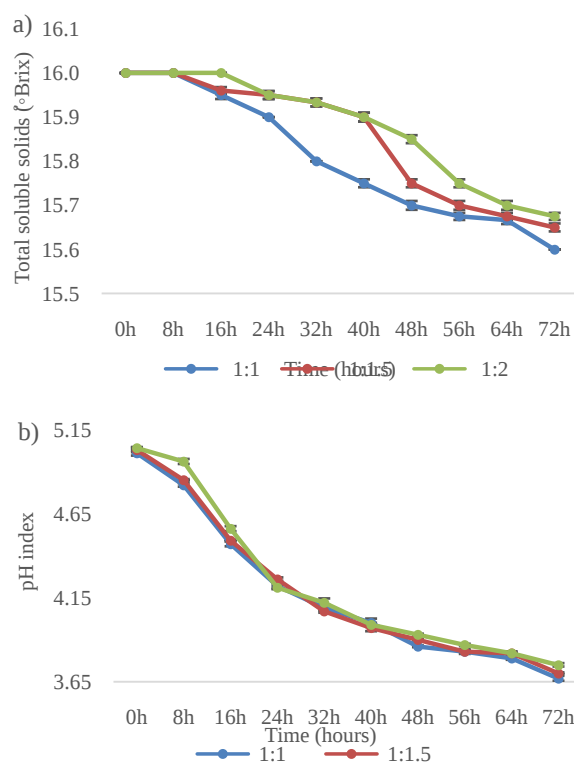
Specifically, for fermented soursop juice products, the

72-hour fermented juice sample was characterized by the natural soursop aroma with the lightest fermented aroma and a harmonious flavor of sour, sweet and astringent, giving the highest overall acceptability (7.17 ± 0.64), with statistical differences when compared with other fermentation times including 80 and 88 hours.

Thus, it can be concluded that the 72-hour fermentation time (48 hours of lactic fermentation combined with 24 hours of alcoholic fermentation) was suitable for the fermented soursop juice product cocultured by *L. plantarum* and *S. bayanus* bacteria strains with good nutritional and sensory values. The above results are consistent with the study of Q. Zhong et al. (2023) choosing a fermentation time of 72 hours for a mango juice product fermented with LAB bacteria (*L. plantarum*, *L. casei*) and non-saccharomyces yeast (*Rhodotorula glutinis* or *Metschnikowia pulcherrima*) [23]. In addition, the study of W. Laosee et al. (2022) with a mixed fruit juice product of watermelon, pineapple and honey also chose a combined fermentation time of 72 hours (48 hours of lactic fermentation by *L. plantarum* or *L. salivarius* followed by 24 hours of alcoholic fermentation by *S. boulardii*), consistent with the conclusion of choosing 72 hours in the fermentation time experiment [24].

3.2. The Influence of Different Dilution Ratio on Product Quality During Cocultured Fermentation

Several studies have shown that diluting fruit juice with water and stabilizing important pre-fermentation parameters such as TSS and pH with sucrose helps create a stable, homogeneous and ideal environment for the growth of yeast and LAB bacteria during fermentation [41]. Furthermore, when fruit juice is diluted, yeast and LAB bacteria can easily access nutrients, leading to improved fermentation efficiency.



