CHAPTER 1

INTRODUCTION

1.1 Introduction

High blood cholesterol, or hypercholesterolemia, is one of the major risk factors for the development of cardiovascular diseases. According to the Malaysia Acute Coronary Care Performance Report 2016 (2017), cardiovascular risk factor occurrence is increasing, with around 80% of hypercholesterolemia cases formerly unidentified. Data by the Malaysian National Health and Morbidity Survey has shown about 20% of young adults (aged 18 to 19 years) have a blood total cholesterol level of more than 5.2 mmol/L, which is the cut-off point for hypercholesterolemia (Management of Dyslipidaemia, 2017). Indeed, cardiovascular diseases related to the heart and blood vessels, for example, ischaemic heart disease and stroke. Both diseases are the topmost illnesses, which are responsible for 16% and 11% of the deaths around the globe, respectively, in 2019 (World Health Organization, 2020b).

More recently, ischaemic heart diseases remained as the principal causes of medically certified death in 2019 (Department of Statistics Malaysia, 2020). Furthermore, a study population of more than two million subjects proved that increased levels of cholesterol were associated with a higher risk of cardiovascular diseases, while decreased levels of cholesterol were associated with a lowered risk of cardiovascular diseases among young adults (Jeong et al., 2018). Even though many drug therapies are available, there are still growing numbers of individuals affected by these ailments

(World Health Organization, 2021). A Malaysian National Health and Morbidity survey conducted in 2015 concluded that approximately 47% of the adult population is hypercholesterolemic.

Excess fat and cholesterol consumption, as well as lipid metabolism within the human body, can all contribute to high cholesterol levels (Lew et al., 2018). Thus, regulating the processes associated with these biomolecules' metabolisms may have beneficial effects in controlling or lowering blood cholesterol levels. Treatments that are available may come at a high cost and may also have undesirable side effects (Guo et al., 2012). Thus, maintaining the serum cholesterol level at the optimal range could possibly cause a significant reduction in the incidence of the diseases. One of the key factors that could influence the serum cholesterol level is lifestyle changes, such as switching to a healthy diet. Efforts have been channelled to identify other diets that contain components that can reduce blood cholesterol levels, such as diets containing probiotics.

Probiotics are valuable living microorganisms that have been used for a long time due to their various health benefits (Behnsen et al., 2013). As consumers become more aware of the benefits of probiotics, the demand for probiotic foods is rising fast. Thus, many attempts have been made to produce food that is supplemented with probiotics (Perera et al., 2017). The development of probiotic products can be divided into two groups, which are probiotic supplements and probiotic foods, with the majority of them being fermented foods. Despite both types being able to promote better health, probiotic foods are more preferable due to consumer preference as well as the food’s buffering properties throughout the digestive tract, its nutrient content and also its synergistic effects that maintain the probiotic growth, activity and efficacy (Homayoni Rad et al., 2014). They are produced from various sources, such as cereal grain, dairy products,

fruits, vegetables, meats, seafood, and fish. However, as processing and storage of food may affect the quality and viability of probiotics, it leads to more research into finding probiotics that occur naturally in foods or fermented foods.

The interesting fact about probiotics is that they can compete with pathogens in the intestinal environment for adhesion sites before they can exert various health effects on the host (Kerry et al., 2018). Thus, it provides a benefit to reduce the possibility of foodborne illness. In addition, probiotics also offer a variety of health benefits, such as preventing and/or treating allergies, dental caries, intestinal inflammation and lactose mal digestion, of which each different species has a different effect (Perera et al., 2017). Plentiful studies have also been done to evaluate the potential of probiotics isolated from fermented foods to heal or prevent diseases (Song et al., 2015; Lim et al., 2017; Le and Yang, 2018). The major group of probiotics are lactic acid bacteria (LAB), which undergo fermentation with substrates and produce lactic acid. The probiotic effects that regulate the metabolism of cholesterol and lower the lipid content are primarily strain-specific, which means not all probiotics can modulate cholesterol levels in the bloodstream. Examples of species that could modulate the cholesterol levels are *Lactobacillus plantarum* (Rapsang and Joshi, 2013; Ma et al., 2019), *Lactobacillus helveticus* (Damodharan et al., 2016), *Lactobacillus acidophilus* (Gao and Li, 2018), *Enterococcus faecalis* (Gao and Li, 2018) and *Lactobacillus paracasei* (Dehkohneh et al., 2019).

In Malaysia, there are many types of fermented aquatic-based products such as cincaluk and belacan that are produced from shrimp, budu which uses anchovies as raw materials, and bosou, which is formed by fermenting small fishes. The first three products are widely consumed by different layers of society in Malaysia as seasonings or condiments. Meanwhile, bosou is a traditional food of the Kadazandusun tribe in

Sabah. It resembles pekasam that can be found in peninsular Malaysia, but with dissimilar ingredients. A number of studies have reported various outcomes related to the products due to their potential as carriers of various beneficial microorganisms, such as microbiological loads the presence of lactic acid bacteria (Zareian et al., 2012; Hajar and Hamid, 2013) and fibrinolytic bacteria (Zakaria et al., 2015). However, to date, there have been no reports investigating potential cholesterol modulation activities by probiotics isolated from Malaysian fermented foods. Thus, the aim of this research was to isolate probiotic LAB strains from Malaysian fermented foods and to evaluate their potential cholesterol-lowering activities using in-vitro studies.

The findings of this study will benefit society because hypercholesterolemia has been identified as the primary cause of multiple cardiovascular diseases. The increasing prevalence and mortality related to this condition justifies the need for other effective approaches. Thus, data from this research may provide additional information regarding the cholesterol modulation properties of probiotic lactic acid bacteria that can be found in Malaysian fermented foods. According to Kerry et al. (2018), an easy, uncomplicated, inexpensive, accessible, and intrinsic approach to achieving healthiness is important in the present era; thus, probiotics have shown so many promising results. Since all the proposed samples could be taken raw, the potential probiotics could be directed to the target cells of the digestive tract more promisingly. Besides that, the proposed research may increase the commercial values of each sample, which may contribute to the positive build-up of small-scale industries in Malaysia.

1.2 Objectives

The objectives of this study were:

i. To isolate, characterise, and identify probiotic lactic acid bacteria from Malaysian fermented foods namely belacan, bosou, cooked budu, non-cooked budu and cincaluk;

ii. To determine the effects of probiotic lactic acid bacteria in relation to the in-vitro cholesterol removal model;

iii. To evaluate the adhesion and aggregation ability of probiotic lactic acid bacteria against HT-29 cell lines;

iv. To analyse the expressions of genes involved in cholesterol metabolic pathway (NR1H3, LXR, NPC1L1, and CD36), in response to presence of probiotic lactic acid bacteria using HT-29 cell lines.