Substitution Effects of Coconut Milk with Soymilk on Sensory Acceptance and Shelf Life of 'Nasi Lemak'

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNL, ZN and AY designed the study, performed literature searches, and prepared the study protocol. Authors NAHM and PLK performed the study protocol, literature searches and statistical analysis. Author MNL wrote the first draft of the manuscript and authors ZN and AY contributed in improvement of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: 'Nasi lemak' is one of the most favourite rice-based products for Malaysians. One of the main ingredients used to prepare 'Nasi lemak' is coconut milk. The aim of this study was to develop 'Nasi lemak' where coconut milk was substituted with soymilk to provide healthier choice for consumers.
Study Design: In this study, five formulations of 'nasi lemak' with different percentages of ratio between coconut milk and soymilk (100:0, 75:25, 50:50, 25:75, 0:100) were studied.

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

Methodology: The work scope of this study was to examine the effects of the coconut milk to soymilk substitution on sensory preference and shelf life of 'nasi lemak'. In terms of shelf life study, Aerobic Plate Count, Yeast and Mould Count, and *Bacillus cereus* Count were determined throughout storage of samples for 24 hours at ambient temperature ($28^\circ \pm 2^\circ C$) and 7 days at chilling temperature ($4^\circ \pm 2^\circ C$).

Results: Fresh 'nasi lemak' samples were subjected to preference test and it was found that up to 25% of substitution of coconut milk with soymilk, there was no significant difference ($P>0.05$) found with control sample. For shelf life studies, there were no significant differences ($P>0.05$) found for Aerobic Plate Count and Yeast and Mould count among samples stored at ambient temperature, except for *B. cereus* Count. However, significant differences ($P<0.05$) were found in all three counts: Aerobic Plate Count, Yeast and Mould count and *B. cereus* Count among samples stored at chilling temperature.

Conclusion: The sample with 75% coconut milk ratio with 25% soymilk is highly recommended to obtain good acceptance compared to control sample (100% coconut milk) with acceptable shelf life (21 hours) at ambient temperature and 6-days at chilling temperature.

1. INTRODUCTION

Malaysia is famous with variety of food. One of the most famous traditional foods in Malaysia is 'Nasi Lemak'. A serving of 'nasi lemak' consists of a plate of white rice cooked together with coconut milk and accompanied with 'sambal', chilli paste mixed with salted anchovies. The presence of coconut milk in 'nasi lemak' contributes to high calorie content due to its high fat content. Excessive intake of high-fat and high-calorie food is harmful to health [1].

Coconut milk, also known as 'santan' in Malaysia and Indonesia and 'gata' in the Philippines, is defined as the liquid product obtained by grating the solid endosperm of a coconut fruit, with or without addition of water. Coconut milk is usually used as an ingredient in various traditional recipes [2]. The main components of coconut milk apart from water are fat and protein. The high amount of fat and protein contained in it makes coconut milk prone to microbial spoilage [3].

As there is increasing demand for healthier and safer product, the effect of substitution of coconut milk with soymilk in 'nasi lemak' is an alternative way to reduce its calorie content. Substitution of coconut milk with soymilk in 'nasi lemak' may affect the sensory acceptance, microbiological quality and shelf life of this product. Shelf life is an important parameter to evaluate the safety of a food product during storage. Therefore, the objective of this study was to determine effect substitution of coconut milk with soymilk on sensory acceptance and microbiological shelf life of 'nasi lemak'.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Experimental Design

Materials used in this study were rice, soybean, fresh coconut milk, salt, ginger, banana leaves, and pandan leaves. All these materials were purchased from local shops around Kuala Terengganu. Soymilk was prepared freshly from soybean and used to substitute coconut milk to cook the rice following normal procedure for preparation of 'nasi lemak'. 'Nasi lemak' was prepared in a controlled and aseptic environment at the Food Service Laboratory, University Malaysia of Terengganu. Different ratios between coconut milk and soymilk were employed as follows: Sample A, 100:0 (control), Sample B, 75:25, Sample C, 50:50, Sample D, 25:75, and Sample E, 0:100 (Table 1). Where there was a ratio between coconut milk and soymilk in production of a sample as shown in Table 1, some amount of coconut milk was replaced with soymilk following the formulation and the mixture of both ingredients was used in the production of 'nasi lemak' in similar way used in producing the control sample which only used coconut milk. Dependent variables in this study were sensory acceptance and shelf life of samples. In terms of shelf life, Aerobic Plate Count, Yeast and Mould Count, and *Bacillus cereus* Count of samples stored at ambient and chilling temperatures were determined.

