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Probiotic Innovation: Optimizing Coconut Water Kefir for Health and Consumer Acceptance

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***Abstract*— Coconut water kefir is a non-dairy probiotic beverage produced through the fermentation of coconut water using kefir starter cultures. Unlike milk kefir, coconut water kefir exhibits distinct physicochemical and microbiological characteristics, necessitating specific quality optimization. This study evaluated the effects of sugar addition timing (before or after 24 h incubation) and storage duration (0–4 weeks) on the chemical (total acid, total sugar, pH, alcohol), physical (viscosity), microbiological (lactic acid bacteria, yeast, total microbes, *Escherichia coli*), and organoleptic properties of coconut water kefir. A quantitative descriptive approach with two replications was applied. The results indicated that sugar addition after incubation combined with two weeks of refrigerated storage provided the most balanced overall quality, considering physicochemical stability, probiotic viability, microbiological safety, and sensory acceptance. Under these conditions, the kefir exhibited total acidity of 0.16%, total sugar of 7.60%, pH 4.00, alcohol content of 0.33%, viscosity of 1.10 cP, lactic acid bacteria of 7.8×10^7 CFU mL^{-1}, yeast of 1.7×10^6 CFU mL^{-1}, total microbes of 2.6×10^6 CFU mL^{-1}, and was negative for *E. coli*. Although the highest overall organoleptic score was observed at week 0, the two-week storage condition was selected as optimal due to its more favorable balance between functional quality and consumer acceptance (overall score 3.20 on a 5-point scale). These findings highlight the distinct characteristics of coconut water kefir compared to milk kefir and support the need for developing specific quality standards for water-based kefir beverages.**

***Keywords*— Coconut water kefir; added sugar; storage duration; probiotics; optimization**

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I. INTRODUCTION

Coconut water kefir is a probiotic drink rich in vitamins, minerals, and electrolytes, produced by fermenting coconut water with a kefir starter containing lactic acid bacteria (LAB), acetic acid bacteria, and yeast. Unlike traditional kefir, which uses kefir grains and milk as substrates, coconut water kefir utilizes starter and coconut water, producing bioactive compounds such as lactic acid, acetic acid, mannitol, CO_2, glycerol, and alcohol in low amounts [1]. This drink has a sweet-sour, effervescent taste and a light texture, making it an attractive isotonic alternative for consumers seeking a functional beverage with health benefits, such as potential benefits related to gut health, immune modulation, and antimicrobial activity [2].

The fermentation process of coconut water kefir is influenced by several key factors, including sugar concentration, sugar addition time, and storage duration. The addition of sugar, either before or after incubation, affects microbial metabolism, resulting in variations in acidity, alcohol content, viscosity, and organoleptic profile [3]. Previous studies have shown that longer fermentation increases lactic acid and alcohol, lowers pH, and affects texture and flavor [1]. However, the lactic acid

content of coconut water kefir (0.1–0.3%) is generally lower than that of milk kefir (minimum 0.6%; [4]), and its alcohol content remains below 0.5% [5], making it a safe and attractive non-alcoholic beverage for health-conscious consumers.

The lack of specific standards for coconut water kefir has led to reliance on milk kefir standards, which are not entirely relevant due to the different nutritional composition of coconut water (lower in lactose and protein) compared to milk [6]. This affects the physicochemical and microbiological characteristics, such as viscosity (1.00–1.70 cP vs. 2–5 cP in milk kefir) and LAB count (10^7–10^8 CFU mL^{-1}), which still meet the requirements for probiotics [2]. Therefore, the development of new standards for coconut water kefir is essential to ensure quality, safety, and consumer acceptance.

