

Original Research Article

Effects of Coconut Milk Substitution with Soymilk on the Shelf Life of 'Nasi Lemak'

ABSTRACT

Aims: 'Nasi lemak' is one of the most favourite foods for many people in Malaysia. The main ingredients in the preparation of 'Nasi lemak' are coconut milk, where fresh coconut milk is used to produce a scrumptious 'Nasi lemak'. The aim of this study was to develop 'Nasi lemak' where percentages of coconut milk were substituted with soymilk as alternative and healthier choice for consumers.

Study design: In this study, five different formulations of 'Nasi lemak' with different percentages ratio of coconut milk to soymilk (100:0, 75:25, 50:50, 25:75, 0:100) were studied.

Place and Duration of Study: The experiments were conducted under control environment in the Food Service Laboratory and Food Microbiology Laboratory, University of Malaysia Terengganu, between July 2011 to April 2012.

Methodology: The work scope of this study was to examine the effects of the shelf life of these new formulations, where Aerobic Plate Count, Yeast and Mould Count, and *Bacillus cereus* count, were determined throughout storage for 24-hours at ambient temperatures (28°C) and 7-days at chilled temperature (4°C).

Results: Based on the statistical analysis for shelf life studies, there were no significant different ($P>0.05$) of Aerobic Plate Count and Yeast and Mould count between each formulation for all samples stored at ambient temperature except for *B. cereus* count. However, there were significant different ($P<0.05$) of Aerobic Plate Count, Yeast and Mould count and *B. cereus* count between each formulation for all samples stored at chilled temperature.

Conclusion: This study strongly indicates that different percentages ratio of coconut milk to soymilk had significantly affected the shelf life of 'Nasi lemak' at chilled temperature.

Keywords: *Nasi lemak, shelf life, coconut milk, soymilk, microbiological quality*

1. INTRODUCTION

Malaysia is one of the countries that famous with variety of food. One of the most famous traditional foods in Malaysia is Nasi Lemak. The basic ingredients of Nasi lemak are a plate of white rice cooked together with coconut milk, and accompanying with 'sambal', a chilli paste mixed with salted anchovies. The presence of coconut milk in Nasi lemak contributed to high calorie content because of it has higher saturated fat and it is harmful to health if it were taken excessively. In addition, saturated fat intake appears to be a risk factor of insulin resistance which is important in the pathogenesis of diabetes and cardiovascular disease [1].

Coconut milk also known as “santan” in Malaysia and Indonesia, and “gata” in the Philippines are defined as the liquid products obtained by grating the solid endosperm, with or without addition of water. The coconut milk is usually used as a food ingredient in various traditional cooking recipes [2]. The main components of coconut milk fat and protein. It has higher fat content and increasing of its oxidative potential will increase the risk of microbial spoilage [3]. Besides that, other factors that may affect the presence of microbes in food are high protein foods such as meat, milk, eggs and fish, lipid and carbohydrates [4].

As there is a demand for healthier and safer product, substitution of coconut milk with soymilk in ‘Nasi lemak’ is one way of methods to reduce the calorie content in ‘Nasi lemak’. The concentration of soymilk substitution to coconut milk in ‘Nasi Lemak’ may affect the microbiological quality and shelf life of this product. Shelf life of product is an important parameter to evaluate the safety of food product from potential hazards during storage of the food product. As there is no literature available regarding the shelf life of ‘Nasi lemak’ as affected by the substitution of coconut milk with soymilk, this is the first study that provide the information on the shelf life of soymilk substitution in ‘Nasi Lemak’. The objective of this study was to determine the effect of coconut milk substitution with soymilk on the shelf life of ‘Nasi Lemak’.