Table 1. Formulation for 1 portion of 'nasi lemak' with different substitution ratios

	A(100:0)%	B(25:75)%	C(50:50)%	D(75:25)%	E(0:100)%
Rice	49.7	49.7	49.7	49.7	49.7
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 soymilk	0	12.45	8.30	4.15	16.6
Thin soymilk	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 [4]

2.2 Preparation of 'Nasi Lemak'

'Nasi lemak' was prepared using the modified method from [4]. Firstly, 100 g of rice was cleaned and rinsed. Then, the rice was soaked in 250 ml of water with ratio of 1:2.5 overnight. After an overnight soaking, the water was drained off. A steamer was prepared and the bottom of the steamer was covered with banana leaves with approximate size of 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 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 mixture of water and thick coconut milk (15 ml water + 60 ml thin 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 next steamed for 15 minutes. Then, 37.5 ml of thick coconut milk and salt were added to the rice and mixed until they were fully absorbed. Again, the rice was steamed for another 15 minutes or until the rice was fully cooked. The products were kept in separate sterile and microwavable polypropylene rectangle cases with clear colour (11.8 cm (L) x 17.2 cm (W) x 6.5 cm (H) for shelf life study.

2.3 Preparation of Soymilk

Soymilk was prepared by using the method modified from [5]. Firstly, dry soybean was cleaned and the weight was measured. After that, soybean was soaked in water with the ratio of 1:3 for about 8 to 10 hours. Next, soybean was grinded with water in the ratio of 1:10 for thick soymilk and 1:11 for thin soymilk by using a blender. Slurry produced was then filtered through a muslin cloth, followed by pasteurization at boiling temperature for about 20 min. Then, soymilk was allowed to cool and kept in the chiller for storage. Temperature and time used in the process were controlled as they are important to avoid under or over cooking of the product.

2.4 Sensory Preference Test

Sensory preference test was carried out by 35 untrained panelists on 5 attributes: colour, texture (mouth-feel), odor, taste and overall preference by using ranking method. Samples were ranked by panelists according to their preference for each attributes. All samples were labeled with random three-digit codes and presented in a randomize arrangement. Data collected was calculated using Least Significant Difference test (LSD).

2.5 Microbiological Analysis

25 g of 'nasi lemak' was weighed and placed in a sterile stomacher bag. The sample was then homogenized in 225 ml of peptone water making serial 10-fold dilutions. These serial dilutions that contained samples were further analyzed. In order to enumerate the microbial count of samples, 0.1 ml portions of appropriate dilutions were transferred onto triplicate plates. A sterile L-spreader was used to spread the diluents on the surface of prepared media (Plate Count Agar [PCA], acidified Potato Dextrose Agar [PDA] and Mannitol Egg Yolk Polymyxin Agar [MYP] agar). Aerobic Plate Count (APC) was obtained from PCA plates that were incubated at 35°C for 24 h while Yeasts and Mould Count was collected from acidified PDA plates incubated at 25°C in a dark place for 5 days. For *Bacillus cereus* Count, MYP plates were incubated at 35°C for 24 h. All media were purchased from Merck, Germany. Data were presented as log_{10} CFU per gram [6].

2.6 Statistical Analysis

Microbiological data were presented as growth curves of CFU/g vs. time and mean values and standard deviation of microbial counts ($\log_{10}$ CFU/g) obtained were used for statistical analysis using one-way ANOVA and followed by Tukey test. Significant difference was determined at $P<0.05$. The software used for statistical analysis was Minitab (Version 14.0).

3. RESULTS AND DISCUSSION

3.1 Sensory Preference of 'Nasi Lemak'

Table 2 shows that in terms of colour, there were no significant differences ($P>0.05$) among samples whose coconut milk had been replaced with soymilk up to 75% substitution. Sample produced with 100% soymilk had the lowest preference due to its yellowish color that was less preferred.

For odor, up to 50% of substitution of coconut milk with soymilk was found to not give significant effect ($P>0.05$) on samples' odor preference. Meanwhile for texture, substitution of coconut milk with soymilk up to 75% gave no significant differences ($P>0.05$) in samples' preferences. The least preferred sample for texture was the one produced with 100% soymilk. Soymilk has higher protein content and denatured protein has more hydrophobic unfolding proteins. Thus, sample with more soymilk had harder texture.

