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Effect of low Temperature Storage Period on some Quality Attributes of Soursop (*Annona muricata* L.) Juice Fermented using *Lactobacillus plantarum* and *Saccharomyces bayanus*

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Abstract

Soursop fruit (*Annona muricata* L.) is known as a nutritional and biological potential tropical fruit. To our knowledge, no study has been conducted on the changes in quality attributes of multi-strain fermented soursop juice during both ambient and refrigerated temperatures storage. Consequently, this study aimed to investigate the changes of physicochemical properties during 30 days refrigerated storage (4°C) of fermented soursop juice. Following the symbiosis fermentation procedure of *Lactobacillus plantarum* LB-1 and *Saccharomyces bayanus* FD-3 for 72 hours, soursop juice then pasteurized at 65°C, 15 minutes. Under low temperature storage conditions, the symbiosis soursop juice retains preeminent quality attributes for 30 days: pH (3.66 ± 0.02), total soluble solids (15.10 ± 0.02°Brix), total sugar (14.493 ± 0.042 g/100mL), reducing sugar (5.501 ± 0.011 g/100mL), total titratable acidity (0.501 ± 0.011%), ethanol content (0.47 ± 0.02%), vitamin C (16.43 ± 0.41 mg/100mL), total phenolic concentration (79.74 ± 0.44 mgGAE/100mL). The 30-day storage product exhibited the microbial quality met the required standards for non-alcoholic beverages of Vietnamese Ministry of Health, with total viable cell counts of $0.63x10^2$ CFU/mL, yeast and mold count of $0.45x10^2$ CFU/mL and no detection of coliform and lactic acid bacteria. The sensory attribute of soursop juice was rated moderated like (7.01 ± 0.37) of overall acceptability at day 30 of storage, showing a slight change compared to day 0 (7.41 ± 0.46). The results of this study have confirmed that the potential shelf life of the combined fermented soursop juice can be extended up to 30 days under refrigerated storage conditions, which is expected to be the basis for fully completing the production process to create commercialization potential, helping to enhance the value of soursop, diversify products and promote economic development in the cultivation area.

Article History

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Keywords

Lactobacillus plantarum; Low Temperature Storage; Multi-Strain Fermentation; Quality; *Saccharomyces bayanus*; Soursop.

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Abbreviations

ABV : Alcohol by Volume
CFU/mL : Colony Forming Units per milliliter
DNS : 3,5-Dinitrosalicylic acid
F-C : Folin-Ciocalteu
GAE : Gallic acid equivalence
LAE : Lactic acid equivalence
LAB : Lactic acid bacteria
MRS : de Man, Rogosa and Sharpe media
PCA : Plate Count Agar media
PDA : Potato Dextrose Agar media
TSS : Total soluble solids
TTA : Total titratable acidity

Introduction

Soursop, a tropical fruit plant, is classified as part of the genus Annona of the Annonaceae family.[1] It is commonly grown in tropical and subtropical areas around the world, particularly in northern and southern America, the Atlantis, Africa, Pacific Islands and the Southeast Asia regions.[2] Locally in Vietnam, soursop is substantially cultivated in the southern provinces in which the Hau Giang soursop cooperative is typical of outstanding development recently. The average harvest yield is estimated to be around 15 to 17 tons per hectare.[3]

Soursop is a climacteric fruit with promising nutritional valuation. Soursop is an exquisite source of carbohydrate (including sugar and fiber), vital vitamins (mainly vitamin C and vitamin B such as vitamin B6, folate, B1, B2), along with a great amount of acid (including citric acid, isocitric acid, malic acid, ascorbic acid).[4-6] In addition, soursop has the potentials in prevention and support of treating problems related to types of cancers,[7] cardiovascular diseases,[8] mental disorders[9] with the presence of biologically valuable compounds including annonaceous acetogenin compounds, essential oils , phenolics and alkaloids substances.

In recognition of their practical advantages, fermented foods—especially non-dairy fermented beverages—have lately gained acceptability and popularity among community.[10] Meanwhile, symbiosis fermentation method, the coexistence of diverse microorganisms, for instance, lactic acid bacteria (LAB) or acetic acid bacteria (AAB) in the combination of yeasts or molds,[11] is increasingly popular in the beverage industry and commonly used to enhance the functional potential of fruit juice products. From the research articles of various authors, this combined fermentation of LAB and yeasts brings 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.*[12-13] 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.[14] Some studies have demonstrated that this symbiotic fermentation inhibits low-density lipoprotein (LDL) oxidation[15] and induces beneficial growth of volatile components, especially esters, which are characteristic for moderate floral and fruity flavors.[16] Ultimately, early inoculation of yeasts and some LAB strains (*L. plantarum*) with antibacterial properties can help protect fruit based beverages from contamination by undesirable lactobacilli and cocci,[17] which leading to the stability and prolongation in storage.

