Hindawi
Journal of Food Processing and Preservation
Volume 2024, Article ID 8841207, 17 pages
https://doi.org/10.1155/2024/8841207

Research Article

Effect of Fermentation Conditions (Dilution Ratio, Medium pH, Total Soluble Solids, and *Saccharomyces cerevisiae* Yeast Ratio) on the Ability to Ferment Cider from Tamarillo (*Solanum betaceum*) Fruit

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Received 8 November 2023; Revised 22 January 2024; Accepted 25 January 2024; Published 2 February 2024

Academic Editor: Prakash Bhuyar

Tamarillo (*Solanum betaceum*) is a nutrition-rich product containing antioxidant components and preventive properties against cancer risk. However, there is currently a scarcity of research on processing techniques to diversify products and extend the preservation time of the active compounds in tamarillo. In this study, we focused on developing a cider processing procedure from tamarillo by fermentation with *Saccharomyces cerevisiae* (Angel RV002). Fermentation conditions, such as the dilution ratio (66 : 34–34 : 66%, *w*/*w*), medium pH (3.5–5), total soluble solids (TSS 10–26°Brix), and yeast ratio (0.6–1.2 g/L) were investigated. Ethanol concentration, pH, TSS, titratable acidity, total sugar content, and reducing sugar content were evaluated from day 0 to day 5 of fermentation. At a 50 : 50 (%, *w*/*w*) dilution ratio of the tamarillo juice with water, pH 4.5, TSS of 22°Brix, and the addition of yeast 0.6 g/L to the fermentation process, the ethanol concentration reached 7.54 ± 0.11 (%, *v*/*v*) after 4 days of fermentation. Additionally, the product maintained a moderately low pH (pH 4.16). The final product exhibited a high sugar content and dissolved nutrients. The results of this study are expected to serve as a basis for the production of tamarillo cider, contributing to the diversification of the product, enhancing the value of tamarillo, and promoting economic development in the region of cultivation.

1. Introduction

Tamarillo (*Solanum betaceum*) is a small tree native to South America. New Zealand, Ecuador, Argentina, and southern Mexico are common locations where this fruit is cultivated and grown [1]. Tamarillo is available in three varieties: Laird's Large, Amber, and Mulligan [2]. As a subtropical fruit, tamarillo has a unique flavor and color and is nutritionally rich in nutrients such as vitamin A (540–2475 IU), protein (1.5–2.5 g/100 g), and vitamin C (19.7–57.8 mg/100 g) and minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, and zinc [1, 3]. Total phenolic content (2.4–6.2 g chlorogenic acid equivalents/100 gDW), carotenoids (4.91 mg/100 gDW), and anthocyanins (672 mg/100 gDW) are considered as the main bioactive components in tamarillo. Organic acids contribute to the distinctive flavor of tamarillo [3, 4]. A previous report highlighted the combination of components such as citric, itaconic, cis-aconitic, malic, and 4-toluic acid, which comprise more than 97% of the total organic acids measured in each sample, creating the distinctive flavor profile of tamarillo [5]. Simultaneously, certain volatile components within the tamarillo also contribute to the distinctive nature of this raw material, such as methyl butanoate (8.5%), methyl hexanoate (36.9%), methyl octanoate (0.2%),

(Z)-3-hexen-1-ol (1.6%), ethyl butanoate (0.55%), ethyl hexanoate (0.51%), and nonanal (0.27%) [6]. Tamarillo has many applications in weight management and improving health [4]. Extracts of polyphenols (such as vanillic acid, p-coumaric acid, and epigallocatechin-3-gallate) from plant sources, together with tamarillo and tomato peel, have shown efficacy in reducing the impact of cancer [3, 4]. Due to the benefits of tamarillo, there have been numerous studies aimed at diversifying tamarillo products in both the Vietnamese and international markets. For example, in Tamil Nadu (India), dried tamarillo fruit powder has been introduced as an ingredient in various products such as sauces, chili sauce, and soups and as a food option for children [7, 8]. In Indonesia, tamarillo yogurt was produced as a health-enhancing product [9].

Fermented fruit juice, also known as cider, is a product obtained by fermenting fruit juice. For the fermentation process, fruit juice is typically extracted directly and the fiber content of the fruit is removed. In addition, concentrated fruit juices can also serve as convenient raw materials for the fermentation process by reconstituting them [10]. Currently, fermented fruit products are a popular trend in consumption. Many previous studies have focused on fermenting various types of fruits, such as apples, to create a product known as "cider" [11], or there have been studies on fermenting pineapple fruit to produce fermented products [12]. Regarding tamarillo, some studies have reported on the lactic fermentation process to create yogurt products using this raw material as well [13, 14]. Currently, the process of fermenting tamarillo to create a "cider" product has not yet appeared in the Vietnamese market, in previous studies, and in a few other countries. The production of cider from tamarillo enhances product diversity in the market and broadens consumer choices. Additionally, the fermentation process extends the preservation of nutritional content in fresh fruit and intensifies its flavor profile. This addresses the commercial challenge of tamarillo's low market value and perishability due to insufficient timely consumption.

Furthermore, factors such as pH, temperature, yeast concentration, and sugar content can potentially improve or inhibit fermentation efficiency. This significantly impacts product quality and production economics. A previous report demonstrated that the fermentation process under conditions of pH 6, yeast concentration of 10% (*v*/*v*), and a temperature of 30°C for 48 hours resulted in high efficiency in the production of ethanol [15]. The sugar beet fermentation process has been reported to achieve optimal conditions at a yeast concentration of 0.2 g/L and a sugar concentration of less than 225 g/L, resulting in high ethanol productivity (>15 g/L/day) [16]. A yogurt fermentation process influenced by the concentration of carrot juice has also been reported. The results obtained indicate that increasing the concentration of carrot juice raises the pH of the mixture but decreases the formation of acid in the food after fermentation [17]. However, it has been observed that excessively high or low pH levels can reduce yeast activity. The influence of pH on ethanol production efficiency has also been previously reported. A medium containing a 30% total soluble solids and a pH of 5–5.5 has been shown to result in optimal ethanol production efficiency [18].

In the current study, a tamarillo cider production process has been proposed based on investigations into fermentation conditions such as the dilution ratio, pH of the fermentation medium, total soluble solids (TSS), and the participation of yeast in the fermentation process. The selection of these conditions is based on achieving a balance between parameters such as ethanol concentration, pH, TSS, titratable acidity, total sugar content, and reducing sugar content. The expected result of this study is to provide a foundation for the production of tamarillo cider, contribute to the diversification of the product, improve the value of tamarillo, and promote economic development in the cultivation region.

2. Material and Methods

2.1. Materials. About 90 kg tamarillo (Magic-S) was harvested in Lam Dong Province, Vietnam (11.5753°N, 108.1429°E). Fresh raw materials need to meet the standards of juicy ripening (21–24 weeks) and glossy skin, with a characteristic smell and aroma. Tamarillo was transported to Nguyen Tat Thanh University and were stored at 4°C with maximum time being 4 days.

2.2. Chemicals and Additives. Sodium hydroxide (NaOH, ≥96%), hydrochloric acid (HCl, 35–36%), 3,5-dinitrosalicylic acid ($C_7H_4N_2O_7$, DNS, ≥98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%), and Folin-Ciocalteu (FCR, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Some additives such as citric acid ($C_6H_8O_6$, >99%; moisture content < 0.3%), sugar ($C_6H_{12}O_6$, >99.5%; moisture content < 0.1%; and RSC < 0.1%), and *Saccharomyces cerevisiae* RV002 (Angel) were purchased from Vietnam.

2.3. Fermentation. Tamarillo was washed with water to remove dirt and surface microorganisms, and the skin was immediately removed. The fruit was then crushed, excluding the seeds, and the seeds were manually squeezed without breaking them. The tamarillo mixture was diluted with water at ratios of 66 : 34, 50 : 50, 40 : 60, and 34 : 66 (tamarillo/water (%), *w*/*w*). Citric acid ($C_6H_8O_7$) and Na_2CO_3 were used to adjust the pH of the mixture to a range of 3.5–5. Refined sugar was used to adjust the total soluble solids (TSS) of the medium to a range of 10–26°Brix. The pectinase enzyme (0.2%) was used for hydrolysis, and $Na_2S_2O_5$ (0.05 g/L) was used for sterilization for 24 hours at a temperature of 37 ± 2°C. The mixture was then fermented using the yeast strain *Saccharomyces cerevisiae* RV002 (Angel) for 1 to 5 days at a temperature of 37 ± 2°C. The yeast was activated at a concentration of 0.6–1.2 g/L for 30 minutes at 38 ± 1°C. The sample was filtered through a filter cloth with a 0.05 mm mesh size to remove residual sediment and microbial remains. Afterwards, the sample was left to settle in stable conditions at a temperature of 18 ± 2°C. The fermented tamarillo water was then bottled and sterilized (Figure 1).

2.4. Determination of Ethanol Concentration. The ethanol concentration was determined using a density meter (Snap 51, Anton Paar, US) after simple distillation of the sample.