Different pattern of preferences could be seen in taste where only sample with 25% substitution of coconut milk with soymilk that was not significantly different ($P>0.05$) with control sample. A similar trend was also observed in overall preference where only the sample with 25% soymilk was not significantly different ($P>0.05$) with control sample. Higher level of substitution gave more beany flavour to 'nasi lemak' produced, thus samples with higher levels of substitution were less preferred.

3.2 Aerobic Plate Count of 'Nasi Lemak'

Aerobic Plate Count [APC] is used to indicate level of microorganisms in a product [7]. Obtaining an estimate 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 a study conducted by [8], it was reported that cooked rice could be stored in refrigerating temperature for 6 to 7 days and about 6 months if stored frozen. For raw white rice, if it is stored in tightly closed container, it was reported to be able to be stored at room temperature and used within one year while brown rice and wild rice have shorter shelf life of 6 months only [8].

For cooked rice, the safety level can be determined based on regulations applied on cooked, ready-to-eat foods which was 10^6 CFU/g for APC based on the Food Standards Australia/New Zealand [9]. Similar value is also stated in the Microbiological Standard for Malaysia Food Law 1983 and Food Regulation 1985 [10].

Fig. 1 shows that all samples of 'nasi lemak' stored at ambient temperature had reached the end of their safety limit after 24-hours storage at ambient temperature (26±2ºC). Sample A reached the end of its shelf life faster than other samples in only 18 hours while samples B, D and E reached the end of their shelf lives after 21 hours at ambient temperature. Sample C reached the end of shelf life before 24-hours storage at ambient temperature.

Table 2. Sensory preference of freshly cooked 'nasi lemak'

Formulation (CM:SM)	Attributes				
	Colour	Odor	Texture (mouth-feel)	Taste	Overall preference
A (100:0)	90[c]	75[c]	79[d]	63[d]	57[d]
B (75:25)	82[c]	76[c]	81[d]	79[cd]	78[cd]
C (50:50)	90[bc]	87[bc]	101[bcd]	103[bc]	105[bc]
D (25:75)	109[abc]	127[a]	98[cd]	109[abc]	114[ab]
E (0:100)	124[a]	130[a]	136[a]	140[a]	141[a]

LSD= 32

- *Values with different superscript letters within the same column are significantly different (P< 0.05).*
- *The score for each attribute is based on a 1-5 scale where 1 = most preferable and 5= least preferable*
- *LSD = Least Significant Difference*
- *CM (coconut milk) and SM (soymilk)*

Next, Table 3 shows that there were no significant differences ($P > 0.05$) in APC of samples after 24-hour storage at ambient temperature.

Fig. 2 shows the APC of samples with different ratios of coconut milk to soymilk in 'nasi lemak'

that were stored at chilling temperature ($4 \pm 2°C$). It can be observed that the samples A, B, D and E had reached the end of safe shelf life before 6 days while sample C was found to be the safest sample since it had reached the end of safe shelf life only after 6 days of storage [9].
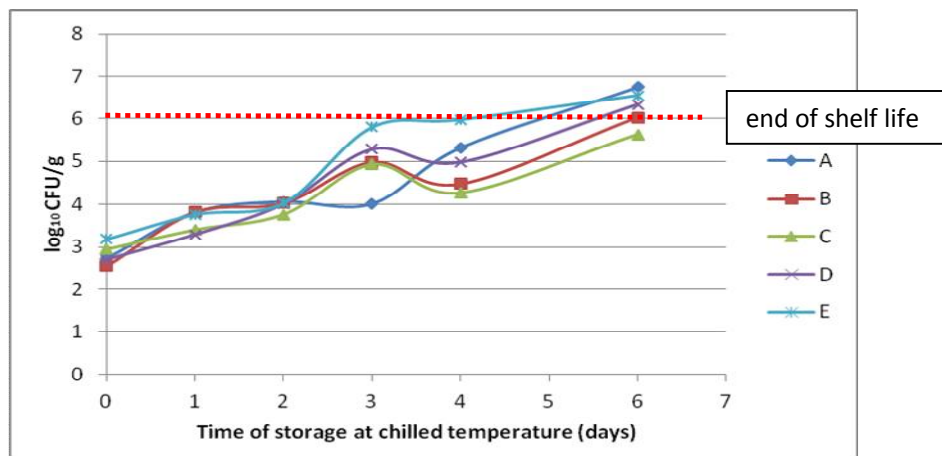
Table 3. Effect of substitution of coconut milk with soymilk on Aerobic Plate Count ($\log_{10}$ CFU/g) of 'nasi lemak' after 24-hour storage at ambient temperature

