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Effect of Time Fermentation on the Microbiological Properties of Coconut Water Kefir

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ABSTRACT

Coconut water is one of the popular drinks in tropical countries, it has been used widely as an isotonic drink, is nutrient-rich, and has other beneficial functions. Many advantages of coconut water also make this product explored by many researchers as a synbiotic or even probiotic drink. This research investigates the impact of time fermentation on the microbiological properties of coconut water kefir. The study employs various time intervals during the fermentation process to explore changes in mesophilic bacteria, lactic acid bacteria, and total yeast and mold. By analyzing the microbiota present at different fermentation times (6, 12, 18, 24, and 48 hours), this research aims to elucidate how time affects the number of beneficial potential probiotic strains, such as lactic acid bacteria and yeast. The findings of this study showed that 24 hours of fermentation time gave the highest number of lactic acid bacteria (7.23 Log CFU/mL), the highest number of mesophilic bacteria (7.52 Log CFU/mL), and total yeast and mold (7.14 Log CFU/mL). Additionally, the inhibitory effects of coconut water kefir on two different kinds of pathogenic bacteria were examined using well diffusion, even though there was no inhibition zone. In conclusion, 24 hours is the best fermentation time to produce coconut water kefir which showed the highest increase of lactic acid bacteria, mesophilic bacteria, and total yeast and mold.

Keywords: ***Coconut Water Kefir, Time Fermentation, Microbiological Properties***

1. INTRODUCTION

Coconut water, the clear liquid found inside young coconuts, has gained widespread recognition for it is refreshing taste and numerous health benefits. It has long been celebrated in tropical regions for its natural hydration properties [1], moreover, it is often referred to as "nature's sports drink" due to its isotonic nature, which closely resembles the electrolyte composition of human bodily fluids, making it an excellent choice for rehydration. Moreover, other research shows the function of coconut water as an isotonic fluid in athletes [2], beyond being a popular tropical beverage, coconut water has found new roles as a versatile ingredient in various culinary creations functional beverages, and treatment of diarrhea [3], coconut water can also be used in the production of various innovative value-added products such as lassi (tender coconut beverage), yogurt, sugar, nata, vinegar, and coconut water kefir [1], [4], one of its intriguing applications is as a medium for fermenting and producing a probiotic-rich beverage known as coconut water kefir. Many aspects, including increased consumer awareness of the health benefits of fermented foods, an increase in cases of lactose intolerance, and the popularity of cuisine trends like veganism, promote the creation of non-dairy fermented drinks like coconut water kefir [5].

In recent years, coconut water kefir has become a star in the world of probiotics and functional foods. The fermentation of coconut water to create kefir opens up a realm of possibilities for harnessing its inherent qualities. Kefir, typically produced from dairy milk, is a fermented beverage teeming with beneficial probiotic bacteria and yeast strains [6], [7].

When these microorganisms are introduced to coconut water, they initiate a transformation that not only preserves the natural goodness of coconut water but also enhances its nutritional profile. These grains are used to ferment sugar-water mixtures and are now collectively referred to as "water kefir grains" or "sugary kefir grains" [8], the main microorganisms in the water kefir grain are lactic acid bacteria, yeast, and acetic acid bacteria. The broad usage of water kefir for preventing IBD (inflammatory bowel disease) [9], improving intestinal permeability [10], and reducing constipation and diarrhea [11] were mentioned in other articles.

Certainly, research exploring the fermentation time for coconut water kefir is a valuable area of this study. There isn't much study on coconut water kefir, but milk kefir is the subject of the majority of studies. These studies cover whatever changes in the bacterial community during fermentation [12], [13], to the creation of sensory-friendly beverage items [14], [15], the duration of fermentation plays a crucial role in determining the microbial composition of coconut water kefir. The result will determine the optimal duration that maximizes probiotic populations for enhanced health benefits. The findings of the study will serve as one of the requirements for producing coconut water kefir for microbusinesses utilizing traditional water kefir grains.

2. METHOD

The research involved three stages, including preparation of tools and materials, fermentation, and microbiological analysis. As part of the microbiological analysis, the number of lactic acid bacteria is calculated, as well as the number of total microbes, yeast molds, and an inhibition test against *Bacillus cereus* and *Listeria monocytogenes* for the two types of pathogenic bacteria.

Fermentation Process

The methodology employed in this study involved the collection of young coconut water in its fresh condition. There were no specific criteria for selecting the coconuts, as they were obtained from nearby traders and remained unopened to prevent any potential contamination. Water kefir grains (WKG) were used to ferment coconut water.

The coconuts were opened to retrieve the coconut water, and 500 mL of the water was then collected in a sterile 700 mL-capacity container. No sterilization or pasteurization processes were applied to the coconut water. After the coconut water was obtained, 50 grams of water kefir grains (10% w/v) was added to each container of coconut water. These containers were then labeled and subjected to different fermentation times according to the planned experimental design. The fermentation times chosen for harvesting the coconut water kefir were 6, 12, 18, 24, and 48 hours. After the fermentation process, sterile glass bottles with a capacity of 500 mL were chosen as appropriate containers for storing the harvested coconut water kefir.