This study aims to optimize the time of sugar addition (before or after 24-hour incubation) and storage duration (0–4 weeks) to improve the chemical (total acid, total sugar, pH, alcohol), physical (viscosity), microbiological (number of LAB and yeast, safety from *E. coli*), and organoleptic (taste, flavor, effervescence, color, and overall acceptability) characteristics of coconut water kefir. The results of this study are expected to provide a basis for quality standards for coconut water kefir, increase its commercial potential as a functional probiotic beverage, and support its health benefits, such as improved gut health and immunity.

II. Material and Methods

A. Material

Fresh coconut water was obtained by manually drilling a sterile stainless-steel hole into intact young green coconuts (6–9 days post-harvest) purchased from a local traditional market in East Denpasar, Bali. Only coconuts with no visible cracks, mold growth, or physical damage were selected to minimize pre-existing microbial contamination. Distilled water and also sucrose (Rose Brand) were purchased from a local chemical supplier in Denpasar.

The kefir starter used in this study was in the form of kefir grains, which were purchased from an official verified online store (Shopee-GIO Nutrifarma). According to information provided by the seller, the starter contains a symbiotic community of lactic acid bacteria (LAB) including *Lactobacillus* spp. and yeasts such as *Saccharomyces cerevisiae*, though no further strain-level characterization was provided. After receiving them from the seller, the kefir grains were washed with boiled (cooled) water until clean and then drained, after which 100 ml of fresh or UHT milk was added. The milk was changed after 3–4 hours and replaced three times. The grains were then stored at 4°C for at least 24 h before use.

All reagents used for analysis were of analytical grade: 0.1 N Sodium Hydroxide (NaOH) solution, prepared by dissolving the appropriate amount of analytical grade NaOH pellets in distilled water. Acetone (99.5% purity) was also used. A 1% (w/v) phenolphthalein indicator solution was prepared by dissolving phenolphthalein powder in 95% (v/v) ethanol.

All equipment used for sample preparation and fermentation (including glass fermentation jars, pipettes, stainless-steel drilling tools, and measuring cylinders) were sterilized prior to use as follows:

1). Heat-stable glassware and metal tools were autoclaved at 121°C, 15 psi for 15 minutes; 2). All work surfaces and non-heat-stable equipment were wiped thoroughly with 70% (v/v) ethanol to eliminate ambient microbial contamination; 3). All inoculation and fermentation setup processes were conducted in a disinfected laminar airflow hood to minimize airborne microbial contamination.

B. Methods

Coconut Water Kefir Production

Fresh coconut water was filtered through sterile double-layer cheesecloth to remove any solid debris, and then pasteurized at 60°C for 30 seconds in a thermostatically controlled water bath. This mild pasteurization temperature was selected to reduce the indigenous microbial load while preserving the heat-sensitive bioactive compounds (such as vitamins and enzymes) and the delicate sensory profile of fresh coconut water, which can be compromised at higher temperatures. After heating, the coconut water was rapidly cooled to 28 ± 1°C in an ice-water bath.

Kefir starter (kefir grains), which had been previously stored in a refrigerator according to the manufacturer's instructions, was adapted in pasteurized coconut water at 25°C for 20 minutes with gentle stirring, and then allowed to stand for another 10 minutes. The kefir starter was added at a concentration of 10% (w/v), and the mixture was incubated at room temperature for 24 hours.

A 60% (w/v) sucrose stock solution was prepared by dissolving 600 g of sucrose in distilled water to a final volume of 1 L. This stock solution was then added to the pasteurized coconut water at 15% (v/v), either before fermentation or after 24 hours of incubation, following protocols adapted from [7]. This corresponds to 150 mL of the stock solution per liter of coconut water. This resulted in a final sucrose concentration of 90 g/L (9% w/v) in the fermentation broth.

After incubation, the kefir was filtered using a sterile food-grade filter cloth to remove biomass and residual starter particles. The filtration process was conducted under hygienic laboratory conditions to minimize microbial contamination. The filtered kefir was then stored at 5 °C for up to 4 weeks. Cold storage was applied to slow down microbial metabolic activity and fermentation processes, thereby maintaining product quality during storage. No additional treatment was applied to completely stop fermentation; however, the low storage temperature effectively reduced further acid and alcohol production during the storage period prior to analysis.