Although the final product has good nutritional, sensory and biological value, maintaining these values as well as ensure its safety during storage is also important due to the specific property of a food product that cannot reach the consumer immediately after production. There are several important constraints affecting the storage quality of food products such as relative humidity, gas composition, light and temperature.[19] To overcome these factors, fruit juices need to be treated, packaged and stored under appropriate conditions to protect, preserve, maintain quality, safety and prolong shelf life because the quality attributes of processed fruit juices are susceptible to change during long-term storage.[20,30] Different scholars have reported on the effects of different processing, packaging and storage conditions on the quality of different fermented fruit juice products including apple,[21,22] dragon fruit,[23,24] or sorghum.[25]

Regarding the research subject of soursop, currently, previous studies have merely focused on unfermented pasteurized soursop juice. Studies have shown that the optimal storage condition for soursop juice is at

refrigerated temperature (4-6°C) when compared to ambient (28-33°C) and cold (–20°C) temperature while applying different pasteurization conditions. Specifically, the temperature of 4-6°C helps to maintain the quality of soursop juice stably for 8 weeks when combining the use of additives (0.1% sodium Benzoate) with pasteurization at 65°C, 15 minutes[41], and even up to 12 weeks when the pasteurization temperature is increased to 79°C[58] and 83°C.[59] Nevertheless, there is a limited amount of research on fermented soursop juice.

A single study conducted by Akpeji (2017) investigated the quality changes of soursop juice fermented with the lactic acid bacteria (LAB) strain *Pediococcus pentosaceus*. The synergistic effect of the LAB strain and storage at 4°C was found to maintain the nutritional, sensory, and probiotic properties of the juice up to the 30th day of storage,[29] while the unfermented juice sample exhibited a notable decline in sensory quality after day 21.

To our knowledge, no study has been conducted on the changes in quality and functional attributes of multi-strain fermented soursop juice using lactic acid bacteria and yeast during storage at both ambient and refrigerated temperatures. Hence, current research aimed to study the effects of refrigerated storage (4°C) for 30 days on pH, Brix, total sugar, reducing sugar and total titratable acidity contents as well as microbial safety and sensory properties of soursop juice when processed by mid-heat pasteurization (65°C for 15 minutes).

Materials and Methods

Materials

Chemicals: Enzyme pectinase was acquired from Angel Yeast Co., Ltd (with the enzyme activity of 60000U·g). The Folin-Ciocalteu (F-C) reagent (≥ 99.8%), 3,5-Dinitrosalicylic acid (DNS) (≥ 98%) and gallic acid (GA) anhydrous (≥ 99.9%), were supplied by Merck Millipore (Darmstadt, Germany). Orther comprised experimental chemicals were guaranteed to meet analytical standards.

Starter culture, freeze-dried LAB strain *Lactobacillus plantarum* LB-1 was obtained from Chr. Hansen company (Hørsholm, Denmark) and commercial dried yeast strain *Saccharomyces bayanus* FD-3 was obtained from Fermentis unit of Lesaffre company (Nord-Pas-De-Calais, France). The medium used to activate these starter cultures was pasteurized soursop juice (65°C, 15 minutes) which was cooled to averagely 30-40°C. These cultures were activated consecutively for 15 minutes prior to the commencement of the experiment.

The material, soursop (*Annona muricata*), was purchased from Thuan Hoa soursop cooperative, Hau Giang province, Vietnam, and transferred to the laboratory within 48 hours with the affinitive weight, maturity and the absence of possible crushes and defects. The following pretreatment process to receive soursop juice is according to the process in our previous research.[18]

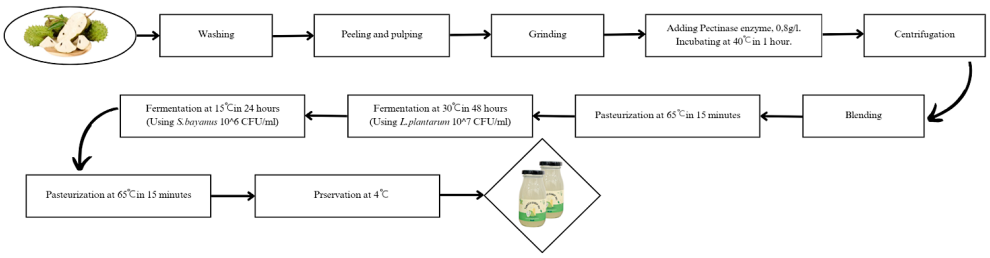

Fig. 1: Soursop juice preparation process

Juice Beverage Formulation, Fermentation and Pasteurization

Symbiosis fermented soursop juice was made by the combination of lactic acid fermentation (in the presence of *Lactobacillus plantarum* LB-1) and alcoholic fermentation (in the presence of *Saccharomyces bayanus* FD-3).

The fermentation process is carried out as follows. Primarily, the extracted soursop juice was regulated to obtain 16°Brix while maintaining the dilution ratio of 1: 2 between soursop juice and water (SJ:W) and pH 5.0, then pasteurized for 15 minutes at 65°C. The fixed *L. plantarum* concentration of 10^7 CFU/mL was introduced into the pretreated soursop juice (after

activation). The process of lactic acid fermentation was carried out at room temperature during 48 hours. Successively, the fixed *S. bayanus* concentration of 10^6 CFU/mL was introduced and the fermentation were carried out at 15°C during 24 hours.[18] The processing of fermented juice is concluded by centrifugation at 4000 rpm for 30 minutes to separate the yeast residue and pasteurization at 65°C for 15 minutes. The product was stored at 4°C to keep in the best condition (Figure 1).