2. MATERIAL AND METHODS

2.1 Sample preparation and experimental design

Samples preparation of ‘Nasi lemak’ used in this analysis were rice, soybeans, fresh coconut milk, salt, ginger, banana leaves, salt and pandan leaves. All these materials were purchased from local shops around Kuala Terengganu. The samples of ‘Nasi Lemak’ were prepared in the controlled environment in the Food Service Laboratory, Universiti Malaysia Terengganu using different ratio between coconut milk and soymilk as follows: (Sample A, 100:0, Sample B, 75:25, Sample C, 50:50, Sample D, 25:75, Sample E, 0:100) and their formulations are shown in Table 1. During the preparation of samples, the aseptic technique was applied and all utensils were sterilised using hot water. ‘Nasi lemak’ with 100% of coconut milk (Sample A) acted as a control. Dependent variables in this experiment were the shelf life data of Aerobic Plate Count, Yeast and Mould count, and *Bacillus cereus* count that stored at ambient and chilled temperatures.

Table 1: Formulation for 1 portion of 100 gram rice in ratio coconut milk:soymilk

	Sample A (100:0)%	Sample B (25:75)%	Sample C (50:50)%	Sample D (75:25)%	Sample E (0:100)%
Rice	49.7	49.7	49.7	49.7	9.7
Thick coconut milk	16.6	4.15	8.30	12.45	0
Thin coconut milk	33.2	8.30	16.6	24.9	0
Thick soy milk	0	12.45	8.30	4.14	16.6
Thin soy milk	0	24.9	16.6	8.30	33.2
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Source: Modified from [5]

2.2 Preparation of Nasi Lemak

‘Nasi lemak’ samples were prepared using the modified method from [5]. Firstly, a 100 g of rice was cleaned and rinsed. Then, the rice was soaked in 250 ml of water for overnight

with ratio of 1:2.5. After an overnight of soaking the rice in the water, the water was drained off. The bottom of steamer was covered with the banana leaves (size approximately 20 cm x 20 cm) and the steamer was then heated up. Next, the rice was placed on the banana leaves. After that, three pieces of pandan leaves (screw pine leaves) and ½ inch ginger (15 g) were added inside the steamer. Then, one teaspoon of salt was sprinkled on the surface of rice and the rice was steamed for 10 minutes. After 10 minutes, the rice was removed from the steamer when it was half cooked. The half-cooked rice was added with 75 ml of water (15 ml of water + 60 ml of thin layer coconut milk) in a separated bowl by using a fork until the water was thoroughly absorbed (about 3-4 minutes). Then, one teaspoon of salt was added into the cooked rice and was mixed properly. The rice was continuously steamed for another 15 minutes or until the rice was completely cooked. Then, 37.5 ml of thick coconut milk and salt were added to the rice until they were fully absorbed. Again, the rice was continuously steamed for another 15 minutes or until the rice was fully cooked. The rice must be kept hot.

2.3 Preparation of soymilk

Soymilk was prepared by using the method modified from [6]. Firstly, the dry soybeans were cleaned and the weight was measured. Then, the water used to soak the soy bean with ratio soybean to water of 1:3 was measured. After that, the soybean was soaked in water overnight for about 8 to 10 hours. The soaked soybeans was then grinded with water in the ratio of 1:10 for thick soymilk and 1:11 for thin soymilk by using blender. Next, the slurry was filtered through a muslin cloth, followed by heating for 10 minutes until it was boiled and then, simmered it for about 20 minutes. Then, the soymilk was allowed to cool and kept in the chiller for storage. The temperature and the time were controlled as they are important to avoid over cooking of the product which may decrease the quality of the product.

