# CHAPTER 3

# ISOLATION OF PREDOMINANT LACTIC ACID BACTERIA AND DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF THE MALAYSIAN FERMENTED FOODS

## 3.1 Introduction

Varieties of fermented foods and beverages are consumed tremendously across the world and have become very common in which almost all ethnic groups have their own recipes passed from generation to generation (Hui et al., 2004). The fermentation process changes the basic characteristics of food or raw materials into palatable and acceptable products. It has its own prestige and values as the process could increase the product shelf life as well as its qualities, for instance, feel, smell, taste, and colour (Marco et al., 2017). Furthermore, the fermentation process may also enhance the nutritional value of the food and retain the viable microorganisms and their metabolites, which may have a health impact on the human host (Hui et al., 2004; Motarjemi, 2002). The fermentation process could also contribute to food safety if other facilities related to food preservation are unavailable (Motarjemi, 2002). The technique is listed as one of the oldest and most cost-effective approaches to preserving food (Ahmed et al., 2013). Numerous studies have been done on the myriad benefits of fermented food (Alu'datt et al., 2018; Kaprasob et al., 2018; Rezac et al., 2018; Haitham et al., 2017).

Fermentation can occur naturally or can be initiated by the addition of starter culture to the raw materials (Pongsetkul et al., 2014). Fermented foods are produced

from a variety of ingredients, including cereal grains, vegetables and bamboo shoots, legumes, roots and tubers, dairy products, meats, and sea creatures (Tamang et al., 2016). The raw materials used are commonly shrimp and fish. The fermented products derived from marine animals are widely accessible and consumed around the globe as flavour enhancers or condiments. Examples of common fermented shrimp products in the world are ronto and terasi of Indonesia (Khairina et al., 2016; Huda, 2012), saewoojeot of Korea (Kim et al., 2014), kapi and Koong-Som of Thailand (Faithong et al., 2010), as well as cincaluk and belacan of Malaysia. Meanwhile, some of the fermented products derived from fish are shidal of India (Ahmed et al., 2013), prahok of Cambodia (Chuon et al., 2014), hous-kasef of Saudi Arabia (Gassem, 2017), jeotgal of Korea (Koo et al., 2016), along with pekasam, budu and bosou of Malaysia.

The presence of beneficial bacteria in the Malaysian fermented shrimp and fish should be in line with the good nutrient content offered by the food to improve human health synergistically. The differences in raw materials and other ingredients used in each product and the fermentation steps could lead to the dissimilar nutrient composition of the final products (Pongsetkul et al., 2014). Available reports on the proximate composition of belacan (Sharif et al., 2008) showed that the protein contents did not comply with the protein requirement stated in the Malaysian Food Act 1983 (Act 1981) and Regulations (1985), whilst no recent reports are available on the proximate composition of cincaluk other than the study done by Tee et al. (1997). In addition, apart from several previous works (Beddows et al., 1979; Ghazali et al., 2011; Mohd. Khairi et al., 2014; Sim et al., 2015), there was no study that reported the comparison of physicochemical properties of both cooked and non-cooked budu, respectively. In the case of bosou, no single study has reported its scientific

characteristics, neither its physicochemical properties nor the isolation of predominant lactic acid bacteria (LAB).

To date, only a single study has been done to isolate LAB from belacan (Haitham, 2017) and cincaluk (Hajar and Hamid, 2013), respectively. In contrast, there is several research on budu which reveals the presence of LAB such as *Lactobacillus paracasei, Lactobacillus fermentum, Lactobacillus casei, Lactobacillus plantarum*, and *Lactococcus lactis* subp *lactis* (Liasi et al., 2009; Abbasiliasi et al., 2011; Sim et al., 2012; Khalil et al., 2018), but with a variety of ways of budu preparation, either the ingredients or the techniques involved. Thus, this study was conducted to to isolate the predominant lactic acid bacteria of the Malaysian fermented shrimp and fish foods, as well as to determine the physicochemical properties of these foods, including the proximate composition, water activity, sodium content, pH, and colour measurement. .

## 3.2 Materials and Methods

### 3.2.1 Sample Collection

Belacan and cincaluk were bought from a supermarket in Senawang, Negeri Sembilan. Bosou was purchased from a seller at a market known as Tamu Donggongon, Pekan Donggongon, Penampang, Sabah. The budu in two different preparations, which are cooked and non-cooked, was received as a gift from Perusahaan Warisan Ketereh, Kampung Ketil, Pengkalan Kubor, Tumpat, Kelantan. The details of each sample are provided in Table 3.1. All samples were transported to the Food Microbiology Laboratory, Universiti Sains Islam Malaysia, Nilai, Negeri Sembilan, Malaysia. Samples were then stored in airtight sample containers and kept in the refrigerator (4°C) until analysis.

