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Effect of Different Fermentation Durations on the Viability of *Lactobacillus Reuteri* DSM 17938 in Coconut Yogurt

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Abstract

The purpose of this research is to investigated the impact of fermentation duration on the viability of Lactic Acid Bacteria (LAB), with particular emphasis on *Lactobacillus reuteri* DSM 17938, in coconut yogurt. The experiment utilized three different fermentation durations (24, 36, 48 hours) to assess the proliferation of LAB in coconut yogurt. The results revealed a consistent increase in the viable LAB count with extended fermentation time, providing evidence for a linear relationship between these variables. However, the one-way ANOVA statistical analysis did not identify these differences as statistically significant. Despite this, the study reaffirms the viability of LAB, specifically *L. reuteri* DSM 17938, in coconut yogurt across varying fermentation periods.

This research contributes to the understanding of LAB behavior in coconut yogurt and their proliferation across different fermentation durations. Although the findings suggest a direct correlation between fermentation time and LAB population, the lack of statistical significance indicates a complex interplay of factors affecting LAB viability. The limitations, such as inability to isolate and identify the specific strain of *L. reuteri* DSM 17938 in the total LAB count, point to areas for future research. Despite these, the study sets a foundation for enhancing the quality and health benefits of probiotic foods by providing valuable insights into optimal fermentation durations for promoting LAB survival and growth in coconut yogurt production.

Keywords: Lactic Acid Bacteria (LAB), *Lactobacillus reuteri* DSM 17938, Coconut Yogurt

Introduction

This study embarks on an exploration of the intersection of plant-based milk alternatives and probiotics, with a focus on the potential of coconut milk as a medium for cultivation of *Lactobacillus reuteri* DSM 17938, a probiotic bacteria species (Mauro et al.,2019; Mu et al., 2018). Coconut milk, acclaimed for its rich texture and significant health benefit, including high nutritional content and potential for promoting beneficial gut microbiota, becomes the focus of our investigation. This research is designed within the broader contest of functional foods, products that provide health benefits beyond basic nutrition (Vergari et al., 2010; Ndlife, 2014). The expansion of this field reflects an increasing consumer interest in nutrition and health, and probiotics, particularly those incorporated into food products, are at the forefront of these developments (Markowial, 2017).

The challenge addressed in this study pertains to the production of probiotic fermented foods, such as coconut yogurt (Yuliana et al., 2010). While the interest in such products is on the rise, their manufacturing is not without hurdles (Mortazavian et al., 2017). The primary concern is the viability of probiotics during the fermentation process. Additionally, when moving away from traditional diary substrates to plant-based alternatives like coconut milk, the process becomes more complex due to inherent differences in the fermentation (Lee et al., 2013). In light of these challenges, the study aims to ascertain the optimal duration of fermentation to ensure the highest probiotic cell count in coconut yogurt.

The core objective of the research is to investigate the effect of different fermentation durations on the viability of *Lactobacillus reuteri* DSM 17938 in coconut yogurt at 37^{o}C for a period of up to 48 hours. The hypothesis suggests that prolonged fermentation, from 24 to 48 hours, will result in a significant increase in the viability of the bacteria, peaking at 48 hours (Ferdousi et al., 2013)

This research carries significant potential implication for both the scientific community and the food industry. If the hypothesis is confirmed, the findings may provide valuable insights for improving the production of high-quality, probiotic-rich

coconut yogurt, thereby contributing to the broader understanding of probiotic's growth and survival in fermented foods (Mauro & Garcia, 2019). This knowledge could lead to the development of improved or new probiotic products.

Ultimately, this investigation into coconut milk fermentation hopes to not only advance scientific understanding and manufacturing practices but also assist health-conscious consumers in creating their own homemade coconut probiotic yogurt. By deciphering the optimal fermentation duration for the highest viable cell count, this study has the potential to enhance the health-boosting benefits of this popular functional food (Ferdousi et al., 2013)