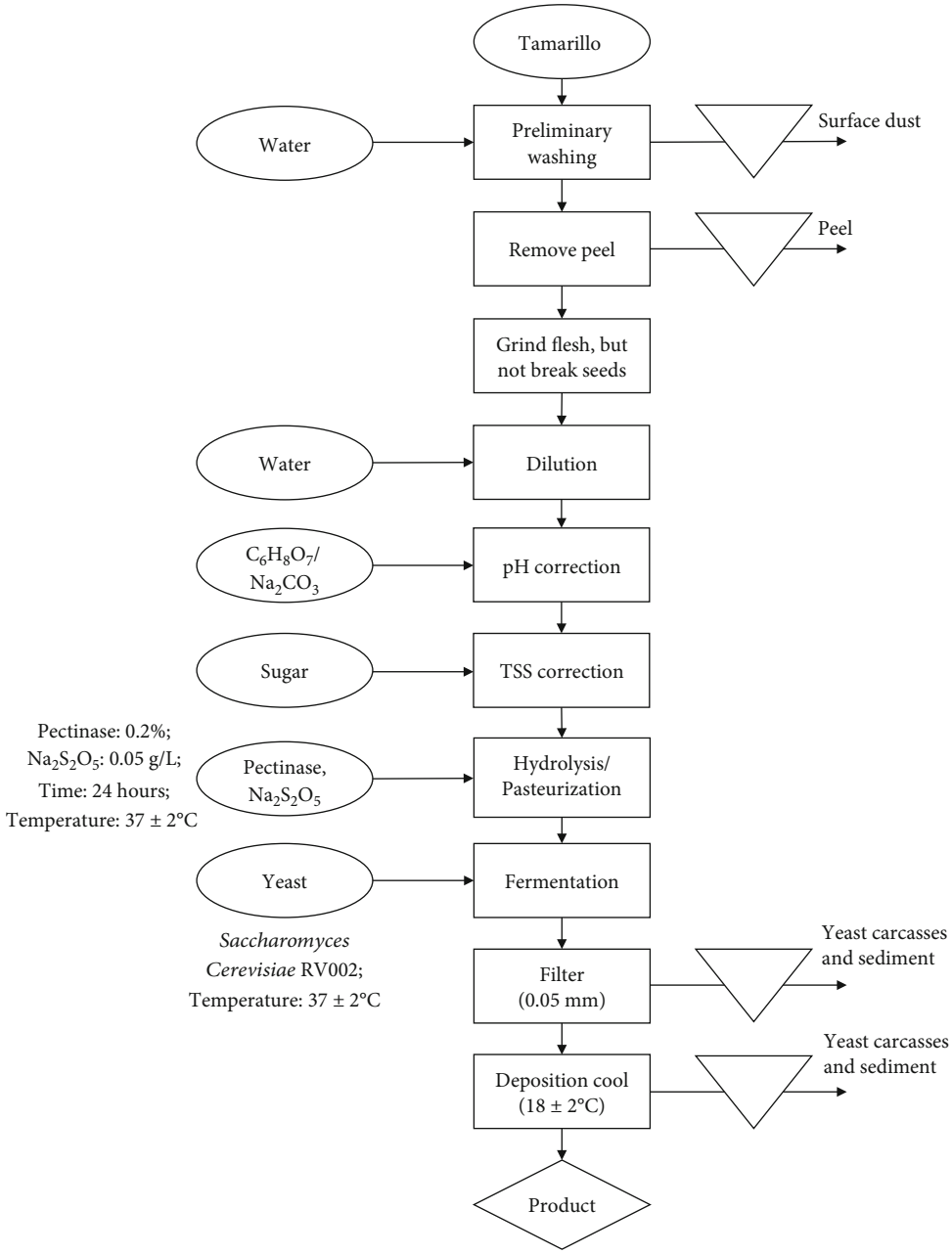

Figure 1: Process of tamarillo fermentation.

2.5. Determination of Total Soluble Solids. The total soluble solids were determined using a refractometer (W/ ATC 0-32:0.2% Brix 95000-022, Alla France, France) based on the principle of light refraction after passing light through the sample.

2.6. Determination of pH. pH measurements were determined using the HI2211 pH/ORP meter manufactured by Hanna Instruments Ltd. [19].

2.7. Determination of Titratable Acidity. The original sample was centrifuged at a speed of 5000 revolutions per minute (rpm), and the supernatant, which does not contain sediment, was diluted fivefold. Subsequently, it was titrated using a 0.1 N sodium hydroxide (NaOH) solution with the assistance of an Eco Titrator, an electrochemical titration device from Switzerland.

2.8. Determination of Total Sugar Content. Each 1 mL of the original sample was mixed with 40 mL of 2% HCl and heated at a temperature of 110°C for 60 minutes. The sample mixture was rapidly cooled and adjusted to a pH of 7 using 10% NaOH and 2% HCl. The mixture was then made up to a volume of 100 mL with distilled water and filtered through a Whatman filter paper (11 μm). The filtered solution was diluted 3–5 times (depending on the sugar concentration in the sample). Each 1 mL of the diluted sample was mixed with 2 mL of DNS solution and boiled for 5 minutes,

followed by cooling. The absorbance at a wavelength of 540 nm was then measured.

The following is the standard equation: $y = 2.8537x - 0.1206$ ($R^2 = 0.9999$).

2.9. Determination of Reducing Sugar Content. Each 10 mL of the original sample is mixed with 50 mL of distilled water. The mixture is stirred thoroughly and filtered through Whatman filter paper (11 μm). The filtrate is further diluted 3-5 times (depending on the sugar concentration in the sample). Every 1 mL of the diluted solution is mixed with 2 mL of DNS solution. The mixture is heated in a water bath for 5 minutes and then allowed to cool. Subsequently, the solution is measured at a wavelength of 540 nm.

The following is the standard equation: $y = 2.8537x - 0.1206$ ($R^2 = 0.9999$).

2.10. Determination of Total Polyphenol Content. A 5 g sample was ground using a blender and then filtered through a filter towel (210 μm) and filter paper (11 μm). Subsequently, a 0.1 mL sample was taken and mixed with 0.5 mL of 10% Folin-Ciocalteu solution and 0.4 mL of 7.5% Na_2CO_3 in an incubation tube. The mixture was vigorously shaken and left to incubate in the dark for 1 hour. After incubation, the optical measurement was performed at a wavelength of 765 nm, and the results were compared with the standard curve of gallic acid for quantification [20].

2.11. DPPH Radical Scavenging Activity. A 5 g sample was ground in a blender with 100 mL of alcohol, followed by filtration through filter towels (210 μm) and filter paper (11 μm). Subsequently, a 0.5 mL portion of the resulting mixture was combined with 1.5 mL of DPPH solution. The mixture was then incubated at $30 \pm 2^{\circ}$C for 30 minutes under low-light conditions and subsequently measured at a wavelength of 517 nm [20].

2.12. Determination of Total Ascorbic Acid. The total ascorbic acid was determined based on the oxidation of ascorbic acid to dehydroascorbic acid with 2,6-dichlorophenolindophenol (DCPIP). DCPIP will be converted to a colorless leuco derivative. The optimal reaction occurs at pH 3-4, where an excess drop of blue DCPIP in this environment turns the solution pink. Each 5 grams of the sample was ground in 100 mL of distilled water and filtered through a cloth filter (210 μm) and a filter paper (11 μm). Subsequently, 10 mL of the filtrate was mixed with 1 mL of 0.04% HCl and titrated with a DCPIP solution [21].

2.13. Statistical Analysis. In this study, each experiment was repeated three times, and the results were presented as the mean value ± standard deviation. The data were computed using Microsoft Office Excel 2016. The ANOVA was conducted with a confidence level of 95% to compare the differences among the treatments using the LSD test. Graphs were plotted using Origin 9 [22].

3. Results and Discussion

3.1. Tamarillo Components. Geographical differences can impact the physicochemical characteristics of plants. The pH value found in the red tamarillo harvested in Vietnam was determined to be pH 3.94 ± 0.06. This result aligns with a general understanding of the pH values of New Zealand tamarillo varieties, which range from pH 3.7 to 4.1 (Table 1). Specifically, the red tamarillo variety was recorded with a pH of 3.80 ± 0.01 [23]. However, the pH of Ecuador and Spain tamarillo has been found to be lower, ranging from pH 3.2 to 3.6 [24]. On the other hand, Wang and Zhu reported similar findings regarding the total soluble solid (TSS) content in the red tamarillo, which ranged from 10.9 to 12.1°Brix (fresh basis) [4]. However, TSS of the red tamarillo of New Zealand has been found to be lower, with a TSS value of 10.6°Brix (fresh basis) [23]. Vasco et al. also reported on the TSS content in purple-red tamarillo harvested in Ecuador and Spain, which ranged from 11 to 12°Brix [24]. In terms of reducing sugar content (RSC), tamarillo harvested in Vietnam has been found to be lower compared to Whangarei fruits in the Northland region of New Zealand. In the New Zealand red tamarillo, the RSC was reported to be 34.4 ± 2.1 mg/gDW, while the red tamarillo harvested in Vietnam only reached 19.36 ± 0.28 mg/gDW [23]. In Vietnam, the total sugar content (TSC) in red tamarillo is lower compared to the varieties of red tamarillo worldwide, reaching only 24.51 ± 0.12 mg/gDW. On the contrary, TSC ranging from 28.1 to 52.0 mg/gDW has been found in tamarillo of 23 different varieties (both yellow and red) and in various countries [4].