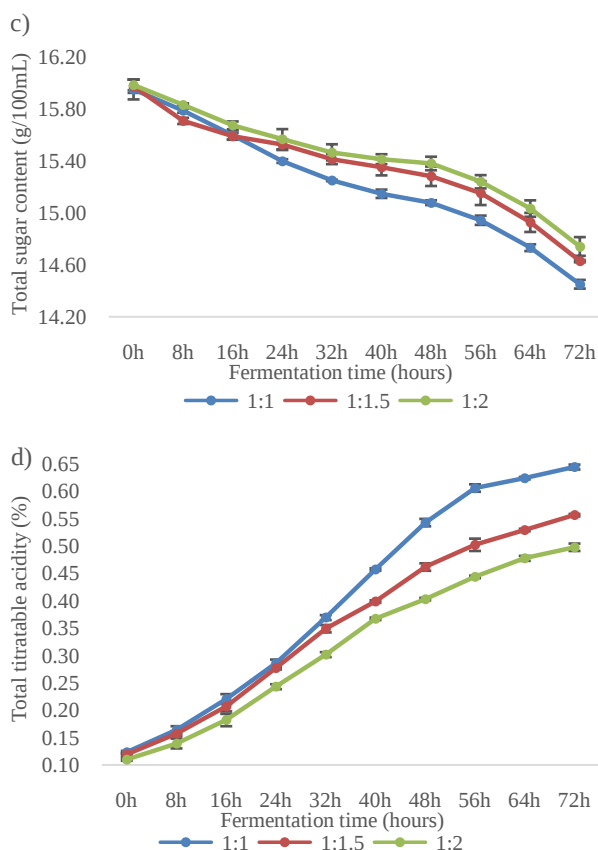


Figure 2. Changes in some physico-chemical quality parameters of multi-strain fermented soursop juice while apply different dilution ratio of soursop juice (SJ) and water (W). (a) Total soluble solids (°Brix), (b) pH, (c) Total sugar content (g/100mL), (d) Total titratable acidity (%)

During fermentation, the total solids concentration in all three samples tended to decrease gradually. The 1:1 sample had the fastest rate of decrease in total solids content. At the end of fermentation, the 1:1 sample had a solids concentration of 15.6 °Brix. The 1:1.5 sample had an average rate of decrease, the final total solids content was lower than the 1:2 sample but higher than the 1:1 sample. The 1:2 sample had the slowest rate of decrease, and the highest final total solids content was 15.68 °Brix. The fermentation process combining two strains of lactic acid bacteria and yeast for 72 hours reduced the pH from 5.0 to 3.69 ± 0.015 for the 1:1 sample, 3.71 ± 0.005 for the 1:1.5 sample, and 3.74 ± 0.010 for the 1:2 sample. However, the pH changes with different total solids contents did not show statistical differences.

The 1:1 sample had the fastest rate of increase in total acid content and the fastest rate of decrease in total sugar. At the end of fermentation, the 1:1 sample had an acid content calculated as lactic acid of 0.644 ± 0.005 % and a total sugar content of 14.45 ± 0.033 g/100ml. The 1:1.5 sample had a slower rate of increase in total acid content and a slower rate of decrease in total sugar content. At the end of fermentation, the total acid content reached 0.556 ± 0.002 % and the remaining 14.628 ± 0.009 g/100ml. The 1:2 sample had the slowest rate of increase and reached the lowest total acid value of 0.498 ± 0.007 % and the highest final total sugar content after fermentation of 14.742 ± 0.072 g/100ml. However, the change in the ratio of fruit juice: sugar juice did not show any statistical

difference in the total sugar content index after 72 hours of fermentation.

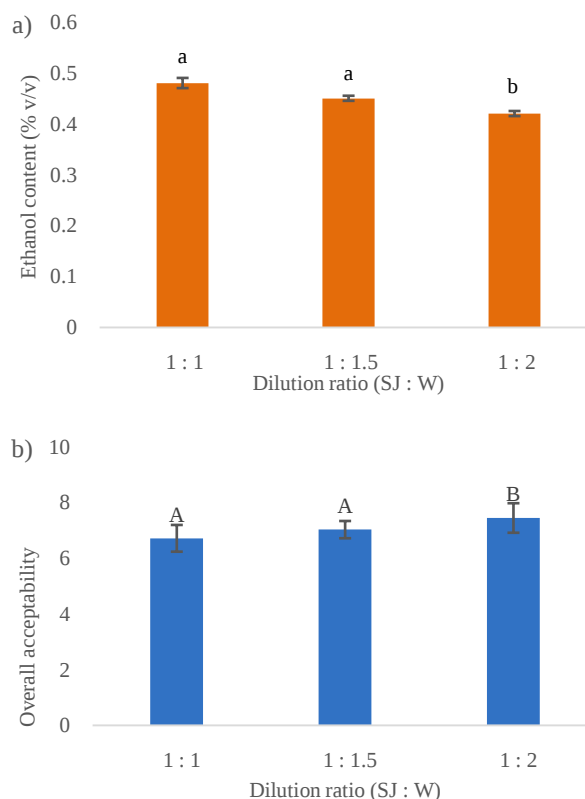


Figure 3. Ethanol content (a) and sensory evaluation (b) of multi-strain fermented soursop juice while apply different dilution ratio of soursop juice (SJ) and water (W)

