CHAPTER I

INTRODUCTION

Palm stearin is obtained by fractionation of palm oil after crystallization at a controlled temperature. Palm stearin is limited in edible application due to its high melting point, which ranging from 44°C to 56°C, giving the product a low plasticity and incomplete melting at body temperature (Soares et al., 2009; Reshma et al., 2008). Thus, modification of palm stearin has been introduced to alter its physical and chemical properties to broaden its functionality and usage where then a new product called structured lipid are produced. Structured lipid is defined as triacylglycerol (TAG) that have changes in its fatty acid composition through incorporation of new fatty acids, restructured to change the position of fatty acids or synthesized to yield novel TAG (Esteban et al., 2011). Besides, structured lipid or TAG is normally refers to those oil and fats containing polyunsaturated fatty acid (PUFA) and medium- or short-chain fatty acids.

Polyunsaturated fatty acids (PUFA) give health benefits however the two or more double bonds present in the chain makes them easily being oxidized during the reaction process. Thus monounsaturated fatty acid (MUFA) is more stable as compared to PUFA and gives health benefits as well. Therefore, oleic acid (MUFA) comes to be favorable for the lipid modification in this study. Rathouský et al. (2011) supported that its double bond influence on the mechanisms of photocatalytic oxidation, oleic acid is more appropriate for the mentioned simulation than saturated fatty acid. This fatty acid rearrangement very often causes a lowering of the melting point (Soares et al., 2009).

Hydrogenation has been the most commonly employed method for modification of edible fats however partial hydrogenation resulted in *trans* fatty acids residues (Da Silva et al., 2010). Thus, researchers continue in exploring the approach of chemical and enzymatic interesterification in lipid modification. Farfán et al. (2013) have produced dietary fats by developing binary blends of linseed oil (LO) and palm stearin (PS) using chemical and enzymatic interesterification. LO was chosen as it contains high omega-3(~50%) and PS was chosen because of its high melting point resulting on rich of omega-3 fat stock. While in 2009, Costales-Rodríguez and his co-researchers modified palm stearin by blending with soybean oil (70/30 wt%) using both chemical and enzymatic interesterification to produce low *trans*-margarine formulation.

Chemical interesterification method uses an inorganic catalyst and usually done with the oil under vacuum or a nitrogen blanket to prevent oxidative degradation (Zainal and Yusoff, 1999). Chemical interesterification is less expensive, however leads to random distribution of fatty acids on the triacylglycerol (Da Silva et al., 2010; Rousseau and Marangoni, 1998). This random distribution result on low possibility to achieve the desired fatty acid rearrangement. Besides, the process also usually carried out in high temperatures (above 100°C) where it can lead to deterioration in finished product (Zainal and Yusof, 1999).

Enzymatic interesterification differs as it employs the enzyme that poses regiospecificity as biocatalyst whereby the reaction can specifically incorporated desired fatty acids to the glycerol backbone. Through enzymatic interesterification, the reaction can be specifically known as exchange of acyl groups between ester and an acid (acidolysis), an ester and an alcohol (alcoholysis) or between two esters (transesterification) (Rodrigues and Fernandez-Lafuente, 2010). Oftenly, acidolysis reaction of saturated fatty acid (the one that contribute to high melting points and solid structure) is replaced with short chain fatty acid or unsaturated fatty acids and catalyze by lipase enzyme. The modification then altered the physicochemical properties of the lipid such as lower the melting point and increase in unsaturated fatty acids and short chain fatty acids.

Lipases often used as biocatalyst in enzymatic interesterification. Generally, in acidolysis reaction, lipases act as a biocatalyst to break down the triacylglycerol chain specifically at *sn*-1, 3 positions then promote desirable TAG rearrangement and favorable structured lipid can be formed. A study from Xu (2000) stated that specific-structured TAG of such kind which are produced by exploiting the *sn*-1,3 regiospecificity lipase are currently attracting more concern. However, either in batch reaction or continuous operation, a single lipase is generally employed as the biocatalyst and little attention has been given to dual or multiple lipases system to reveal their synergistic effects (Ibrahim et al., 2007). Ibrahim et al. (2007) proved that the co-immobilization action from the carrier of the immobilized lipase towards the free lipase was proposed to be one of the reasons leading to synergistic effect between the lipases in the esterification between palm stearin and coconut oil. Therefore, in this research, a dual lipase system of immobilized enzyme (TL IM) and non-immobilized lipase (lipase Amano AK) was believed to promote a synergistic effect and optimized the acidolysis of palm stearin and oleic acid. However, the synergistic effect may also depend on other factors such as lipase species mixture, their ratios and other parameters (Ibrahim et al., 2007). The synergistic effect of two used lipases can be measured by its degree of interesterification of the acidolysis reaction.

In this study, the acidolysis reaction by dual lipase system was enhanced by Response Surface Methodology (RSM). The optimization was used to measure the optimum parameters and to promote the reaction so that later on, a favorable value for corresponding responses can be obtained. These optimum parameters are not only assisting the industry to produce healthier fat stocks but also give them cost benefit for large scale production. On the other hand, determination of physical and chemical properties was performed on the purified modified palm stearin and being compared with palm stearin. This analysis was then used to indicate the value of modified palm stearin in producing edible plastic fats.

Based on the research overview, the problem statements are as follows;

1. Palm stearin has high melting point ranging from 44°C to 56°C. Thus it has low plasticity and incomplete melting at body temperature according to Pantzaris (2000).

2. Single lipase system often used in enzymatic interesterification and little attention has been put on dual lipase system.
3. Optimization of acidolysis reaction between palm stearin and oleic acid need to be done to measure the reaction optimum condition.

Resulting from the all above, thus, the objectives of the research were as follows;

1. To optimize the parameters of acidolysis reaction between palm stearin with oleic acid by response surface methodology (RSM).
2. To study the synergistic effect of two occurring lipases in acidolysis reaction of palm stearin with oleic acid.
3. To determine the physical and chemical properties of purified modified palm stearin.