Regarding antioxidant activity, Vietnam's tamarillo contains 68.04 ± 0.05 mgAA/100 gFW (fresh weight). However, according to Vasco et al., the TAA in yellow and red tamarillo harvested in Ecuador ranged from 16 to 24 mg/100 gFW [25]. In another country, the tamarillo harvested from New Zealand, as reported by Lister et al., showed higher TAA compared to the raw material harvested from Ecuador. In the yellow tamarillo, it was found that TAA was 24.7 and 31 mg/100 gFW, while in the red tamarillo, it was 34.3 and 29.8 mg/100 gFW [26]. Previous studies have reported evaluations of the TAA content in tamarillo from various countries. In the Argentinian tamarillo, the average TAA in the fruit was 153 mg/100 gFW [27]. In the Malaysian tamarillo, 8.27 mg/100 gFW of TAA was found [28].

In addition, polyphenols are also key components with antioxidant activity. The red tamarillo from Vietnam has been found to contain 164.35 ± 0.01 mgGAE/100 gDW (milligrams of gallic acid equivalents per 100 grams of dry weight) of the total polyphenol content (TPC). On the other hand, TPC in yellow tamarillo from Ecuador ranged from 308 to 557 ± 14 mgGAE/100 gDW, depending on the specific extraction method used [24, 29]. For the same yellow tamarillo variety, but harvested from New Zealand, it has been found to have a TPC twice as high as the yellow tamarillo variety grown in Ecuador, with a value of 1060 mgGAE/100 gDW [26]. For the red tamarillo variety from Ecuador, it has been reported to have a TPC two to three times higher than that of the yellow tamarillo variety, with corresponding values ranging from 570

Table 1: Tamarillo components in Vietnam.

No.	Criteria	Results	Units
1	pH	3.94 ± 0.06	—
2	Total soluble solids	11.00 ± 0.01	°Brix
3	Reducing sugar content	19.36 ± 0.28	mg/gDW
4	Total sugar content	24.51 ± 0.12	mg/gDW
5	Total ascorbic acid	68.04 ± 0.05	mgAA/100 g
6	Total polyphenol content	164.35 ± 0.01	mgGAE/100 g
7	DPPH radical scavenging activity	64.66 ± 0.01	% IC_{50} (μg/mL)

to 1413 ± 50 mgGAE/100 gDW [24, 29]. However, the red tamarillo variety from New Zealand has reached a TPC of 1564 mgGAE/100 gDW, which is only half of the TPC of the yellow tamarillo variety [26]. Indeed, multiple research groups have reported TPC in red tamarillo harvested in various countries. In Argentina, TPC in red tamarillo was found to be 2314 ± 357 mgGAE/100 gDW [27]. The red tamarillo variety from Malaysia has been reported to have a TPC ranging from 183 to 763 ± 50 mgGAE/100 gDW, depending on the specific extraction method used [28, 30]. In Taiwan, the red tamarillo variety has been found to have a significantly higher TPC compared to other countries such as Argentina, New Zealand, Ecuador, and Malaysia. TPC in the Taiwanese red tamarillo is reported to be 2.6 to 8.01 times higher, with a value of 6110 ± 10 mgGAE/100 gDW [31].

A report has indicated that the antioxidant activity of red tamarillo, evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, was measured at 47.38 μg/mL. Furthermore, red tamarillo was observed to exhibit higher antioxidant activity compared to red tamarillo harvested in Vietnam [30].

3.2. The Influence of the Dilution Process on Product Quality during Fermentation

3.2.1. Ethanol Concentration. The dilution of fruit juice prior to fermentation is an important method in the production of alcoholic beverages. The optimal activity of yeast is primarily dependent on the pH, temperature, and sugar content present in the fermentation medium [15]. Diluting the fruit juice facilitates the stabilization of TSS and pH of the mixture using refined sugar, creating a stable, homogeneous, and ideal medium for yeast development during fermentation [17]. Furthermore, when fruit juice is diluted, yeast can easily access nutrients, resulting in improved fermentation efficiency [16]. In this study, dilution ratios of 66 : 34, 50 : 50, 40 : 60, and 34 : 66 (%, w/w) between fruit juice and water were investigated to examine the appropriate dilution level for the tamarillo fermentation process.

The dilution ratio of tamarillo juice and water significantly affected the ethanol concentration of the product ($p < 0.05$). The difference became more pronounced with increasing fermentation time from day 1 to day 5 ($p < 0.05$) (Figure 2). After 1 day of fermentation, the ethanol concentration tended to increase rapidly, with the highest increase observed in the 34% tamarillo juice and the 66% water dilution ratio (34 : 66%, w/w), reaching the 3.15 ± 0.03% ethanol concentration. No significant differences were observed among the remaining three dilution ratios ($p > 0.05$), as shown in Figure 2. However, significant differences were observed among the dilution ratios from day 2 onward ($p < 0.05$). The increase in ethanol concentration rate for the 66 : 34 (%, w/w) dilution ratio showed no significant changes during the 5-day period ($p > 0.05$). A lower water content in the mixture corresponded to higher TSS, so a lower amount of sugar was added to reach a TSS of 10°Brix. A low sugar content in the fermentation mixture was a disadvantageous factor for the conversion of sugar to ethanol [32]. On the other hand, a higher concentration of tamarillo juice in the mixture corresponded to higher concentrations of dense components, which hindered the accessibility of nutrients and sugars in the mixture [33]. However, the fermentation time from day 1 to day 4 tended to be positively correlated with the ethanol production capacity for all three fermentation processes with the remaining dilution ratios. This was due to the higher amount of sugar added, which increased the conversion of sugar by yeast into ethanol [34]. Furthermore, the ethanol concentration of the mixture containing 50% tamarillo juice and 50% water (50 : 50%, w/w) remained unchanged after 4 days of fermentation, as glucose and fructose levels in the juice were depleted, resulting in a minor conversion of sugar to ethanol ($p < 0.05$) [12]. Both the 40 : 60 and 34 : 66 (%, w/w) dilution ratios contained a high sugar content, and the ethanol concentration tended to continuously increase after 5 days of fermentation due to the yeast sugar conversion. The fastest increase in ethanol concentration was observed in the 34 : 66 (%, w/w) dilution ratio, reaching the highest ethanol concentration on day 5 (7.15 ± 0.08%). At this dilution ratio, the mixture contained a high sugar content, which reduced nutritional competition among the yeast and increased the rate of sugar conversion to ethanol [35].

3.2.2. Titratable Acidity and pH. Changes in TA and pH of the fermentation medium directly affect the growth and efficiency of yeast fermentation [36]. If the TA and pH of the fermentation medium are outside the optimal range for yeast, it can inhibit their activity or even lead to the death of yeast. Additionally, uncontrolled acidity and pH can affect the flavor or quality of the final product. Therefore, maintaining appropriate levels of TA and pH is crucial to the successful fermentation process and the desired sensory characteristics of the product [37].

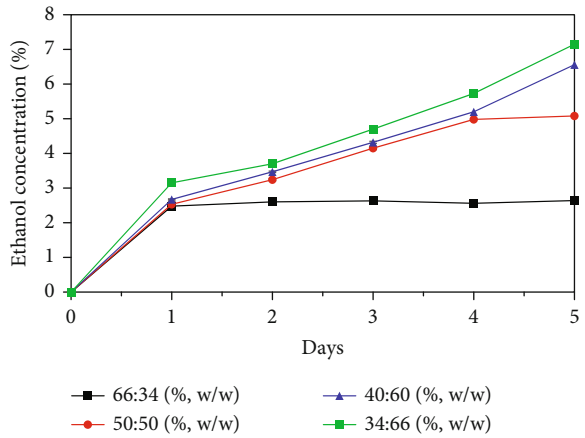

FIGURE 2: Effect of dilution ratio and fermentation time on ethanol concentration.

The dilution ratio had a significant influence on the variation in titratable acidity (TA) during fermentation time ($p < 0.05$) (Figure 3). At all dilution ratios, TA showed a rapid increase from day 1 to day 4 and tended to decrease beyond day 4. However, the different dilution ratios significantly affected the rate of increase in TA over time ($p < 0.05$). The initial difference in TA on day 0 could be explained by the higher water content and the higher pH in the diluted tamarillo mixture. Citric acid was added to adjust the pH to 4.5. Furthermore, a higher water content in the mixture led to a higher amount of added sugar to reach TSS 10°Brix. After 1 day of fermentation, the increased rate of TA between dilution ratios was not significant ($p < 0.05$). However, from day 2 of fermentation, the 34 : 66 (%, *w*/*w*) dilution ratio exhibited a significantly faster increase in TA compared to the other dilution ratios, reaching the highest values on day 3 (2.65 ± 0.09 g/L) and day 4 (2.78 ± 0.23 g/L). The rapid increase in TA was attributed to the high sugar content in tamarillo juice, which promoted the consumption and metabolism of organic matter by yeast, resulting in increased acid production [38]. For the 40 : 60 (%, *w*/*w*) dilution ratio, the increased rate of TA was also rapid and reached its peak on day 2 (1.74 ± 0.05 g/L). This was due to the presence of additional organic acids in the pH-adjusted tamarillo juice, which the yeast utilized as the main energy source for producing other acids. However, a slower increase in TA was observed on the following fermentation days. The rapid increase in TA led to a lower pH of the fermentation medium, which fell below the optimal range for yeast growth (4.5–5.0). Furthermore, the lower sugar content compared to the 34 : 66 (%, *w*/*w*) dilution ratio resulted in increased competition for nutrients between yeast, leading to a reduction in the acid production rate. The dilution ratios 66 : 34 and 50 : 50 (%, *w*/*w*) showed a significant increase in TA after 4 days of fermentation, with a slightly lower TA compared to the dilution ratio 34 : 66 (%, *w*/*w*). A higher tamarillo juice content in the dilution ratios resulted in a higher concentration of nonionized acids (such as malic acid, oxalic acid, and citric acid). The fermentation process was not the primary cause of the higher