Physicochemical, Microbiological, and Sensory Analysis

Weekly testing assesses the chemical (acid, sugar, pH, alcohol), physical (viscosity), and microbiological (lactic acid bacteria) characteristics.

Total acid

The total acidity was measured by titration [8]. A 25 mL aliquot of the homogenized sample was transferred into a 250 mL Erlenmeyer flask and mixed with three drops of 1% (w/v) phenolphthalein indicator solution. The sample was then titrated with a standardized 0.1 N sodium hydroxide (NaOH) solution until a stable light pink color persisted for at least 30 seconds, indicating the titration endpoint. The total titratable acidity was calculated and expressed as percent lactic acid (% w/v) using the following formula:

$$Total\ acid\ (\%) = \frac{\text{V NaOH x N NaOH x MW lactic acid x DF}}{V\ sample \text{ x } 1000} \text{ x } 100 \quad \text{eq (1)}$$

where: V_NaOH is volume of NaOH use in titration (mL); N_ NaOH is normality of NaOH solution (0.1 N); MW_lactic acid is molecular weight of lactic acid ($C_3H_6O_3$)(90.08 g/mol); DF is the dilution factor; V_sample is volume of sample used (25 mL).

Total Sugar and Alcohol

Total sugar was measured using a refractometer, and alcohol content was measured using an alcohol meter, both validated by distillation [9]. A 100 mL sample was distilled with 100 mL of water until 50 mL of distillate was collected. The specific gravity of alcohol was calculated as:

$$\text{Specific gravity(alcohol)} = \frac{Weight(pycnometer+distillate) - Weight(empty\ pycnometer)}{Weight(\text{pycnometer+water}) - Weight(empty\ pycnometer)} \quad \text{eq (2)}$$

Organoleptic analysis of coconut water kefir was carried out using a hedonic rating method with 20 semi-trained panelists. The sample consisted of two types of coconut water kefir. The attributes tested were taste, flavor, effervescence, color, and overall acceptability. The panelists gave a score on a scale of 1-5, where (1) they disliked it extremely; (2) disliked it slightly; (3) neither liked nor disliked it; (4) liked it slightly; and (5) liked it extremely [10].

Viscosity

Viscosity was measured using an Ostwald viscometer [11]. The flow time of a 10 mL sample was recorded, and viscosity was calculated using the following formula :

$$\text{Viscosity} = \text{Viscosity(water)} \times \frac{Density(sample) \times Time(sample)}{Density(\text{water}) \times Time(water)} \quad \text{eq (3)}$$

Microbial Analysis

Lactic acid bacteria (LAB) counts were determined weekly following standard procedures for mesophilic lactic acid bacteria [12]. Samples were serially diluted in 0.1% sterile peptone water, and 1 mL aliquots were pour-plated onto de Man, Rogosa and Sharpe (MRS) agar supplemented with 0.1% L-cysteine hydrochloride. Plates were incubated anaerobically at 37°C for 72 hours using anaerobic jars (AnaeroGen™ system). Colony-forming units were enumerated and expressed as $\log_{10}$ CFU mL^{-1}.

Detection of *Escherichia coli* was performed using the most probable number (MPN) method according to standard procedures [13]. Samples were enriched in Lauryl Sulfate Tryptose (LST) broth with inverted Durham tubes at 37°C for 24–48 h, followed by subculture into EC broth at 44°C for 24 h. Positive tubes were confirmed by streaking onto Eosin Methylene Blue (EMB) agar. Typical metallic-green sheen colonies were counted as presumptive *E. coli*. Results were reported as MPN mL^{-1} (limit of detection <3 MPN mL^{-1}). All analyses were performed in duplicate.