2.4 Microbiological analysis

25 g of *Nasi lemak* samples were weighed and placed in a sterile stomacher bag. The samples were then homogenized in 225 ml of peptone water solution using a stomacher making 10^{-1} dilution factor. One milliliter of the homogenized 10^{-1} diluted sample was transferred and mixed with 9 ml of peptone water solution to provide 10^{-2} dilution. The step was repeated until the desired dilutions were made. These serial dilutions containing samples were further analyzed. In order to enumerate the microbial count of samples, 0.1 ml portions of appropriate dilution were transferred onto duplicate plates of the appropriate culture medium and sterile L-spreader was used to spread the diluent on the surface of prepared media (PCA, acidified PDA and MYP). This method is known as spread plate method. Aerobic plate count (APC) was obtained from the following plate count agar (PCA, Merck), incubated at 35°C for 24 h; Yeasts and Mould count on Acidified Potato Dextrose agar (PDA, Merck), incubated at 25°C for 5 days at dark place and *Bacillus cereus* count on the MYP Agar (MYP, Oxoid) incubated at 35°C for 24 h (Gibbons et al., 2006). Enumeration of agar plates for all microbial analyses were followed counting rules, and these data were calculated as CFU/g [7].

2.5 Statistical Analysis

All data were analysed using one-way ANOVA, and post-hoc test used was Tukey test. The data were presented as growth curves of CFU/g vs. time. Mean and standard deviation of microbial counts (CFU/g) obtained were used for statistical analysis. Level of significant difference was at $P < 0.05$. The software that used to analysis the data was Minitab (Version 14.0).

3. RESULTS AND DISCUSSION

3.1 Shelf life study of 'Nasi Lemak' as assessed by Aerobic Plate Count

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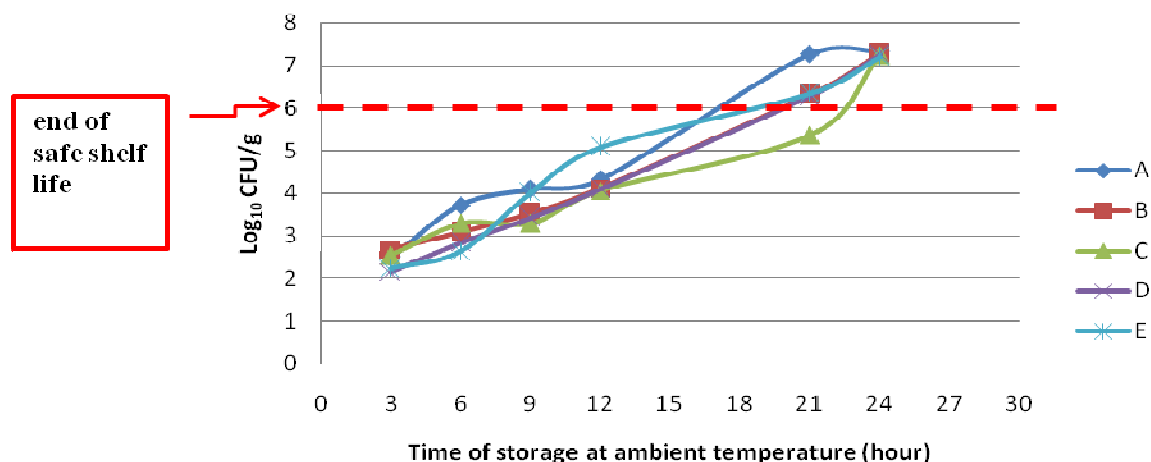


Figure 1: Aerobic plate count (CFU/g) of 'Nasi Lemak' samples with different ratio between coconut milk (CM) to soymilk (SM) stored at ambient temperature

Figure 1 shows the samples (A,B,C,D and, E) of 'Nasi Lemak' stored at ambient temperature had reached the end of the safe shelf life after 24-hours of storage at ambient temperature (26±2°C). At the end of the experiment, sample A had reached the unsatisfactory level faster than other samples. While, other samples of B, D and E had reached the end of safe shelf life after 21-hours storage at ambient temperature. The safest sample was found in the sample C, where it had reached the end of safe shelf life after 24-hours storage at ambient temperature. Referring to Table 2, there were no significant different ($P>0.05$) in Aerobic Plate Counts (APC) of *Nasi Lemak* samples that stored at ambient temperature. The unsatisfactory level for safety of ready-to-eat (RTE) foods that were fully cooked without further handling or processing before consumption was 10^6 log CFU/g and above based on the Food Standards Australia/New Zealand [8]. Besides, based on Microbiological Standard for Malaysian Food Law 1983 and Food Regulation 1985 [9], the limit for APC was 6 log₁₀ CFU/g.