**Table 3.1:** Malaysian Fermented Food Samples Used in The Determination of Physicochemical Properties and Isolation of Predominant Lactic Acid Bacteria

| Sample | Manufacturer | Ingredients | Production date | Expiration date |
|---|---|---|---|---|
| Belacan | 360 Horizonz Sdn. Bhd. | Small shrimps and salt | Not stated | 13/07/2020 |
| Bosou | Home-based product | Small freshwater fishes, cold cooked rice, salt and pangi seeds | 10/01/2019 | 10/01/2020 |
| Cooked Budu | Perusahaan Warisan Ketereh | Anchovies, salt, sugar, colouring and seasoning | 07/01/2019 | 31/12/2020 |
| Non-cooked Budu | Perusahaan Warisan Ketereh | Anchovies and salt | 07/01/2019 | 31/12/2020 |
| Cincaluk | Seri Rombang Industries | Small shrimps, sugar and salt | Not stated | 31/12/2020 |

### 3.2.2 Isolation and Preliminary Selection of LAB

The isolation of LAB for all samples was done separately using MRS agar (de Man, Rogosa, Sharpe; Oxoid, UK). The samples were mixed homogenously at a ratio of 1:9 sample to MRS broth (Oxoid, UK) before a serial dilution using MRS broth up to $10^5$ was prepared from the mixture. The 100µL of the homogenized mixture was spread on MRS agar plates containing 0.8% calcium carbonate ($CaCO_3$) and incubated for 48 h at 37°C. After 48 h of incubation, the white and creamy colonies with different morphologies and dissolved calcium circles were randomly chosen and transferred onto other MRS agar plates until pure colonies were obtained.

All isolates were tested for catalase activity using the slide method. A small amount of isolate was picked up using a sterile inoculating loop and placed on the surface of the slide before a single drop of 3% hydrogen peroxide ($H_2O_2$) solution was applied to the isolates. The fluid was observed for the immediate and drastic formation of gas bubbles, which indicated a positive catalase test, whereas those without bubble formation indicated a negative catalase test.

Each pure isolate with a negative catalase test was microscopically examined for Gram's staining reaction and cell morphology. Only catalase-negative and Gram-positive isolates were selected to be tested for their ability to grow in both aerobic and anaerobic conditions. All pure isolates were stored in MRS broth with 20% (v/v) glycerol at -80°C. Isolates were activated before each experiment by subculturing twice on MRS agar and inoculating in MRS broth.

### 3.2.3 Proximate Analysis of Samples

Proximate analysis of samples was carried out using a standard method of the Association of Official Analytical Chemists (AOAC, 2005). All the chemicals involved in the study are analytical grades, which include petroleum ether, sulphuric acid, and copper Kjeltabs.

#### 3.2.3.1 Moisture Analysis

The moisture content of samples was determined by drying samples in an oven at 105°C until constant weights of the crucible with samples were obtained. The percentage of moisture content was measured by calculating the difference between the wet weight and dry weight of the sample, using the following equation:

$$\text{Moisture content (\%)} = [(W_w - W_d) / Ww] \times 100$$

Where:

$W_w$: the weight of wet samples;

$W_d$: the weight of dried samples.

### 3.2.3.2 Ash Analysis

The total ash content of samples was resolved according to AOAC (2005), Method 920.153, utilising conventional dry-ashing. Initially, 4.0 to 5.0 g of dried samples placed in a crucible were burnt and ashed in a muffle furnace at 525°C for 24 h. Each crucible containing grey mass (ash) was then cooled in a desiccator before it was weighed. The percentage of total crude ash was determined using the following equation:

$$\text{Crude ash (\%) on dry weight basis} = (W_a/W_b) \times 100$$

Where:

$W_a$: the weight of samples after ashing;

$W_b$: the weight of samples before ashing.

### 3.2.3.3 Protein Analysis

The protein content of samples was measured using a nitrogen assay based on the Kjeldahl method (AOAC, 2005), Method 928.08, which included digestion and distillation processes (Vapodest, C. Gerhardt). The percentage of crude protein was expressed as a total nitrogen percentage and multiplied by a factor of 6.25 (nitrogen-protein conversion factor for fish and seafood products) as the following equation:

$$\text{Crude protein (\%) on dry weight basis} = \text{Nitrogen (\%) in samples} \times 6.25$$

### 3.2.3.4 Fat Analysis

Fat was estimated using Soxhlet extraction with petroleum ether as the solvent. The fat content of each sample was determined after oven-drying the extracted fats overnight (Soxtherm® extractor, C. Gerhardt). The fat content of the samples was calculated as the following equation:

$$\text{Fat (\%) on dry weight basis} = (W_f/W_d) \times 100$$

Where:

$W_f$: the weight of fat in samples;

$W_d$: the weight of dried samples.

### 3.2.3.5 Carbohydrate analysis

Total carbohydrate was calculated using the difference between the value of each nutrient based on the equation below:

$$\text{Carbohydrates (\%)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Protein (\%)} + \text{Fat (\%)}]$$

## 3.2.4 Chemical Properties of Samples

### 3.2.4.1 Water Activity Measurement

The water activity (Aw) of each sample was determined using an Aqua Lab Dew Point Water Activity Meter 4TE (Decagon, US) at about 25°C. The samples were placed into the cup before it was distributed according to the manufacturer's instructions.