The alcohol content produced during fermentation in three samples with dilution ratios of 1:2, 1:1 and 1:1.5 is shown in Figure 3. The alcohol content in sample 1:2 was 0.42 ± 0.01 %, sample 1:1.5 was 0.45 ± 0.01 % and sample 1:1 was 0.48 ± 0.02 %. The ratio 1:1 showed the largest increase in ethanol content but showed a small difference when compared to the two dilution ratios of 1:1.5 and 1:2. The results showed similarities with the study of Cao Xuan Thuy et al. (2024), after the first 24 hours of alcoholic fermentation with *S.cerevisiae* RV002 strain, the increase in ethanol concentration was observed without much difference at different dilution ratios of tamarillo juice: water including 34:66, 66:34, 50:50, 40:60 % w/w. However, the difference in ethanol concentration increased when the fermentation process was extended up to 5 days because with the prolonged fermentation time, the competition for nutrients of the starter strain occurred [11]. Sensory evaluation for the results shown in Figure 4 can be seen that although the fermented sample with a dilution ratio of 1:1 gave optimal fermentation performance of multi-strain starter, the overall acceptability of this dilution ratio is the lowest of three dilution ratio (6.71 ± 0.88). Meanwhile, fermented samples with dilution ratios of 1: 1.5 and 1: 2 result in higher overall acceptability. The SJ:W ratio of 1:2 had the greatest overall acceptance (7.44 ± 0.93), indicating a statistical difference from the other two ratios.

Thus, the results of basic physicochemical indicators and sensory evaluation methods show that when compared

with the 1:1 dilution ratio, the fermented sample with the dilution ratio of 1:1.5 and 1:2 fully meets the product requirements for nutritional values including total acid below 0.6%, ethanol concentration below 0.5% and receives higher overall acceptability than the 1:1 dilution sample. Therefore, the ratio of 1:2 is suitable for producing fermented soursop juice from the *L. plantarum* bacteria strain combined with the *S. bayanus* yeast strain to optimize production costs and bring a juice product with a harmonious taste. The above results are consistent with the study of Safiah et al. (2020) [35], the fermentation process of fermented soursop juice by the probiotic strain *L. plantarum* also selected a dilution ratio of 1:2 juice: water.

3.3. The Influence of Different Total Soluble Solids on Product Quality During Cocultured Fermentation

Sugar is the main substrate source for lactic acid bacteria and yeast to carry out the fermentation process to create lactic acid and alcohol, so the high or low content of lactic acid or ethanol produced will depend on the sugar content used in the fermented fruit juice [42]. Therefore, the initial concentration of soluble solids must be suitable to provide enough substrate for the activity of lactic acid bacteria and yeast. In this study, the concentration of soluble solids in the range of 14, 16, 18°Brix was investigated, which is the common solids content used for fruit juice fermentation.

