CHAPTER 1

INTRODUCTION

1.1 Research Background

Protein is a vital macronutrient which serves as a source of amino acids and energy for the human body. Beside on its basic function, some food proteins are believed to exhibits some biofunctional benefits towards the human health. The biofunctional benefits can be obtained through the released of bioactive peptides encrypted in the sequence of amino acids within the protein molecules. Bioactive peptide is defined as peptides which trigger biological responses *in vivo* beyond nutritional function that are crucial to the human health and well-being. The biological responses performed by bioactive peptides depends upon the composition and sequence of amino acids, they may influence some major body systems, including enhancement of immunomodulatory attributes to combat infection by pathogenic microorganisms (Pina-Perez & Ferrus Perez, 2018).

Antimicrobial peptides (AMPs) refers as specific protein fragments which often the first line of defense against invading pathogens and play an important role in innate immunity (Park, Cho, Kim, Kim, Kim, & Kim, 2004). AMPs can be obtained from several living organisms, such as from amphibians, insects, mammals, plants, and even some species of microorganisms through enzymatic hydrolysis by proteases (Wang & Wang, 2004). The discovery of natural antimicrobial agents is important for the field of life sciences, especially in food science. This is due to the fact that the utilization of synthetic antimicrobial agents might have undesirable side effect towards the human body and environment (Osman, Goda, Abdel-Hamid, Badran, & Otte, 2016). In addition, the growing incidence of drug-resistant infections also demands for the discovery of conventional and natural antimicrobial therapies to control those infectious diseases (Bahar & Ren, 2013; Mur, Kenton, Lyod, Ougham, & Prats, 2008). AMPs are believed to be capable of fulfilling this demand as they may display several antimicrobial activities

towards pathogenic microorganisms, such as antibacterial, antivirus, antifungal, and antiparasitic (Salas, Badillo-Corona, Ramírez-Sotelo, & Oliver-Salvador, 2015).

AMPs usually have two distinct modes of action as an antimicrobial agent. The first action is through the disruption of microorganism's cellular membrane leading to membrane rupture. The second action is through the inhibition of intracellular function of the microorganism's cell leading to cell death. However, both general mode of actions shares the common initial action which is through the binding of the AMPs with the cellular membrane of the microorganism (Zhang & Gallo, 2016).

Plant protein hydrolysate represent a wide spectrum of options for the production of antimicrobial peptides that can be obtained and isolated from several plant species. Naturally, plant AMPs are the defense component and as a defense response induced upon the infection of pathogenic microorganisms in plants (Pelegrini, Sarto, Silva, Franco, & Grossi-de-Sa, 2011). AMPs from plants have been isolated from the stems, roots, flowers, seeds and leaves of wide variety of plant species (Nawrot, Barylski, Nowicki, Broniarczyk, Buchwald, & Goździcka-Józefiak, 2014). Research back then in 1972 from Fernandez De Caleya et al., (1972), showed that AMPs isolated from wheat endosperm crude purothionin exhibits antimicrobial activity against *Pseudomonas solanacearum*, *Xanthomonas phaseoli*, *Xanthomonas campestris*, *Corynebacterium fascians*, and *Erwinia amylovora*. In the last 10 years, a study from Chandrashekhara et al., (2010), showed that pearl millet seed thionin exhibits antifungal activity against *Sclerospora graminicola*. Another study from De Beer and Viver (2011), showed that AMPs from a species of flower (*Heliophila coronopifolia)* exhibits antifungal activity towards *Botyris cinerea* and *Fusarium solani*. During the last 10 years, there has been growing discovery of bioactive peptides isolated from plants species being studied, such as the total hydrolysates and/or peptide fractions from chickpea, soybean, pea, mungbean and other common beans which demonstrate antioxidant and angiotensin-I converting enzyme activities

(Ruiz-Ruiz, Dàvila-Ortiz, Chel-Guerrero, & Betancur-Ancona, 2011; Ariza-Ortega, Zenón-Briones, Castrejón-Flores, Yàñez-Fernández, Gómez-Gómez, & Oliver-Salvador, 2014). Since then studies about the bioactive peptides isolated from plants, especially the antimicrobial activity of plant protein has been one of the research interest.