Juice Stability Assessment

The shelf life of the treated soursop juice stored at 4°C was assessed over a period of 30 days. The soursop juice was analyzed for changes in total soluble solids (TSS) concentration, total sugar content, reducing sugar content, total titratable acidity (TTA) level, and pH index on the 0, 5, 10, 15, 20, 25 and 30 days of storage.

After 30 days preservation, microbiological analyses, the ethanol content, sensory evaluation, total phenolics and vitamin C content of the product was also performed concurrently. Each of the chemical analysis experiments were conducted three times and each of microbiological analysis experiments were conducted duplicate.

Chemical Constituents Analysis

TTA, pH and TSS

The technique outlined by Association of Official Analytical Chemist (AOAC) (2010) was used to determine TTA.[31] The process is as follows: mix 5 mL of sample with 20 mL of distilled water. Three to four phenolphthalein droplets addition were utilized as the indication. Each 25 mL solution was titrated with 0.1N NaOH solution until the tested solution became and remained pink for 30 seconds.

The pH index was employed by using a pH meter (Thermo Scientific STARA1117, Thermo Fisher, USA) and TSS was measured by using a portable refractometer.

Reducing Sugars Content and Total Sugars Content

By using Miller's technique, the reducing sugars were measured.[32] The solution 3 mL of DNS and 0.5 mL sample of soursop juice (diluted with distilled water if needed), is boiled for five minutes at 100°C, then cooled for ten minutes in an ice water bath. Using an UV-vis spectrophotometer (PD-3000UV, Apel Co., Japan), the solution was measured absorbance at 540 nm. In order to create the standard curve, glucose concentrations (from 0.1 to 1 g/L) were utilized.

With the alteration of hydrolyzing juice sample with 2% HCl (at 100°C, 45 minutes) and neutralizing it with 10% NaOH before starting the procedure, the total sugars content was derived using the same Miller's method.

Total Phenolic Content

With respect to Obanda *et al.* (1997) study[33] and a slight modification, Folin-Ciocalteu (F-C) assay was employed to evaluate the total phenolic content. A test tube holding 1 mL F-C reagent was precisely filled with 0.2 mL diluted soursop juice. After vortexing and incubating for three minutes, 0.8 mL of 7.5% Na_2CO_3 was inserted, and the mixture continued to react for an hour in the absence of light at ambient temperature. The solution's absorbance was measured by an UV-vis spectrophotometer (PD-3000UV, Apel Co., Japan) at 765 nm. Gallic acid was used in a concentration range of 25 – 125 mg/L to create the standard curve.

Vitamin C

The iodine titration method[34] was used to determine the content of vitamin C. To make a starch indicator solution, dissolve 1 g of starch in 200 mL of boiling water and let it cool. While iodine solution 0.01N was made by combining 5.00 g of KI and 0.268 g of KIO_3 to 200 mL distilled water, followed by the addition of 30 mL H_2SO_4 3M and brought the total volume to 500 mL.

Using three to four drops of 1% starch indicator solution, 5 mL of 1% ascorbic acid standard solution was titrated by iodine solution until the purple color appeared. The same procedure was used to titrate a 5 mL juice sample. The ascorbic acid concentration of the juice sample was calculated as following equation.

Ascorbic acid = V1/V2×1000 (mg/100mL)

Where V1 is titre (mL) from the titration of juice sample solution, V2 is titre (mL) from the titration of standard ascorbic acid solution.

The 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).[35] 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.

Microbiological Analysis

Microbiological analyses are conducted to evaluate the quality and ensure the safety of soursop juice throughout the storage period of 30 days at 4°C. Every analysis was carried out in duplicate.

Lactic Acid Bacteria Count in Symbiosis Fermented Soursop Juice

The microbial analysis was obtained by the spread plate technique. Serial dilutions were accomplish by initially homogenizing a precisel 1 mL juice sample in a sterilized tube contained 9 mL peptone solution (0.1M concentration), then took 0.1 mL from 0.1M solution and homogenized into 0.9 mL sterilized peptone solution (0.01M concentration). The process repeated subsequently to achieve the different diluted concentration. 0.1 mL of different diluted concentrations (of juice sample) were transferred into de Man, Rogosa and Sharpe (MRS) agar plate and incubated anaerobically for 48 hours at 37°C.[36] Distinct colonies were counted after the incubation period, multiplied by reciprocal of the corresponding dilution factor to 0.1 mL inoculated volume of sample (in each plate), and represented as colony forming units per milliliter of juice (CFU/mL).