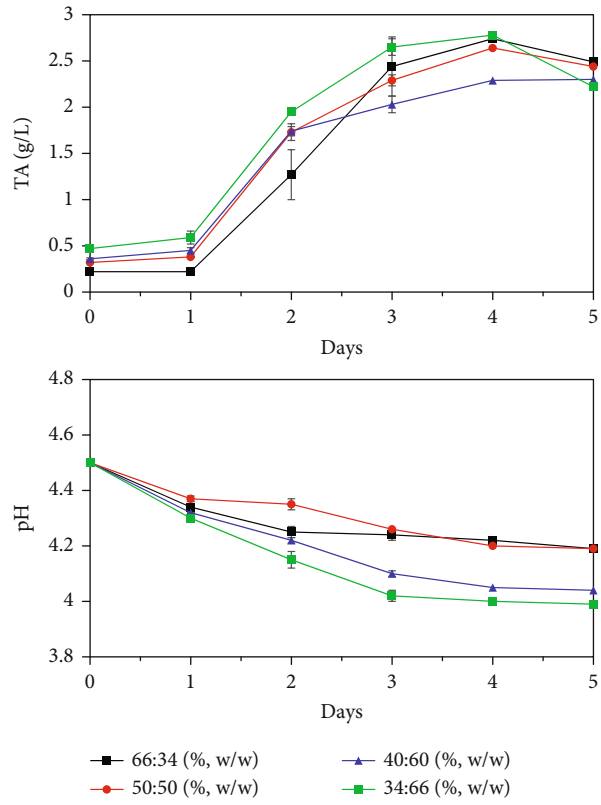

FIGURE 3: The influence of dilution ratio and fermentation time on titratable acidity and pH.

TA values in these two conditions, as no significant acid production by fermentation was observed to decrease the pH. The low sugar content in the 66 : 34 and 50 : 50 (%, *w*/*w*) dilution ratios resulted in less efficient conversion of sugar into acid molecules by yeast. In conclusion, the variation in TA was largely dependent on the sugar content in the tamarillo juice mixture and later on the pH of the fermentation medium. Furthermore, after the significant increase in TA, a slight decrease in TA was observed after day 5 for all four dilution ratios. However, the pH value did not show a significant variation ($p > 0.05$). Similar results were reported regarding the decrease in pH after 3 days of fermentation and its stability to increase further until day 4 in tarhana fermentation. Additionally, TA did not increase during the extended fermentation time from day 3 to day 4 [39]. The cocoa pulp fermentation process previously revealed an increase in titratable acidity (TA) after 3 days of fermentation, followed by a subsequent decrease in the following days [40]. A similar report indicated a 17–23% increase in total acidity (TA) and a 7.74% decrease in pH after 72 hours of fermentation in tarhana [41].

3.2.3. Total Soluble Solids. Fermentation is a biological process in which organic matter is transformed by bacteria or yeast into various products such as acids, CO_2, ethanol, and heat [42]. The dilution ratio between juice and water can influence the rate of reduction in TSS.

The results showed that the rate of reduction of TSS increased progressively with dilution ratios of 66 : 34, 40 : 60, and 34 : 66 (%, *w*/*w*) (Figure 4). After the first day of fermentation, the TSS reduction rate was rapid for all four dilution ratios. However, as the fermentation time continued to day 5, the TSS reduction rate gradually decreased. The rapid decline in TSS after the first day of fermentation could be explained by the phenomenon of rapid consumption of soluble solids by microorganisms in the medium [43]. During the initial stage of fermentation, the microorganisms in the system consume the available organic matter. This was the phase of initial degradation of organic matter, resulting in a rapid decrease in TSS due to the consumption of soluble solids. Therefore, after the first day, the rate of TSS reduction was rapid. However, as the fermentation process continued, the amount of organic matter available in the medium decreased. The microorganisms no longer had an abundant source of organic matter for consumption, and thus, the rate of reduction in TSS decreased with time. This could be attributed to the limitation of available organic matter, and microorganisms either started consuming different substances or ceased activity due to the lack of organic matter. In conclusion, increasing the fermentation time to day 5 led to a gradual decrease in the rate of reduction of TSS due to the depletion of organic matter and changes in microbial activity during the fermentation process. On the other hand, water plays a crucial role in microbial activity. When the dilution ratio with water was high (34 : 66), the microorganisms had an easily accessible and digestible medium. This created favorable conditions for the fermentation process and increased the rate of reduction of TSS. Higher water dilution ratios (34 : 66) resulted in a higher amount of supplemented sugar. Sugar provided a source of energy for the microorganisms during fermentation. With increased sugar supplementation, microorganisms have additional carbon sources to enhance their biological activity and produce high-quality products such as enzymes or organic acids [44]. This also contributed to a higher rate of TSS reduction. However, reducing the water ratio and increasing the juice ratio until reaching a 50 : 50 ratio showed a slower rate of reduction in TSS compared to the 66 : 34 ratio. This could be due to the fact that the activity of the microorganisms relied heavily on medium with high sugar or high water content for rapid access to and consumption of nutrients. At this ratio, the amount of sugar and water was at a moderate level, impeding the access of microorganisms to nutrients. Therefore, the efficiency was not high when fermentation was performed under a 50 : 50 dilution condition. A previous report revealed a decrease of approximately 21% in sucrose content after 5 days of black tea Kombucha fermentation—a type of yeast culture grown in a solution of tea and sugar [45]. A report on the fruit juice pressing fermentation process has revealed a decrease in TSS from 15.94°Brix to 9.00°Brix [43].

3.2.4. Total Sugar Content and Reducing Sugar Content. Tamarillo is a fruit with a relatively high sugar content. Additionally, the adjustment of TSS during the fermentation process has significantly increased the sugar content in the mixture. As the water ratio increases, the amount of sugar required to supplement the juice also increases. The difference in sugar content in the juice mixture can impact changes in TSC and RSC of the product over fermentation time [46].

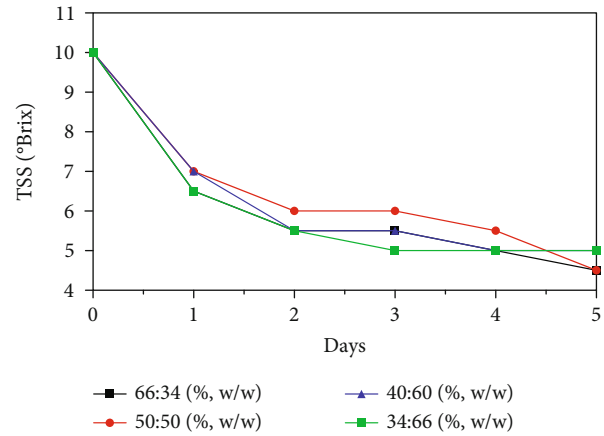

Figure 4: Effect of dilution ratio and fermentation time on total soluble solids.

The results revealed that the reduced sugar content accounted for 62% to 81% of the total sugar content. The addition of sugar to the samples with increasing water content resulted in an increasing trend in both total sugar and reducing sugar levels (Figure 5). The strong activity of the microbial population occurred within the first day of fermentation. The microorganisms used all types of sugars present in the mixture for their metabolic processes. From the second day of fermentation onward, the rate of reduction in RSC and TSC was slowed down in the investigated dilution ratios. However, higher levels of added sugar corresponded to higher rates of reduction in RSC and TSC. This could be attributed to the availability of carbon sources for microbial activity. When a large amount of sugar was added, the microorganisms had an abundant carbon source to sustain their metabolic processes and proliferation. This created favorable conditions for microbial activity, leading to increased rates of reduction in RSC and TSC. Furthermore, sugar acts as a catalyst for enzyme activity during the fermentation process. Enzymes facilitate the breakdown of sugars into by-products and the production of necessary compounds for the fermentation process. With a higher sugar content, enzyme activity is enhanced, resulting in a faster fermentation process and higher rates of RSC and TSC [47]. In addition, a high sugar concentration creates a strong osmotic medium in which the solute concentration (sugar) is higher in the extracellular medium compared to the microbial cytoplasm. This imposes osmotic stress on the microorganisms, forcing them to continue to take up sugar from the surrounding medium to maintain equilibrium [48]. Therefore, yeast will rapidly consume sugar to reduce osmotic pressure, resulting in a higher decrease in the rates of both RSC and TSC.

