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Research Article

Katuk Leaf, Carrot, and Pineapple Mixed Juice: A New Media for the Non-dairy Probiotic Drink

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Abstract: Mixed vegetable and fruit juices are promising for developing non-dairy probiotic beverages. This study investigated the physicochemical properties, sensory acceptance, and the viability of *Lactobacillus casei* 0090-NRRL B-1992 during refrigerated storage (4 °C) in a juice blend comprising Katuk (*Sauropus androgynus*) leaves, carrots, and pineapple. Four juice formulations with varying dilution ratios were prepared and evaluated over a 21-day storage period (0, 7, 14, and 21 days). Results indicated a gradual decline in both total Lactic Acid Bacteria (LAB) count and LAB viability percentage, as well as a decrease in total soluble solids throughout storage. Nevertheless, all treatments maintained LAB counts above the minimum threshold recommended for probiotic efficacy ($\geq 10^6$ CFU/mL). The total lactic acid content of the probiotic juices ranged from 0.19% to 1.36%, and this increase corresponded with a pH reduction from 4.81 to 3.41, reflecting enhanced LAB fermentative activity during cold storage. Sensory evaluation revealed an improvement in aroma and overall acceptability scores, particularly after 14 days of storage, from moderately liked to liked. Among the formulations, the 2 : 1 juice-to-water dilution ratio was most preferred and demonstrated potential as a candidate for further development as a probiotic mixed juice beverage.

***Keywords*:** Katuk leaf, carrot, pineapple, mixed juice, non-dairy probiotic drink

1. Introduction

The advancement of scientific research has been accompanied by a growing consumer interest in functional foods, particularly probiotic products [1]. Probiotic beverages derived from fruits and vegetables represent a promising alternative to dairy-based fermented products due to their abundant essential nutrients, including vitamins, minerals, antioxidants, and fermentable carbohydrates that support probiotic growth [2, 3]. Beyond their nutritional value, selecting appropriate fruit and vegetable combinations is crucial to ensure desirable sensory qualities and consumer acceptability. In this study, a novel probiotic beverage was formulated using Katuk (*Sauropus androgynus*) leaves, carrots, and pineapple juice, fermented with *Lactobacillus casei*, a strain known to tolerate and remain viable at pH levels between 3.7 and 4.3 [4]. Katuk leaves, native to Indonesia and traditionally consumed for their medicinal properties, are rich in bioactive compounds such as tannins, saponins, flavonoids, and alkaloids, which have been used in ethnomedicine to manage gastrointestinal ailments, including ulcers and constipation [5]. Carrot and pineapple juices contribute additional bioactive compounds and fermentable sugars, making the mixed juice blend a suitable medium

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to support probiotic growth and activity. Combining these three ingredients is hypothesized to produce a bioactive-rich beverage that goes beyond fundamental nutritional value and is a sensorially acceptable, non-dairy probiotic drink.

Dilution of fruit or vegetable juice is often required to improve the sensory properties of probiotic beverages and enhance consumer acceptability. However, excessive dilution with water can reduce the Total Soluble Solids (TSS), which may limit the availability of essential nutrients required for probiotic growth and metabolism. Yuliana et al. [6] reported using durian juice with a TSS of approximately 10 °Brix in producing a probiotic beverage, highlighting the importance of maintaining sufficient nutrient content. The significance of studying dilution ratios has also been emphasized in previous research involving kedondong juice fermentation [7], probiotic drinks made from date fruit [8], tamarillo cider production [9], and the impact of grape juice dilution on oenological fermentation outcomes [10].

Probiotic viability and functional performance are critical parameters affecting probiotic products' quality, stability, and efficacy [11-13]. During cold storage, probiotic beverages undergo various microbiological, physicochemical, and sensory changes. For example, Nematollahi et al. [14] observed a progressive pH decrease in cornelian cherry probiotic juice over 28 days of refrigeration, attributed to continued growth and metabolic activity of *Lactobacillus casei*. Similarly, Yuliana et al. [6] reported that durian-based probiotic drinks stored under refrigeration for four weeks exhibited reductions in sugar content and pH, a quadratic increase in total acidity and Lactic Acid Bacteria (LAB) populations, along with shifts in sensory attributes and a decline in LAB viability. These findings underline the importance of optimizing formulation and storage conditions to ensure the stability and functionality of probiotic beverages.

Based on the aforementioned considerations, investigating the changes that occur during the storage of probiotic beverages is essential to ensure that product quality remains within the recommended probiotic standards while preserving favorable sensory attributes. Therefore, this study aimed to evaluate the effects of dilution ratio and storage duration on the microbiological and sensory properties of a mixed probiotic juice formulated from Katuk leaves, carrot, and pineapple.

2. Materials and methods

2.1 *Materials*

The materials used in this study were Katuk leaves (*Sauropus androgynus*), carrots, and honey pineapples, sourced from local traders in Bandar Lampung, Indonesia. The selected vegetables and fruits consisted of young and mature Katuk leaves, fresh and healthy carrots, and ripe Queen-variety honey pineapples with an orange-yellow color, suitable for consumption. Another material used was *Lactobacillus casei* 0090-NRRL B-1922 culture, obtained from the Inter-University Center for Food and Nutrition, Gadjah Mada University (UGM).

