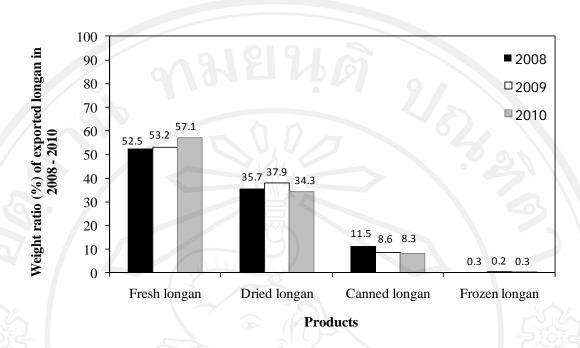
#### **CHAPTER 1**

### INTRODUCTION

## 1.1 Importance and significance of the problems

Gasoline fuels has long been considered as significant driving force to the economic advancement of Thailand, especially, in the transportation sector which consumes a large quality of fuels. The present energy situation of country since 2006 has indicated the rising and continuous demands of fuels while relying on the varying foreign supply of crude oil (KAPI, 2006; EXIM BANK, 2006). The leaded to the energy crisis within Thailand. The Royal Thai Government urgently needs to develop the alternative energy source which can be produced within the country to minimize importation (KAPI, 2006). Recently, a number of countries have shown interests in the ethanol production research which can be used as a substitutive energy source from natural raw materials (Sin, 1999). Cellulose is the major composition of these natural materials. It is the organic compound belongs to carbohydrate group which is generally found in plant cells. Cellulose is formed by the small units of glucose ( $\beta$ -D-glucose) linking with  $\beta$ -1, 4 linkage bond in a long chain. Cellulose degradation results in glucose which can be used in the fermentation process to produce ethanol (Premjit *et al.*, 2007; Szczodrak, 1998).

Longan is the important economic fruit of Thailand which is frequently planted in the north. In each year, a number of longan products are released to the market as evident in Figure 1.1. The highest exportation volume of longan was in the form of fresh longan which was then followed by dried longan, canned longan and frozen longan, respectively. The biggest export market was China (DOA, 2008; BAAC, 2008). Poapongsakorn *et al.* (2002) reported to Food and Agriculture Organization (FAO) of the United Nation (UN) that Thailand was one of the biggest dried longan exporters after China and Viet Nam (OAE, 2009).



**Figure 1.1** Weight ratio (%) of exported longan comparing with fresh longan weight in 2008 – 2010 (OAE, 2011)

**Note:** Conversion rate of fresh longan weight (kg):dried longan weight (kg) as 3.3:1

The reason that fresh longan had to be processed was its short shelf life (Choo, 2000). The government had thus categorized this fruit in the goods categories which required processing steps with the annual exportation value of no less than 5 billion Baht (OAE, 2009; DOA, 2008).

However, the problems of substandard quality such as exportation of immature longan or dried longan which was covered in molds had led to the low confidence of Thai longan products from foreign cotraders (Boonmak *et al.*, 2005). The illegal mixing of 2003 – 2004 dried longan which contamination of insects and molds to the new batch (2008) of fresh dried longan had led to the reputation damage of Thailand in foreign dried longan market. A number of dried longan ordering had stopped and led to the plummeted prices. Some even proposed the destruction of expired dried longan from the stock (NPPDO, 2009) or converted it to the renewable biomass. The latter was the final resolution in which the Ministerial Cabinet had

agreed upon. The mentioned project was handled by Ministry of Science and Technology and completed in May, 2010 (RYT9, 2010).

The principle composition of fresh and dried longan flesh was carbohydrate (Angasit *et al.*, 1999) which was the combination of 3 main sugars, namely, sucrose, glucose and fructose (Panyathep, 2005). These sugars were used as carbon source to support the microbial growth (Pratong *et al.*, 2007). The microbes were used to eliminate low quality dried longan by converting into high value products instead of wastage disposal. One common example was high level ethanol production from expired dried longan which was mixed with molasses prior to fermentation by yeast *Candida utilis* and *Saccharomyces cerevisiae* (Kumtip *et al.*, 2009). Low quality dried longan still possessed high level of sugars that could be used in ethanol or butanol production. Boiling extraction of dried longan would result in sugars which could be used for fermentation to achieve alcohol and left over dried longan flesh. The latter must be utilized for useful proposes to achieve zero waste process as shown in Figure 1.2.

