CHAPTER 3

RESEARCH METHODOLOGY

3.1 Sample Preparation

Ingredients for nasi lemak: White rice (Jasmine Super 5), coconut milk (Kara), pandan leaves, ginger, lemongrass, red onion, salt and sugar were purchased from a local market in Selangor. The nasi lemak was prepared in triplicate, in a controlled and hygienic manner in Santan HACCP-certified central kitchen. The fresh nasi lemak was prepared as follows: Sample A, a control group of nasi lemak without a acidity regulator. The sample was prepared by mixing the nasi lemak rice with Soluex 220301 acidity regulator with a ratio of 0.5% and B2 was prepared by adding Soluex 220301 acidity regulator to the rice with a ratio of 1.0%. Meanwhile, sample C1 was prepared by adding Soluex 220302 acidity regulator to the rice with h a ratio of 0.5%. Sample C2 was prepared by adding Soluex 220302 acidity regulator with a ratio of 1.0%. The formula is tabulated in Table 3.1. All samples are to be stored at a chill temperature of 4 to 8 degrees celsius for 24 days. Microbial analysis and sensory evaluation were conducted every seven days in triplicate.

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Table 3.1: Formulation for one batch of Nasi Lemak for all levels of treatments

	Formulation for one batch of Nasi Lemak for all levels of treatments				
	Sample A (Control)	Sample B		Sample C	
	No additive	(B1) 0.5%	(B2) 1%	(C1) 0.5%	(C2) 1%
	Rice (2.8kg)	Rice	Rice	Rice	Rice
Acidity Regulator		Acidity Regulator A (14g)	Acidity Regulator A (28g)	Acidity Regulator B (14g)	Acidity Regulator B (28g)
	Water (4kg)	Water (4kg)	Water (4kg)	Water (4kg)	Water (4kg)
	Santan (0.65kg)	Santan (0.65kg)	Santan (0.65kg)	Santan (0.65kg)	Santan (0.65kg)
	Daun Pandan (0.2kg)	Daun Pandan (0.2kg)	Daun Pandan (0.2kg)	Daun Pandan (0.2kg)	Daun Pandan (0.2kg)
Ingredients	Lemongrass (0.15kg)	Lemongrass (0.15kg)	Lemongrass (0.15kg)	Lemongrass (0.15kg)	Lemongrass (0.15kg)
	Ginger (0.15kg)	Ginger (0.15kg)	Ginger (0.15kg)	Ginger (0.15kg)	Ginger (0.15kg)
	Red Onion (0.08kg)	Red Onion (0.08kg)	Red Onion (0.08kg)	Red Onion (0.08kg)	Red Onion (0.08kg)
	Sugar (0.06kg)	Sugar (0.06kg)	Sugar (0.06kg)	Sugar (0.06kg)	Sugar (0.06kg)
	Salt (0.1kg)	Salt (0.1kg)	Salt (0.1kg)	Salt (0.1kg)	Salt (0.1kg)

3.2 Preparation of Nasi Lemak

A 5kg rice steamer with no brand, manufactured by Hangzhou XIB JiulongKitchen Tool from China was used. and rice net was put into a clean and dry rice pot. Raw white rice was weighed 2.80 kg by using a 30kg digital scale. The scale was first tared to zero before the rice was weighed as per the recipe. Then, the rice was thoroughly rinsed 3 times before cooking. Next, 0.20kg of daun pandan, 0.15kg lemongrass, 0.15kg ginger, 0.08 kg red onion, 0.65kg santan Kara, 4kg water, 0.1kg salt and 0.06kg sugar were added and mixed in the rice pot. The acidity regulator at different concentrations as tabulated in Table 3.1 was added together during the

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mixing of all ingredients. Rice was cooked until the light 'cook' indicator at the rice cooker panel changed from red to green. Once cooked, it was left warm for five minutes before flaking the rice well and left it rest for another five minutes. The cooked nasi lemak was transferred into the chiller. During meal assembly, the cooked nasi lemak was packed into individual packaging. This individual packaging is referring to the normal airline aluminium rectangle foil tray and aluminium lid. Refer Figure 3.2 for an example of a inflight foil tray and aluminium lid.

Figure 3.2 (a): Example of a inflight foil tray and aluminium lid.

In the Nasi Lemak set assembly, it will include the half of the hard-boiled egg, chicken rendang, onion sambal, fried groundnuts and anchovies. Finally, the packed Nasi Lemak was kept at a chill temperature until further analysis.

3.3 Preparation of Acidity Regulator

Acidity regulators with brand names Soluex 220301 and Soluex 220302 were properly weighted to prepare the 0.5% and 1% concentration by using the 10kg digital scale for each of Sample B and Sample C according to the formula calculated in Table

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3.1. Sample B1 is a mixture of the Nasi Lemak with Soluex 220301 with 0.5% concentration. Sample B2 is a mixture of the Nasi Lemak with Soluex 220301 with 1% concentration. Meanwhile, Sample C1 is a mixture of the Nasi Lemak with Soluex 220302 with 0.5% concentration and C2 is a mixture of the Nasi Lemak with Soluex 220302 with 1% concentration.