2.2 *Methods*

The study used a Randomized Complete Block Design (RCBD) with a factorial arrangement of two factors and three replications, where the day of replication was used as the blocking factor. Factor 1: Proportion of juice to water (P), with four levels: P0: Control (1 : 0), P1: 4 : 1, P2: 2 : 1, P3: 4 : 3 (v/v). Factor 2: Storage duration (S), with four levels: S0: 0 days, S1: 7 days, S2: 14 days, S3: 21 days. Preliminary research determined that the optimal formulation of mixed juice (Katuk leaves: carrots: pineapples) was 30 : 90 : 80 (w/w/w), characterized by a slightly sour taste with a somewhat bitter aftertaste, a distinctive aroma of Katuk and pineapple, and a very dark green color. Overall, the study had 48 treatment units. The ratio of ingredients used is seen in Table 1.

Table 1. Ratio of ingredients used (Katuk leaves, carrots, pineapple, and water)

Code of treatments	Amount of ingredients					°Brix	Juice: water ratio
	Katuk leaves (g)	Carrots (g)	Pineapple (g)	Juice amount (mL)	Water (mL)		
P0	210	630	560	400	0	± 13.2	1 : 0
P1	150	450	400	400	100	± 11.8	4 : 1
P2	120	360	320	300	150	± 10.2	2 : 1
P3	120	360	320	320	240	± 9.3	4 : 3
Total	600	1,800	1,600	1,420	490		

2.3 *Preparation of starter culture*

The starter culture preparation was conducted using a modified method based on Yuliana et al. [6]. The procedure was as follows: A test tube containing the bacterial culture (*Lactobacillus casei* 0090-NRRL B-1922) was transferred into sterile MRS Broth. One milliliter of the MRS Broth was inoculated into 10 mL of sterile 5% (w/v) skim milk and incubated at 37 °C for 48 hours. This culture was referred to as the primary culture. Four percent (v/v) of the primary culture was inoculated into 10 mL of sterile 5% (w/v) skim milk and incubated at 37 °C for 24 hours. This method resulted in the intermediate culture. As much as 4 percent (v/v) of the intermediate culture was inoculated into 10 mL of sterile 5% (w/v) skim milk supplemented with 3% (w/v) sterile glucose. The culture was incubated at 37 °C for 48 hours, making the working culture ready for use.

2.4 *Preparation process for probiotic mixed juice*

Katuk leaves, carrots, and pineapples were cleaned to remove physical debris, thoroughly washed, and weighed according to the specified formulation ratio of 30 : 90 : 80 (g/g/g), respectively. The Katuk leaves were blanched before the juicing process. This blanching was to inactivate undesirable green microorganisms and deactivate enzymes like peroxidase and polyphenol oxidase that cause browning, color loss, and off-flavors. This process helps improve the safety and preserve the sensory quality of the probiotic juice. All ingredients were then processed into juice using a juicer to produce a mixed juice. The mixed juice was diluted with water in varying proportions according to the juice-to-water ratio treatments, which included 1 : 0, 4 : 1, 2 : 1, and 4 : 3 (v/v). The diluted juice was packaged into 100 mL glass bottles, with 5% (w/v) glucose added to each sample. The juice was pasteurized at 75 °C for 15 minutes, then cooled to approximately 37 °C. A 4% (v/v) inoculation was performed using the working culture of *L. casei* 0090-NRRL B-1922 with a cell density of approximately Log 10.88, 10.88, and 11.08 CFU/mL. The glass bottle lids were covered with aluminum foil, and the samples were incubated at 37 °C for 2 hours. After incubation, the products were stored in a refrigerator at approximately 4 °C. The parameters observed in this study were total Lactic Acid Bacteria (LAB), LAB viability, total lactic acid, pH, total soluble solids, and sensory evaluation.

2.5 *Total LAB and LAB viability*

Lactic Acid Bacteria (LAB) probiotics were enumerated using de Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany). Serial dilutions of the samples were prepared and plated on MRS agar, followed by incubation at 37 °C for 48 hours under anaerobic conditions [6]. The number of LAB was determined by counting Colony-Forming Units (CFU). LAB viability was assessed by comparing CFU counts before and after storage, and the reduction was used to calculate the percentage of LAB viability.

2.6 *pH, total lactic acid, and sensory evaluation*

The pH of probiotic juice was recorded using a digital pH meter (Hanna HI 2211-02, RI/USA). Total lactic acid was determined using the titration method described by Yuliana et al. [15]. Total Soluble Solids (TSS), expressed as Brix of the samples, were measured using a refractometer (Abbe Hergestellt in der DDR, Germany). Sensory evaluation of the fermented mixed juice drink was performed by a panel of 30 students who scored the aroma and overall acceptability of the sample from different storage times [16]. The samples were coded by a different 3-digit number and were served in a randomized order, while the panel was asked to evaluate them based on a 1-5 preference scale.

2.7 *Statistical analysis*

The collected data were tested for ANOVA and further analyzed using orthogonal polynomial and orthogonal contrast tests at 1% and 5% significance levels.