Table 2: Significant difference in APC of 'Nasi Lemak' for all the samples at ambient temperature after 24 hours storage

Aerobic plate count	APC after 24 hours				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
	7.33 ± 0.01 ^a	7.28 ± 0.07 ^a	7.24 ± 0.04 ^a	7.26 ± 0.03 ^a	7.20 ± 0.08 ^a

Data was reported in mean ± standard deviation (n=2). Under each attribute, means followed by same letter are not significantly difference ($p>0.05$).

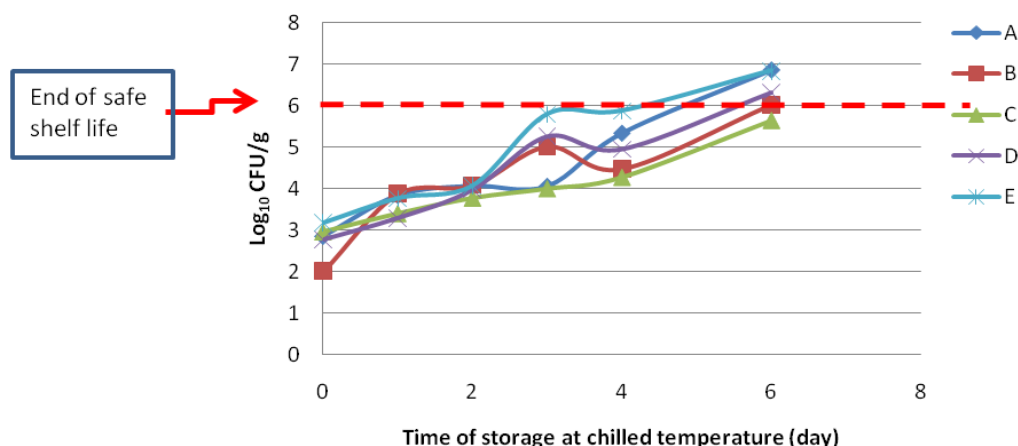


Figure 2: Aerobic plate count (CFU/g) of *Nasi Lemak* samples with different ratio between coconut milk to soymilk stored at chilled temperature

Figure 2 shows the Aerobic Plate Count (APC) for all samples with different ratio of coconut milk to soymilk in 'Nasi Lemak' that stored at chilled temperature ($4 \pm 1^\circ\text{C}$). Figure 2, samples A, B, D and E had reached the end of safe shelf life before 6-days of storage at chilled temperature. While, it was found that sample C was the safest sample where it had reached the end of safe shelf life after 6-days of storage [8]. Table 3 shows there was significant different ($P < 0.05$) in Aerobic Plate Count of sample C compared to other samples (A, B, D and E) stored at chilled temperature. This result indicated that the ratio 50:50 of coconut milk to soymilk had significant difference in affecting the shelf life of 'Nasi lemak' stored at chilled temperature.

While performing microbial analyses, samples were also observed through physical observation. From physical observation, all samples were still looked in good conditions until 7-day of storage at chilled temperature, however, surprisingly the Aerobic Plate Count had already reached the unsatisfactory level for human consumption. This results show that physical observation only is not sufficient to determine safe products. A strict microbiological standard is applied in Japan, where APC lower than 5-log_{10} CFU/g is considered safe for consumption [10]. The Food Safety Institute of Ireland also had recommended the APC value of $<10^5$ CFU/g for cooked rice [11]. Other study had indicated a higher count of APC observed in rice. A study by [12], they had discovered that Indonesian RTE fried rice had APC as high as 10^7 CFU/g. A study by [13] had reported that only APC value as high as 10^6 CFU/g was given a satisfactory microbial maximum limit for analysed fried rice samples. If the APC is higher than that, it had exceeded the maximum limit for ready-to-eat rice samples.