One way to solve the problems would be emphasized on sugars extraction from dried longan as carbon source for microbial growth with the potential of production ethanol. In addition, the remnant microbial biomass also contained pyruvate decarboxylase (PDC) enzyme which could be utilized for the biotransformation of benzaldehyde and pyruvate to valuable chemicals such as phenylacetylcarbinol (PAC) in a well – known Knoll procedure (Hildebrandt and Klavehn, 1934; Hildebrandt and Klavehn, 1932). Chemicals methods would follow to convert phenylacetylcarbinol into high value pharmaceutical compound – ephedrine which could be used to relieve asthma symptom. This was achieved by amination process (CH<sub>3</sub>NH<sub>2</sub> -) that was catalyzed by Platinum (Pt). Conversion to pseudoephedrine – a nasal decongestant – was also possible by acetic anhydride, hydrochloric acid (Ac<sub>2</sub>O/HCl) and water (Rosche *et al.*, 2002) as shown in Figure 1.3. The selling prices of ephedrine hydrochloride and pseudoephedrine hydrochloride in Thailand were 4,450 and 2,000 Baht per kg, respectively (FDA Thailand, 2007).

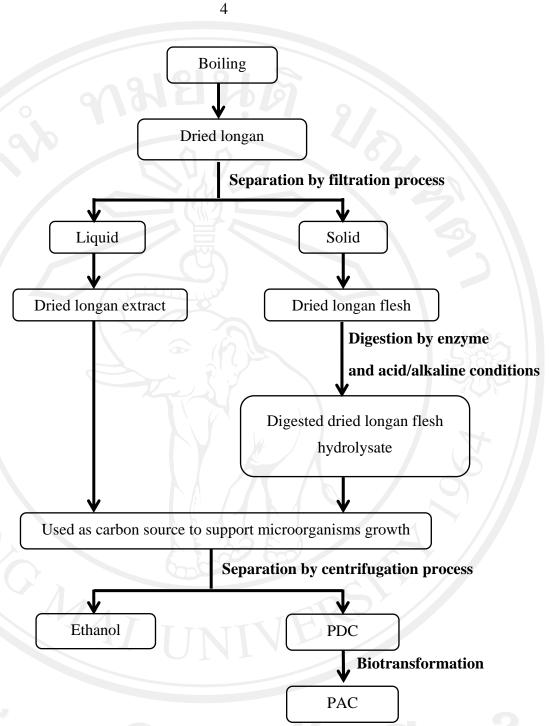


Figure 1.2 Summary of research direction to fully utilize dried longan in ethanol and PAC production

### **BIOTRANSFORMATION:**

# CHEMICAL SYNTHESIS:

$$\begin{array}{c} \text{OH} \\ \text{OH} \\ \text{CH}_3 \\ \text{OH} \\ \text{CH}_3 \\ \text{IPt]} \\ \text{H}_2\text{N-CH}_3 \\ \end{array} \begin{array}{c} \text{OH} \\ \text{CH}_3 \\ \text{NHCH}_3 \\ \end{array} \begin{array}{c} \text{1) Ac}_2\text{O/HCl} \\ \text{2) H}_2\text{O} \\ \end{array} \begin{array}{c} \text{CH}_3 \\ \text{NHCH}_3 \\ \text{2) H}_2\text{O} \\ \end{array} \begin{array}{c} \text{CH}_3 \\ \text{NHCH}_3 \\ \text{CH}_3 \\ \text{NHCH}_3 \\ \end{array} \\ \begin{array}{c} \text{CH}_3 \\ \text{NHCH}_3 \\ \text{CH}_3 \\ \text$$

Figure 1.3 Biotransformation of benzaldehyde and pyruvate into phenylacetylcarbinol (Leksawasdi, 2004)

## 1.2 Objectives

- 1.2.1 To determine the appropriate conditions for digesting post extracted dried longan flesh with low sugar level so that the higher level of sugars content can be achieved and utilize as an alternative carbon source for the microorganism with ethanol producing capability.
- 1.2.2 To compare the growth kinetics of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400, as well as UNSW 709700 using dried longan extract and dried longan flesh residue digested with the appropriate condition from 1.2.1 as the carbon source.
  - 1.2.3 To compare PAC production levels from whole cells obtained from three microbial strains in 1.2.2 using the two phase emulsion system.

## 1.3 Experted benefits

- 1.3.1 Obtain the appropriate digestion condition for low sugared dried longan flesh residue in order to achieve higher sugar level which can be used as carbon source for the ethanol production microorganisms.
- 1.3.2 Obtain growth kinetics of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400, as well as UNSW 709700 using dried longan extract and dried longan flesh residue extract digested by the appropriate conditions obtained from 1.3.1 as the carbon source.
- 1.3.3 Obtain the comparison information of PAC production levels between whole cells from three microbial strains from 1.3.2 in the emulsion two phase system.

## 1.4 Scope of the study

The carbon sources used for the microorganism cultivation were dried longan extract and dried longan flesh residue extract which were cv. Daw in the area of Chiang Mai and Lamphun provinces.