3. Results and discussion

3.1 *LAB*

Figure 1 illustrates that the total Lactic Acid Bacteria (LAB) count in all dilution treatments exhibited a quadratic trend during refrigerated storage. The peak LAB populations were observed as follows: 11.1 log CFU/mL at 9.14 days for treatment P0 (no dilution), 10.04 log CFU/mL at 10.49 days for P1, 10.9 log CFU/mL at 10.54 days for P2, and 10.78 log CFU/mL at 11.47 days for P3. During the initial 7 days of storage, the P0 treatment (undiluted juice) exhibited the highest LAB counts, likely due to its higher nutrient concentration that supported more vigorous microbial growth. However, by day 14, the LAB count in P0 declined more rapidly than in the diluted treatments. This condition suggests that the culture had entered the decline phase earlier, potentially due to nutrient depletion or accumulation of inhibitory metabolites. Although dilution was associated with reduced total LAB counts, all treatments maintained LAB levels above the minimum threshold for probiotic efficacy ($\geq 10^6$ CFU/mL). These results indicate that although P0 exhibited the highest initial LAB concentration, the diluted treatments (P1-P3) showed more stable microbial viability over time and achieved superior sensory attributes, highlighting their greater suitability for developing functional probiotic beverages.

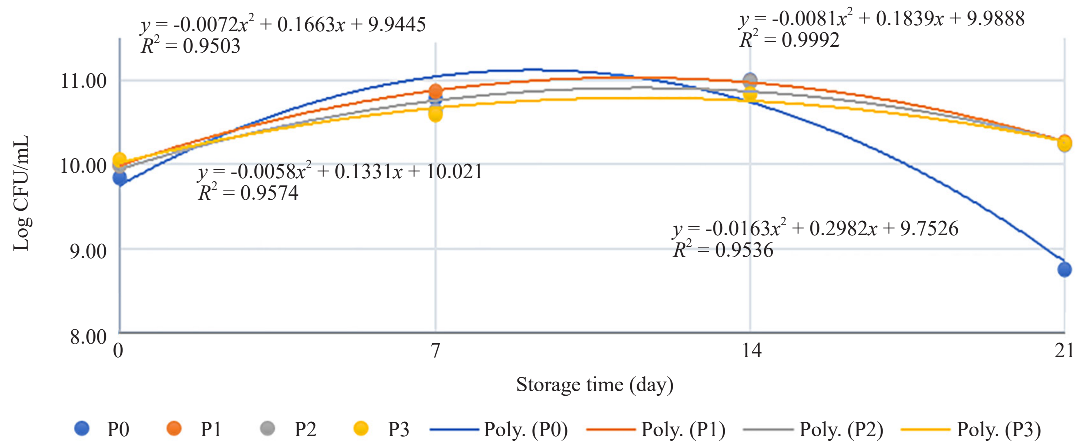

Figure 1. Total LAB of the probiotic juice mixture during the cold storage. Proportions of juice to water (v/v) are (1 : 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4:3) for P3. P0 vs P1, P2, P3**, P1 vs P2, P3^{ns}, P2 vs P3^{ns}. (ns = not significant, ** = significant at alpha 1% by contrast test)

These findings are consistent with previous studies by [9, 10, 17], which demonstrated that appropriate dilution levels can create more favorable conditions for microbial fermentation by optimizing the availability of nutrients and reducing stress factors on the starter cultures. In the present study, total LAB counts increased significantly ($p < 0.01$) by day 7, plateaued on day 14, and began to decline by day 21, following a typical microbial growth curve comprising the lag, exponential, stationary, and death phases. The post-peak decline in LAB viability is in line with the observations of Yuliana et al. [6], Koirala and Anal [1], and Güney and Güngörmüşler [18], who reported similar reductions in LAB populations during extended cold storage of probiotic beverages. The decrease in LAB counts may be attributed to several factors, including the accumulation of lactic acid and the associated drop in pH, which can create inhospitable conditions for LAB survival. Additionally, nutrient depletion, metabolic stress, and inhibitory substrates or fermentation byproducts can further compromise LAB viability [6, 11]. Despite this decline, all treatments maintained total LAB counts above the minimum threshold for probiotic functionality ($\geq 10^6$ CFU/mL) as recommended by Codex Alimentarius [19]. This number suggests that the mixed juice formulations provided sufficient nutrient support during refrigerated storage to sustain LAB viability and limited metabolic activity. However, the gradual depletion of fermentable sugars over time may have slowed bacterial proliferation, transitioning cultures into a maintenance phase rather than active growth [11].

3.2 *LAB viability*

Figure 2 illustrates that LAB viability across all dilution treatments followed a quadratic trend during refrigerated storage. This pattern reflects the overall microbial growth curve, wherein an initial increase in LAB viability was followed by a gradual decline, influenced by changes in the fermentation environment. The quadratic trend corresponds with concurrent increases in lactic acid concentrations and reductions in pH, which, while initially favorable to LAB proliferation, eventually created stress conditions that impaired cell viability. In the undiluted treatment (P0), LAB viability peaked at 100.4% on day 5 of storage. However, this treatment subsequently experienced the steepest decline in viability, dropping to 69.7% by the end of the storage period. This sharp reduction may be attributed to accelerated nutrient depletion and metabolite accumulation due to higher microbial activity in the undiluted medium. In contrast, the diluted treatments exhibited more gradual declines in viability, likely due to a more balanced nutrient environment and reduced acid stress, allowing for better maintenance of LAB survival over time. These results underscore the importance of optimizing dilution levels in probiotic beverage formulations to support initial LAB growth and preserve cell viability during extended storage.