Total Bacteria Count in Symbiosis Fermented Soursop Juice

Fermented fruit juice sample was diluted decimally, and 0.1 mL of different diluted concentrations were transferred into plate count agar (PCA) media to calculate the total bacterial count (in duplicate).[37] After incubating 48 hours at the temperature of 30-32°C, 20-200 colonies plates were counted and calculated to achieve the result (reported as CFU/mL).

Coliform, Yeast and Mold Counts in Symbiosis Fermented Soursop Juice

Spread plate technique was used to aqquired the coliform, yeast and mold counts of the soursop juice product. On MacConkey Agar, coliform count was determined, and the medium was incubated for 24 hours at 37°C.[38] Coliform bacteria are often represented by dark red colonies. The yeast and mold in the symbiosis fermented juice was calculated on potato dextrose agar media (PDA) supplemented with 0.01% chloramphenicol (in order to restrict the development of bacteria) after incubating 48 hours at 30°C.[36]

Sensory Analysis

The sensory evaluation was conducted with fifty panelists, ages 18 to 28, participated in the panel to rate the fermented soursop juice. Samples of 10mL 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 9:00 and 10:00 am. The samples were assessed on a nine-category hedonic scale to determine their overall acceptance.[39] Sensory scores corresponding to: 1= extreme dislike, 2 = very much dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = moderately like, 8 = very much like, 9 = extremely like.

Statistical Analysis

After the fermentation process, it was divided into 21 samples (each containing 100 mL of pasteurized symbiosis soursop juice in 250 mL sterilized glass bottle). On the day 0, 5, 10, 15, 20, 25 and 30, three samples were taken to be analyzed, and each experiment was repeated 3 times.

The experiment outcomes were indicated as Mean (of triplicate analysis) ± standard deviation (SD) in tables and graphs. Statistical data was processed using Microsoft Excel to perform One-way ANOVA at a confidence level of 5% ($p < 0.05$). The significant sample-to-sample difference of the samples from each storage days was identified using Duncan's multirange tests performed by Statistical Package for Social Sciences (SPSS), and results with statistical differences are denoted by different letters.

Results

Changes in Some Physicochemical Parameters of the Product during Storage.

Change of pH and TTA content

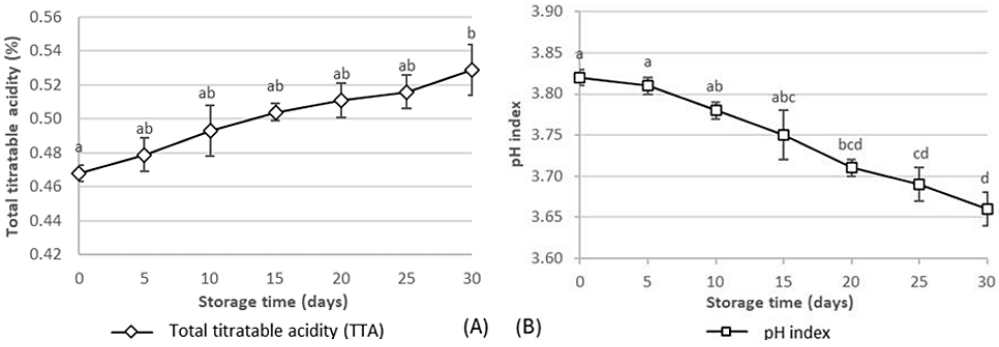

Fig. 2: Changes in certain physico-chemical factors of symbiosis fermented soursop juice during storage. (A) TTA (%), (B) pH.

Change of TSS

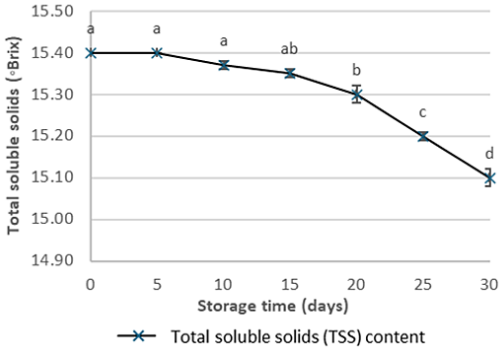

Fig. 3: Changes in TSS of symbiosis fermented soursop juice during storage.

Change of total sugar and reducing sugar content

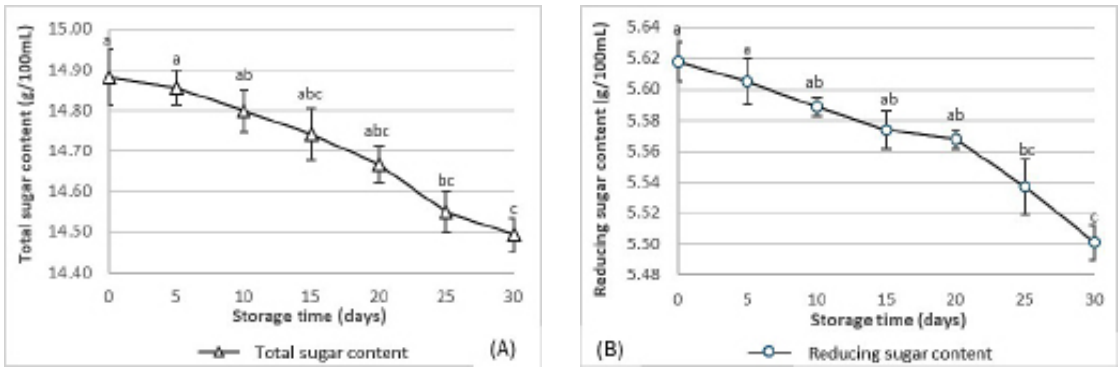

Fig. 4: Changes in certain physico-chemical factors of symbiosis fermented soursop juice during storage. (A) Total sugar content, (B) Reducing sugar content.