3.3. The Influence of pH on Product Quality during Fermentation

3.3.1. Ethanol Concentration. pH has a significant influence on the fermentation process. It affects enzyme activity,

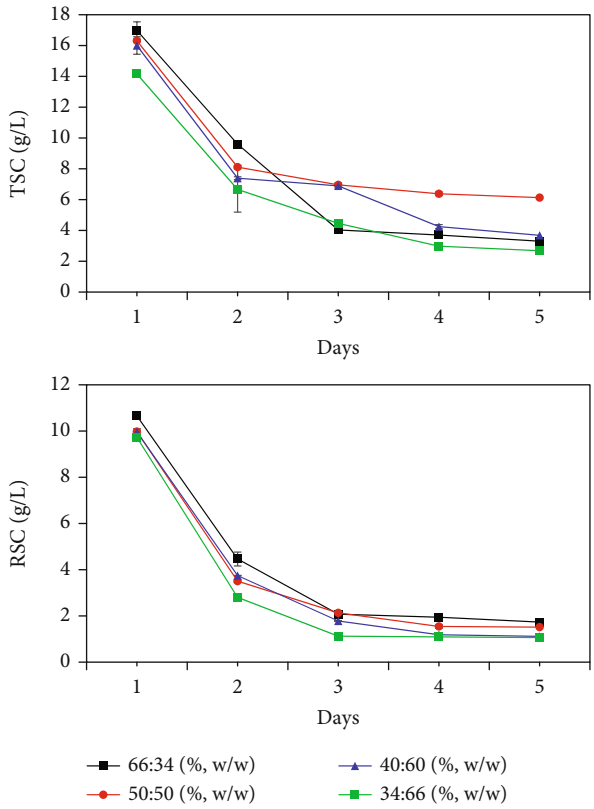

Figure 5: Effect of dilution ratio and fermentation time on RSC and TSC.

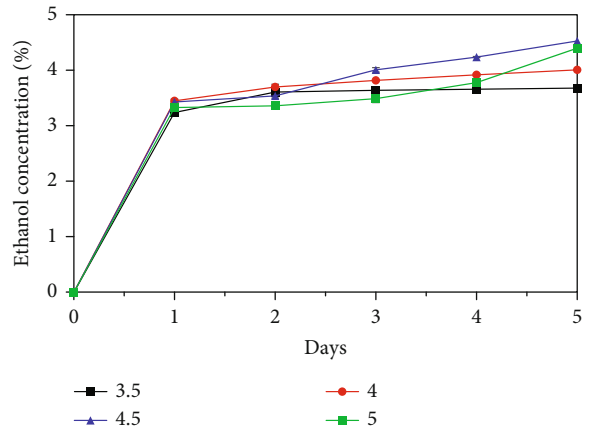

Figure 6: Effect of initial juice pH and fermentation time on alcohol concentration.

chemical equilibrium, microorganisms, and the chemical properties of the medium [49]. Each enzyme has an optimal pH for optimal performance, and microorganisms can only thrive and function well at specific pH levels [18]. Changes in pH can affect the speed and efficiency of the fermentation process, as well as the dispersion and solubility of chemical substances. Therefore, maintaining the appropriate pH is crucial to ensure a favorable fermentation process.

The fermentation process at pH 3.5–5 significantly affects the ethanol concentration after 5 days of fermentation, with a statistically significant impact ($p < 0.05$). After the first day of fermentation at pH 3.5, the ethanol concentration increased from 0 to 3.24% (Figure 6). Continuing the fermentation process, the ethanol concentration continued to increase significantly until the fifth day, reaching a concentration of 3.68%. Similarly, for the fermentation process at pH 5, the ethanol concentration tended to increase from 0 to 3.33% after the first day of fermentation. The ethanol concentration continued to increase until the fifth day (4.4%). The highest increased rate in ethanol after 5 days of fermentation was observed at pH 4.5, reaching a concentration of 4.53%. At pH 3.5, *Saccharomyces cerevisiae*, the yeast involved in the fermentation process, can encounter difficulties in enzymatic activity and metabolic processes [50]. This may be due to an overly acidic medium, which inhibits the enzyme's activity. As a result, the conversion rate of organic compounds and ethanol production tended to be slower at pH 3.5 compared to other pH levels. At pH 4, the medium conditions are closer to the optimal pH for *Saccharomyces cerevisiae* and enzymes. This creates favorable conditions for the activity of microorganism and the fermentation process [36]. As a result, the rate of ethanol production increases rapidly after the first day of fermentation at pH 4. At pH 4.5, the conditions are near the optimal value for some enzymes and the participation of *Saccharomyces cerevisiae* in the fermentation process [36, 51]. This provides the best medium for enzyme activity and organic compound metabolism. Therefore, at pH 4.5, microorganisms have the best conditions for ethanol production, and the ethanol concentration tends to increase most rapidly during fermentation. However, the ethanol concentration increases rapidly on the first day because the yeast has consumed all available oxygen in the medium and switched to anaerobic respiration. The yeast is a facultative anaerobe, and in anaerobic conditions, it primarily produces ethanol. In summary, pH significantly affects the rate of ethanol production during fermentation. pH values closer to the optimal range for enzyme activity and *Saccharomyces cerevisiae* create favorable conditions and lead to a faster increase in the ethanol concentration after the first day. A previous report has revealed yeast activity during fermentation at a pH range of 2.5 to 3.5, which is lower than the pH range of 4 to 6 [50]. A fermentation process with *Saccharomyces cerevisiae* BY4742 at pH 4 has revealed a high efficiency of ethanol production when investigated in the pH range of 3 to 6 [36]. An ethanol production process of *Chlamydomonas reinhardtii* has revealed high efficiency when carried out at pH 4.5 [51].

3.3.2. Changes in pH during Fermentation. The fermentation process can induce alterations in pH in the medium. The pH of the medium plays a crucial role in determining the rate of enzymatic activity of the yeast [52]. This phenomenon is associated with the metabolic process of yeast in generating acids and increasing the concentration of H^+ ion.

The results of the investigation indicate that time has a statistically significant influence on pH throughout the fermentation process ($p < 0.05$). After fermentation at pH 3.5–5, there is a notable decreasing trend as the fermentation

time increases (Figure 7). The slope of the pH curve during the fermentation process is comparable between pH 4 and 5. However, the slope of pH 3.5 shows a significantly slower variation. The sluggish change in pH at a pH level of 3.5 during fermentation can be explained by the complex interaction between yeast and fermentation medium. At pH 3.5, the medium is more acidic, which inhibits yeast activity, resulting in reduced reaction rates and low acid production, leading to a slow pH transformation [53]. Additionally, the equilibrium between acid and base in the acidic medium at pH 3.5 delays pH variations due to the time required for interactions and equilibrium changes. Fermentation bacteria face difficulties in accessing and utilizing nutrients at pH 3.5, resulting in a lower fermentation rate and slower pH changes. Furthermore, acidic medium at pH 3.5 significantly affects enzyme activity, further slowing the fermentation process and reducing the slope of the pH curve [54].

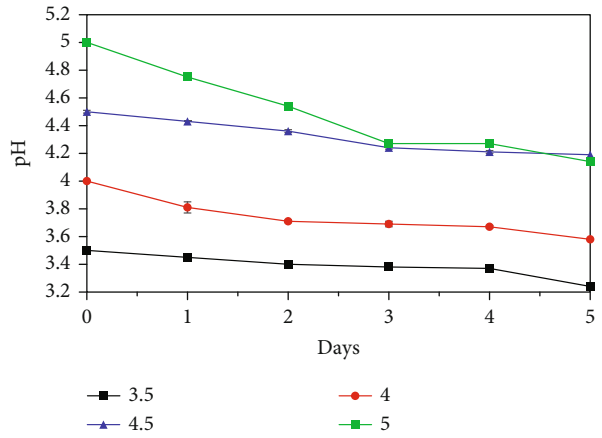

Figure 7: Variation of pH with fermentation time at different pH conditions.

3.3.3. Total Sugar Content and Reducing Sugar Content. pH influences the activity of microorganisms, the metabolism of organic compounds, and the products of fermentation. pH helps optimize enzyme activity and the rate of metabolism of organic compounds [55]. The presence of sugars in the medium plays a vital role as the primary source of nutrients that provide energy for yeast. The decrease in sugar content in the solution may be related to the ability of yeast to function under different pH conditions [36]. Therefore, an appropriate pH adjustment is crucial in the fermentation process.

The influence of pH and time on RSC (reducing sugar content) and TSC (total sugar content) is statistically significant ($p < 0.05$). After fermentation at pH levels ranging from 3.5 to 5, the remaining highest TSC is 1.83 g/L at pH 3.5, while the lowest is 1.01 g/L at pH 5 (Figure 8). The general trend for the variations of RSC and TSC over time is a decrease. However, the rate of decrease in RSC and TSC depends on specific time points during the fermentation process. During the first day of fermentation, 72% of the RSC were equally reduced in all investigated pH levels. However, the TSC reduction rate gradually increases from pH 3.5 to 5. At pH 3.5, approximately 64% of TSC was reduced compared to TSC on day 0. At pH 5, the degree of reduction in TSC increased to 69% compared to TSC on day 0. This indicated that in addition to utilizing reducing sugars at higher pH levels, nonreducing sugars also contributed to the metabolic process. The yeast prioritized the use of reducing sugars (sugars susceptible to oxidation) for fermentation until RSC remained at around 28%. At this point, the yeast switched to utilizing nonreducing sugars to continue the fermentation process. This was possibly due to the fact that reducing sugars provided a higher energy content and were more easily metabolized. Additionally, when RSC decreased to around 28%, the yeast started using nonreducing sugars to sustain the fermentation process. This could be related to changes in fermentation medium, including an increase in pH, a decrease in the concentration of reducing agents, and alterations in enzymatic activity. However, as RSC decreased to low levels, the distribution of RSC became more diverse, reducing the yeast's ability to access the nutrient source. Instead, nonreducing sugars at this stage were more easily accessible and consumed. Furthermore, the prolonged fermentation time to the fifth day clearly demonstrated the extent of the reduction in RSC and TSC. At pH 5, RSC and TSC experienced the greatest reduction after 5 days of fermentation. The degree of reduction in RSC and TSC was proportional to the investigated pH range of 3.5 to 5. At pH 3.5, RSC and TSC were less affected by the reduction. This may be related to the inhibitory effect of higher acid medium on the consumption and metabolism of carbohydrates by yeast. A previous report on the Burans red wine fermentation process showed a slight increase in sugar reduction at higher pH levels [56]. Another report on the banana wine fermentation process also yielded similar results [57].