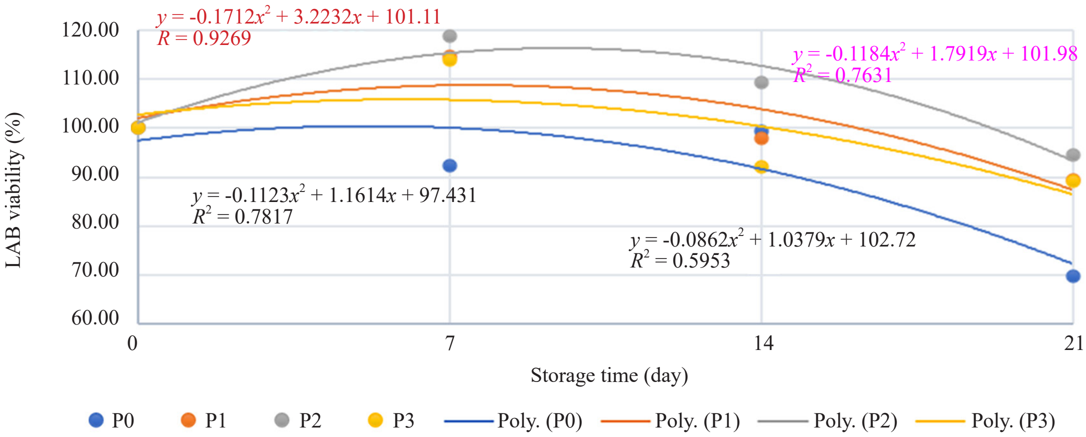

Figure 2. LAB viability of probiotic juice mixture drink during cold storage. Proportions of juice to water (v/v) are (1 : 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4 : 3) for P3. P0 vs P1, P2, P3**, P1 vs P2, P3^{ns}, P2 vs P3** (ns = not significant, ** = significant at alpha 1% by contrast test)

The undiluted treatment (P0) exhibited a significantly faster and more substantial decline in LAB viability ($p < 0.01$) compared to the diluted treatments. This decline is likely attributed to the rapid accumulation of lactic acid and the consequent sharp reduction in pH, both known to create increasingly stressful conditions for probiotic survival.

In contrast, the diluted treatments (P1, P2, and P3) demonstrated higher peak viabilities and more gradual declines, indicating improved conditions for maintaining probiotic cell integrity over time. Specifically, LAB viability in P1, P2, and P3 declined slightly from 100% to 89.4%, 94.4%, and 89.2%, respectively. These differences underscore the critical role of dilution in modulating the fermentation environment. The reduced acidity in the diluted treatments is likely due to lower lactic acid production, resulting in a less inhibitory environment that supports better LAB survival. This observation aligns with findings by Sionek et al. [13], who noted that the accumulation of organic acids produced by LAB can progressively lower pH and inhibit bacterial growth during prolonged storage. Similarly, [6, 11] reported that nutrient depletion and metabolic byproducts, such as organic acids, can disrupt LAB metabolism and viability. Overall, these results demonstrate that undiluted juices (P0) may promote rapid initial microbial growth but are also more susceptible to conditions compromising long-term viability. In contrast, appropriate dilution can buffer the fermentation environment, mitigate the inhibitory effects of acid accumulation, and thereby enhance the stability of probiotic viability during cold storage.

3.3 *Total lactic acid and pH*

During lactic acid fermentation, Lactic Acid Bacteria (LAB) metabolize fermentable sugars into lactic acid as the primary byproduct, reducing the substrate's pH [12, 13]. In this study, the total lactic acid content of the probiotic mixed juice ranged from 0.19% to 1.36% throughout the cold storage period. As shown in Figure 3, total lactic acid levels increased in a quadratic pattern during refrigerated storage. This trend aligns with previous reports that lactic acid concentrations in probiotic beverages typically rise during cold storage, driven by ongoing LAB metabolic activity [6, 11, 13]. The observed increase in lactic acid content supports the notion that LAB remain metabolically active even under cold conditions, particularly in the early storage stages, contributing to continued acid production and the gradual decline in pH. These findings highlight the dynamic nature of post-fermentation changes in probiotic beverages and emphasize the importance of monitoring organic acid production to maintain product stability and microbial viability.