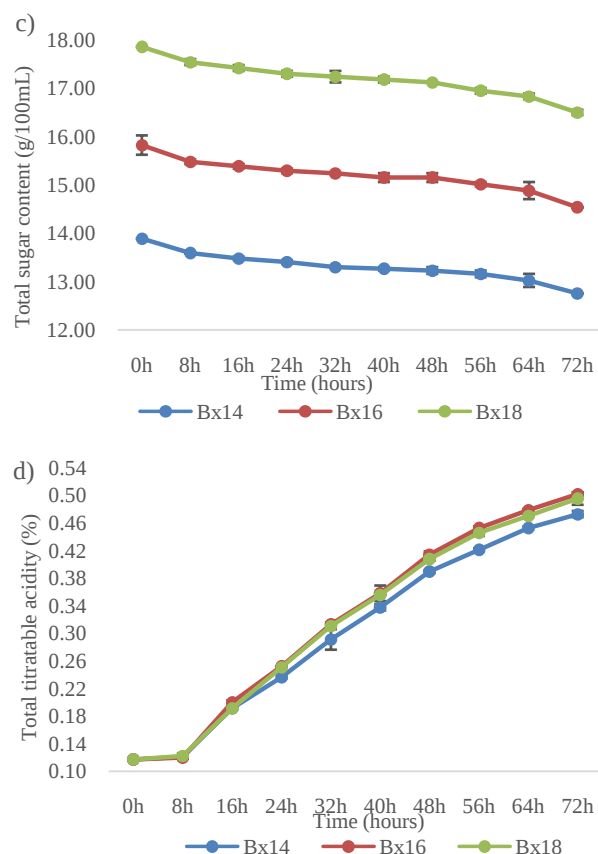
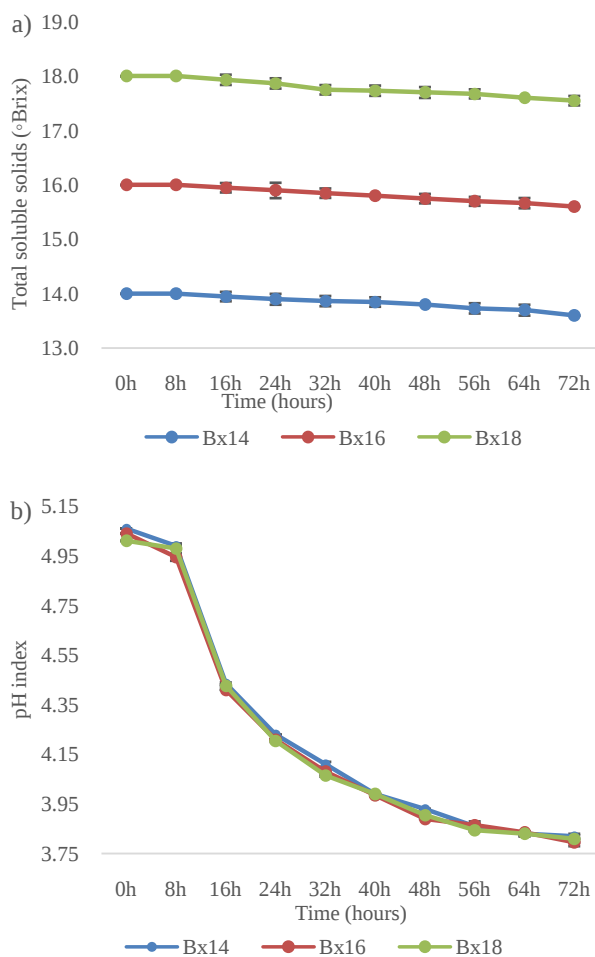


Figure 4. Changes in some physico-chemical quality parameters of multi-strain fermented soursop juice while apply different total soluble solids (TSS). (a) Total soluble solid (°Brix), (b) pH, (c) Total sugar content (g/100mL), (d) Total titratable acidity (%)

During the fermentation process, the total solids concentration in all three samples (Bx14, Bx16 and Bx18) tended to decrease gradually. Sample Bx18 showed the most significant change in total solids content during both lactic acid fermentation and alcoholic fermentation. Meanwhile, samples Bx14 and Bx16 showed similar changes in total solids content. Specifically, after 48 hours of lactic fermentation, sample Bx14 decreased by 0.2 °Brix, sample Bx16 decreased by 0.25 °Brix, and sample Bx18 decreased by 0.3 °Brix. In the next 24 hours of fermentation, combining lactic acid bacteria and yeast, samples Bx14 and Bx16 simultaneously decreased by 0.4 °Brix, while sample Bx18 decreased by 0.45 °Brix. The fermentation process combining 2 strains of lactic acid bacteria and yeast for 72 hours reduced the pH from 5.0 to 3.82 ± 0.010 for sample Bx14, 3.80 ± 0.015 for sample Bx16, and 3.81 ± 0.014 for sample Bx18. However, the change in pH value did not show statistical differences when fermented with different total solids contents.

When examining the change in total sugar content over time in the three samples, it is indicated that the total sugar content in all three samples decreased gradually over time and the rate of decrease in total sugar content in the cocultured fermentation stage of 48 - 72h was faster than the period from 0 - 48h of the first lactic fermentation. At the end of 72h of fermentation, the remaining total sugar content in sample Bx14 was 12.755 ± 0.036 g/100ml, sample Bx16 was 14.535 ± 0.048 g/100ml and sample Bx18 was 16.502 ± 0.054 g/100ml. Sample Bx14 reduced 1.134g of total sugar while Bx16 reduced 1.290g and

Bx18 reduced the total sugar content the most, at 1.355g. From this, it can be seen that the higher the content of soluble solids added, the faster the fermentation process occurs and the faster the reduction rate of total sugar. This can be explained by the fact that high sugar concentrations create a strong osmotic environment in which the extracellular medium has a greater concentration of the solute (sugar) than the microbial cytoplasm. As a result, microbial cells experience osmotic stress, which compels them to keep absorbing sugar from the surrounding media in order to stay in equilibrium state [11]. Therefore, yeast and LAB bacteria will quickly consume sugar to reduce osmotic pressure, leading to a higher reduction rate of total sugar.