3.4. The Influence of the Total Soluble Solids on Product Quality during Fermentation

3.4.1. Ethanol Concentration. The addition of sugar during the fermentation process not only enhances the sensory attributes of the product but also serves as a carbon source for the yeast to convert into ethanol and CO_2. This process typically occurs in a low pH medium. Therefore, the alteration of the sugar content in the fermentation medium can also affect the efficiency of the fermentation process.

Fixation of the initial TSS at different sugar concentrations significantly affects ethanol production during the fermentation process ($p < 0.05$). The initial TSS of the juice increased from 10 to 26%, which corresponded to a higher sugar content and resulted in a higher ethanol concentration over time during fermentation (Figure 9). The highest ethanol concentration was observed on the fifth day of fermentation using juice with adjusted TSS of 26°Brix (8.45 ± 0.02%). A higher sugar content enhances the accessibility to nutrient sources and reduces competition between yeast in nutrient consumption. This promotes carbohydrate metabolism in juice with higher TSS. On the first day of fermentation, the difference in the ethanol production rate between the TSS juices ranging from 10 to 26°Brix was relatively low. However, as the fermentation time increased, the juice with lower

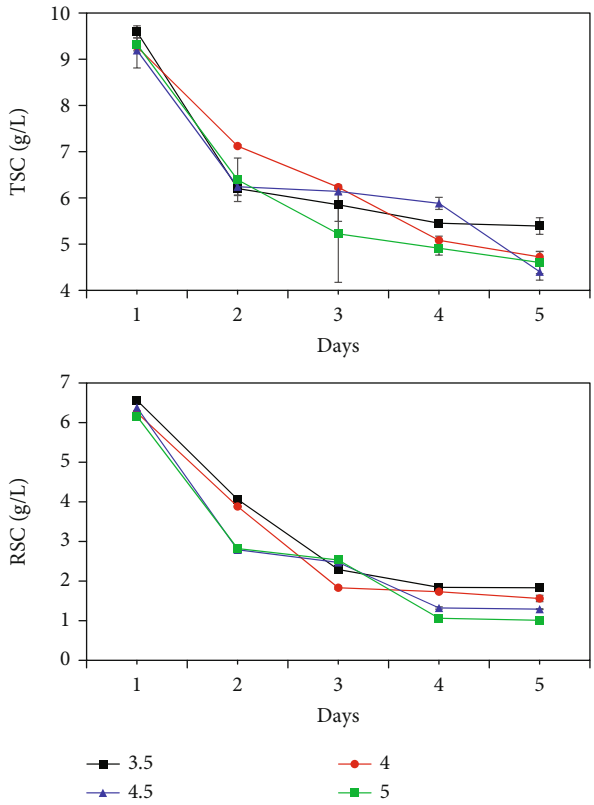

Figure 8: Effect of initial juice pH and fermentation time on RSC and TSC.

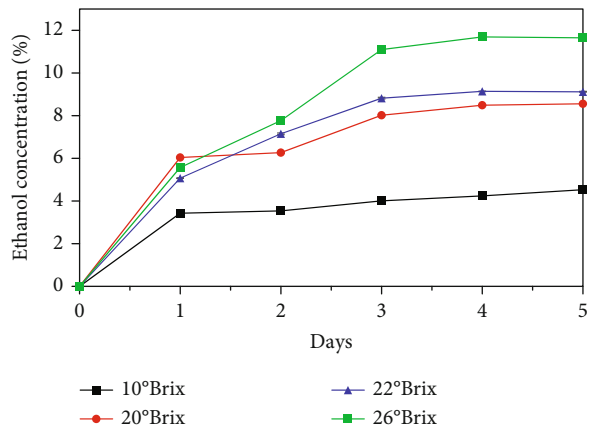

Figure 9: Effect of total soluble solids in initial juice and fermentation time on alcohol concentration.

TSS (10°Brix) showed a slower ethanol production rate. A consistent and rapid increase in ethanol concentration was observed in juices with a gradual increase in TSS from 10 to 26°Brix. This may be attributed to limited nutrient availability and increased competition for nutrients among yeast in juice with lower TSS (10°Brix) towards the end of the fermentation process, resulting in restricted ethanol production [58]. In contrast, for the fermentation process using juice with higher TSS (26°Brix), the supply of nutrients was sufficient for the growth and consumption of yeast, allowing the production of ethanol to continue until the fifth day. Therefore, the rate of increase in ethanol concentration remained high with prolonged fermentation time. However, the ethanol production rate gradually slowed from day 3 to day 5 in all juices investigated with different TSS. This could be due to the aging of yeast and the increasingly acidic medium, which reduced the carbohydrate metabolism for the production of ethanol [59].

3.4.2. pH. TSS represents nutrients, sugars, and other soluble compounds in the product. However, during the fermentation process, sugar is considered the main nutrient for yeast and is used as a substance to adjust the TSS of the juice [60]. This nutrient source is often depleted over time due to the breakdown and consumption by yeast to produce acid and CO_2.

In the first two days of the fermentation process, a significant reduction in pH was evident between juices with different levels of TSS ($p < 0.05$). In juice with 26°Brix, TSS exhibited the fastest pH decrease, from 4.5 to 4.04, in the first two days of fermentation (Figure 10). On the other hand, when the TSS of the juice was adjusted from 26°Brix to 22°Brix, the rate of pH reduction also decreased significantly after two days of fermentation ($p < 0.05$). The most distinct difference was observed when evaluating the pH changes during fermentation between juices with TSS of 10°Brix and 22°Brix. The reduction in pH in the fermentation process of the juice with TSS of 10°Brix did not show the abrupt decrease seen in the juices with TSS ranging from 20 to 26°Brix. A consistent pH reduction was observed over five days of fermentation in the juice with TSS of 10°Brix. Additionally, the pH of the juice reached its highest value of 4.2 after five days of fermentation. However, the pH changes from day 2 to day 5 of the fermentation process showed a slowing trend until there was no significant pH change. This phenomenon may occur due to factors such as nutrient depletion in the medium, yeast aging, and the inhibitory effect of low pH medium [61]. These factors contribute to inhibition of the uptake and metabolism of nutrients by yeast, which is a primary cause of acid production and reduction pH. Additionally, the predominant acid in cider is acetic acid, which is mainly produced by the oxidation-reduction reaction between two acetaldehyde molecules or by the activity of acetic bacteria during fermentation [62]. Acetic bacteria use sugars and produce acetic acid, but towards the end of fermentation, the acid content gradually decreases as organic acids combine with higher alcohols to form aromatic esters [63]. Another reason is the depletion of nutrient content in the medium, which leads yeast to utilize acids as their primary energy source to maintain their viability [64]. On the other hand, after the first day of fermentation, there were no statistically significant difference ($p > 0.05$) between juices with TSS of 20°Brix and 22°Brix. However, the juice with fixed TSS at 20°Brix exhibited a higher pH reduction rate in the first two days of fermentation compared to the juice with fixed TSS at 22°Brix. This result may be attributed to the competition for nutrients and the time required for the nutrient accessibility by the yeast. At TSS 22°Brix, the concentrated nutrient content creates a barrier for yeast to consume nutrients, resulting in

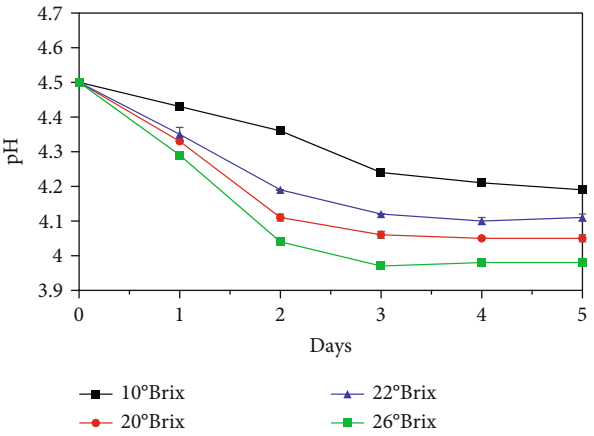

Figure 10: Effect of total soluble solids in initial juice and fermentation time on pH.

the simultaneous participation of yeast to increase the production of fermentation by-products (acids and CO_2), leading to a reduction in the fermentation process. On the contrary, this trend is observed in the juice fermentation process with TSS of 20°Brix.

