CHAPTER 1

INTRODUCTION

Milk proteins are rich in bioactive peptides that are embedded within its primary structure (Raveschot et al., 2018). The bioactivities of peptides in milk have increased attention as potential ingredients for health promoting functional foods targeted at diet related chronic diseases such as cardiovascular disease, diabetic type II, and obesity (Abubakr et al., 2012a). These peptides can be released by proteolysis during gastrointestinal transit (digestive enzyme) or during food processing either by fermentation of proteolytic starter cultures or using enzymes derived from microorganisms and plants (Korhonen & Pihlanto, 2006).

Lactic acid bacteria (LAB) are unique bacteria that can adapt in both aerobic (aerotolerant) and non-aerobic surroundings with one of their specific metabolites having a proteolytic system and most of the LAB have probiotic properties. The LAB with proteolysis can degrade proteins into smaller proteins, peptides, and/or amino acids with numerous bioactivity (Varshavsky, 2001). While probiotic LAB may benefit gut microbial communities which lead for certain health issues improvement such as immune system improvement by helping the body to resist and fight against infection (Wedajo, 2015).

The fermentation of milk by LAB resulted in generation of bioactive peptides with biological actions such as angiotensin I-converting enzyme (ACE) inhibitory, antibacterial activity (Minervini et al., 2003), immunomodulatory (LeBlanc et al., 2004), and antioxidant activities (Abubakr et al., 2012a). Antioxidants prevent oxidation and act as natural preservatives in foods. Synthetic antioxidants that are available in food industry such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) have been reported to have negative effect for human health (Nandhini et al., 2012; Sah et al., 2016), thus, replacement of synthetic antioxidants to the natural antioxidants source are preferable.

Several methods were used to determine the antioxidant activity of a substance such as scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity, and ferrous ions chelating activity (FICA) assay (Abramovic et al., 2018; Bamdad et al., 2017; Lin et al., 2014). The DPPH radical scavenging method is widely used to evaluate antioxidant activities due to its simplicity, rapidity, sensitivity, and reproducibility compared with others (Milardovic´ et al., 2006). Meanwhile, FICA measures the ability of the substance to chelate ferrous ions indicating the sample tested has chelating power and may act as antioxidant (Lim et al., 2007). Both assays analyse antioxidant activity of different sample types including milk, and milk products (Bamdad et al., 2017; Li et al., 2015; Maleki et al., 2015).

Fermentation of milk with LAB were reported to generate whey with antioxidative activity (Abubakr et al., 2012a; Namdari & Nejati, 2016; Osuntoki & Korie, 2010) and were dependent on the LAB strains used and not directly connected to fermentation time (Virtanen et al., 2007). Indeed, skimmed milk fermentation with *Leuconostoc mesenteroides* ssp. *cremoris* strains, *Lactobacillus jensenii* ATCC 25258, and *L. acidophilus* ATCC 4356 found scavenging activity (27 to 53 % v/v) after 24 h fermentation by 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay (Virtanen et al., 2007). Fermentation of skimmed milk by Malaysian local isolates of *L. plantarum* 1 and *Leu. mesenteroides* were reported to produce whey with good antioxidative activity using both DPPH scavenging activity, and FICA assays (Abubakr et al., 2012a). In addition, a good antioxidant activity of cow milk yogurt with 63.99 % (v/v) at 0 day after 12 h fermentation have also been appeared by *L. acidophilus* during storage at 4 °C for 15 days using DPPH assay but slightly decreased to 52.64 % (v/v) (Gjorgievski et al., 2014). Previously, fermentation of buffalo milk by *L. lactis* and *L. delbeurkii* was reported to produce whey with higher antioxidant activity (21.91 to 32.73 %, v/v) expressed inhibition activity using DPPH assay (Vankudre et al., 2015).

Buffalo milk is extensively used and produced in the international milk industry (The World Dairy Situation, 2010). Approximately, 85 % of total world's milk production comes from cow milk followed by buffalo milk and other minor dairy species (Guha et al 2021). Similar to other milks, buffalo milk is nutritious and has a higher protein level compared to cow milk (El-Salam & El-Shibiny, 2011). As protein content in buffalo milk is higher than cow milk, more production of peptides with bioactivities should be generated from this type of milk. Combination characteristic of proteolytic and probiotic LAB in the LAB selection as starter culture in buffalo milk fermentation to degrade milk protein into peptides was preferable to preserve functionality of LAB. The LAB functionality from the beginning until the end of the fermentation process to generate bioactive peptides including antioxidative peptides is the crucial part.

Therefore, the aimed of this study was to evaluate the antioxidative activity of whey buffalo milk produced by fermentation of local isolated LAB using scavenging DPPH free radical and FICA assays. Thus, the objectives were to

i. screen the proteolytic activity of LAB on skimmed milk agar (SMA) and to determine the antioxidative activity of the whey skimmed milk generated by proteolysis LAB using DPPH free radical activity and FICA assays.

ii. evaluate probiotic potential of LAB, the effect of culturing methods, and fermentation time on antioxidative activity of whey buffalo milk by LAB using DPPH and FICA assays.

iii. identify LAB strains responsible in producing whey buffalo milk with antioxidative activity, and to determine their antioxidative peptides of whey buffalo milk using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) method.