Table 3: Significant difference in APC of 'Nasi Lemak' for all the samples at chilled temperature after 6 days storage

Aerobic plate count	APC after 6 days				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
	6.83 ± 0.03^a	6.03 ± 0.02^a	5.63 ± 0.01^b	6.32 ± 0.00^a	6.84 ± 0.01^a

Data was reported in mean $\pm$ standard deviation ($n=2$). Under each attribute, means followed by same letter are not significantly difference at ($p > 0.05$).

Aerobic Plate Count (APC) is used to indicate the level of microorganisms in a product [14] and it is also used to indicate the hygiene status starting from food production. Obtaining an estimate of the number of microorganisms in a food product will aid in evaluating sanitary practices during processing and handling, as well as determining potential sources of microbial contamination. In other studies by [15], they had reported that the cooked rice may be stored in the refrigerator temperature for 6-days to 7-days and 6-months in the freezer temperature. However, raw white rice in tightly closed containers can be stored at room temperature and use within one year but, brown rice and wild rice stored at room temperature will have shorter shelf life (6 months) [15].

3.2 Shelf life study of *Nasi Lemak* as assessed by Yeast and Mould Count

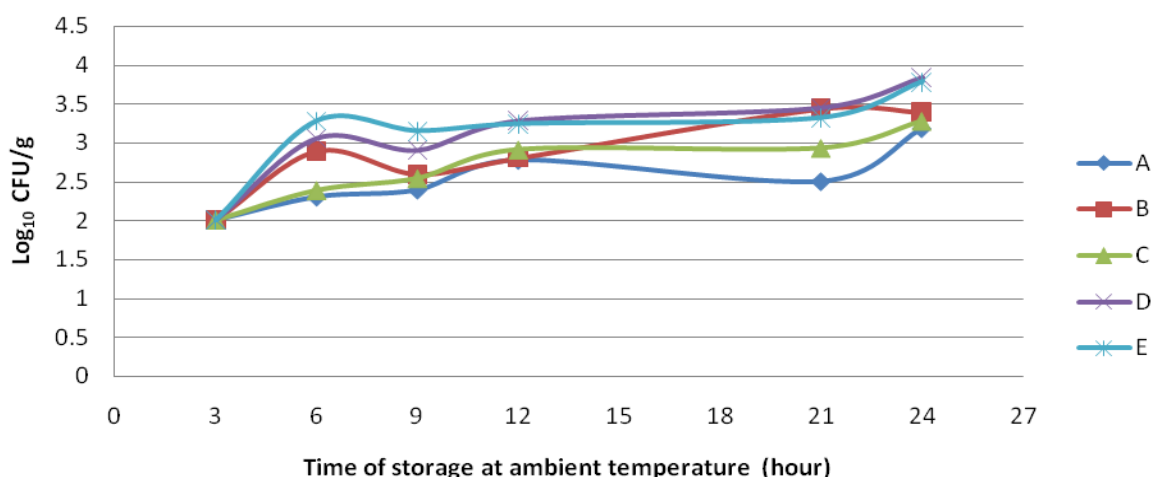


Figure 3: Yeast and mould count (CFU/g) of *Nasi Lemak* samples with different ratio between coconut milk to soymilk stored at ambient temperature

Figure 3 shows the yeast and mould count for all samples of *Nasi Lemak* that stored at ambient temperature for 24-hour, where all samples were within the satisfactory level, which below than 1.0×10^5 CFU/g that is the limit for the end of safe shelf life for yeast and mould [16]. Samples E and D had higher yeast and mould count compared with samples A, B, and C because both samples contained more percentage of soymilk compared to samples of A, B and C. Table 4 shows there is no significant different ($P > 0.05$) of yeast and mould count between all samples.