3.4.3. Total Sugar Content and Reducing Sugar Content. Reducing sugars refer to sugars that have the ability to undergo reduction, such as glucose, fructose, and lactose. Additionally, some disaccharides like sucrose and maltose are also referred to as sugars, although they do not possess reducing properties. Total sugar content refers to the combined amount of various sugars present in a food product. During the fermentation process, yeast utilize sugars as a nutrient source for their metabolic activities [56]. However, depending on the fermentation conditions, there can be variations in both the total sugar content and the content of reducing sugars.

Changes in sugar content in the juice after the fermentation process are depicted in Figure 11. Both the total sugar content and the reducing sugar content showed a significant decrease after 5 days of fermentation. A sharp reduction in TSC and RSC was observed during the first 2 days of fermentation for juices with different concentrations of dissolved solids (10–26°Brix). Data on the reduction of TSC and RSC during the fermentation process revealed that after 5 days of fermentation, TSC decreased by approximately 70.60%, 81.14%, and 83.08% compared to the initial TSC in juices with TSS of 26°Brix, 22°Brix, and 20°Brix, respectively. Furthermore, the reducing sugar content showed a decreasing trend over time during the fermentation process ($p < 0.05$). After the fermentation process (5 days), RSC decreased by approximately 74.28%, 77.82%, and 78.75% compared to the initial RSC in juices with TSS of 26°Brix, 22°Brix, and 20°Brix, respectively—results that were proportional to the reduction in TSC. This indicates that the fermentation process in juices with higher TSC/RSC resulted in a slower decrease in sugar content after 5 days of fermentation. However, the fermentation process for juices with low TSC (10%) showed the lowest reduction rate in TSC

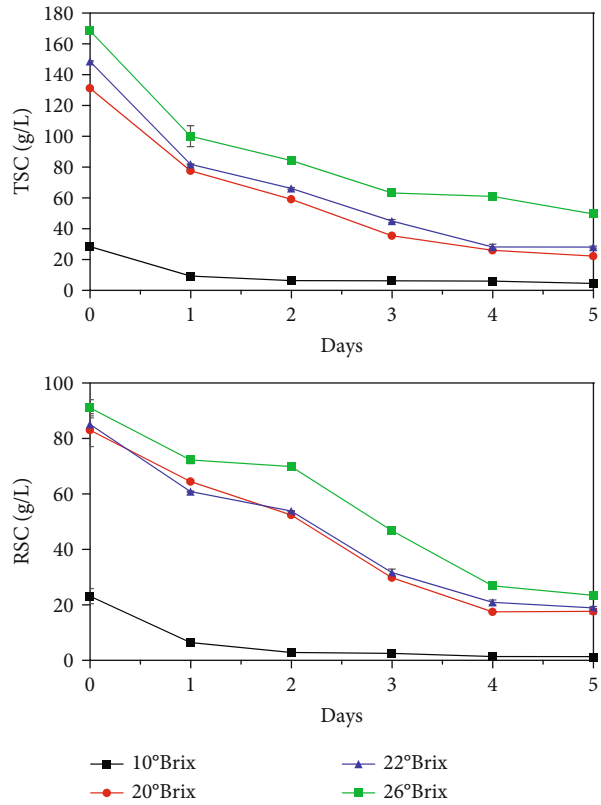

Figure 11: Effect of total soluble solids in initial juice and fermentation time on RSC and TSC.

(67.57%) compared to the initial TSC after 5 days of fermentation. The accessibility of nutrients by fungi is an important factor in achieving fermentation efficiency and the ability to reduce TSC. Additionally, competition for nutrients is also a factor contributing to the reduced efficiency of nutrient breakdown and fermentation. In juices with TSS of 10°Brix, the low TSC leads to low nutrient accessibility and high competition for nutrients, resulting in slower reduction of TSC in raw material compared to fermentation processes in juices with TSS > 10°Brix. Furthermore, the fermentation process in juices with high TSS (26°Brix) can impede the accessibility of nutrients due to the high concentration of dense solids. Previous studies on the fermentation processes of plum wine, mulberry wine, and apricot wine have reported similar results of increased sugar reduction when increasing TSS from 10 to 20°Brix in this study [56, 65]. A previous report has revealed that fungi utilize reducing sugars, such as glucose, as a primary source of nutrients during the fermentation process under anaerobic conditions [66].

3.5. The Influence of the Yeast Biomass on Product Quality

3.5.1. Ethanol Concentration. Saccharomyces cerevisiae, commonly known as yeast, is often employed to carry out the sugar reduction process for alcohol production during the fermentation of wine [67]. The addition of yeast in the fermentation process can significantly impact the efficiency,

economy, and uniform growth of the fermentation. Excessive or insufficient yeast levels can affect the overall fermentation performance.

The results revealed a statistically significant influence of fermentation time and yeast biomass on the ethanol concentration of the fermentation broth ($p < 0.05$). Increasing the yeast biomass from 0.6 g/L to 1.2 g/L resulted in a gradual increase in the ethanol production rate (Figure 12). However, the increase in yeast biomass from 0.6 g/L to 0.8 g/L did not have a significant impact on the ethanol production rate. After 1 day of fermentation, the ethanol concentration reached $4.75 \pm 0.03\%$ and $4.79 \pm 0.08\%$ for the fermentation with yeast biomass of 0.6 g/L and 0.8 g/L, respectively. Continuing the fermentation process until the 5th day, the ethanol concentration for the fermentation with a yeast biomass of 0.8 g/L reached $8.06 \pm 0.01\%$, which was higher than the fermentation with a yeast biomass of 0.6 g/L ($7.66 \pm 0.02\%$). However, the ethanol concentration increased rapidly and significantly when the yeast biomass was further increased to 1.0 g/L during the fermentation process. After 2 days of fermentation, the ethanol concentration increased rapidly and reached $7.15 \pm 0.04\%$. For yeast biomass of 0.6 g/L and 0.8 g/L, the ethanol concentration only reached around 5.35–5.57% after 2 days of fermentation. After 5 days of fermentation with an additional yeast biomass of 1.0 g/L, the ethanol concentration reached $9.12 \pm 0.02\%$. Similarly, increasing the yeast biomass to 1.2 g/L for the fermentation process resulted in a relatively high ethanol concentration after 5 days of fermentation ($11.12 \pm 0.02\%$). Increasing the yeast biomass was synonymous with increasing the number of yeast cells involved in the fermentation process. With a higher number of yeast cells, the fermentation process proceeded faster due to the increased participation of yeast cells. Yeast cells possess the ability to convert monosaccharides and disaccharides into ethanol and other by-products during fermentation [68]. When the yeast biomass was increased, the fermentation capacity was enhanced due to the higher number of yeast cells, leading to an increased ethanol production rate [69]. Furthermore, increasing the yeast biomass also increased the conversion rate of sugars to ethanol, which contributed to the enhanced ethanol production [36].

3.5.2. pH. The enhanced ability of yeast cells to metabolize and convert sugars is directly proportional to the increased production of by-products such as acid and CO_2 during the fermentation process [70]. Acids are one of the factors that contribute to the decrease in pH in the fermentation broth. The yeast biomass is a crucial factor that determines the capacity of yeast cells to metabolize and convert sugars present in the fermentation broth.

The results showed that the pH value exhibited a significant downward trend over time during the fermentation process ($p < 0.05$), while the yeast biomass increased ($p < 0.05$). After one day of fermentation, the pH of the pressed juice experienced a sharp decrease when supplemented with four different yeast biomasses (Figure 13). At a concentration of 1.2 g/L, the pH value decreased the most, remaining at 4.38 ± 0.02 after one day of fermentation. Additionally, the pH continued to decline significantly until

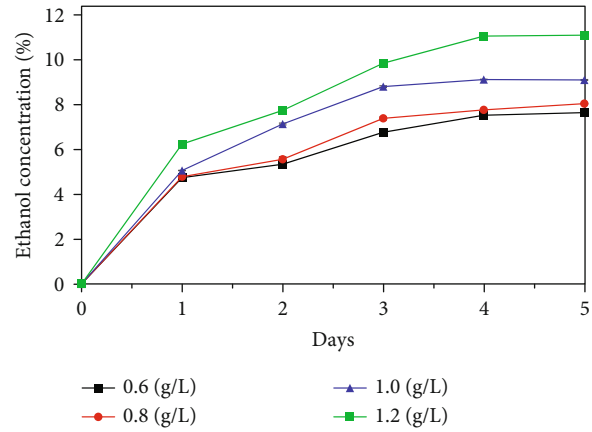

Figure 12: Effect of yeast biomass and fermentation time on alcohol content.

the fourth day, reaching 4.27 ± 0.02. The rate of pH decreases slightly diminished as the fermentation time extended to the fifth day (pH 4.16 ± 0.03). The fermentation process of the pressed juice with a yeast biomass of 1.0 g/L demonstrated a slower pH reduction rate compared to the fermentation process with a yeast biomass of 1.2 g/L. From the third to the fifth day of fermentation, the pH tended to decrease gradually and stabilize at 4.11 ± 0.02. However, the pressed juice fermented with yeast biomass ranging from 0.6 to 0.8 g/L did not exhibit rapid pH reduction throughout the fermentation process. Furthermore, there was no significant difference in pH value between these two yeast biomasses during the fermentation process ($p > 0.05$). As the yeast biomass increased, the reproduction and growth of yeast cells also increased. Cell reproduction occurred through the conversion of sugars into metabolites, including ethanol. One of the primary by-products of this process was CO_2, which is combined with water in the fermentation medium to form carbonic acid (H_2CO_3). Carbonic acid decomposed into bicarbonate ions (HCO_3^-) and hydrogen ions (H^+), contributing to the reduction of the medium's pH [71]. Additionally, yeast has the ability to produce organic acids such as acetic acid and lactic acid during the fermentation process [72]. The release of these acids into the fermentation medium also contributed to pH reduction. However, at yeast biomasses ranging from 0.6 to 0.8 g/L, the pH reduction rate was slow and not significantly different, possibly due to the influence of balancing factors and the improved automatic pH regulation ability of the system. The level of yeast cell reproduction and acid secretion within this range was insufficient to create substantial changes in pH. However, when the yeast biomass was increased to 1.0 g/L and 1.2 g/L, the pH reduction rate increased due to the higher number of yeast cells and their reproductive capacity, resulting in a significant increase in acid production. The elevation of yeast biomass in the fermentation medium led to a greater acid production and consequently a faster pH reduction.