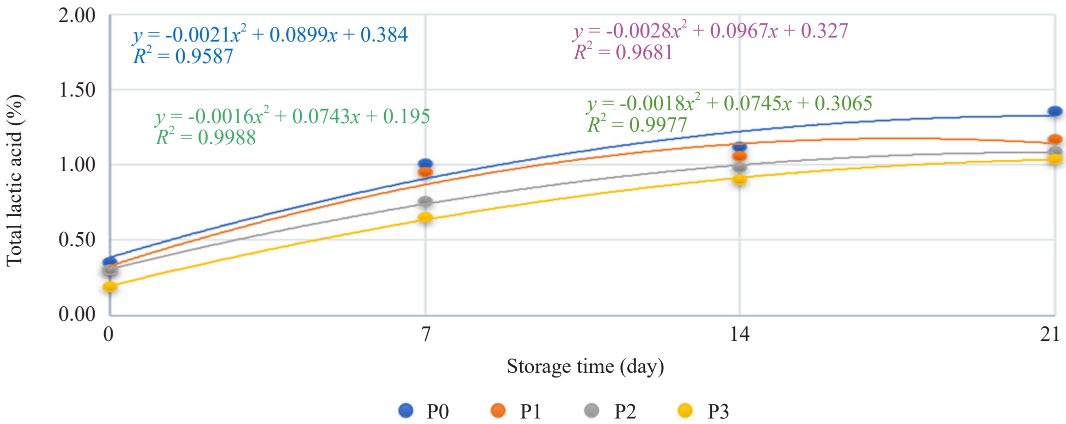

Figure 3. Total lactic acid (%) of the probiotic juice mixture drink during the cold storage. Proportions of juice to water (v/v) are (1 : 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4 : 3) for P3. P0 vs P1, P2, P3**, P1 vs P2, P3 **, P2 vs P3^{ns} (Note: ns = not significant, ** = significant at alpha 1% by contrast test)

The increase in total lactic acid content during cold storage was accompanied by a gradual decrease in pH, indicating sustained metabolic activity of LAB. The declining pH reflects the acidic environment resulting from lactic acid production, which not only contributes to the characteristic sourness of the beverage but also enhances its preservative properties. Variations in lactic acid accumulation and pH reduction across treatments were influenced by the degree of dilution, highlighting the impact of juice composition on microbial metabolism. In this study, the pH of the mixed probiotic juice ranged from 4.81 to 3.41 across treatments and storage durations. As shown in Figure 4, the pH of all formulations decreased following a quadratic trend during cold storage. The lowest pH values were recorded in P0 (3.33 on day 16.06), P1 (3.43 on day 16.04), P2 (3.41 on day 16.58), and P3 (3.40 on day 15.94), respectively. The most significant pH decline occurred within the first 7 days, followed by a more gradual reduction through day 21. This pattern corresponds with the typical microbial growth curve, where active LAB proliferation and fermentation occur in early storage, resulting in increased acid production. These findings align with previous studies reporting similar trends in lactic acid production and pH reduction during the refrigerated storage of probiotic beverages [6, 11, 14]. Additional literature confirms that vegetable- and fruit-based probiotic drinks typically exhibit pH values ranging from 2.8-3.5 [20], 3.6-3.7 [21], 4.6-4.9 [12], and 4.28-5.4 [22], which are consistent with the range observed in this study. The progressive acidification during storage confirms ongoing LAB activity and supports the functional role of LAB in extending shelf life and maintaining the sensory characteristics of probiotic products. Notably, the pH values remained within the acceptable range for probiotic beverages, ensuring the viability of LAB and the overall quality and safety of the product throughout the storage period.

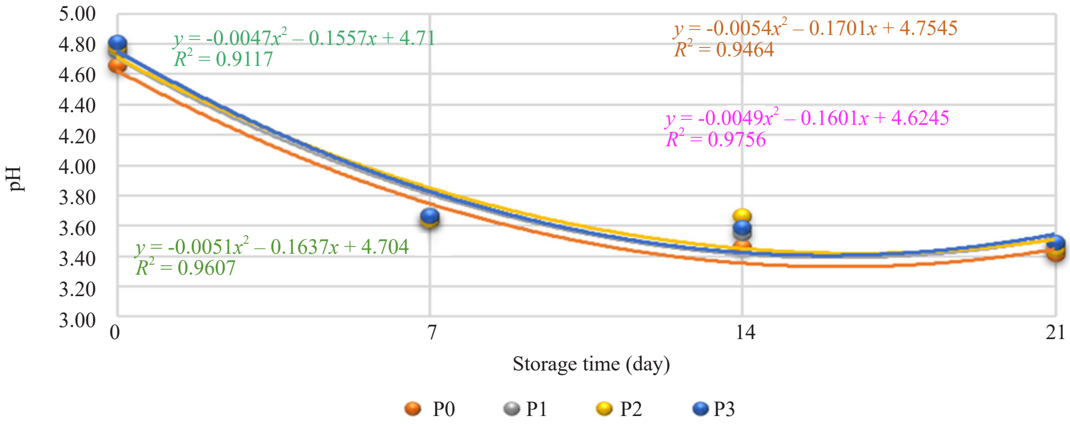

Figure 4. pH of probiotic juice mixture drink during cold storage. Proportions of juice to water (v/v) are (1: 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4 : 3) for P3. P0 vs P1, P2, P3**, P1 vs P2, P3^{ns} , P2 vs P3^{ns} (ns = not significant, ** = significant at alpha 1% by contrast test