### 3.2.4.2 Sodium Content Measurement

Initially, the samples were ground until they formed a fine, homogenous powder and could pass through a 0.25 mm sieve. The indigestion tube was then filled with 20 mL of concentrated nitric acid and 10 mL of concentrated hydrocloric acid for each sample (1 g). The mixture was boiled gently until the solution turned colourless. Lastly, the distilled water was added to bring the volume of the solution to 100 mL. The sodium content of samples was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) methods.

### 3.2.4.3 pH Analysis

The measurement of pH of samples was done using a pH metre (Mettler Toledo, US). Prior to analysis, 10 g of belacan sample was mixed with 2 mL distilled water to form a paste-like structure (Vijayakumar and Adedeji, 2017), and the pH of bosou, budu (cooked and non-cooked), and cincaluk were measured directly to the sample in a small beaker.

### 3.2.5 Colour Measurement

Colour determination of samples was done using a colourimeter (LabScan® XE Spectrophotometer, HunterLab, US) based on the *L*a*b** colour scale system. The *L** value represents lightness/darkness, *a** value represent redness/greenness, and *b** value represents yellowness/blueness and was measured according to the manufacturer's instructions.

### 3.2.6 Statistical Analysis

All quantitative results were calculated as mean ± standard deviation (mean ± SD), respectively. Statistical analysis of the data was carried out using SPSS (Statistical Package for the Social Sciences) version 26.0. The selection of analysis was dependent on normality and equal variance of data. ANOVA was used to analyse data with a normal distribution and equal variance, while Kruskal-Wallis was used for data that did not meet these conditions. Data were considered statistically significant if the probability value was less than 0.05 ($P < 0.05$).

### 3.3. Results

#### 3.3.1 Isolation and Preliminary Selection of LAB

In the present study, 59 LAB were isolated from belacan, bosou, and non-cooked budu. The presumptions were made based on their ability to hydrolyse $CaCO_3$ on the MRS agar along with their morphological characteristics, such as round, white or creamy, and raised or convex elevation. Besides, the isolates were also negative in the catalase test and were stained positive with rod-shaped in Gram's reaction (Figure 3.1). The isolates were labelled as BE1 to BE17 (isolates from belacan), BO1 to BO17 (isolates from bosou) and BUM1 to BUM25 (isolates from non-cooked budu). All the isolates were facultative anaerobes, which was proved by their ability to grow aerobically and anaerobically, with no significant difference between both conditions ($P$>0.05). However, no presumptive LAB with catalase-negative and Gram-positive was isolated from both cooked budu and cincaluk samples.

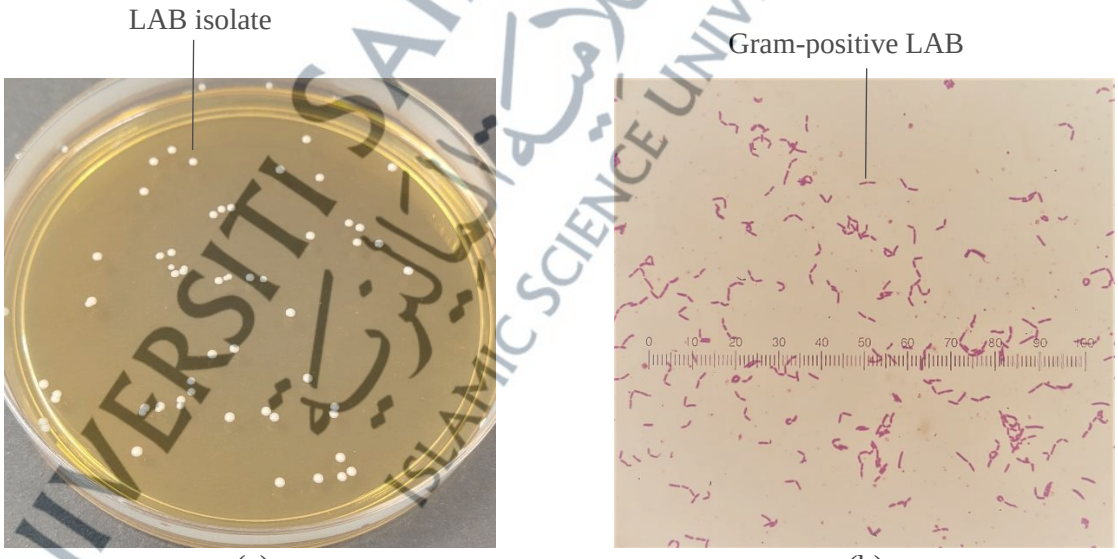


**Figure 3.1:** Morphology of LAB a) Grown on MRS Agar and b) Observed Under Light Microscope (Magnification 100×)

### 3.3.2 Proximate Analysis

The proximate composition of all fermented food samples is presented in Table 3.2. Moisture content ranged from 32.16% to 72.02%, with belacan marked the lowest moisture content while bosou had the highest percentage. The moisture content of belacan was significantly lower ($P < 0.05$) compared to non-cooked budu and bosou. Bosou also contained significantly higher moisture content ($P < 0.05$) when compared to cooked budu.