Table 4: Significant difference in yeast and mould count of 'Nasi Lemak' for all the samples at ambient temperature after 24 hours storage

Yeast and mould count	YM after 24 hours				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
	3.19 ± 0.11^a	3.39 ± 0.13^a	3.28 ± 0.03^a	3.84 ± 0.23^a	3.79 ± 0.03^a

Data was reported in mean $\pm$ standard deviation ($n=2$). Under each attribute, means followed by same letter are not significantly difference ($p > 0.05$).

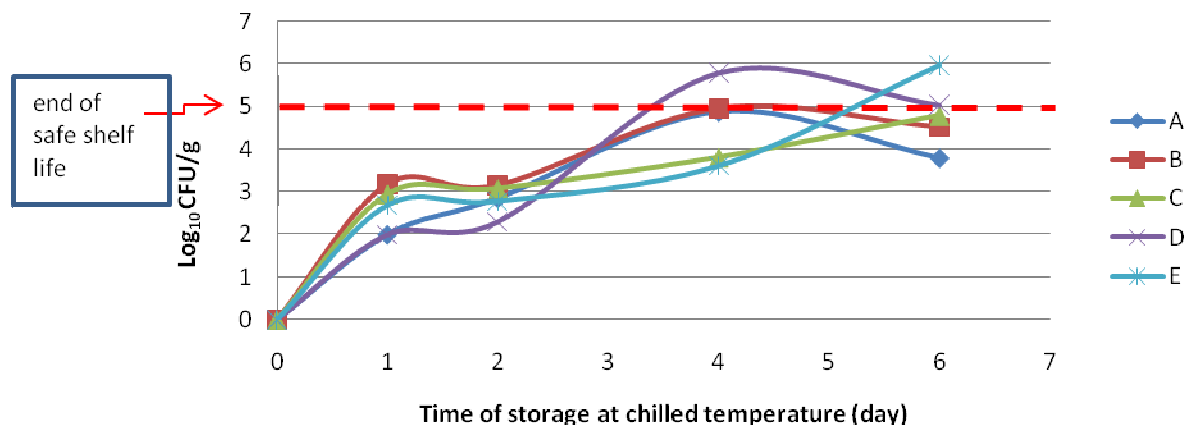


Figure 4: Yeast and Mould count (CFU/g) of 'Nasi Lemak' samples with different ratio between coconut milk to soymilk stored at chilled temperature

Table 5: Significant difference ($P < 0.05$) in Yeast and Mould of 'Nasi Lemak' for all the samples at chilled temperature after 6 days of storage

Yeast and mould count	YM after 6 days of storage				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
	3.77 ± 0.04^d	4.46 ± 0.08^c	4.82 ± 0.04^b	5.01 ± 0.03^b	5.99 ± 0.02^a

Data was reported in mean $\pm$ standard deviation ($n=2$). Under each attribute, means followed by same letter are not significantly difference ($p > 0.05$).

Figure 4 shows the number of log₁₀ CFU/g for yeast and mould count of 'Nasi lemak' samples at chilled temperature ($4 \pm 1^\circ\text{C}$). Sample D was the earliest sample to reach the unsatisfactory level, 10^5 CFU/g after 3-days storage at chilled temperature ($4 \pm 1^\circ\text{C}$). While sample C was within the satisfactory level of yeast and mold count. Referring to Table 5, there were significant differences ($P < 0.05$) in Yeast and Mould for 'Nasi lemak' samples stored at chilled temperature. It is believed that spoilage of yeast and mould may enter into 'Nasi lemak' through ingredients soybean, coconut and spices [7].

Yeast and Mould count is used to detect the presence of yeast and mould in a sample. Mould can grow in foods that are high acid and low moisture while during freezing method, it does not destroy the mould [3]. Yeasts are commonly found on plants, grains, fruits, and other foods containing sugar and yeast can cause food to spoil but does not cause foodborne illness. The yeast and mould count had slower growth in comparison to bacteria and they were often out-competed [17]. Yeast and mould can invade and grow on virtually any type of food at any time [7]. Furthermore, many moulds can grow well at refrigerator, which is the reason why moulds commonly cause spoilage of refrigerated foods [3].

