CHAPTER I

INTRODUCTION

1.1 Motivations

World energy consumption has been continuously increasing for several decades until limitation of fossil fuel become a threat to human. Consequently, alternative fuels have been developed recently worldwide. Among alternative fuels, biomass is one of an interest since it is renewable and generates less pollution than fossil fuel, i.e. in case of biodiesel. According to Lin and Lin (2006), fat and oil from animal, vegetable, or waste cooking can be used to produce biodiesel. There are many plants that contain high amounts of oil and they can be used as an energy source; for example, coconuts, palms, rape seed, peanut and physic nuts (Bajpai and Tyagi, 2006). However, there is a controversial on food demand and crisis that might occur from competition of plant for food or for fuel. Therefore, plant that is non-edible like Jatropha or physic nut is purposed for being a solution.

Jatropha curcas or the physic nut is a plant in the Euphobiaceae family. It is a native plant of Central and South American countries (Heller, 1996; the Department of Alternative Energy Development and Efficiency of Thailand et al., 2006; Jongschaap et al., 2007). This crop has wide variations in the morphological characteristics in its stems, leaves, flowers, fruits, and seeds. It contains 30 – 40% of oil by weight in their seed. Four kilograms of seed can produce 1 liter of oil (Koedklai, 2007). This oil can be used directly or indirectly in engines. In addition, the seed meal is nutrient rich. There is crude protein about 26.0% (Makkar et al., 1997). The amount of crude protein that contents in *J. curcas* kernel is higher than that of soybean (Chivandi, Kachigunda and Fushai, 2005). For this reason, *J. curcas* kernels after extracted oil are expected to be a raw material for feedstock production. Unfortunately, most varieties of *J. curcas* seeds contain some toxic compounds. A significant toxic compound found in Jatropha seeds is phorbol esters (Jongschaap et al., 2007). According to the Division of Occupational Health and Safety, phorbol

esters are toxic and suspected carcinogens. They are easily absorbed into the body by the dermal route and the ingestion. The possible effects of contact with phorbol esters are the severe irritation of tissues (the skin, eyes, mucous membrane, and lungs) and induced sensitivity. Consequently, the oil extracted *J. curcas* kernel will be not safety to be as any kind of food, if the toxin has not been removed.

In Thailand and other countries, the conventional method to extract oil from *J. curcas* seed is compression (Division of Occupational Health and Safety, 2006). Nevertheless, this method cannot extract all the oil from the seed. In addition, some toxin is still found in extracted oil and residue meal. Consequently, several techniques have been developed. In this present work, surfactant aqueous-based solution is proposed for using as an extracting solvent to separate phorbol esters from *J. curcas* meals. This technique is based on the fact that by nature a surfactant has hydrophilic head and hydrophobic tail which make its molecule to be soluble in both aqueous and hydrophobic oil environment; hence, it is able to reduce interfacial tension between two phases. In addition, once micelle is form, hydrophobic compound can be trapped within the micelle. Thus it is believed that both oil and phorbol esters can be removed from residue meals after extraction (Lin and Wang, 2003; Lin and Lin, 2006; Lif and Holmberg, 2006; and Zhao et al., 2006).

This study focuses on *J. curcas* kernel meals after oil extraction from *J. curcas* seeds by using surfactant technology. Investigation on various parameters of extraction that yields less phorbol esters in *J. curcas* kernel meals will be carried out. Moreover, the phorbol esters extraction method for quantitative determination will be examined what extraction method will give the highest percent recovery.

1.2 Objectives

The main objective of this study was to evaluate the method that used surfactant solution for phorbol esters removal from the *Jatropha curcas* residual meals. The sub-objectives to support the main objectives were:

1. To develop the phorbol esters extraction method for quantitative determination from the *Jatropha curcas* kernels and meals.

2. To compare the amount of phorbol esters in *Jatropha curcas* meals from the different oil extraction method.
3. To determine the optimal condition for the phorbol esters extraction from *Jatropha curcas* pressed meals using surfactant aqueous-based solution.

1.3 Hypotheses

1. Sonication can enhance an efficiency of phorbol esters extraction from the *Jatropha curcas* kernels and meals by methanol.
2. Surfactant aqueous-based solution can be a solvent for phorbol esters extraction from the *Jatropha curcas* kernels and meals.

1.4 Working Scope

This study focuses on two parts; in the first part was to develop the sample preparation method for phorbol esters extraction, and the second part aimed to determine phorbol esters in the different *Jatropha curcas* kernels; raw kernel, defatted kernel by Soxhlet-hexane, pressed kernel and defatted kernel by surfactant solution. In addition, the systems of surfactant aqueous-based solution were evaluated for the removal of phorbol esters in the meals. Diagram of overall study is shown in Figure 1. The scope of this study is described below.

1.4.1 Develop method for phorbol esters extraction

The solvent generally used for phorbol esters extraction from the *Jatropha curcas* sample is methanol (Hass and Mittelbach, 2000). In this study, the methanol was selected for extraction by techniques of shaking-extraction and ultrasonic extraction. The shaking-extraction was performed in the orbital shaker at different rate to find the optimum shaking rate yielded the highest phorbol esters extraction efficiency. The ultrasonic extraction was performed by Transsonic analoguous ultrasonic cleaning unit series T700/H from Elma® that generated ultrasonic frequency at 35 kHz and 320 watt of ultrasonic peak output. The satisfied extraction method from this part was used for determination phorbol esters in part II.