	Formulation (CM:SM)*				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
Aerobic plate count after 24-hour storage at ambient temperature ($\log_{10}$ CFU/g)	7.33 ± 0.06^a	7.27 ± 0.02^a	7.24 ± 0.03^a	7.25 ± 0.02^a	7.21 ± 0.02^a

*Data represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05). *CM (coconut milk) and SM (soymilk)*
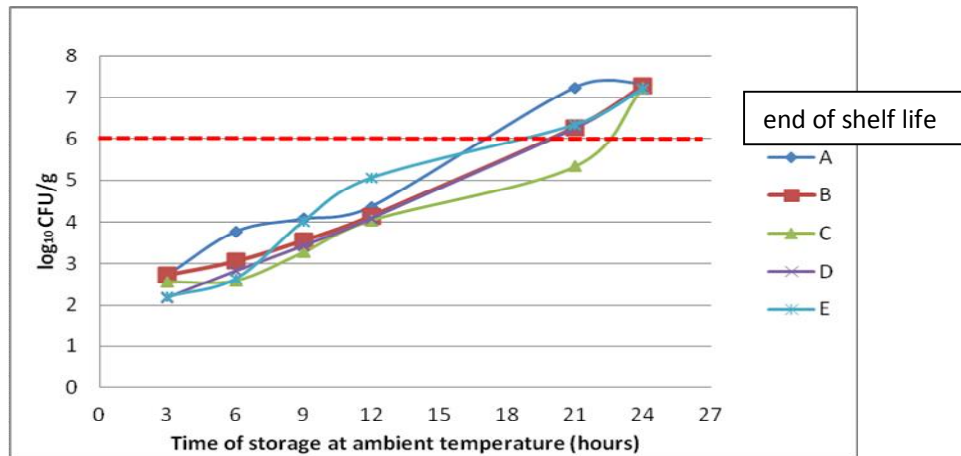
Fig. 1. Effect of substitution of coconut milk with soymilk on Aerobic Plate Count ($\log_{10}$ CFU/g) of 'nasi lemak' stored at ambient temperature

Fig. 2. Effect of substitution of coconut milk with soymilk on Aerobic Plate Count ($\log_{10}$ CFU/g) of 'nasi lemak' stored at chilling temperature

Table 4 shows that there was significant differences ($P<0.05$) in APC of sample C compared to other samples after 6 days of storage at chilling temperature.

While performing microbial analyses, samples were also observed for their physical characteristics. All samples still looked good until 7 days of storage at chilling temperature; however, the samples' APCs had already reached the unsatisfactory level for human consumption. This results show that physical observation only is not sufficient to determine safety of a product.

3.3 Yeast and Mould Count of 'Nasi Lemak'

Yeast and mould can invade and grow on virtually any type of food at any time [6]. Determination of Yeast and Mould Count is used to detect the presence of yeast and mould in a sample. Specifically, mould can grow in foods that have high acidity and low moisture [11]. Meanwhile, yeast is commonly found on plants, grains, fruits, and other foods containing sugar and it can cause food to spoil but it lacks the risk of foodborne illnesses. Yeast and mould have slower growth in comparison to bacteria and they were often out-competed [12]. However, many moulds can grow well in refrigerated temperature

and becomes the common cause of spoilage in refrigerated foods [11].

Fig. 3 shows yeast and mould count [YMC] for all samples stored for 24 hours at ambient temperature, where they were all within the satisfactory level of lower than 1.0×10^5 CFU/g, which is considered as is the end of safe shelf life for yeast and mould count in ready-to-eat (RTE) products [13]. Samples E and D had higher YMC compared to samples A, B, and C since they contained more soymilk than coconut milk.

Table 5 reveals that there are no significant differences ($P>0.05$) of yeast and mould count between all samples.

Fig. 4 shows the YMC values of 'nasi lemak' samples stored at chilling temperature ($4\pm1°C$). Sample D was the first sample to reach the unsatisfactory level of 10^5 CFU/g after 3 days while sample C managed to stay within the satisfactory level for YMC even after 6 days of storage.