Results also found that ash was a major component in all samples except bosou. The bosou only contained 18.29% of ash, which was significantly lower ($P < 0.05$) than belacan and cooked budu. Cooked budu also recorded the highest percentage of ash (57.74%), thus marking a significantly different value ($P < 0.05$) from cincaluk.

The protein content of the fermented food samples varied narrowly between 31.83% and 37.15%. Among the five samples studied, bosou contained the highest protein content (37.15%), which was also found to be the major component in the sample, whilst belacan recorded the lowest protein, which was 31.83%. However, there was not a significant difference ($P > 0.05$) in protein among the fermented food samples.

The fat content in all samples became the minor proximate component of each sample, respectively. Bosou contained 17.52% fat content, which was the highest fat content among the other samples. In contrast, the fat content in the remaining samples was very low, with a range of between 1.01% and 2.38% only. The values were also not significantly different ($P > 0.05$) among each other. The fat content in bosou was not significantly different ($P > 0.05$) from non-cooked budu and cincaluk but was significantly higher ($P < 0.05$) from belacan and cooked budu.

Besides protein and fat content, bosou also documented the highest carbohydrate content with values of 27.04%. The percentage was followed by cincaluk (19.43%), belacan (11.01%), non-cooked budu (6.84%), and cooked budu (6.62%).

**Table 3.2:** Proximate Composition (%) of Malaysian Fermented Food

| Samples | Moisture (%) | Ash (%) d.w. | Protein (%) d.w. | Fat (%) d.w. |
|---|---|---|---|---|
| Belacan | $32.16 \pm 2.00^{ab}$ | $56.15 \pm 0.56^{bc}$ | $31.83 \pm 0.66^{a}$ | $1.01 \pm 0.23^{a}$ |
| Bosou | $72.02 \pm 0.71^{b}$ | $18.29 \pm 0.98^{a}$ | $37.15 \pm 3.18^{a}$ | $17.52 \pm 1.46^{b}$ |
| Cooked budu | $66.09 \pm 0.37^{a}$ | $57.74 \pm 1.14^{c}$ | $34.27 \pm 0.42^{a}$ | $1.37 \pm 0.35^{a}$ |
| Non-cooked budu | $68.77 \pm 0.22^{b}$ | $55.37 \pm 0.24^{abc}$ | $35.80 \pm 0.56^{a}$ | $1.99 \pm 0.23^{ab}$ |
| Cincaluk | $67.44 \pm 0.52^{ab}$ | $43.97 \pm 1.39^{ab}$ | $34.22 \pm 0.47^{a}$ | $2.38 \pm 0.95^{ab}$ |

Value represents the mean of triplicates ± SD.
d.w.: dry weight basis
For each column, different superscripts lowercase letters indicate significantly different at $P<0.05$

### 3.3.3 Chemical Properties of Samples

Table 3.3 shows the chemical properties of the fermented food samples, which include water activity, sodium content, and pH value. The result showed that the water activity value of fermented food products ranged from 0.723 to 0.939. The lowest water activity value was found in belacan (0.723), followed by cooked budu (0.795), non-cooked budu (0.821), cincaluk (0.823) and bosou (0.939). The salt present in all fermented foods improves both the shelf-life and the taste of fermented food products. In the assessment of sodium content, it was found that belacan contained the highest sodium content (10.10g/100g) which doubled the value of cooked budu (5.33g/100g), non-cooked budu (5.04g/100g) and cincaluk (5.25g/100g). Meanwhile, bosou had the lowest sodium content (1.02g/100g), which is approximately one-tenth of belacan's value. From Table 3.3, it could also be seen that the pH of the various fermented food samples ranged between 3.86 and 7.31. Belacan was the only fermented food sample which recorded a slightly basic pH (7.31) while the remaining samples had an acidic

pH of 5.05, 4.96, 4.96, and 3.86 for cooked budu, non-cooked budu, cincaluk, and bosou, respectively.

**Table 3.3:** Water Activity, Sodium Content, and pH Values of Malaysian Fermented Food

| Samples | Water activity | Sodium content (g/100g) | pH |
|---|---|---|---|
| Belacan | 0.723 | 10.10 | 7.31 |
| Bosou | 0.939 | 1.02 | 3.86 |
| Cooked budu | 0.795 | 5.33 | 5.05 |
| Non-cooked budu | 0.821 | 5.04 | 4.96 |
| Cincaluk | 0.823 | 5.25 | 4.96 |