Lactic fermentation is the process of metabolizing sugar to produce lactic acid, reducing the pH value and increasing the total acid value. Therefore, during the first 48 hours of lactic fermentation, the acid content increased significantly and uniformly from 0.117% to 0.390±0.002 (with sample Bx14), 0.392±0.005 (with sample Bx16) and 0.394±0.002 (with sample Bx18), without showing statistical differences. From Figure 5(d), it can be seen that from 48 to 72 hours, the combined fermentation of LAB and yeast still increased the acid content in all three samples but more slowly. After the end of fermentation, the acid in the three samples Bx14, Bx16 and Bx18 were 0.473±0.005%, 0.502±0.002% and 0.495±0.009%, respectively.

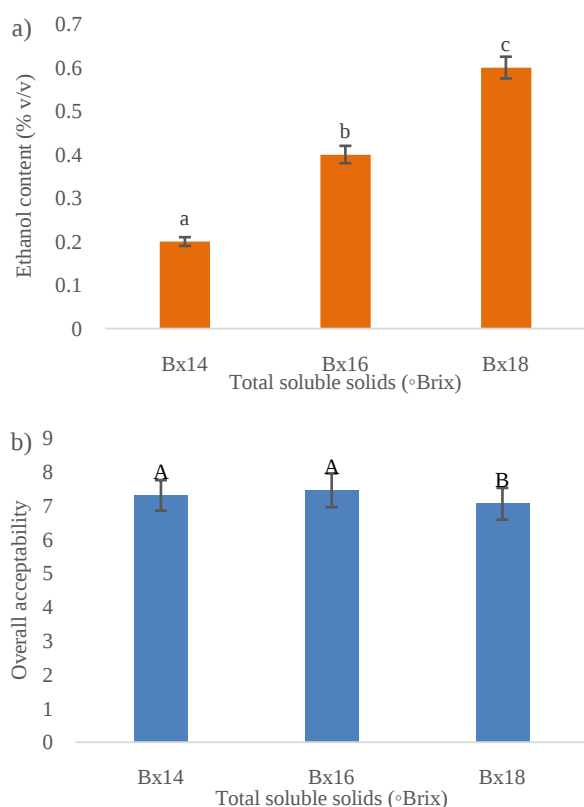


Figure 5. Ethanol content (a) and overall acceptability (b) of multi-strain fermented soursop juice while apply different total soluble solids (TSS)

The alcohol content produced during fermentation in the three samples after fermentation was different. The alcohol content of sample Bx14 was 0.2±0.01%, sample Bx16 was 0.4±0.02% and sample Bx18 was 0.6±0.03%. From Figure 5, sensory results indicated that the total

soluble solid content of 16 has the highest overall sensory acceptability (7.46 ± 0.80), showing a statistical difference compared to the other TSS contents. The overall acceptability of Bx was 7.31 ± 0.75 while that of Bx18 was the lowest at 7.06 ± 0.77 .

In conclusion, there is not much difference in the variation of pH, Brix, acid and sugar content consumed between different total solids contents. However, in the 18°Brix sample, the final total sugar content was quite high (16.502 ± 0.054 mg/100mL), making the product have an unpleasantly sweet taste. The 14°Brix and 16°Brix samples do not have too much final total sugar content, when the senses give a moderate sweetness. With the higher overall acceptability, the TSS 16°Brix is most suitable for the cocultured fermentation process, giving the product a harmonious and balanced flavor.