Research Design and Data Analysis

A quantitative experimental design was employed to investigate the effects of two independent variables: (1) time of sugar addition (added at the beginning of fermentation vs. after 24 hours) and (2) storage duration (0, 1, 2, 3, and 4 weeks). The experiment was conducted with two replications (n=2) for each treatment combination. Due to the limited number of replications, the statistical power of the study was constrained. This limitation was acknowledged, and the results should be interpreted with caution, particularly when detecting subtle differences between treatments.

Data were analyzed using SPSS version 21. For each measured parameter (e.g., pH, total acid, microbial count), a two-way Analysis of Variance (ANOVA) was performed to assess the main effects of sugar addition time and storage duration, as well as their interaction. Post-hoc comparisons were conducted using the Least Significant Difference (LSD) test at a significance level of $\alpha = 0.05$. Given the low replication, the results of the ANOVA and LSD tests are presented as indicative trends rather than definitive conclusions. Further studies with higher replication are recommended to confirm these findings.

III. Results and Discussion

Table 1 presents the analyzed data for the chemical and physical characteristics of coconut water kefir. **Table 2** shows the organoleptic profile of coconut water kefir.

TABLE 1
CHEMICAL AND PHYSICAL CHARACTERISTICS OF COCONUT WATER KEFIR BY SUGAR ADDITION, TIMING, AND STORAGE DURATION

Storage Time (Weeks)	Sugar addition	Total Acid (%)	Total Sugar (%)	pH	Alcohol (%)	Viscosity (cP)
0	Post-incubation	0.16 ns	7.90 ns	4.00 ns	0.33*	1.10 ns
	Pre-incubation	0.34*	7.80 ns	4.50 ns	0.45*	1.00 ns
1	Post-incubation	0.30 ns	7.70 ns	4.05 ns	0.34*	1.20 ns

	Pre-incubation	0.34*	7.70 ns	4.55 ns	0.50*	1.20 ns
2	Post-incubation	0.43*	7.60 ns	4.05 ns	0.35*	1.20 ns
	Pre-incubation	0.45*	7.50 ns	4.60 ns	0.60*	1.10 ns
3	Post-incubation	0.48*	7.50 ns	4.10 ns	0.40*	1.40*
	Pre-incubation	0.56*	7.40 ns	4.70 ns	0.70*	1.40*
4	Post-incubation	0.66*	7.50 ns	4.15 ns	0.40*	1.40*
	Pre-incubation	0.56*	7.40	4.80 ns	0.75*	1.70*

Notes:
- Post-incubation: Sugar added after 24-hour incubation; Pre-incubation: Sugar added before incubation.
- ns: Not significant; *: Significant at 5% (LSD test).
- Data represent the means of two replicates.

TABLE 2
ORGANOLEPTIC SCORES OF COCONUT WATER KEFIR BY SUGAR ADDITION, TIMING, AND STORAGE DURATION

Storage Time (Weeks)	Sugar addition	Taste	Flavor	Fizziness	Color	Overall
0	Post-incubation	3.40 ns	3.60*	3.20 *	3.40 ns	3.80 ns
	Pre-incubation	3.40 ns	3.40*	3.40 ns	3.40ns ns	3.80 ns
1	Post-incubation	4.40 ns	3.60*	3.40 *	3.40 ns	3.60 ns
	Pre-incubation	3.00 ns	3.60*	3.20 ns	3.40 ns	3.60 ns
2	Post-incubation	3.20 ns	3.60*	3.20 ns	3.60*	3.40 ns
	Pre-incubation	3.20 ns	2.60*	3.20 ns	3.60*	3.40 *
3	Post-incubation	3.20 ns	2.40*	3.20 ns	3.80*	2.80 ns
	Pre-incubation	2.80 ns	2.40*	3.20 ns	3.80*	2.80 *
4	Post-incubation	2.60*	2.40*	2.40 *	3.40 ns	2.20 ns
	Pre-incubation	2.60*	2.40*	2.40 *	3.40 ns	2.20 ns

Notes:
- Post-incubation: Sugar added after 24-hour incubation; Pre-incubation: Sugar added before incubation.
- ns: Not significant; * : Significant at 5% (LSD test).
- Scores range from 1 (dislike extremely) to 5 (like extremely), based on 20 semi-trained panelists.
- Data represent the means of two replicates.