Evaluation of Some Quality Indicators of Soursop Juice Products after 30-Day Storage Time

Table 1: Physicochemical and microbiological characteristics of soursop juice during 30-day storage period

Days	Physicochemical parameters			Microbiological parameters			
	Vitamin C (mg/100 mL)	Total phenolic content (mg GAE/100mL)	Ethanol content (%)	Viable cell counts (1x 10^1 CFU/mL)	Lactic acid bacteria (CFU/mL)	Coliform (CFU/mL)	Yeast and mold (1x10^1 CFU/mL)
Day 0	29.26 ± 0.79^a	95.35 ± 0.64^a	0.42 ± 0.01^a	Not detected	Not detected	Not detected	Not detected
Day 5	27.84 ± 0.26^{ab}	93.18 ± 0.31^b	0.43 ± 0.02^{ab}	Not detected	Not detected	Not detected	Not detected
Day 10	24.99 ± 0.39^b	92.10 ± 0.34^c	0.44 ± 0.02^{abc}	0.10 ± 0.02^a	Not detected	Not detected	0.08 ± 0.03^a
Day 15	20.79 ± 0.30^c	90.23 ± 0.29^d	0.44 ± 0.01^{abc}	0.24 ± 0.05^b	Not detected	Not detected	0.17 ± 0.03^b
Day 20	17.73 ± 0.42^d	86.38 ± 0.41^e	0.45 ± 0.01^{abc}	0.37 ± 0.04^c	Not detected	Not detected	0.32 ± 0.02^c
Day 25	17.22 ± 0.46^e	83.47 ± 0.22^f	0.47 ± 0.00^{bc}	0.51 ± 0.03^d	Not detected	Not detected	0.40 ±0.02^d
Day 30	16.43 ± 0.41^f	79.74 ± 0.44^g	0.47 ± 0.02^c	0.63 ± 0.04^e	Not detected	Not detected	0.45 ± 0.02^e

* Mean sharing different letters in the same column are significantly different ($p < 0.05$).

Table 2: Sensory quality of soursop juice during 30-day storage period on a nine-category hedonic scale

Parameters	Results at with respect to days						
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Color	7.01 ± 0.21^a	7.03 ± 0.17^a	7.01 ± 0.31^a	6.87 ± 0.18^a	6.71 ± 0.24^a	6.53 ± 0.28^a	6.59 ± 0.32^a
Apperance	7.21 ± 0.19^a	7.15 ± 0.33^{ab}	7.11 ± 0.30^{ab}	7.02 ± 0.20^{ab}	7.00 ± 0.40^{ab}	6.87 ± 0.22^{ab}	6.65 ± 0.27^a
Aroma	7.53 ± 0.16^a	7.44 ± 0.25^a	7.23 ± 0.28^{ab}	7.17 ± 0.17^{abc}	7.04 ± 0.16^{bc}	6.84 ± 0.29^c	6.53 ± 0.21^d
Taste	7.88 ± 0.35^a	7.72 ± 0.19^a	7.78 ± 0.25^a	7.69 ± 0.30^a	7.41 ± 0.16^a	7.33 ± 0.37^a	7.24 ± 0.31^a
Overall acceptability	7.41 ± 0.46^a	7.40 ± 0.26^a	7.23 ± 0.20^a	7.20 ± 0.29^a	7.09 ± 0.32^a	7.12 ± 0.25^a	7.01 ± 0.37^a

* Mean sharing different letters in the same row are significantly different ($p < 0.05$).

* Sensory scores corresponding to: 1= extreme dislike, 2 = very much dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = moderately like, 8 = very much like, 9 = extremely like

Discussion

Changes in Some Physicochemical Parameters of the Product during Storage.

Change of pH and TTA content

According to the production process of fermented soursop juice, various stages—namely fermentation, pasteurization, and storage—induce changes in the pH and total titratable acidity (TTA) due to distinct mechanisms. The fermentation stage increases the TTA value as a result of organic acid production, and decreases the pH due to the interaction between CO_2 and water, which releases hydrogen ions (H^+) and bicarbonate ions (HCO_3^-), facilitated by microorganisms, particularly *L. plantarum* and *S. bayanus*.[53] The pasteurization stage slightly lowers the pH, as the elevated temperature accelerates the hydrolysis of pectin molecules, producing acidic compounds such as galacturonic acid.[42] After pasteurization, the activity of both microorganisms and enzymes is largely restricted, resulting in a low microbial activity product.[54] Consequently, the cold storage stage stabilize the TTA and pH over time by slowing and limiting microbial activity. However, cold storage does not prevent certain natural chemical reactions, for instance, the hydrolysis of pectin[42] and sugar,[55] as well as the oxidation of vitamin C,[56] which release acidic by-products, further lowering the pH and increasing the TTA.

