CHAPTER 1

INTRODUCTION

1.1. Background of research

Food allergy is an abnormal response from the body's immune system which triggered by exposing food allergen to the body. Food allergen is a term for food substances that able to trigger allergic reaction. In the exposure of the food allergen, food allergic reaction has several outcomes ranging from mild until severe and cause anaphylaxis reaction which can eventually lead to death. Based on the Center for Disease Control and Prevention (CDC) data, among all food major allergens in the US children, peanut has the highest prevalence (CDC, 2010).

Due to higher prevalence and severity of anaphylaxis reaction of peanut allergy, it increases the awareness of investigating peanut allergy. Commonly, peanut allergy occurs among Western Populations; however, there is an increasing number of peanut allergy in Asian Populations recently, especially in Singapore, Philipines, and China (Kagan *et al..* 2003, Hrihane *et al..* 2007, Shek *et al..* 2010, Sicherer *et al..* 2010, Chen *et al.*. 2011, Osborne *et al..* 2011, Tang *et al..* 2012, Lao-Araya Ped Int. 2012). Peanut allergy also considered as a life-threatening type I hypersensitivity reaction food allergy which is strictly IgE-mediated reaction. Mostly, it is caused by the major peanut allergens, such as Ara h 1, Ara h 2, and Ara h 3. These three major peanut allergens are able to sensitize immune system and cause secretion pro-inflammatory cytokines which lead to the manifestation of peanut allergic reaction. Moroever, recent studies shows among all of the major peanut allergens Ara h 2 has the highest specificity with reactivity to sera from 85% peanut allergic patients (Hales *et al.*, 2004). Thus, Ara h 2 is chosen to be the preferred candidate for diagnostic reagent. It also shows reactivity to IgE in more than 90% of peanut allergic patients (Stanley *et al.*,

1997). In details, Ara h 2 has two isoforms which are Ara h 2.01 (17kDa) and Ara h 2.02 (19kDa). Ara h 2.02 has additional 12 amino acids which contain an additional IgE epitope compare with Ara h 2.01 and exhibit higher binding and a more potent cross-linker of IgE.

To date, there is no effective cure for peanut allergy. Peanut-strict avoidance and self-inject epinephrine are the only ways to help peanut allergic patients to prevent undesirable outcome (CDC, 2010). Therefore, a proper diagnostic tool is needed for better health application, especially for those who had historical allergy background. Current diagnostic tools used by health service providers includes medical history, skin prick test (SPT), and food allergy blood test (IgE immunoassay). The SPT and IgE immunoassay are using three major peanut allergens (Ara h 1, Ara h 2, and Ara h 3) to detect the sensitization of immune system (WAO, 2007). However, the current SPT and IgE immunoassay has disadvantages due to the use of peanut crude extract which result in inconsistent reagent content and low predictive value (false positive) (Schmitt *et al.,* 2010). The native protein allergen on the other hand is difficult to purify, costly and low in yield (WAO, 2007).

To circumvent the disadvantages of using crude or native allergen extract, new alternatives can be explored which involves understanding peanut allergens at the molecular level in order to develop a specific diagnostic tool and appropriate therapeutic strategies for peanut allergy. Thus, recombinant technology can be an alternative to produce peanut allergen in a host cell for component resolved diagnostic (CRD) reagent for peanut allergy. Moreover, the recombinant technology approach provides several advantages compare with crude extract or native allergen, such as high yield of desired protein, cost saving and low batch variation.

Study of recombinant peanut allergen of Ara h 2.01 and Ara h 2.02 had been reported previously in bacterial expression system. In 2007, Glenting *et al.* successfully produced Ara h 2.01 using *Lactococcus lactis,* a generally recognized as safe (GRAS) microorganism. However, Ara h 2.01 used have a weaker affinity towards specific IgE

compared to Ara h 2.02 due to lack of an additional IgE epitope. Recently, Lew and Lim (2016) have successfully produced yAra h 2.02 by using *E. coli* as the host cells; but, the protein was insoluble and may contain endotoxin contamination such as lipopolysaccharide from the gram negative bacteria. Despite being insoluble, the protein was purified, refolded and was found to be able to sensitize Balb/c mice to elicit the production of Ara h 2.02-specific IgE. Although the result was good, the use of *E. coli* for recombinant allergen production presents other problems such as lack of post-translational modifications (PTM) which is important especially for use as human therapeutic agent. Another expression vector comprising of eukaryotic yeast cells allows PTM and secretion of synthesized protein, thus allowing for production of a soluble recombinant allergen. In addition, there is no reported study of the heterologous expression of a yAra h 2.02 gene in an eukaryote such as yeast which may provide soluble protein of substantial yield. Codon optimized gene is designed according to the optimal codon preference of the host in order to obtain high-level expression of a heterologous gene derived from another source, in this case from a plant.

The pPICZα expression system in *Pichia pastoris* yeast is chosen for expression of Ara h 2.02 peanut allergen. *Pichia pastoris* is a methylotrophic yeast, having the ability to utilize methanol as carbon source, employing a strong AOX1 promoter using methanol as an inducer for protein expression (Ahmad *et al.,* 2014). Moreover, *Pichia pastoris* allows the production of either intracellular or secreted protein that is high in yield due to a strong inducible promoter. Codon optimisation of the Ara h 2.02 gene according to the codon usage bias of the host cells can potentially increase protein expression. Hence, the use of *Pichia pastoris* as recombinant host with yAra h 2.02 gene is an ideal combination to produce high yield recombinant allergen reagent for diagnostic and further research on peanut allergy.

1.2. Objective of research

Production of recombinant Ara h 2.02 allergen in an eukaryote yeast expression system may provide secreted and thus soluble allergen protein suitable for development of diagnostic and therapeutic reagent for peanut allergy. As such, this research aims:

- To generate an expression construct of codon optimised Ara h 2.02 in pPICZα for eventual expression in *P. pastoris*