C. Total Acid

Analysis of total acid data in coconut water kefir showed no significant difference between weeks 0 and 1 (0.16–0.34%), but a significant increase was observed from weeks 2 to 4 (0.43–0.66%). The timing of sugar addition affected acid production, starting from week 2 (**Table 1**). This increase is due to lactic acid bacteria (LAB) in the kefir starter metabolizing sugars into lactic acid, which is the dominant acid in kefir [1]. Longer fermentation increases LAB activity, as glucose and fructose in coconut water are converted through glycolysis and homolactic fermentation, producing lactic acid and lowering the pH [14]. The lower acid content (0.16% at 2 weeks) compared to standard milk kefir (0.6%; [4]) reflects the lower lactose content of coconut water, limiting the availability of substrate for LAB compared to milk kefir [15]. However, these levels are consistent with water kefir studies, where total acidity typically ranges from 0.1–0.3% [16]. The addition of sugar after incubation resulted in lower acidity (0.16% vs. 0.45% when added before incubation at 2 weeks), likely due to delayed sugar availability, which reduced initial LAB metabolism. Nutrient availability (e.g., total sugars), pH (4.00–4.80), and storage at 5°C favored microbial growth, which increased acidity over time [17]. These findings suggest that, while coconut water kefir may not meet the acidity standards of milk kefir, its acidity is sufficient for probiotic function and flavor, which has implications for the development of specific standards for water-based kefir.

D. Viscosity

Viscosity did not appear to differ significantly from week 0 to 2 (1.00–1.20 cP) (**Table 1**) However, it increased significantly from week 3 to 4 (1.40–1.70 cP). This increase is primarily driven by the metabolic activity of lactic acid bacteria (LAB), particularly *Lactobacillus* species, which produce exopolysaccharides (EPS) during fermentation [7]. EPS formation is stimulated by sugar availability. However, the timing of sugar addition played a crucial role in the viscosity profile.

The results indicated that the pre-incubation treatment resulted in a higher viscosity (1.70 cP) compared to the post-incubation treatment (1.40 cP) at week 4. This suggests that the availability of sucrose from the beginning of the fermentation process provided a sustained substrate for LAB. As a result, they could synthesize and accumulate EPS over a longer duration throughout the storage period. While the post-incubation treatment provided additional energy, the delayed availability may have restricted the window for optimal EPS production compared to the pre-incubation group.

The slight decrease in viscosity observed at week 2 in the pre-incubation treatment (1.10 cP) may be attributed to the initial breakdown of sugars and degradation of the soluble proteins. This occurred before EPS accumulation became the dominant factor. Total solids, protein content, and acid production also influence viscosity (e.g., acid-induced protein coagulation) [18]. However, the overall viscosity of coconut water kefir remained lower than that of milk kefir (2–5 cP) due to the lower inherent protein and solids content of the substrate [6]. These results suggest that optimizing the timing of sugar addition is critical for modulating EPS synthesis to achieve a desirable mouthfeel and consistency for commercial probiotic beverages.

E. Alcohol contents

Alcohol content increased significantly in line with fermentation time (0.33–0.75%) and was influenced by the timing of sugar addition (**Table 1**). Yeasts in kefir starters, such as Saccharomyces species, ferment glucose and fructose into ethanol via the Embden-Meyerhof pathway, with longer fermentations increasing yeast activity [19]. Adding sugar after incubation resulted in lower alcohol content (0.33% at 2 weeks

vs. 0.60% before incubation), as the delayed availability of sugar limited initial yeast metabolism and reduced ethanol production. Alcohol content remained below 0.5% after incubation, which is consistent with the typical range of water kefir (0.2–0.5%; [1]) and is considered safe for non-alcoholic beverage classification (<1% in most regulations; [5]). Acidity (pH 4.00–4.80), starter composition, and storage at 5°C favor yeast metabolism, which increases alcohol over time [20], [21].