The concentrations of 0.5% and 1% were selected based on a preliminary study that was conducted prior to this research. The preliminary result showed that the addition of acidity regulator Soluex below 0.5% into the Nasi Lemak mixture had an insignificant effect on the shelf life. Hence, the study focused on the concentration of 0.5% and 1%.

3.4 Sensory Preference Test

The Sensory preference test was carried out by nine trained panelists a team of Product Development & Quality Assurance of an airline catering team at RedQ, Kuala Lumpur International Airport (KLIA) Terminal 2 on five attributes. The sensory evaluation was conducted by a panel of trained food panelists, who are considered trained due to their job role requiring them to conduct daily meal tests to ensure meal quality consistency is maintained in every production. The attributes tested are color, texture (mouth-feel), odor, taste and overall preference by using a ranking method. Samples were ranked by panelists according to their preference for each attribute. All samples were labeled with random three-digit codes and presented in a randomized arrangement. Data collected was calculated using the Least Significant Difference test (LSD).

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3.5 Microbiological Analysis

Sample Preparation

Nasi lemak samples cooked from the kitchen were packed in individual sterile plastic bags, transferred directly to the laboratory in a clean ice box with dry ice inside, for an immediate bacteriological testing.

3.5.1 Total plate count

The total plate count was determined by preparing the initial dilution from each sample using a 25g sample and 225mL 0.1% peptone. Samples were rehydrated at room temperature for 15 minutes for homogenizing. Serial ten-fold dilutions were prepared using 0.1% peptone solution. All microbial media were purchased from the appointed vendor by FST, USIM lab unless otherwise stated.

By Australian Standard (1991) 1766.2.1, homogenates and dilutions were plated onto plate count agar (PCA) using the plate pour technique. PCA plates were prepared according to the manufacturer's instructions. Plates were incubated under aerobic conditions at 30°C for 72 hours and all bacteria colonies were counted. The number of colony-forming units per gram of sample was determined.

3.5.2 Enumeration of *Escherichia coli*

Following Australian Standard (1991) 1766.2.3, homogenates and subcultures were subcultured into 10mL lauryl triptone broth (LTB). The broth was incubated at 37°C and examined after 24 and 48 hours. Positive tubes (gas bubbles in the concavity of Durham tube) were subcultured onto eosin methylene blue (EMB) agar for

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confirmation and into EC Broth (ECB) for *Escherichia coli* detection. EMB agar plates were incubated under aerobic conditions at 37°C for 24 hours and ECB tubes at 44.5°C for 24 hours and 48 hours. EMB plates were examined for typical *Escherichia colii* colonies. Typical colonies are subcultured into Trytone water and incubate at 44.5°C for 24 hours. Tubes were tested for indole production using Kovac's indole reagent for the presence of *Escherichia coli* and MPN *Escherichia coli* enumerated. The MPN of *Escherichia coli* per gram of sample is determined.

3.5.3 Enumeration of *Salmonella*

The conventional technique was employed for *Salmonella* enumeration. Initially, a broth and agar were prepared by suspending 15g of peptone water in 100mL of distilled water. The solution was thoroughly mixed and transferred to final containers, then sterilized using an autoclave machine at 15lbs pressure (121°C) for 15 minutes. The subsequent step involved the preparation of selenite cysteine broth. Four grams of sodium biselenite were dissolved in 1 liter of distilled water, followed by the addition of 19g of Selenite Cysteine (SC) broth base. This mixture was sterilized by placing it in free-flowing steam for 15 minutes. Simultaneously, 56.8g of Xylose Lysine Deoxycholate (XLD) agar was suspended in 1 liter of distilled water. The mixture was heated and then transferred to a 50°C water bath.

Each 25g of rice sample was placed into a stomacher bag, and 225ml of peptone water was added before stomaching for two minutes using a stomacher machine. The enrichment-containing bag was then placed in a beaker and incubated at 35°C for 24 hours. Subsequently, 1ml of the enriched sample was transferred into the

SC broth tube, and the inoculated tubes were incubated at 35°C for an additional 24 hours.

Finally, a loopful of the SC enrichment was streaked onto XLD agar plates. These streaked plates were incubated at 35°C for 24 hours, and characteristic colonies, including black-centered colonies on the XLD agar, were observed.

3.5.4 Enumeration of *Staphylococcus aureus*

Methods used to detect and enumerate S. aureus is by using Direct Plate Count method. For each dilution plated, 1 ml sample suspension was aseptically transferred to 3 plates of Mannitol Salt agar, distributing 1 ml of inoculum equitably to 3 plates. The inoculum was spread over surface of agar plate, using sterile bent glass streaking rod. Plates were retained in upright position until inoculum was absorbed by agar. Plates were inverted and incubated for 48 hr at 37°C. Plates containing that have colonies were observed with typical appearance of *S. aureus*. Colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle.

3.6 Statistical Analysis

Microbiological data were presented as growth curves of CFU/g vs. time and mean values and standard deviation of microbial counts (log10 CFU/g) obtained were used for statistical analysis using one-way analysis of variance (ANOVA) and

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followed by Tukey test. The significant difference was determined at $P<0.05$. The software used for statistical analysis was Minitab (Version 14.0).