3.4. Evaluation of Some Quality Indicators of Soursop Juice Products After Multi-Strain Fermentation Process

According to the results from Table 3, during the combined 72-hour fermentation period of LAB strain *L. plantarum* and yeast *S. bayanus*, the pH of soursop juice decreased significantly from 5.0 to 3.82 ± 0.04 after fermentation while TSS decreased from 16°Brix to 15.37 ± 0.17 °Brix. Along with this process, the total titratable acidity also increased, to $0.488 \pm 0.026\%$ and the total sugar content decreased from 15.825 ± 0.076 to 14.881 ± 0.189 g/100mL.

Table 3. Physico-chemical, microbiological and sensory quality characteristics of soursop juice after fermentation

Parameter	Results
Total titratable acidity (TTA) (%)	0.488 ± 0.026
Reducing sugar content (g/100 mL)	5.618 ± 0.143
Total sugar content (g/100 mL)	14.881 ± 0.189
Total soluble solids (°Brix)	15.37 ± 0.17
pH	3.82 ± 0.04
Vitamin C (mg/100 ml)	29.26 ± 0.79
Total phenolic content (mg GAE/100mL)	95.35 ± 0.64
Ethanol content (% v/v)	0.42 ± 0.01
Sensory overall acceptability	7.41 ± 0.46
Viable cell counts (1×10^1 CFU/mL)	ND
Lactic acid bacterial counts (1×10^1 CFU/mL)	ND
Coliform counts (1×10^1 CFU/mL)	ND
Yeast counts (1×10^1 CFU/mL)	ND
Mold counts (1×10^1 CFU/mL)	ND

The nutritional changes in the quality of soursop juice are the result of biochemical changes carried out by the microbial strains used for fermentation. Specifically, the *L. plantarum* strain acted as a hetero-fermentative LAB strain, metabolizing carbohydrates into the main end product of lactic acid along with small amounts of other by-products via the Embden-Meyerhof-Parnas (EMP) pathway, reducing the total solids content specifically sugars and increasing the acid content as well as decreasing the pH value for the studied juice products. Meanwhile, the yeast strain *S. bayanus* used a

combination of glycolysis and the pentose phosphate pathway for hexose metabolism, contributing to the yeast's metabolic needs. The metabolic process transformed sugars in the juice into the main end products of ethanol and CO₂, increasing the ethanol content of the soursop juice product to a value of 0.42 ± 0.01 %v/v.

For the microbiological quality of the product, the microbiological quality criteria including total viable cell count, total lactic acid bacteria count, total yeast, mold, and coliform content were undetected in the final product. This can be explained, account of first, sanitary measures were applied during the juice processing. And more critically, after the fermentation process was completed, the fermented juice product was filtered to remove remaining yeast cells and bacteria by centrifugation at 4000 rpm for 30 minutes. And the cocultured fermented soursop juice production process was completed by pasteurization at 65°C for 15 minutes. This ensures that the final product met microbiological quality for beverage product.

4. Conclusion

Fermented soursop juice by multi-strain starters including *Lactobacillus plantarum* LB-1 and *Saccharomyces bayanus* FD-3 was successfully produced. The cocultured fermentation process studied and applied was a combination of 48 h of lactic fermentation with the addition of 10^7 CFU/mL of *L. plantarum* bacterial strain at 30°C followed by 24 h of combined alcoholic and lactic fermentation with the addition of 10^6 CFU/mL of *S. bayanus* yeast strain at 15°C. The suitable conditions for fermentation of soursop fruit juice were established as the dilution ratio of soursop juice (SJ) and water (W) of 1:2; the total soluble solid of 16°Brix; pH 5.0. Under these suitable fermentation conditions, the final product achieved total titratable acidity of $0.488 \pm 0.026\%$ with total sugar content of 14.881 ± 0.189 g/100mL, TSS of 15.37 ± 0.17 °Brix, pH index of 3.82 ± 0.04 , vitamin C concentration of 29.26 ± 0.79 mg/100mL and total phenolic content of 95.35 ± 0.64 mg GAE /100mL. The fermented soursop juice met the required microbiological quality standards and the sensory quality of the fermented juice was rated moderated like, based on the results of the overall sensory acceptability test with a score of 7.41 ± 0.46 .

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Conflict of Interest

The authors declare no conflict of interest.

List of Abbreviations

SJ: soursop juice
SS: sugar syrup

TSS: total soluble solids

TTA: total titratable acidity

GAE: Gallic acid equivalent

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