### 3.3.4 Colour Measurement

Figure 3.2 shows the Hunter *L, a, b* colourimetric values of the fermented food products. The colour properties of each sample differed, as demonstrated by the difference in the mean *L*a*b** values; nevertheless, all samples exhibited both red and yellow hues. Belacan had the darkest colour (29.42), which is significantly different ($P < 0.05$) from the others, which were followed by cooked budu (33.01), non-cooked budu (38.74), bosou (39.43) and cincaluk (50.23). The luminosity values of all samples were statistically different ($P < 0.05$) from each other, excluding non-cooked budu versus bosou, which recorded fairly comparable values. The *a** (redness/greenness) values for bosou (5.3) and non-cooked budu (4.88) are also almost parallel, while the remaining samples present statistically distinct *a** values ($P < 0.05$) from each other. Bosou had the lowest *b** (yellowness/blueness) value (11.66), whilst cooked budu scored the highest value (24.12). Both values were statistically different from the other fermented food samples ($P < 0.05$). In contrast, the values for belacan, non-cooked budu, and cincaluk are almost equivalent ($P > 0.05$).

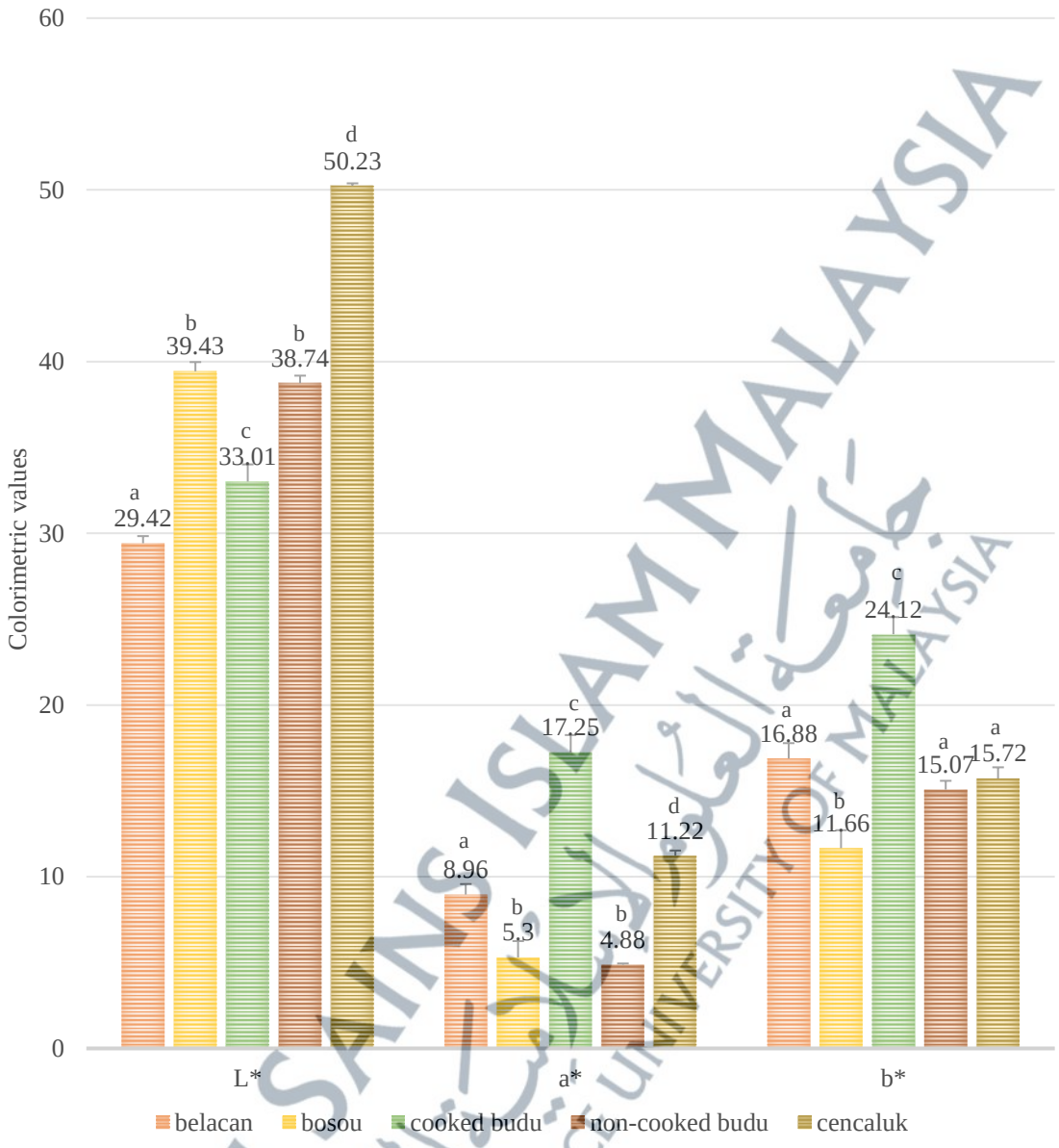


**Figure 3.2:** Hunter *L*, *a*, *b* Colorimetric Values of Malaysian Fermented Food
For each category, different superscripts lowercase letters indicate significantly different at $P<0.05$