It can be observed from Table 6 that there were significant differences ($P<0.05$) in YMC of 'nasi lemak' samples after 6 days of storage at chilling temperature. It was deducted that spoilage of yeast and mould of 'nasi lemak' was most probably due to its ingredients such as coconut and spices [14].

Table 4. Effect of substitution of coconut milk with soymilk on Aerobic Plate Count (log_{10} CFU/g) of 'nasi lemak' after 6 days of storage at chilling temperature

	Formulation (CM:SM)*				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
Aerobic Plate Count after 6-days of storage at chilling temperature (log_{10} CFU/g)	6.75 ± 0.09^a	6.03 ± 0.01^a	5.63 ± 0.03^b	6.34 ± 0.02^a	6.55 ± 0.05^a

*Data represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05). *CM (coconut milk) and SM (soymilk)*

Table 5. Effect of substitution of coconut milk with soymilk on Yeast and Mould Count (log_{10} CFU/g) of 'nasi lemak' after 24-hour storage at ambient temperature

	Formulation (CM:SM)*				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
YMC after 24-hour storage at ambient temperature (log_{10} CFU/g)	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 represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05). *CM (coconut milk) and SM (soymilk)*

3.4 *Bacillus cereus* Count of 'Nasi Lemak'

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* [15]. Research done by [11] mentioned that rice is a well-recognized source of *B. cereus* as most samples contain the organism but usually at low levels.

A report by [16] stated that cooked rice was the first product to be recognized as a cause of food poisoning through contamination with *B. cereus*. Growth and toxin production by psychrotrophic strains can be prevented by storage temperatures of below 4ºC and pH above than 5 [17]. *B. cereus* can cause two distinct forms of foodborne disease: the emetic and diarrhea syndromes. The Food Standard Guidelines of Australia/New Zealand has determined that *B. cereus* count of 10^4 CFU/g and above indicates potentially hazardous level [9].

Table 7 shows the *B. cereus* counts in 'nasi lemak' samples during storage at ambient temperature. *B. cereus* was detected after 3 hours, 6 hours and 12 hours of storage for all samples. However, sample E was found to be free of *B. cereus*. *B. cereus* was able to grow until 12 hours of storage at ambient temperature because it can live in starchy products [17].

Table 8 shows *B. cereus* counts of all samples after storage at chilling temperature. *B. cereus* was detected only after 1 to 2 days of storage and it was not detected anymore after the third day. Thus, the samples produced in this study could be considered to have low contamination of *B. cereus*.

Table 6. Effect of substitution of coconut milk with soymilk on Yeast and Mould Count (log$_{10}$ CFU/g) of 'nasi lemak' after 6 days of storage at chilling temperature

	Formulation (CM:SM)*				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
Yeast and mould count after 6 days of storage at chilling temperature (log$_{10}$ CFU/g)	3.77±0.02[d]	4.46±0.01[c]	4.82±0.01[b]	5.01±0.06[b]	6.00±0.01[a]

*Data represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05). *CM (coconut milk) and SM (soymilk)*

Table 7. *B. cereus* Count (log$_{10}$ CFU/g) of 'nasi lemak' stored at ambient temperature

Storage time (hours)	*Bacillus cereus* count (log$_{10}$ CFU/g)				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
3	2.00± 0.02[a]	1.03±0.03[a]	2.00±0.01[a]	1.98±0.02[a]	ND
6	2.13±0.03[a]	1.98±0.02[a]	2.12±0.02[a]	3.00±0.03[a]	ND
12	2.56±0.02[ab]	2.89±0.02[b]	2.37±0.03[b]	3.07±0.02[b]	ND

Data represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05).
ND = Not detected in 25 g sample

Table 8. *B. cereus* Count (log$_{10}$ CFU/g) of 'nasi lemak' stored at chilling temperature

Storage time (days)	*B. cereus* count (log$_{10}$CFU/g)				
	A(100:0)	B(75:25)	C(50:50)	D(25:75)	E(0:100)
1	2.35±0.49[a]	2.13±0.15[a]	2.03±0.06[a]	2.30±0.17[a]	2.02±0.02[a]
2	3.00±0.02[a]	3.06±0.02[a]	2.17±0.21[c]	3.10±0.10[a]	2.37±0.02[b]