3.4 *TSS*

Figure 5 shows that all treatments' Total Soluble Solids (TSS) decreased linearly during cold storage. The initial TSS levels varied depending on the dilution level, with higher water content in P1, P2, and P3 leading to lower TSS values. These results are consistent with findings by Rakhmawati and Yunianta [7] and Retnowati and Kusnadi [8], who observed that increasing the proportion of water in juice formulations reduces TSS. TSS, which includes total sugars, organic acids, pigments, and soluble proteins, declined progressively during storage in all treatments. Specifically, TSS levels decreased from 13.8 to 11.7 °Brix in P0, 12.3 to 9.7 °Brix in P1, 10.2 to 9.0 °Brix in P2, and 8.8 to 7.7 °Brix in P3. The decline in TSS is primarily attributed to the metabolic activity of LAB, which utilizes sugars as an energy source during fermentation and cold storage. The rate of TSS decline was more pronounced in P0 and P1, at 0.10 and 0.12 °Brix per day, respectively, compared to 0.06 °Brix in P2 and 0.05 °Brix in P3 over 21 days. This difference may be associated with the higher LAB population observed in less diluted treatments, resulting in greater sugar consumption. This relationship supports the hypothesis that a higher LAB count accelerates sugar utilization, thereby increasing the rate of TSS reduction. These findings agree with those of Thakur and Joshi [23], who reported that TSS in probiotic apple juice decreased during refrigerated storage due to total and reduced sugars consumption by LAB. Overall, the P0 treatment consistently exhibited the highest TSS values, while increasing dilution (P1-P3) resulted in lower TSS, initially and throughout storage.

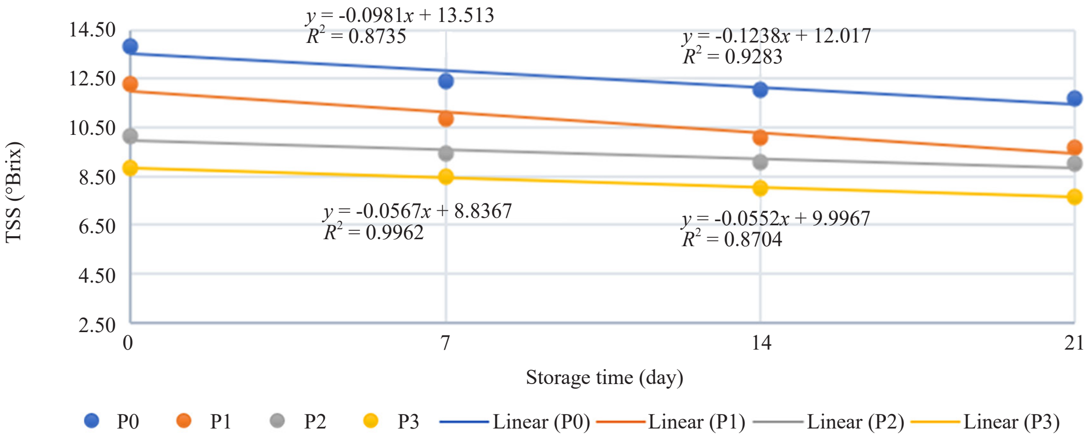

Figure 5. Total Soluble Solids (ºBrix) of probiotic juice mixture drink during the cold storage. Proportions of juice to water (v/v) are (1 : 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4 : 3) for P3. P0 vs P1, P2, P3**, P1 vs P2, P3**, P2 vs P3** (** = significantly different at alpha 1% by contrast test)

3.5 *Aroma and overall acceptance*

Figure 6 illustrates that the aroma scores of all treatments exhibited a quadratic trend during cold storage. This pattern reflects initial improvement in aroma perception during early storage, likely due to ongoing fermentation and metabolite formation, followed by a plateau or slight decline toward the end of the storage period. A significant difference in aroma preference was observed between treatment P0 (undiluted) and the diluted treatments (P1, P2, P3), with P0 consistently receiving lower aroma scores. Treatment P0 recorded aroma scores ranging from 2.8 (dislike) to 4.08 (like), whereas P1 ranged from 3.1 (slightly like) to 3.9 (like). Although aroma scores for P1, P2, and P3 were not significantly different, P2 demonstrated a statistically significant difference compared to P3, suggesting slight variations in the volatile profile or sensory intensity. The lower aroma acceptability of P0 is attributed to the strong grassy odour derived from undiluted Katuk leaf juice. This undesirable aroma is primarily associated with naphthalene, a volatile compound identified in Katuk leaves [24]. Similar findings were reported by Utami and Anjani [25], who noted that the grassy smell of Katuk leaves negatively influenced sensory ratings. The dilution appears to mitigate the impact of strong vegetal notes from Katuk leaves, resulting in higher aroma preference scores in the diluted treatments. These results suggest that adjusting the juice-to-water ratio affects physicochemical properties and plays a crucial role in optimizing sensory acceptance, particularly aroma, during cold storage of probiotic vegetable-fruit beverages.