### 3.4 Discussion

The LAB is a group of Gram-positive and catalase-negative bacteria with no spore-forming that undergo fermentation with substrates and produce lactic acid as their major end product. Some of the common LAB genera isolated from fermented foods are *Lactobacillus, Leuconostoc, Enterococcus, Lactococcus,* and *Pediococcus* (Tan et al., 2017). The presence of these bacteria in fermented food is not uncommon as they are usually the major microorganisms found in the food, such as *Lactobacillus plantarum* isolated from Malaysian fermented durian or tempoyak (Ahmad et al., 2018) and Korean kimchi (Huang et al., 2019), as well as *Lactobacillus brevis* and *Streptococcus anginosus* isolated from Malaysian fermented fish, pekasam (Tan et al., 2017). The genus of LAB strains isolated from each sample may vary depending on the raw materials used, the process involved, and the stage of food fermentation (Yuliana et al., 2018).

In this study, several fermented food products available in Malaysia were used for the isolation of LAB. The results showed that the presumptive LAB was successfully isolated from belacan, non-cooked budu, and bosou. The LAB may derive from the shrimp or fish used as the raw materials or the environment (Lee et al., 2018). Thus far, this study is the first finding of the isolation of LAB in bosou. However, no presumptive LAB strain was isolated from both, cincaluk and cooked budu samples.

The study on the presence of LAB in cincaluk was limited. The study by Zareian et al. (2012), which focused mainly on LAB with glutamic acid production, isolated 27 and 49 presumptive LAB isolates from cincaluk and budu, respectively, without informing the exact genus of each isolate. The presence of *Staphylococcus piscifermentans,* the LAB strain from cincaluk was reported in a previous study (Hajar and Hamid, 2013). Although water activity is a major factor that modulates the growth

of microorganisms in a food product (Haitham, 2017), the difference in these values does not correlate to the successful isolation of LAB in each sample. Bacteria can maintain their viability regardless of the water activity, but water activity above 0.8 is adequate for them to grow (Vesterlund et al., 2012). Cooked budu and cincaluk recorded water activity values that theoretically could support the viability and growth of microorganisms, but LAB failed to be isolated from these samples. These findings are not surprising as other studies are reporting the absence of LAB bacteria in their shrimp paste products despite the total bacterial count being up to $10^5$ colony forming units/g (Daroonpunt et al., 2016).

Several factors could contribute to the absence of LAB in cooked budu and cincaluk. The cooking procedure at the end of the fermentation process would wipe out the potential occurrence of LAB in cooked budu. The preservation procedure done during food production, such as ultraviolet radiation, could retain the original organoleptic properties while reducing the microbial load of the food (Bhat, 2016). This could lead to the incapacity to isolate the LAB. Other than that, the presence of other microorganisms such as yeast can set aside the existence of LAB. For example, the study by Cissé et al. (2019) on fermented indigenous foods of Burkina Faso, West Africa has molecularly identified different types of yeast in their samples, such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Candida tropicalis.* Theoretically, harmful bacteria may not survive in the fermented food due to the high salt concentration and acidic pH of the sample (Lee et al., 2018).

Fermented food is categorised as functional food with a positive effect on health as it is closely related to the presence of beneficial microorganisms (Bell et al., 2017). The nutrient values in the fermented food may be altered positively or negatively due to the catabolism and anabolism processes done by the microorganisms, which lead to

the breakdown of complex compounds and the production of new compounds (Hasan et al., 2014). As belacan, bosou, budu, and cincaluk are broadly consumed by Malaysians in their daily meals, there is a need to investigate the nutritional values of each product that may tacitly give health effects to the consumers.

Water was an important component of all the fermented foods. The moisture content of cincaluk obtained in this study (67.44%) was slightly lower than the study done by Tee et al. (1997). The moisture value of belacan was almost in the same range as kapi (Faithong et al., 2010) and saewoojeot (Kim et al., 2014), products from Thailand and Korea that are approximately similar to belacan, and it complies with the water content stated in the Malaysian Food Act 1983 (Act 1981) and Regulations (1985) (< 40%).

Meanwhile, the moisture content value of cooked budu, non-cooked budu, and bosou was relatively high compared to products from different countries, such as shidal, which ranged between 37.74% and 44.24% (Ahmed et al., 2013), and hout-kasef, with a moisture level of 47.96%. The percentages for rusip were slightly similar to those reported by Koesoemawardani et al. (2018), with a percentage ranging from 66.52% to 67.27%. The findings for both cooked budu and non-cooked budu were also different from the previous study of budu, with values ranging from 58.13% to 62.93% (Ghazali et al., 2011; Sim et al., 2015).

The high moisture content of bosou might be contributed by the addition of the carbon source, which is cooked rice in the ingredient. The probability is supported by Faithong et al. (2010), which suggested that palm-sap-sugar concentrate that is mixed with shrimp to produce koong-som may cause the highest range of moisture content of the products, compared to the other types of samples. The variation of percentage might also be attributed to the washing procedure of raw materials, water retention that

happens during the fermentation process, and the fermentation period (Rapsang and Joshi, 2013; Kim et al., 2014; Mohd Khairi et al., 2014).