Microbiological Analysis

Microbiological analysis involves the use of specific agar media, such as MRS (de Mann Rogosa Sharpe, Merck, Germany) agar for counting lactic acid bacteria, PCA (Plate Count Agar, Oxoid, UK) media for enumerating total mesophilic bacteria, and PDA (Potato Dextrose Agar, Himedia, India) for quantifying yeast and mold numbers. During the enumeration stage, a dilution process was performed by adding a 1 mL sample of coconut water kefir into a diluent solution under aseptic conditions, and decimal dilutions were performed for the enumeration of total mesophilic bacteria lactic acid bacteria (LAB) and yeasts and molds. Sterile NaCl 0.85% (Sodium chloride, Merck, Germany) was used to prepare the dilutions for the microbial analysis. On agar plates, all mesophilic bacteria were counted (PCA). 1 mL of the dilution sample was added to the medium after dilution continued until a calculable dilution was obtained. After 24 hours, the samples were counted for the number of bacterial colonies that were growing (lactic acid bacteria and total mesophilic bacteria); if no colonies were growing, the samples were incubated once more for a total of 48 hours, incubated at 30±1°C. In the meantime, the incubation process for the mold and yeast for 3-5 days at a temperature of 25±1°C was followed

by the computation of the number of mold and yeast colonies. The Bacteriological Analytical Method (BAM) counting range, which was 25 to 250 colonies, was used to determine the number of colonies counted. For each sampling period sampling was performed in duplicate, and from each sample at least three technical replicates were made.

The good diffusion assay was chosen as the preferred technique for evaluating the inhibitory activity of coconut water kefir samples against *Listeria monocytogenes* and *Bacillus cereus*. The prepared samples are delivered to a different laboratory for examination. The SPSS Inc.25 program was used to statistically analyze the data from LAB, mesophilic bacteria, and yeast count. Following a test to see if the variance was homogeneous (Levene's test of equality of error variances), the Mann-Whitney U test (p 0.05, ANOVA) was used to see whether there was a significant difference between the values.

3. RESULT AND DISCUSSIONS

Lactic acid bacteria (LAB) enumeration is a critical aspect of microbiological analysis in various fields, LAB are a group of bacteria known for their ability to produce lactic acid as a metabolic byproduct, and they are commonly found in fermented foods, dairy products, and the human gut. Figure 1 shows the amount of lactic acid bacteria in this research from each fermentation time.

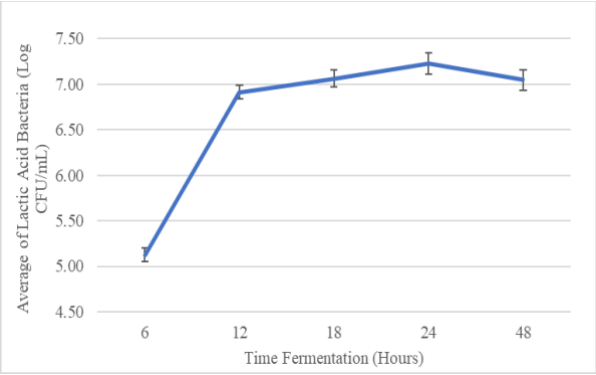

Figure 1. The number of lactic acid bacteria from coconut water kefir during fermentation time

Shows that the majority of the LAB colonies examined were able to increase during the fermentation process, as was expected. After 24 hours of fermentation, there was a decline that reached 7.04 log CFU/mL. This finding was demonstrated by rises in cell counts and corresponding drops in pH, as stated in other publications [16], according to one of the research findings [17], after the first six hours of fermentation, bacteria significantly utilized the amount of sugar that was present in the media. In this study, sugar wasn't used when making coconut water kefir. Consequently, a variety of nutrients that are naturally present in coconut water are among the available nutrients. Meanwhile, according to some sources, the temperature and duration of the fermentation process can affect the water kefir ecosystem's composition [18], additionally, the length of storage also affects the microbial composition of water kefir [14], bacterial growth will become slower and may eventually reach a state of near-zero growth, known as the stationary phase. It's important to note that the specific effects of nutrient limitation on bacterial growth can vary depending on the bacterial species.

Figure 2 shows more results in terms of the total number of mesophilic bacteria that were present during the fermentation period. It has been noted that the cell count initially rises and then gradually decreases with time fermentation, and it can be brought on by traces of nitrogen molecules or by the oxygen that is already there. An increase in microbial cells, a rise in acid content, and a concomitant decline in the amount of soluble carbohydrates are the three factors that contribute to the reduction in the number of bacteria.