Research Methodology

In this research, an experimental research design was employed to investigate the influence of fermentation duration on the viability of Lactic Acid Bacteria (LAB), specifically *L. reuteri* DSM 17938, within coconut yogurt with the viability of *L. reuteri* DSM 17938 being the dependent variable and is measured in colony forming units per gram (CFU/g). Utilizing a non-probability sampling technique, yogurt samples were collected at three distinct fermentation durations of 24, 36, and 48 hours with the fermentation duration being the independent variable. The experiment involved the preparation of three batches of coconut yogurt, each incubated for up to 48 hours, with samples collected at each fermentation time point, in total 9 samples were collected. This procedure was replicated three times to enhance the reliability of the findings. Control variables, such fermentation temperature at 37^{o}C and fermentation duration of 48 hours, were maintained to ensure experimental integrity and validity of the results. The data collected were subjected to the One-way Analysis of Variance (ANOVA) to identify any statistically significant variations in the viability of *L. reuteri* DSM 17938 across the different fermentation durations. Ethical considerations were minimal due to the absence of human and animal subjects, but laboratory safety protocols were strictly followed throughout the experiment to prevent potential hazards. The microbiological analysis of the samples was carried out at the Biodiversity Research Centre of the Thailand Institute of Scientific and Technological Research. The analytical process employed was based on the American Public Health Association's Compendium of Methods for the Microbiology Examination of Foods (5th edition), a widely accepted

methodology known for its effectiveness in enumerating viable microorganisms in food products. (Tortorella, 2015)

Results

Table 1 Characteristic of the samples (n=9 samples)

Sample #	Batch	Test Samples	Sample Description	Size: g/cup
1	1	B1, 24h	Replication 1, coconut yogurt sampling at 24 h after fermentation	60g
2	1	B1, 36h	Replication 1, coconut yogurt sampling at 36 h after fermentation	60g
3	1	B1, 48h	Replication 1, coconut yogurt sampling at 48 h after fermentation	60g
4	2	B2, 24h	Replication 2, coconut yogurt sampling at 24 h after fermentation	60g
5	2	B2, 36h	Replication 2, coconut yogurt sampling at 36 h after fermentation	60g
6	2	B2, 48h	Replication 2, coconut yogurt sampling at 48 h after fermentation	60g
7	3	B3, 24h	Replication 3, coconut yogurt sampling at 24 h after fermentation	60g
8	3	B3, 36h	Replication 3, coconut yogurt sampling at 36 h after fermentation	60g
9	3	B3, 48h	Replication 3, coconut yogurt sampling at 48 h after fermentation	60g

Table 1 shows general information of all the 9 samples in the research project. Each sample size is weight 60g/cup.

Table 2 Results for the microbiological analysis of the viability of *L. reuteri* DSM 17938 in the 9 samples presented in CFU/g conducted by the Biodiversity Research Centre of Thailand Institute of Scientific and Technological Research Laboratory

Sample #	Batch	Test Samples	Amount of 'Lactic Acid Bacteria' detected (CFU/g)
1	1	B1, 24h	$9.17x10^7$
2	1	B1, 36h	$9.50x10^7$
3	1	B1, 48h	$1.09x10^8$
4	2	B2, 24h	$9.50x10^7$
5	2	B2, 36h	$9.77x10^7$
6	2	B2, 48h	$1.02x10^8$
7	3	B3, 24h	$7.50x10^7$
8	3	B3, 36h	$8.97x10^7$
9	3	B3, 48h	$1.08x10^8$

Table 2 represent the results which indicate the viable count of Lactic Acid Bacteria (LAB) detected in each sample. The colony-forming unites (CFU) were determined and expressed as CFU/g, which stands for colony-forming units per gram.

It is important to note a limitation for the APHA method used by the Biodiversity Research Centre of Thailand Institute of Scientific and Technology Research, while this research focus was on the *L. reuteri* DSM 17938 species, the laboratory was only able to present results as total Lactic Acid Bacteria (LAB) count, rather than specifically enumerating the target strain. Nonetheless, the presence of LAB indicated the presence of lactic acid-producing bacteria, including potentially the species of interest, *L. reuteri* DSM 17938.

Table 3 Geometric Mean (G.M.), Minimum, and Maximum of the viable count of Lactic Acid Bacteria (LAB) in CFU/g for each batch (Logarithm base 10)

Statistical	Batch 1 (B1)	Batch 2 (B2)	Batch 3 (B3)
G.M.	7.9925 CFU/g	7.9921 CFU/g	7.9535 CFU/g
Minimum	7.9624 CFU/g	7.9777 CFU/g	7.8751 CFU/g
Maximum	8.0374 CFU/g	8.0086 CFU/g	8.0334 CFU/g

Table 3 shows the geometric mean, minimum, and maximum of the viable count of LAB in CFU/g for each batch presented in logarithm base 10 value, which helps understand the characteristics of the data collected for the microbiological analysis results.