Ash percentage indicated the presence of minerals that were not destroyed after burning the food at a tremendously high temperature (Siti Mahirah et al., 2018). This study found that ash was a significant component in all fermented food samples, except for bosou. According to the Malaysian Food Act 1983 (Act 1981) and Regulations (1985), the ash content in belacan shall be less than 30%, whilst cincaluk shall contain no more than 15% ash. Extrapolation of data to a wet weight basis showed that ash content in belacan and cincaluk was approximately 38.09% and 14.32%, respectively, with the former failing to comply with the regulations. The ash content in both cooked and non-cooked budu, when extrapolated to wet weight, showed a value of 19.58% and 17.29%, respectively.

The values are almost parallel to the ash content (19.60% wet weight) of hous-kasef, the fermented fish samples of Saudi Arabia (Gassem, 2017). The lower percentage of ash in bosou reflects the multiple ingredients used, such as small fish, salt, cold cooked rice, and fermented pangi. As the other fermented food samples used fewer raw materials and high salt content, they contributed to a greater ash ratio (Pongsetkul et al., 2014). Fresh shrimp itself is abundant in mineral contents, which are significant in maintaining various functions of living organisms, and thus may instigate the high percentage of ash in belacan and cincaluk (Ajifolokun et al., 2018). On the contrary, the ash of fresh anchovies on a wet weight basis varied between 1.5% and 2.4% with approximately 0.5% salt content (Shiriskar et al., 2010).

Theoretically, there should be a slight increase in protein percentage when animals undergo the fermentation process (Achinewhu and Oboh, 2002). The increments could be due to the new synthesis of protein by the microorganisms involved

in the fermentation process. However, there could be a likelihood that the values decrease due to the presence of proteolytic enzymes by the animal itself (Beddows et al., 1979) or produced by the microorganisms (Rapsang and Joshi, 2013) that could breakdown the proteins. Besides, an extended fermentation period may also cause rigorous degradation of proteins (Faithong et al., 2010).

According to the Malaysian Food Act 1983 (Act 1981) and Regulations (1985), belacan, budu, and cincaluk must contain at least 30%, 5%, and 10% protein, respectively. The protein content in belacan, when extrapolated to wet weight, showed a value that did not comply with the regulation (21.59%), which is in agreement with the previous report done by Sharif et al. (2008). Contrary to the extrapolated protein content in both cooked and non-cooked budu, both samples obtained values that complied with the standard (11.62% and 11.18%), which is in agreement with the previous reports done by Ghazali et al. (2011) and Mohd Khairi et al. (2014). The extrapolated protein content value for cincaluk (11.14%) is also slightly higher than the stated values. Meanwhile, the protein content in bosou when extrapolated to wet weight showed a slightly lower value (10.39%), which may be driven by the addition of more ingredients in the making of bosou, thus varying the total percentage of each parameter tested. The values stated in this study were notably less than the protein content of fasiekh (25.40% to 33.41% wet weight) reported by Osman et al. (2012).

Fat content is negatively correlated with water content (Ajifolokun et al., 2018). Contrary to the theory, the outcome of the present study found that although there was a difference in the moisture percentage, the fat content in all samples was nearly comparable, not including bosou. The values obtained in this study are in agreement with other fermented shrimps reported by Faithong et al. (2010), Kim et al. (2014), and Khairina et al. (2016). The fat content of fermented shrimp products might be

contributed by the lipid content in the shrimp oil (Pongsetkul et al., 2014). Meanwhile, Osman et al. (2012) found that the fat content of various preparations of fasiekh, the fermented fish samples, ranged between 1.18% and 8.09% wet weight. The fat content in bosou, cooked budu and non-cooked budu, when extrapolated to wet weight, showed a different range of values, which was 4.9%, 0.47%, and 0.62%, respectively. The difference in fat content in bosou, budu, and other fermented fish samples may be attributed to the lipid content of the raw small fish versus anchovies (Majumdar et al., 2016).

Carbohydrate content will be reduced in fermented foods in comparison to fresh materials as the biomolecules are used by microorganisms in the fermentation process (Rapsang and Joshi, 2013). In general, belacan and budu are manufactured without the addition of any carbon source, whereas cincaluk and bosou have which has sugar or cooked rice as one of their ingredients. Thus, the raw materials correlate to the low carbohydrate content in belacan and budu, when compared to cincaluk and bosou. In mass production, the carbohydrate components might be added as exact ingredients or deliberately added to increase the yield and reduce the cost (Faithong et al., 2010).

Meanwhile, water activity is a measure of water available in food that microorganisms can use for growth. Thus, when considering the methods to preserve food, this value is more important than the total amount of water present in a food item. In food preservation methods, active water is more critical for food stability than total water content. The availability of water could be altered by various approaches, such as increasing the solute concentration or removing the water component. Pathogens may not grow in food with a water activity below 0.85 to 0.86 (Shafiur Rahman and Labuza, 2007).