Compared to milk kefir (0.5–1%), the lower alcohol content of coconut water kefir makes it more appealing to health-conscious consumers. These findings highlight the importance of controlling added sugar to minimize alcohol content, ensure compliance with non-alcoholic standards, and increase marketability.

F. Total Lactic Acid Bacteria

Total lactic acid bacteria (LAB) ranged from 7.0×10^7 to 1.3×10^8 CFU mL^{-1} with sugar addition after incubation, which is consistent with probiotic beverage standards (10^6–10^9 CFU mL^{-1}; [2] (**Table 1**)). The kefir starter contains sucrose from milk, which supports the growth of LAB, especially *Lactobacillus casei*, which metabolizes glucose, lactose, and fructose into lactic acid via glycolysis [22]. Adding sugar after incubation increased LAB counts by providing a delayed carbon source, which sustained microbial proliferation during storage. These counts are comparable to those of other water kefirs (10^7–10^8 CFU mL^{-1}; [16] and are sufficient for probiotic health benefits, such as improved gut microbiota and immunity [2]. The absence of *E. coli* confirms microbiological safety.

The nutrients in coconut water (e.g., minerals, sugars) and its low pH (4.00–4.80) create an optimal environment for LAB, unlike substrates with a neutral pH [17]. These results underscore the potential of coconut water kefir as a probiotic delivery medium, with sugar addition after incubation optimizing LAB viability for health applications.

G. Organoleptic Evaluation

Table 2 shows that taste scores ranged from 3.2 to 4.4 ("like slightly" to "like"), with significant differences observed at longer storage durations. The decline in taste scores at weeks 3 and 4 is associated with increased acidity (0.56–0.66%) resulting from lactic and acetic acid production, as well as reduced sweetness due to decreasing sugar content (7.40–7.60%) [23]. The addition of sugar after incubation maintained a balanced sweet–sour profile for up to 2 weeks of storage (score 3.20), which aligns with consumer preferences for probiotic beverages with mild acidity [24].

Flavor scores ranged from 2.4 to 3.6 and were primarily influenced by the formation of volatile compounds such as lactic acid, acetic acid, and acetaldehyde during fermentation. Treatments with sugar addition after incubation generally exhibited higher flavor scores, reflecting moderated acid production and reduced development of overly sour notes compared to pre-incubation treatments [22].

Effervescence scores ranged from 2.4 to 3.4 and were higher in post-incubation treatments for up to 3 weeks of storage, driven by CO_2 production from yeast metabolism, which enhances sensory appeal [25]. In contrast, color scores (3.4–3.8) showed no significant differences among treatments or storage durations, as the kefir consistently retained a transparent, slightly milky appearance typical of water kefir beverages [26].

Overall acceptability scores ranged from 2.2 to 3.8, indicating generally good consumer acceptance, with the 2-week storage condition showing a favorable overall score of 3.40. Although higher overall scores were observed at earlier storage times, the 2-week post-incubation treatment represents an acceptable sensory quality. It also maintains better physicochemical stability and controlled alcohol content compared to longer storage durations. Therefore, this condition reflects a balanced sensory profile that supports its selection as an optimal condition in terms of overall quality rather than the highest sensory score alone.

H. Analysis of Correlation Between Factors

The strength and direction of relationships between variables were identified using a heat map chart (**Figure 1**). This approach facilitates the interpretation of how changes in one parameter are associated with changes in others, providing insights into fermentation dynamics and supporting data-driven decision-making. The heat map illustrates the correlations observed in coconut water kefir under post-incubation conditions.