Table 4 Microbiology results (Logarithm base 10)

Fermentation Duration	Batch 1 (B1)	Batch 2 (B2)	Batch 3 (B3)	Mean ± SD
24 Hours	7.9624 CFU/g	7.9777 CFU/g	7.8751 CFU/g	7.9384 ± 0.0554
36 Hours	7.9777 CFU/g	7.9899 CFU/g	7.9528 CFU/g	7.9735 ± 0.0189
48 Hours	8.0374 CFU/g	8.0086 CFU/g	8.0334 CFU/g	8.0265 ± 0.0156
G.M.	7.9925 CFU/g	7.9921 CFU/g	7.9535 CFU/g	
Standard Deviation	0.0396 CFU/g	0.0156 CFU/g	0.0792 CFU/g	

Table 4 presents the data for the microbiology analysis result presented in CFU/g for batch 1, 2, and 3 during the fermentation duration of 24 hours, 36 hours, and 48 hours. In addition, represent the calculation for the means and the standard deviation of batch 1, 2, and 3.

Table 4.5 Compare mean between fermentation duration by One-way analysis of variance (ANOVA) summary

Fermentation Duration	G.M. ± SD	F-Statistic	P-Value
24 hr	7.9384 ± 0.0554 CFU/g	4.828	0.056
36 hr	7.9735 ± 0.0189 CFU/g		
48 hr	8.0265 ± 0.0156 CFU/g		

Note Homogeniety of variance (Levene statistic value = 1.908, P-value = 0.228)

Table 4 represent the between-group sum of squares, the within-group sum of squares, the between-group degrees of freedom, the within-group degrees of freedom, the between-group mean square, the within-group mean square, the F-statistic value and the P-value.

Let's assume that the significance level is 0.05. The p-value is 0.056, which is greater than the common significance level of 0.05. In statistical hypothesis testing, if the p-value is greater than the predetermined significance level which is 0.05, then this means that fail to reject the null hypothesis. The null hypothesis in this research was that there are no significant differences in the mean values of *L. reuteri* DSM 17938 viability among the different fermentation durations which is 24 hours, 36 hours, and 48 hours.

Therefore, based on the ANOVA results, this research fails to reject the null hypothesis. This suggests that the fermentation duration might not have a significant effect on the viability of *L. reuteri* DSM 17938 in coconut yogurt.

Failing to reject the null hypothesis is not the same as accepting it. It simply means that, based on the sample and the chosen significance level, the evidence was insufficient to conclude that a statistically significant difference exists. There could be a difference, but this research didn't have enough power to detect it, or the difference could be negligible in practical terms.

Discussion and Suggestion

This study aimed to analyze the influence of varying fermentation durations on the viability of Lactic Acid Bacteria (LAB), specifically *L. reuteri* DSM 17938, in coconut yogurt. Although an increase in LAB counts was observed with the prolongation of fermentation time, statistical analysis via One-way ANOVA suggested that these increases were not statistically significant enough to establish a direct correlation. This finding indicates the specific impact of fermentation duration on LAB viability remains complex, potentially due to multiple underlying factors such as variations in preparation or incubation conditions.

The research provided a wealth of data to analyze the effect of fermentation duration on LAB viability and demonstrated the successful growth of LAB in coconut yogurt. However, limitations related to the specific analysis of *L. reuteri* DSM 17938.

While the research question was focused on this particular strain, the microbiological analysis performed by the Biodiversity Research Centre only presented results in term of total Lactic Acid Bacteria (LAB). Therefore, the study relied on the assumption that a significant proportion of the LAB count was attributable to *L. reuteri* DSM 17938. This constraint limited our ability to draw precise conclusion about the behavior and proliferation of this specific strain during different fermentation durations.

Key findings from the analysis highlighted that the coconut yogurt fermented for 48 hours consistently demonstrated a higher LAB count, although the difference was not statistically significant. It underlines the necessity for future studies to consider a larger sample size or a broader range of fermentation durations for a more comprehensive understanding of LAB viability in coconut yogurt.

The study's findings lay the groundwork for future research that can refine the understanding of *L. reuteri* DSM 17938 and other LAB strains in fermented product. Future directions include more specific strain analysis, in-depth exploration of fermentation durations, and an examination of additional variables impacting LAB growth. These recommended future search directions emphasize the broader impact of the study's finding, potentially contributing valuable insights to the scientific community and the food industry,

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