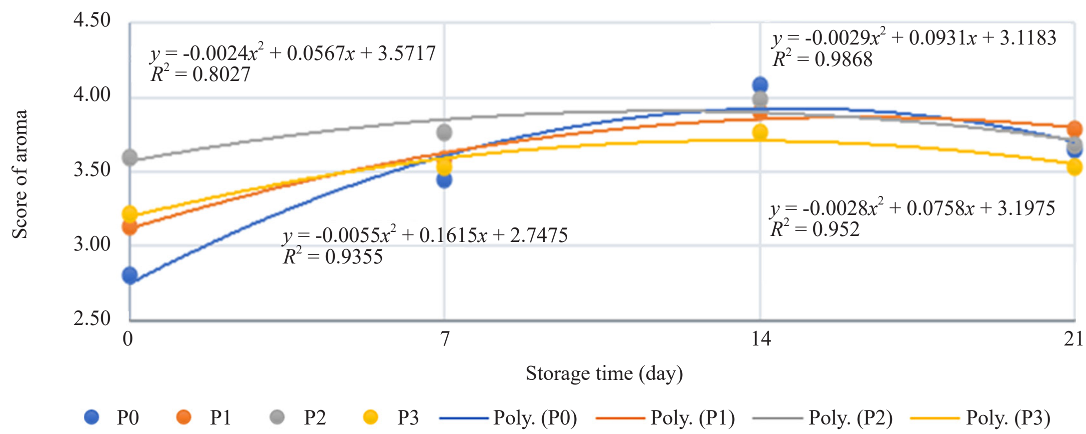

Figure 6. Aroma score of probiotic juice mixture drink during cold storage. (Proportion of juice to water with four level P0 = Control (1 : 0), P1= 4 : 1, P2 = 2 : 1, P3 = 4 : 3 (v/v). P0 vs P1, P2, P3*, P1 vs P2, P3^{ns}, P2 vs P3**(ns = not significant, ** = significant at alpha 1% by contrast test

During cold storage, the aroma scores of the probiotic beverage increased following a quadratic trend. As illustrated in Figure 6, aroma scores rose progressively until the second week of storage, after which they declined in the third week. Treatment P1 achieved the highest aroma score of 3.86 at approximately 16.05 days of cold storage, which was not significantly different from treatments P2 and P3. The peak in aroma acceptability observed in week two corresponds to the optimal growth phase of LAB (as shown in Figure 1), suggesting a link between microbial activity and volatile compound development. These results agree with Yuliana et al. [6] and Malganji et al. [26], who reported similar quadratic trends in sensory scores of fermented beverages during cold storage. Lactic acid bacteria produce various organic acids-such as lactic, malic, pyruvic, and acetic acids-contributing to probiotic beverages' aroma and flavour complexity [27]. In addition to LAB-derived volatiles, the natural aromas of carrot and pineapple also contributed to the sensory profile. Carrot aroma is associated with terpenoids and volatile compounds originating from the pericycle tissue of the roots [28]. Meanwhile, pineapple aroma is primarily influenced by ester compounds such as ethyl acetate, isopentyl acetate, methyl hexanoate, and methyl 3-(methylthio) propanoate [29]. These compounds enhance the fruity and sweet aroma, likely contributing to the improved panelist acceptance of the diluted formulations. The results of the sensory evaluation for overall acceptance (Figure 7) showed scores ranging from 3.05 (slightly like) to 3.98 (like). Similar to the trend observed for aroma, overall acceptance scores followed a quadratic trend of increased acceptance during cold storage across all dilution treatments. This trend suggests that cold storage allows for the development of desirable flavor and aroma compounds up to a certain point, after which over-acidification or off-flavor development may occur, decreasing acceptability. The influence of dilution was also evident, with diluted treatments generally exhibiting higher overall acceptance scores. This score can be attributed to reducing strong vegetal or grassy notes from Katuk leaves, resulting in a more balanced flavor profile. Thus, dilution and storage duration are critical factors influencing consumer acceptance of mixed vegetable-fruit probiotic beverages.

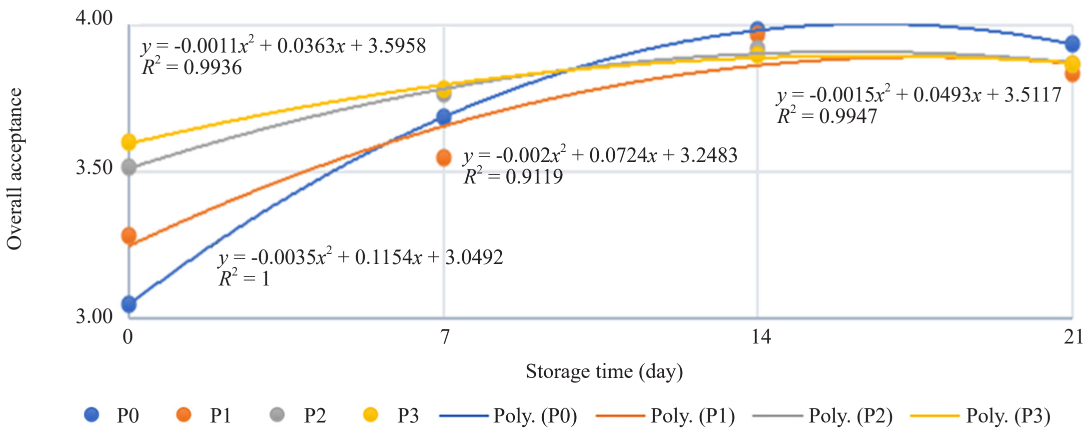

Figure 7. Overall acceptability score of probiotic juice mixture drink during the cold storage. Proportions of juice to water (v/v) are (1 : 0) for P0 = control, (4 : 1) for P1, (2 : 1) for P2, (4 : 3) for P3. P0 vs P1, P2, P3^{tn}, P1 vs P2, P3*, P2 vs P3^{tn} (ns = not significant, ** = significant at alpha 1% by contrast test