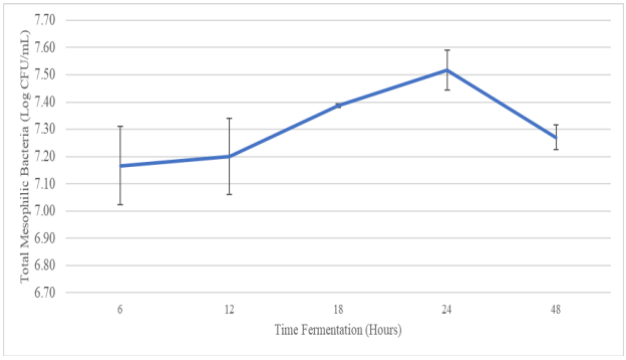

Figure 2. Total mesophilic bacteria enumeration results from coconut water kefir during fermentation time

Mesophilic bacteria grew at moderate temperatures between 20°C and 45°C and with an optimum growth temperature in the range of 30–39°C. However, most probiotic organisms (living microorganisms that reach the intestine and thus confer beneficial effects to the host) are mesophiles; in particular lactic acid bacteria are

largely used in the dairy industries [19], fermentation at 18, 24, and 48 hours has a great number of mesophilic bacteria so the bacteria produced are strongly suspected to consist of probiotic bacteria.

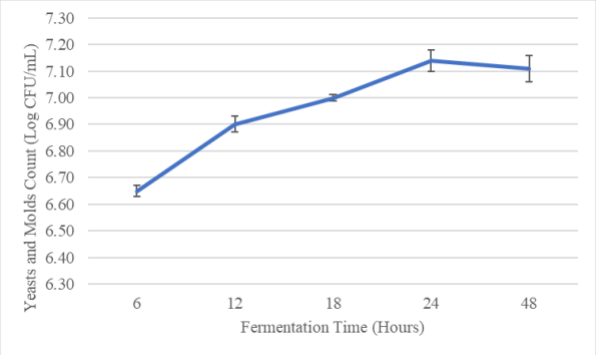

Figure 3. Total yeasts and molds from coconut water kefir during fermentation time

During the fermentation period, the number of molds was counted, as shown in Figure 4. In general, the number of yeasts and molds always showed the lowest value when compared to the number of lactic acid bacteria or total mesophilic microbes. The findings of this study revealed the same pattern and differences in the quantity of yeast, lactic acid bacteria, and total mesophilic flora in milk kefir, which showed that the quantity of yeast was at a lower level than the quantity of LAB [17].

This study's microbiological evaluation consisted of testing coconut water kefir's inhibitory zone against two different kinds of pathogenic bacteria, *Listeria monocytogenes* and *Bacillus cereus.* Both types of bacteria belong to the representative types of Gram-positive and Gram-negative bacteria. In almost all plates, observations of the presence of an inhibition zone revealed that no distinct inhibition clear zone had formed. However, variations in the density of bacteria growing around the well can be seen in Fig.4.

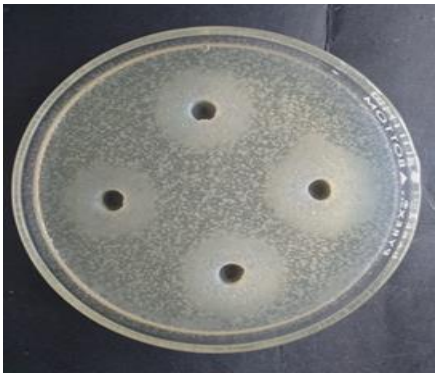

Figure 4. Inhibition test results of Coconut water kefir against *Listeria monocytogenes* and *Bacillus cereus*

These findings point out that the two types of pathogenic bacteria investigated could not be inhibited by coconut water kefir from all fermentation time. However, additional processing to isolate the active components from the observed fermentation results is still required to fully investigate the antibacterial potential of coconut water kefir. Regarding the inhibitory potential of kefir (especially for milk kefir) against various kinds of food-borne pathogens, on *Yersinia enterocolitica* isolate, all milk kefir isolates displayed an inhibition zone, while other types did not show an inhibition zone [20].

However, these results were achieved as a result of numerous LAB isolation procedures from kefir milk. Another paper stated that antimicrobial agent concentrations below the minimum inhibitory concentration, or MIC, can result in the formation of this subinhibitory activity, and kefir's only bacteriostatic antimicrobial activity is likely to be caused by subinhibitory inhibition, which is caused by barriers arising from metabolite components other than organic acids in relatively modest concentrations [21], the findings from this study cannot be directly compared with those from earlier studies due to the limits of research results that particularly describe the antibacterial effects of coconut water kefir on food-borne pathogens.

4. CONCLUSION

According to the study's findings, the number of live bacteria in coconut water kefir can vary depending on how long it ferments. The ideal fermentation period for obtaining the highest amount of lactic acid bacteria is 24 hours. The number of total yeast and mesophilic bacteria similarly followed the same trend, rising at 24

hours of fermentation. The coconut water kefir inhibition test against the test bacteria (*Listeria monocytogenes* and *Bacillus cereus*), on the other hand, failed to reveal a distinct zone of inhibition.

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