Data represent mean and standard deviation from three independent replicates (n=3) for each sample. Mean values followed by similar superscript letter are not significantly different (P>0.05)
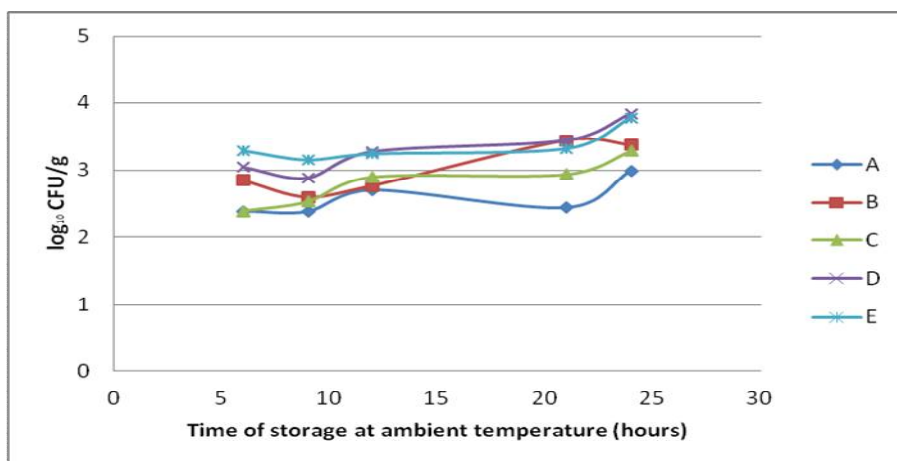
Fig. 3. Effect of substitution of coconut milk with soymilk on Yeast and Mould Count ($\log_{10}$ CFU/g) of 'nasi lemak' stored at ambient temperature
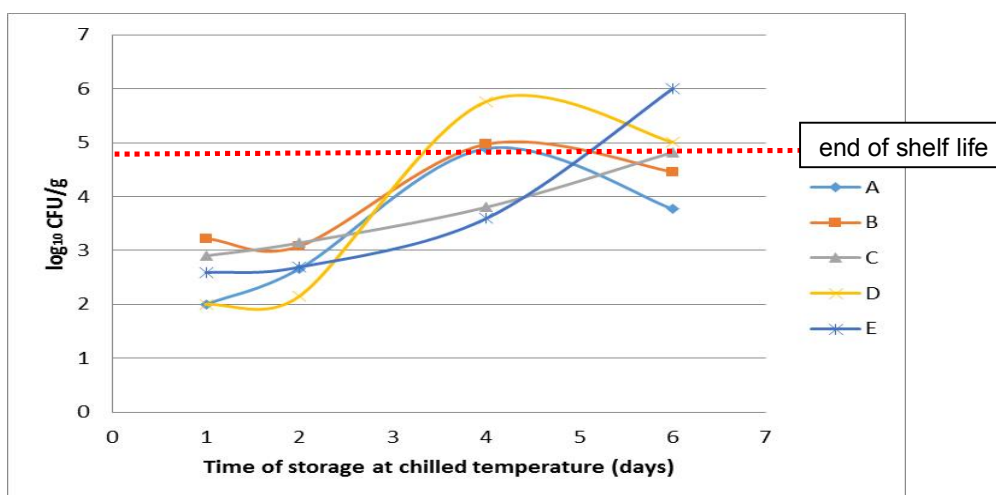

Fig. 4. Effect of substitution of coconut milk with soymilk on Yeast and Mould Count ($\log_{10}$ CFU/g) of 'nasi lemak' stored at chilled temperature

4. CONCLUSION

In conclusion, up to 25% substitution of coconut milk with soymilk in 'nasi lemak' successfully provided the sensory characteristics close to control sample that are preferred by panelists.

In terms of microbiological shelf life, there were no significant differences ($P>0.05$) of aerobic plate count and yeast and mould count among samples when stored at ambient temperature, except for *B. cereus* count. However significant differences ($P<0.05$) were observed when samples were stored at chilled temperature. This study strongly indicates that substitution of coconut milk to soymilk had significantly affected the shelf life of 'nasi lemak' at chilled temperature.

Overall, the sample B with 75% coconut milk ratio with 25% soymilk is highly recommended to obtain good sensory acceptance comparable to control sample (100% coconut milk) with acceptable shelf life (21 hours) at ambient temperature and 6-days at chilling temperature.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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