The overall acceptance scores of the probiotic juice increased quadratically during cold storage. Figure 7 shows a significant difference among P1, P2, and P3 in the increase of overall acceptance scores during cold storage. P2 and P3 exhibited higher overall acceptance scores during cold storage. The overall acceptance scores rose until the second week and then declined in the third week. The quadratic increase in sensory scores was due to LAB activity, which produced flavor- and aroma-forming compounds in the probiotic juice made from Katuk leaves, carrots, and honey pineapple. Malganji et al. [26] similarly reported that the increase in overall acceptance scores during cold storage was consistent with the rise in aroma scores due to the growth and activity of LAB. The overall acceptance scores of the probiotic juice increased quadratically during cold storage, as illustrated in Figure 7. A significant difference ($p < 0.05$) was observed among treatments P1, P2, and P3, with P2 and P3 exhibiting the highest acceptance scores during storage. The increase in overall acceptance was most pronounced during the first two weeks, followed by a gradual decline in the third week.

This pattern suggests an optimal sensory window for consumer preference. The quadratic increase in acceptance scores corresponds with the metabolic activity of Lactic Acid Bacteria (LAB), which contribute to developing desirable flavor and aroma compounds. These include organic acids and various volatiles formed during fermentation, which enhance the complexity and palatability of the beverage. The formulation combined Katuk leaves, carrots, and honey pineapple and offered a unique sensory profile influenced by raw ingredient volatiles and fermentation byproducts. These findings are consistent with the report of Malganji et al. [26], who found that the increase in overall acceptance scores during cold storage was associated with elevated aroma scores resulting from LAB growth and activity. In the present study, diluted treatments (P2 and P3) were preferred, likely due to the moderated intensity of vegetal notes from Katuk leaves and the more balanced sensory attributes. In summary, the results demonstrate that the degree of dilution and storage duration significantly influence consumer acceptance. The second week of storage represents the optimal period for achieving favorable sensory characteristics in probiotic juice made from mixed vegetable and fruit substrates.

3.6 *Determination of the best treatment*

The best treatment in this study was determined based on several criteria: (1) total LAB count meeting the minimum standard for probiotic beverages ($\geq 10^6$ CFU/mL) [19]; (2) LAB viability maintained during cold storage; (3) pH and total lactic acid content within the typical range of probiotic juice; (4) Total Soluble Solids (TSS) compliant with the Indonesian National Standard (SNI 2981 : 2009), which requires a minimum of 8.2 °Brix; and (5) the highest sensory scores for aroma and overall acceptance. All probiotic juice formulations met the minimum LAB count requirement, with total LAB levels ranging from 8.76 to 9.96 log CFU/mL. LAB viability values ranged from 69.7% to 118.6%, indicating that viable probiotic cells were maintained across all treatments. The total lactic acid content ranged from 0.19% to 1.36%, aligning with the values reported in previous studies for vegetable-fruit probiotic beverages, typically within 0.17%-1.23% [6, 28, 29], and within the expected range for titratable acidity (0.5%-1.5%) in probiotic products. The pH values of the probiotic juices ranged from 3.42 to 4.81, consistent with values previously reported in studies on vegetable-fruit-based probiotic drinks (3.34-5.2) [30, 31]. The TSS ranged from 7.7 to 13.8 °Brix, with all treatments meeting the minimum SNI requirement except P3S2 and P3S3. In terms of sensory acceptability, panelists showed a preference for formulations that were not excessively sour. Based on the integrated evaluation of microbiological, physicochemical, and sensory parameters, the P2S2 treatment, corresponding to a 2 : 1 juice-to-water dilution and 14 days of cold storage, was the most optimal formulation. This treatment exhibited the following characteristics: Total LAB: 10.98 log CFU/mL, LAB viability: 109.4%, pH: 3.66, Total lactic acid: 0.98%, TSS: 9.1 °Brix, Aroma score: 3.98 (*like*). Overall acceptance score: 3.92 (*like*). These findings suggest that the P2S2 formulation offers the most favourable balance between probiotic functionality and consumer sensory acceptance. It is a promising candidate for further development into a commercial non-dairy probiotic beverage.

4. Conclusions

Dilution significantly improved the viability of Lactic Acid Bacteria (LAB), as well as the aroma and overall acceptance of the probiotic beverage formulated from Katuk leaves, carrots, and honey pineapple. Storage duration exhibited a quadratic effect on total LAB count, viability, lactic acid content, aroma, and overall sensory acceptance. In contrast, pH decreased quadratically, and Total Soluble Solids (TSS) declined linearly over time. The best treatment was P2S2 (2 : 1 juice-to-water dilution, 14 days of cold storage). This formulation demonstrated high LAB count and viability, appropriate pH and lactic acid levels (not overly sour), TSS values that met the SNI 2981:2009 standard, and superior aroma and overall sensory scores. These findings suggest that the mixed juice of Katuk leaves, carrot, and honey pineapple at a 2 : 1 dilution ratio holds promising potential as a novel non-dairy probiotic beverage.

Authors' contribution

Conceptualization: Neti Yuliana; Formal analysis: Trisna Aulia; Methodology: Tirza Hanum; Supervision: Azhari Rangga; Writing-original draft: Trisna Aulia; and Writing-review & editing: Neti Yuliana.

Conflict of interest

The authors declare that there are no competing interests.

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