The variable statistics in Figure 2 indicated the physicochemical stability of symbiosis fermented soursop juice under 4°C storage during 30 days. Both

pH and TTA content altered in a marginal amount to those determined right at the end of technical fermentation process (day 0), which demontrated the stability throughout the storage condition at cool temperature. Specifically, after 30 days of storage, the pH value of the product decreased from 3.82 ± 0.04 to 3.66 ± 0.02 (a decrease of 0.16 pH index) while TTA value increased slightly from 0.468 ± 0.005 to 0.529 ± 0.015 (an increase of 0.061%), indicating a statistical difference. However, these statistical variation was in slight amount and barely noticeable, confirming stability of multi-strain fermented soursop juice quality during storage.

As the research of Maia *et al.* (2021), pH index and titratable acidity was a crucial indicator for the quality of a product. TTA content or pH fluctuations indicated certain decomposition reactions (such as oxidation, hydrolysis, and fermentation), which generated various substances that enhance the food acidity. Therefore, slightly alterarion in titratable acidity and pH during 30-day storage indicate storage stability, ensuring good product quality maintenance. This can be explained by the combination of effective pasteurization treatment, airtight packaging and low-temp storage condition, which is considered as the standard practices for high-acidity juices to maintain quality over significant period of time.[40]

Under identical pasteurization (65°C for 15 minutes) and storage conditions (refrigerated temperature of 4°C) for 30-day period, the unfermented soursop juice product according to the study of Ndife *et al.* (2014) had a pH value decrease of 0.6 (from 4.10 to 3.50), a decrease of 14.63%.[41] Meanwhile, our study showed that fermented soursop juice co-cultured of *L. plantarum* and *S. bayanus* strains merely decreased the pH value by 0.16 (from 3.82 to 3.66), accounting for 4.19%, showing a significant difference. This difference possibly due to during the fermentation process, the two starter strains competed with unwanted microorganisms and pathogens in the juice, reducing or even destroying their presence in the product, thereby making the pasteurization, packaging and preservation process more effective. Thus, it emphasized the positive effect of symbiosis or co-culture fermentation in combination with pasteurization on stability of soursop juice during storage.[28]

Change of TSS

The evaluation of TSS in Figure 3 indicated the physicochemical stability of symbiosis fermented soursop juice under 4°C storage during 30 days storage. When storing apple juice for 41 days, the study of Karaman *et al.* (2020) detected slight alterations in both pH and Brix for acidic juices with a pH below 4.3.[43] The durability of TSS in litchi juice storage for 30 days was similarly demonstrated by Babbar *et al.* (2015).[44] The results of the above researches were comparable to those of current soursop juice study, showing that the TSS reaction was stable when the storage period was increased to 30 days at low temperature (4°C).

Specifically, during the 30-day storage period, the TSS of the product decreased from 15.40 ± 0.00 to 15.10 ± 0.02, a decrease of a small amount 0.3° Brix corresponding to the reduction of 1.95%. This further emphasized the superiority of the symbiosis fermentation process's influence on the stability of physicochemical parameters when applying the same sterilization, packaging and storage conditions. There is another study of on pasteurized soursop juice showed a equivalent reduction in TSS by around 0.5°Brix after 4 weeks of storage at 4°C.[28] While in comparison with unfermented soursop juice as the study of Ndife *et al.* (2014), the 4-week storage period reduced the TSS value by up to 2.3° Brix corresponding to 16.06% (from 14.32°Brix at beginning to 12.02°Brix after 4 weeks).[41]

Change of Reducing Sugars and Total Sugars Content

According to Erkmen and Bozoglu (2016), the rate of substrate degradation (mainly sugars when fermented with LAB and yeast strains) is also affected by storage temperature and could be better controlled when stored at low temperature. Many studies have shown that temperature changes in foods during low temperature storage can have a greater impact on the growth, sublethal injury and death of micro-organisms as well as limit enzymatic changes in food products. Refrigeration below 5°C effectively slows the growth of many foodborne pathogens.[45]

In this study, the combined fermented soursop juice was applied with a combination of mid-heat pasteurization and low temperature storage. With the

combined fermentation process, the overwhelming growth of *L. plantarum* and *S. bayanus* strains somewhat inhibited the activity of undesirable microorganisms. Then, the soursop juice underwent a 65°C pasteurization process lasting 15 minutes to inactivate the microorganisms and the cold storage regime will help prolong the product's shelf life remains safe and fresh for consumption. However, with the incomplete destruction of microorganisms residing in the juice despite mid-heat pasteurization and combined low temperature storage at 4°C, throughout the long storage process, the growth of microorganisms, especially yeast, possibly initiated the fermentation process for metabolic purposes leading to a decline in juice samples' sugar content.