The analysis revealed a strong positive correlation between Total Acid and Log LAB ($r = 0.92$), as well as between Alcohol and Log Yeast ($r = 0.97$), indicating that increased fermentation activity promotes acid production and microbial growth. These relationships are consistent with the metabolic interactions between lactic acid bacteria and yeasts during kefir fermentation.

Conversely, Overall acceptability showed a strong negative association with alcohol content ($r = -1.00$), indicating that increasing alcohol levels during extended storage were accompanied by a decline in sensory acceptance. This trend explains the reduction in overall scores observed at longer storage durations (**Table 2**; **Figure 2**). Therefore, the two-week storage condition with post-incubation sugar addition represents a balanced condition in which alcohol content remained controlled while acceptable sensory quality was maintained. Under this condition, the kefir maintained a controlled alcohol content (0.33%) while retaining satisfactory consumer acceptance (overall score 3.40).

These findings highlight the importance of regulating fermentation duration to achieve a balanced quality profile in which probiotic viability and safety are maintained without compromising sensory acceptance. This correlation-based approach supports the development of tailored quality standards for coconut water kefir that consider multiple quality attributes simultaneously.

I. Urgency of Developing New Standards for Coconut Water Kefir

This study showed that the characteristics of coconut water kefir, such as total acid (0.16–0.66%), viscosity (1.00–1.70 cP), and alcohol content (0.33–0.75%), were significantly different from milk kefir, which has a minimum standard of 0.6% lactic acid and a viscosity of 2–5 cP [4],[6]. This difference is due to the nutritional composition of coconut water, which is lower in lactose and protein than milk, thus affecting microbial metabolism and physicochemical properties. The use of milk kefir as a reference standard is not entirely relevant because coconut water kefir has a unique profile and characteristics that are closer to water-based beverages such as kombucha [1], [16]. The lack of specific standards for coconut water kefir hinders the development of consistent, high-quality products and may pose challenges for product consistency and regulatory alignment through appropriate regulation.

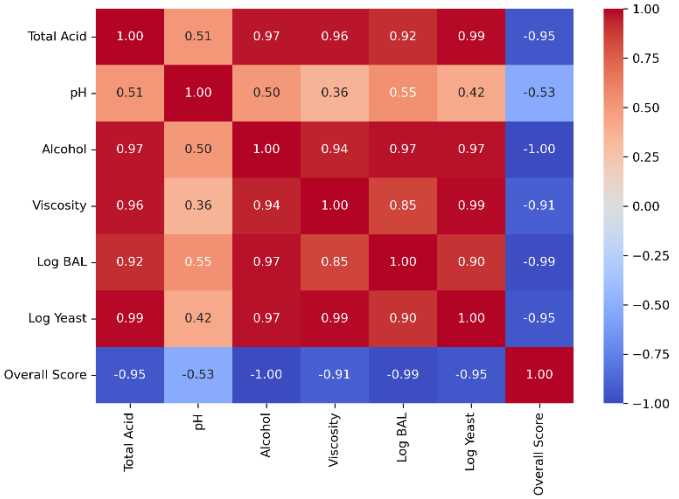

Fig. 1 Correlation heatmap of coconut water kefir characteristics under post-incubation conditions, illustrating relationships among physicochemical, microbiological, and sensory parameters. Strong positive correlations are observed between Total Acid and Log LAB (r = 0.92) and between Alcohol and Log Yeast (r = 0.97), while overall acceptability shows a perfect negative correlation with alcohol content (r = −1.00). These relationships highlight the importance of controlling fermentation dynamics to achieve an optimal balance between functional quality and consumer acceptance.