3.5.3. Total Soluble Solids. The yeast biomass plays a crucial role in influencing the total soluble solids in the medium.

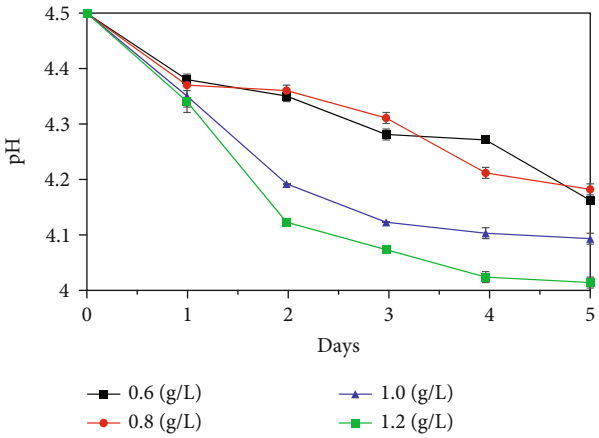

Figure 13: Effect of yeast biomass and fermentation time on pH.

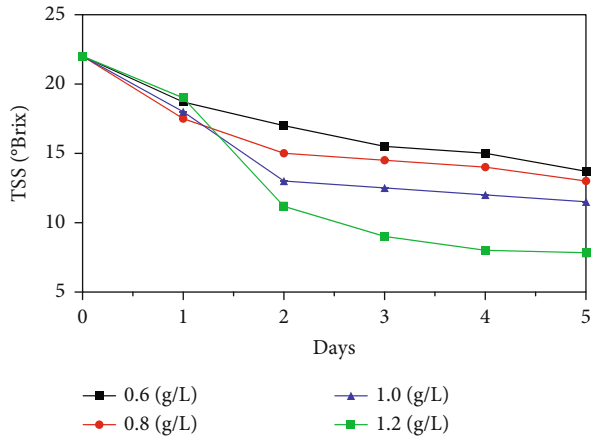

Figure 14: Effect of yeast biomass and fermentation time on total soluble solids.

The yeast biomass can impact factors such as fermentation rate, pH, cell reproduction, and the production of by-products [73]. The correlation between the yeast biomass and TSS is vital for optimizing the fermentation process and achieving high efficiency.

The results of the investigation have shown a significant statistical influence of yeast biomass on TSS. Increasing the yeast biomass has demonstrated a higher rate of TSS reduction. After 2 days of fermentation with a yeast biomass of 1.2 g/L, TSS decreased by 53.47% compared to the initial value, resulting in a remaining TSS of 11.17°Brix (Figure 14). The minimum reduction in TSS was observed during the fermentation process when supplementing the yeast biomass with 0.6 g/L. However, the fermentation process with a yeast biomass of 1.2 g/L extended to the fifth day showed a reduction in TSS of 67.36%, resulting in a remaining TSS of 7.83°Brix. The fermentation process with higher yeast biomass exhibited a faster fermentation rate, and the TSS reduction curve became steeper compared to the fermentation processes with lower yeast biomass. During fermentation, yeast uses TSS as a source of nutrients for growth and metabolism [74]. Specifically, yeast converts sugars into ethanol, organic acids, CO_2, and other by-products [72]. Increasing the yeast biomass to 1.2 g/L accelerates the rate of reduction in TSS because yeast simultaneously participates in the breakdown and metabolism processes.

3.5.4. Total Sugar Content and Reducing Sugar Content. The reduction in sugar content on the substrate is an indication of the breakdown and consumption activity of yeast during the fermentation process [75]. A significant decrease in sugar content is directly proportional to the effectiveness of fermentation and the metabolic activity of yeast [76]. The appropriate biomass of yeast contributes to accelerating the fermentation process by facilitating efficient breakdown and metabolism. However, it can also increase the competition for nutrients among yeast and surrounding medium, leading to a decrease in the overall efficiency of the fermentation process [76].

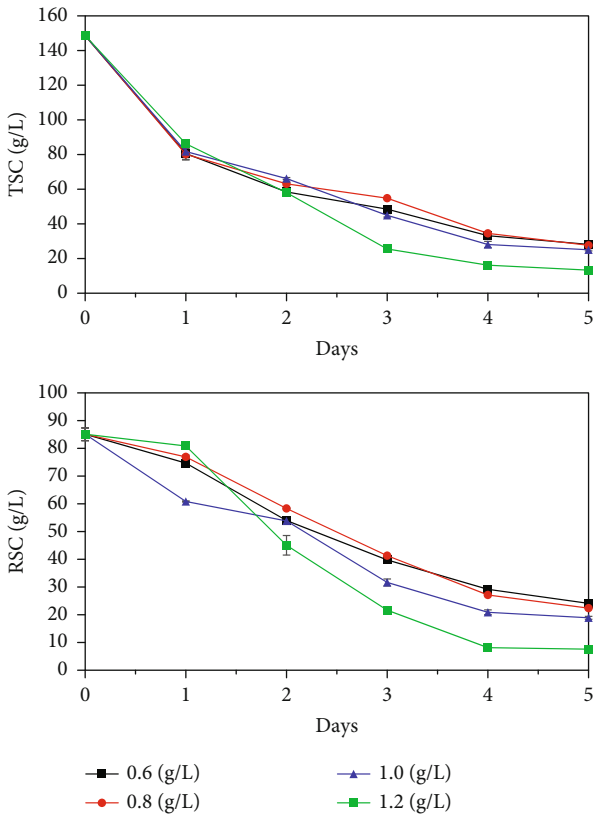

Figure 15: Effect of yeast biomass and fermentation time on RSC and TSC.

The increase in yeast biomass participating in the fermentation process had a statistically significant impact on RSC and TSC throughout the fermentation process ($p < 0.05$) (Figure 15). TSC and RSC showed a decreasing trend over time during fermentation. Furthermore, fermented substrates with yeast biomass ranging from 0.6 g/L to 1.2 g/L exhibited a stronger reduction in TSC and RSC. After 5 days of fermentation with a yeast biomass of 0.6 g/L, the remaining TSC and RSC were 24.07 g/L and 28.1 g/L,

respectively, representing a decrease of 71.70% and 81.1% compared to the initial levels. Increasing the yeast biomass to 1.2 g/L in the fermentation process resulted in a reduction of approximately 91.10% in both RSC and TSC after 5 days of fermentation. Furthermore, after 5 days of fermentation, the remaining TSC and RSC were 7.55 g/L and 13.28 g/L, respectively. A similar result was obtained in a previous report in which an increase in the fermentation time of the cocoa pulp led to a reduction in the sugar content in the final product [40]. A previous report has revealed an inverse correlation between the biomass of yeast involved in the fermentation process and the remaining reducing sugar content in the medium [77].

4. Conclusion

In this study, a tamarillo fermentation process (Magic-S) was developed through investigations on the dilution ratio, pH, TSS, and yeast biomass, the main parameter being the ethanol concentration. Other parameters such as TSC, RSC, TSS, pH, and TA provided scientific data on the variations throughout the fermentation process of tamarillo (Magic-S). The results showed that under fermentation conditions with a 50 : 50 dilution ratio (%, w/w) of juice and water, pH 4.5, TSS 22°Brix, and yeast biomass supplementation of 0.6 g/L, the ethanol concentration reached $7.54 \pm 0.11\%$ after 4 days of fermentation and remained stable as fermentation continued until the 5th day. Furthermore, the pH of the product did not drop too low (pH 4.16). In addition, the TSC, RSC, and TSS after fermentation remained high, ensuring a significant amount of sugars and some dissolved nutrients in the product. The outcome of this study is the development of a new consumer-accepted product that contains the nutritional elements and the characteristic of the tamarillo flavor profile. Furthermore, the results of the study serve as a basis for industrial-scale production to diversify products and enhance the value of tamarillo. The purpose of using this product is to supplement the nutritional components found in tamarillo for the body, helping to prevent certain diseases, especially cancer, due to some active antioxidant properties. In addition, on an industrial production scale, it helps address the short shelf life issue of fresh fruits and increases the options to supplement nutritional components for consumers.

Data Availability

All the data is available within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Nguyen Tat Thanh University and Ho Chi Minh City University of Industry and Trade, Vietnam, by providing the facilities required to perform this work.

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