Specifically, obtaining from Figure 4, during the 30-day storage period, the reducing sugar of the product decreased from 5.618 ± 0.013 to 5.501 ± 0.011, a decrease of 0.117 g/100mL corresponding to 2.08%. Meanwhile, the total sugar content of the product decreased from 14.881 ± 0.069 to 14.493 ± 0.042, a decrease of 0.388 g/100mL corresponding to 2.61%, a relatively small decrease. The results show consistency with the study of Jerry *et al.* (2019) about unfermented soursop juice pasteurized at 83°C, reducing the total soluble solids content of 0.59° Brix from 16.10 to 15.51°Brix,[59] a moderately small amount. This further emphasizes the outstanding influence of the symbiotic fermentation procedure in term of the stability of physicochemical parameters during the storage process, even though this symbiotic fermented juice product was pasteurized at lower temperature (65°C).

Evaluation of some Quality Indicators of Soursop Juice after 30-day Storage Time

In the combination of our previous study[18] of cocultured soursop juice using 2 strains, *L. plantarum*, *S. bayanus* and the additional analyses on days 0 and 30 of storage under refrigerated conditions (4°C) after pasteurized treatment (65°C - 15 minutes), the index of physicochemical, microbiological and sensory quality attributes is indicated and abridged in Table 1 and 2.

Vitamin C is a high sensitivity compound, which is highly degraded by external impacts including oxygen, light, metal ions, processing and temperature during storage. Therefore, undergoing pasteurization treatment at 65°C and despite being stored at low temperature (4°C) still showed a negative impact on vitamin C content, which gradually decreases with storage time. The most common degradation pathway of vitamin C is oxidation to form dehydroascorbic acid, which further breaks down into other acidic compounds, such as oxalic acid, increasing TTA values, negatively affecting the quality of fruit juice products during prolonged storage.[55] Indeed, the vitamin C content decreased by 43.8% from 29.26 ± 0.79 to 16.43 ± 0.41 mg/100mL. This outcome corresponds to other research that demonstrated the detrimental effects of pasteurization duration and temperature on fruit juices' vitamin C content.[46] According to the research of Feszterová *et al.* (2023) with grapefruit and mandarin as the research subjects, these juices were studied in comparison of the post-processing preservation regime at 3 temperatures of 4°C, 23°C and −18°C during 21 days in glass bottles.[56] At a refrigerator temperature of 4°C, it was shown that for grapefruit juice, with an initial vitamin C content of 34.50 mg/100g, after 21 days of storage, it decreased to 22.60 mg/100g (34.49%), mandarin juice reduced a large amount of vitamin C content up to 52.17% from 23.00 to 11.00 mg/100g. At a storage temperature of 23°C, the vitamin C content of grapefruit juice decreased to 22.40 mg/100g (35.07%) and that of mandarin juice decreased to 7.00 mg/100g (69.56%). At a storage temperature of −18°C, the vitamin C content of grapefruit juice decreased to 33.33% and that of mandarin juice decreased to 60.86%. The results showed that the most suitable temperature to ensure the vitamin C value for 24 hours seemed to be the refrigerator temperature (4°C), followed by the freezer temperature (−18°C) and room temperature (23°C) as the most unfavorable condition, showing the similar to the storage temperature condition of symbiosis soursop juice of our study.

During the 30-day storage period, a decrease in phenolic compounds was observed, from 95.35 ± 0.64 to 79.74 ± 0.44 (only 16.37%), demonstrating the relative thermal stability (pasteurization and storage temperatures) of these compounds. The decrease in total phenolic content was triggered by oxidative cleavage of phenolic compounds and their cross-linking with proteins,[23] leading to polyphenol precipitation during storage, which is facilitated by the low pH of the product.

The symbiosis fermentation of soursop juice with a mixture of *L. plantarum* and *S. bayanus* produces ethanol as one of the end products. After 72 hours fermentation, the ethanol content of the product was 0.42±0.01 %, after sterilization treatment at 65°C for 15 minutes and storage at 4°C for 30 days, the product had a slight increase in ethanol content, up to 0.47±0.02 %. The increase in ethanol content can be explained by the effect of pasteurization temperature which accelerated the hydrolysis of esters in fermented soursop juice during storage, resulting in more organic acids and ethanol released in the product.[47] Supported by Patricia research, acetate ester, which is commonly found in soursop, hydrolyzed faster than other ethyl esters.[48]

Along the hydrolysis of ester under the simultaneous effect of pasteurization temperature, a lower pH and significant water availability during the 30-day storage period, although the ethanol concentration did not increase significantly (only 0.05%), the overall balance of flavor and aroma may change, as the fruity and floral aroma brought by ester may decrease, causing the sensory acceptance score of the product to decrease significantly, to 7.01 ± 0.37, a decrease of 0.4 points compared to the product after fermentation (reaching 7.41 ± 0.46).[49] Similarly, other sensory parameters, including appearance and aroma, exhibited a significant decline over the 30-day storage period, with scores decreasing by 0.56 and 1.00 points, respectively, to 6.65 and 6.53, indicating a 'slightly liked', compared to day 0. However, refrigerated storage did not result in significant changes to the color attribute of the soursop juice. Furthermore, the sensory score for flavor parameter showed a statistically significant difference between days 0 and 30, though it remained at 'moderately liked', with scores of 7.88 ± 0.35 and 7.24 ± 0.31, respectively.