Soybean (*Glycine max)* is known as an edible seed which is one of the highly valued crop worldwide due to its nutritional and biofunctional benefits towards the human health. Nutritionally, soybean is a rich source of dietary protein and phytochemicals (Xiao, 2008). In terms of the biofunctionality, there has been several studies shown that soybean exhibits several functional benefits towards the human health, such as reduction in the risk of coronary heart diseases, atherosclerosis, type 2 diabetes; improve bone health; relief menopausal symptoms; and reduce the risk of several types of cancers (Lin, Meijer, Vermeer, & Trautwein, 2004; Anderson, Johnstone, & Cook-Newell, 1995; Moriyama, et al., 2004). According to a statistical data published by the Food and Agriculture Organization of the United Nations (FAO), from 2012 to 2016, the production of soybean worldwide is progressively increase from 241.185.392 tons to 334.894.085 tons. In addition, soybean has been prepared as the food ingredients and/or the food itself for many populations worldwide, especially in Asia in the form of Tofu, Tempeh, Soy milk, Boiled Soybean, and many more. In which soybean has been utilized and being one of the essential crops worldwide.

Pea (*Pisum sativum*) is known as an edible seed contains within a pod which also been prepared as the food ingredients and/or the food itself for many populations worldwide. Commonly, pea seeds are consumed uncooked and/or cooked as a vegetable and available in the market fresh, canned, and frozen. Pea seeds are rich in protein, starch, fiber, vitamins, minerals and polyphenols (Agboola, Mofolasayo, Watts, & Aluko, 2010). In terms of biofunctionality, there is a study showed that the polyphenols content in pea exhibits antioxidant activity (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). According

to a statistical data published by the Food and Agriculture Organization of the United Nations (FAO), from 2012 to 2016, the production of pea worldwide is progressively increase from 17.065.688 tons to 19.877.344 tons. In which the utilization of pea is increasing worldwide.

Soy protein and pea protein contain several motifs of bioactive peptide with biofunctional benefit that can be released using enzymatic hydrolysis. The hydrolysis of soy protein and pea protein could give rise to several interesting biofunctionalities, such as anti-adipogenic activity (Tsou, Lin, Lu, Tsui & Chiang, 2010), lipolysis-stimulating activity (Tsou, Kao, Lu, Kao, & Chiang, 2013), antioxidant activity (Chiang, Shih & Chu, 1999), and Angiotensin-I converting enzyme inhibitory activity (Chiang, Tsou, Tsai & Tsai, 2006). In the last 10 years, several studies also showed that enzymatic hydrolysis followed with fractionation by ultra- and nano-filtration with different molecular weight cut-off (MWCO) membrane of dietary protein hydrolysates might enhance the biofunctionalities of the protein hydrolysates (Tsou, Lin, Chao & Chiang, 2012; Chiang, Tsou, Weng, & Tsai, 2008). Additionally, soy protein and pea protein as well as their hydrolysates have been studied and showed the presence of antimicrobial activity against some species of pathogenic bacteria (Pina-Pérez & Pérez, 2018). However, the effects on enzymatic hydrolysis by trypsin, pepsin and alcalase followed with fractionation by ultra- and nano-filtration into 30kDa and 10kDa MWCO membranes on the antimicrobial activity of soy protein isolate and pea protein isolate have not yet been reported.

1.2 Research Objectives

The general objectives of this bachelor research are:

a) To isolate biofunctional peptides from soy protein isolate (SPI) and pea protein isolate (PPI) by enzymatic hydrolysis with trypsin, pepsin, and Alcalase that supplemented with fractionation by ultra- and nano-filtration using 30kDa and 10kDa molecular weight cut-off (MWCO) membrane columns.
b) To screening of plant protein hydrolysates with the highest antimicrobial activity.

The further (Specific) objective of this bachelor research is:

To screening of peptide fractions from plant protein hydrolysates in 30kDa and 10kDa MWCO columns with the highest antimicrobial activity.

1.3 Research Benefits

This bachelor thesis research serves as a preliminary research platform in the field of functional food discovery and development. In which this kind of research could be expanded further and may support other studies related to protein hydrolysates and antimicrobial peptides. For society, this preliminary research in the discovery of antimicrobial activity of soy protein and pea protein may further assist in the promotion of good health and the prevention of disease infections caused by pathogenic microorganisms. For food science, preliminary research in the discovery of natural antimicrobial agents may promote the utilization of safe, harmless, and cheap materials as both food preservatives and functional foods.