1.4.2 Part II

1.4.2.1 *Jatropha curcas* meals

The concentration of phorbol esters in the Jatropha residual meals obtained from different oil extraction methods were determined and compared. The three oil extraction methods were Soxhlet-hexane extraction by hexane, surfactant solution extraction and mechanical pressing technique. For both Soxhlet-hexane and surfactant extraction meals were prepared in our laboratory by using the Jatropha seeds obtained from PTT Chemical Public Company Limited. The pressed residual meals were also provided by PTT Chemical Public Company Limited.

1.4.2.2 Phorbol esters extraction by surfactant solution

Nonionic surfactants used in this study were fatty alcohol C12 – 14 ethoxylate group and polyoxyethylene (20) sorbitan group (Tween), and anionic surfactant was AOT. Various systems prepared by these surfactants were tested to find a suitable surfactant composition for phorbol esters extraction from the meals. The parameters selected for evaluation the optimum condition were surfactant concentration, contact time and solid:liquid ratio.

1.4.2.3 Phorbol esters determination

Analysis of phorbol esters was performed by HPLC. The concentration of phorbol esters was determined according to the standard 12-o-tetradecanoyl-phorbol-13-acetate or TPA.

1.4.2.4 Quality of the Jatropha meal

After phorbol esters was removed, in order to examine the quality of the meal, crude protein content was determined in order to compare whether extraction method affected the nutrition value of the meal.

1.5 Expected Results

1. Obtain high recovery of phorbol esters extraction method from *Jatropha curcas* kernels that can be used for phorbol esters determination in biological sample.

2. Obtain Jatropha seed meals containing very low phorbol esters concentration (less toxicity) which will be more suitable for other application i.e. animal feed stock.

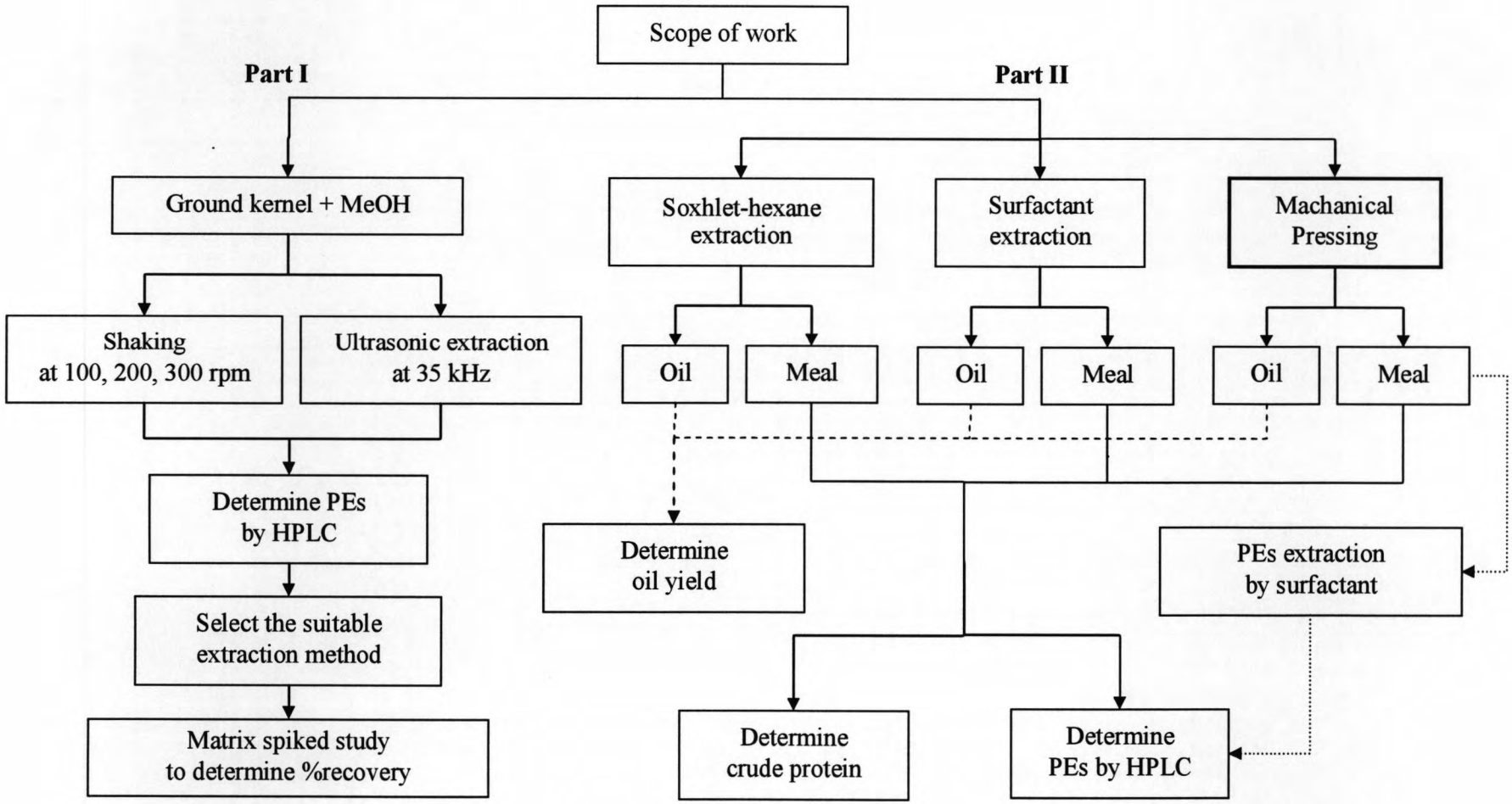

Figure 1 Flow chart of the scope of this study