Microbiological evaluation is vital in assessing the product quality and safety. Microbial growth and the presence of pathogens required to be analyzed since they tend to result in food spoilage and other food security risks during storage to reach consumers.[50] Meanwhile, soursop is a climatic fruit with a short shelf life. Along with the physical properties of the soft fruit skin, soursop is therefore very susceptible to contamination by undesirable microorganisms. A study by Vwioko *et al.* (2013) on soursop juice stored at ambient temperature for 28 days reported the isolation of yeast strains *Saccharomyces cerevisiae*, *Candida tropicalis* consistently during storage. Bacterial strains included *Bacillus* sp., *Acetobacter aceti*, *Staphylococus* sp. appeared sporadically throughout the study, and molds including *Apergillus niger* and *Penicillium chrysogenium* were also present at 20% and above incidence.[26] Fungi were observed to be more dominant in occurrence than bacteria species from the study.

Currently, there have been many reports indicating that soursop juice can be microbial safely maintained by pasteurization alone[50] or can be specially preserved by adding preservatives both natural such as lime,[27] ginger,[51] garlic[26] extracts, and synthetic such as sodium metabisulphite[52] or sodium benzoate.[25] Indeed, by applying a combination of mid-heat pasteurization and low temperature storage (4°C), our study showed promising results of a product that is confirmed microbiologically safe for consumption. The total viable cell count of co-cultured fermented soursop juice was 0.63×10^2 CFU/mL which is lower than pasteurized soursop juice (10^2 CFU/g) stored at 4°C.[28] Regarding mold and yeasts, the result was 0.45×10^2 CFU/mL and lactic acid bacteria and coliforms at undetectable levels, met the microbiological standard requirement for non-alcoholic beverages (alcohol content below 0.5% ABV) of the Vietnamese Ministry of Health which is below 1×10^2 CFU/mL (QCVN 6-2:2010/BYT). This compliance is a combination of hygiene issues that have been ensured during the processing and packaging of the product, where coliforms are a sign of unhygienic conditions, unhygienic activities during or after production and poor quality of water sources used. In addition, the more important thing is that the multi-strain fermentation process contributed to the rapid growth of desirable microorganisms, or starter strains, in the juice product, and suppressed other undesirable food spoilage microorganisms. The co-cultured of yeast and LAB created a relatively acidic juice product with the presence of carbon dioxide, ethanol, and organic acids generated thoughout the fermentation process helped to increase the bacteriostatic and bactericidal activity of the pasteurization process[24] during the 30-day storage period at low temperature. The results of these microbiology parameters show certain similarities with Lagnika *et al.* (2017) study on pasteurized pineapple juice[57] under mild heat pasteurization (65°C, 15 minutes) condition. On the

30th day of storage, the total viable cell count was $2x10^1$ CFU/mL, the total yeast count was $2.8x10^1$ CFU/mL and the total mold count was $2.5x10^1$ CFU/mL, indicating that the combination of pasteurization and refrigerated storage was found to be effective in slowing the growth of microorganisms in pineapple juice. The effectiveness in ensuring microbiological quality was also observed in the study by Nguyen *et al.* (2019) on unfermented soursop juice pasteurized at 95°C, when ensuring microbiologically safe for consumption with a total viable cell count of $1.1x10^1$ CFU/g and no mold and coliform detected when stored at refrigeration (4°C) for 6 weeks.[60]

Conclusion

Soursop, a tropical fruit with notable nutritional and biological potential, was fermented through symbiotic fermentation of *L. plantarum* and *S. bayanus* strains, yielding a product with high nutritional value and favorable organoleptic properties. The juice exhibited minimal physicochemical changes during storage, suggesting stability beyond 30 days. Sensory evaluation showed no significant quality deterioration, likely due to reduced chemical, enzymatic, and microbial activity in cold storage. Microbiological analysis showed the undetectable levels of lactic acid bacteria and coliforms, along with low total viable cell counts, yeast and mold counts, confirming the microbiological safety of the soursop juice for consumption. Future research should explore packaging materials, pasteurization with non-thermal treatments, and the impact of storage on key bioactive compounds, particularly annonaceous acetogenins. Additionally, chemical additives for enhanced stability and shelf life should be investigated.

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Author Contributions

- **Dr. Nguyen Thi Hanh:** Conceptualization, Methodology, Writing – Original Draft, Review, Editing.
- **Nguyen Ngoc Cham:** Data Collection, Analysis
- **Nguyen Thi Trang:** Data Collection, Data Curation
- **Cao Thi Ngoc Anh:** Data Collection

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