It was found that the water activity values for belacan, budu, and cincaluk were lower than the stated range. The results for belacan (0.723) are almost comparable to those of Bruneian fermented paste (0.728) and Korean dried shrimp paste (0.771) (Kim et al., 2014). Meanwhile, the values of water activity obtained for both budu samples (0.795 and 0.821) were consistent with Mohd Khairi et al. (2014), which reported that their tested budu showed water activity values of between 0.819 and 0.840. Although bosou appeared to have high water activity that suits the growth of various microorganisms, another parameter such as acidic pH could counteract this convenient environment.

All fermented foods contain salt, which improves both the shelf life and the taste of the fermented foods. The addition of salt is crucial as it helps to reduce active water and causes the cells to shrink, thus transforming perishable fresh ingredients into products that are more shelf stable (Shafiur Rahman and Labuza, 2007). The sodium content of bosou was paralleled to the sodium content of various fasiekh samples and ranged from 0.83 to 1.02g/100g (Osman et al., 2012). Kapi which have a comparable feature as belacan, also contains a higher percentage of salt compared to Koong-Som, which are slightly akin to cincaluk (Faithong et al., 2010). The sodium contents were also aligned with the ash content for each sample, which also reflects the massive amount of salts added during the making of belacan, budu, and cincaluk. Cooked budu contains a higher level of salt as more salt was added to adjust the final taste before it was distributed to the consumer.

Belacan recorded the basic pH (7.31), which is slightly analogous to kapi (Faithong et al., 2010), Bruneian shrimp paste, and saewoojeot (Kim et al., 2014). The similarity may be due to the use of raw materials and processes that are slightly comparable. All these fermented shrimp products use only shrimp and salt as their

ingredients. The slightly basic pH may be due to the formation of ammonia during the fermentation process (Faithong et al., 2010). Nonetheless, all the other samples in this study presented an acidic pH. Earlier studies of budu reported the acidic pH value to be between 5.17 and 5.6 (Beddows et al., 1979; Mohd Khairi et al., 2014). The pH of the fermented fish products produced in Indonesia and the Philippines was also greater than the samples in this study, which were above 5.55 and 5.96, respectively (Mueda, 2015; Nofiani et al. 2019). Other types of fermented shrimp which contain other raw materials as their ingredients also obtain an acidic pH, such as Koong-Som (Faithong et al., 2010) and Ronto (Khairina et al., 2016), with the latter using rice as a carbohydrate source. The acidic value of bosou might be due to the higher growth of microorganisms stimulated by the addition of cold cooked rice as the carbon source.

Theoretically, pH in fermented foods will be slightly lower as fermentation progression might produce various organic acids, for example, lactic acid, acetic acid, and propionic acid (Erkmen and Bozoglu, 2016). Common microorganisms that can be isolated from fermented foods are yeast and lactic acid bacteria. The incredibly low level of pH in bosou could also be beneficial to counter the high level of water activity that favours the growth of pathogens (Chiang et al., 2006).

The difference in *L*a*b** values of each sample could be due to the variation of the raw materials used in the products. In the case of belacan and cincaluk, although both products make use of shrimp as their main ingredients, the types of shrimps used, the process, and other ingredients added could affect the colour of the end products. In fact, during the manufacturing of cooked budu, the colouring ingredient was added to enhance the colour. Indeed, a previous study on the L*a*b* values of budu were 33.43, 19.53, and 25.54, respectively, which was comparable to the values shown by cooked budu (Mohd Khairi et al., 2014). Not only that, the whole fermentation process, which

involves several chemical reactions, for example, the Maillard reaction, oxidation and hydrolysis processes, could also contribute to further colour developments (Daroonpunt et al., 2016). The duration of fermentation could also have a significant effect on the colour characteristics of fermented products (Mueda, 2015).

### 3.5 Conclusion

A total of 59 presumptive LAB was succesfully isolated from belacan, bosou and non-cooked budu. The presence of bacteria in each sample may be affected by multiple causes such as the raw materials used, the manufacturing procedures, as well as the environments. The physicochemical and colourimetric characteristics of belacan, bosou, cooked budu, non-cooked budu, and cincaluk were varied depending on the ingredients and fermentation procedures involved in the making of each product. In terms of nutrient content, bosou is a more competent candidate to provide good nutrients with low salt content. Nevertheless, the high salt content and low water activity value in other samples would cause the products to be more shelf-stable for an extended period. Cooked budu and non-cooked budu offer relatively comparable nutrient values within one another, as well as low-fat content. The advantages of the cooking process are that it could kill the pathogens or spoilage microorganisms that may grow in the sample. However, this step could also destroy the beneficial microorganisms with nutraceutical properties that may have a health impact on the consumer. The findings in this study can be used as a basis for more advanced research to explore the potential of this fermented food, for example, the probiotic potential of the strains isolated from the samples.