3.3 Shelf life study of 'Nasi Lemak' as assessed by *Bacillus cereus* count

Rice is arguably the most important foodstuff associated with *B. cereus* food-poisoning. Because of its particular cultivation conditions in rice paddies where *B. cereus*

comprises about 10% of the soil microflora, raw rice is generally contaminated to varying degrees with *B. cereus* [18].

Table 6: *B. cereus* count (CFU/g) of *Nasi Lemak* samples at ambient temperature

Storage times (hours)	<i>Bacillus cereus</i> count (log ₁₀ CFU/g)				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
3	2.00± 0.00 ^a	1.00 ± 1.41 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a	ND
6	2.15 ± 0.21 ^a	2.00 ± 0.00 ^a	2.15 ± 0.21 ^a	3.02 ± 0.03 ^a	ND
12	2.54 ± 0.34 ^{ab}	2.89 ± 0.16 ^b	2.35 ± 0.07 ^b	3.08± 0.11 ^a	ND

ND = Not detected in 25 g sample

Data was reported in mean ± standard deviation (n=2). Under each attribute, means followed by same letter are not significantly difference ($p>0.05$).

Table 6 shows the *B. cereus* counts in 'Nasi lemak' samples during the storage at ambient temperature. *B. cereus* contaminations were detected after 3-hours, 6-hours and 12-hours of storage for all the samples (A, B, C and D). However, *B. cereus* was not able to be detected in sample E from the beginning of storage. The common microbes that can be found in rice product is *B. cereus*. Research done by [3], they had mentioned that rice is a well- recognized source of *B. cereus* with most samples containing the organism but usually at low levels. *B. cereus* was able to grow until 12-hours of storage at ambient temperature because *B. cereus* is more predominant in the starchy product [19].

Table 7: *B. cereus* count (CFU/g) of 'Nasi Lemak' samples at chilled temperature

Storage times (days)	<i>B. cereus</i> count (log ₁₀ CFU/g)				
	Sample A (100:0)	Sample B (75:25)	Sample C (50:50)	Sample D (25:75)	Sample E (0:100)
1	2.35± 0.490 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a	2.40 ± 0.13 ^a	2.05 ± 0.08 ^a
2	3.00 ± 0.00 ^a	3.06 ± 0.07 ^a	2.00 ± 0.00 ^c	3.01 ± 107 ^a	2.39 ± 0.13 ^b

Data was reported in mean ± standard deviation (n=2). Under each attribute, means followed by same letter are not significantly difference ($p>0.05$).

Table 7 shows the data of all samples for 'Nasi Lemak' that stored at chilled temperature. *B. cereus* were detected only after 1-day until 2-day of storages. However, *B. cereus* was not detected (ND) by the plate count method at 3-day of storage at 4°C for all samples. The samples were considered had low contaminant of *B. cereus* because less than 10⁴ CFU/g. This is based on the Food Standard Guidelines of Australia/New Zealand that samples with 10⁴ CFU/g and above indicated the potentially hazardous level of the samples based on the Food Standards Australia/New Zealand, 2001 [8]. A report by [20], the cooked rice was the first recognised as a caused of food poisoning through contamination with *B. cereus*. Growth and toxin production by psychrotrophic strains can be prevented by temperatures below 4°C and pH above than 5 [19]. *B. cereus* can give the rise to two distinct forms of foodborne disease, the emetic and diarrhea syndromes.

4. CONCLUSION

In conclusion, there were no significant different ($p>0.05$) of aerobic plate count and yeast and mould count between each formulation for all samples stored at ambient temperature except for *B. cereus* count. However, there were significant different ($p<0.05$) of aerobic plate count, yeast and mould count and *B. cereus* count between each formulation for all samples stored at chilled temperature. This study strongly indicates that different percentages ratio of coconut milk to soymilk had significantly affected the shelf life of 'Nasi lemak' at chilled temperature

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