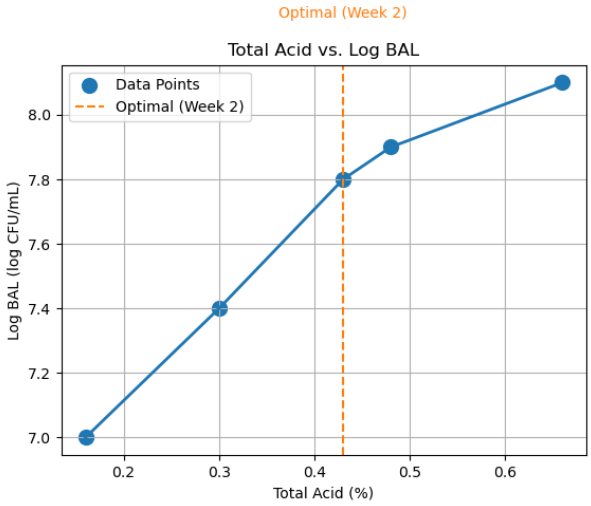

Fig. 2 Relationship between alcohol content and overall organoleptic score of coconut water kefir under post-incubation conditions. A strong negative association (r = −1.00) demonstrates that increasing alcohol content reduces consumer acceptability, supporting the selection of 2 weeks of storage as the condition that provides an optimal balance between functional quality and acceptable sensory attributes rather than the highest organoleptic score.

pH: the range is 4.00–4.80 to support LAB growth and maintain the desired sweet-sour taste profile.

Alcohol content: the maximum is 0.5% to comply with the non-alcoholic beverage classification and ensure appeal to the health-conscious market [1].

Total Lactic Acid Bacteria (LAB): the minimum is 10^7 CFU mL^{-1} to ensure probiotic benefits, such as improved gut health and immunity [2].

Viscosity: the range is 1.00–1.50 cP to achieve a smooth and slightly viscous mouthfeel, according to consumer preferences.

Total sugar: the range is 7.0–8.0% to balance sweet-sour flavors without encouraging excess alcohol production.

Microbiological safety: must be negative for pathogens such as *E. coli* to ensure product safety.

Organoleptic attributes: with a minimum hedonic score is 3.0 (scale 1–5) for taste, flavor, effervescence, and color, ensuring good consumer acceptance.

This standard will support the development of consistent coconut water kefir products, facilitate quality certification, and increase consumer confidence in the health benefits of this beverage. Further research is needed to validate these parameters across different coconut water varieties and kefir starters to ensure an inclusive and flexible standard.

IV. CONCLUSION

The timing of sugar addition and storage duration significantly influenced the physicochemical, microbiological, and sensory characteristics of coconut water kefir. Sugar addition after incubation combined with two weeks of refrigerated storage resulted in the most balanced overall quality. Under these

conditions, the product exhibited moderate acidity (0.16%), controlled alcohol content (0.33%), stable pH (4.00), adequate viscosity (1.10 cP), high lactic acid bacteria viability (7.8×10^7 CFU mL^{-1}), acceptable yeast and total microbial counts, and was free from *Escherichia coli*. The overall organoleptic score (3.20 on a 5-point scale) indicates satisfactory consumer acceptance.

Although higher sensory scores were observed at earlier storage times, the two-week storage condition with post-incubation sugar addition provided a more favorable balance between functional quality, microbiological safety, and sensory acceptance. Therefore, this condition was selected as optimal in terms of overall quality balance rather than achieving the highest individual sensory score.

The findings demonstrate that coconut water kefir exhibits physicochemical and microbiological properties that differ substantially from those of milk kefir, highlighting the limitations of applying milk kefir standards to water-based fermented beverages. This study supports the need to develop specific quality standards for coconut water kefir, including appropriate ranges for total acidity, pH, alcohol content, probiotic viability, viscosity, and sensory acceptance.

Future studies with higher replication levels, different coconut water sources, and alternative starter cultures are recommended to validate and refine these proposed quality parameters. Such efforts will strengthen the scientific basis for standardization and support the development of coconut water kefir as a safe, consistent, and functional probiotic beverage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

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