CHAPTER II

LITERATURE SURVEY

In this project, we are interested in studying the transesterification of menthol and an ester by enzyme lipases in non-conventional media. Therefore, this chapter will include a literature survey of similar types of reaction in systems investigated by various research groups in views of parameters affecting biocatalytic yield and/or activity.

2.1 Effects of type of lipases and acyl donors

Langrand et al., 1988 reviewed the effects of types of lipases on transesterification reactions as shown in table 2.1.

Table 2.1 Ester formation by transesterification reaction of isoamyl alcohol with two types of acyl donors

Lipase	%conversion after 24 hours	
	ethyl acetate	ethyl butyrate
Candida rugosa	15	71
Porcine pancreas	3.5	21

Ester syntheses were carried out in 10 ml of n-heptane. The mixture was incubated at 37 °C for 24 hours.

Kamiya et al., 1995 focused on the resolution of (±)menthol by lipases in organic media. The reaction of this work was an esterification of 5 mM (-)menthol and 10 mM lauric acid by lipase in 10 ml of iso-octane at 35 °C. The enzyme content was 0.1 g/l in all lipases. Six kinds of lipases selected for the study were from *Candida cylindracea*, *Rhizopus sp.*, *Pseudomonas sp.*, *Mucor javanicus*, *Aspergillus niger*, and Porcine pancreas. They found that the lipase from *Candida cylindracea* gave the highest percent conversion of 19.

Five lipases were also tested for their ability to synthesize terpene esters. The reactants used for transesterification were terpene alcohol (geraniol & citronellol) and tributyrin, and the results are shown in table 2.2 (Yee et al., 1995). It was found that *Candida rugosa* gave the highest yield for geraniol substrate, and second highest for citronellol. The reaction was carried out with tributyrin 0.03 mole in 2 ml n-hexane at 30 °C and 200 rpm stirring speed for 24 hours.

Table 2.2 Transesterification of terpene alcohol (geraniol and citronellol) with tributyrin

Lipase	% yield	
	Geraniol	Citronellol
Pseudomonas sp.	15.2	27.5
Candida rugosa	96.2	28.2
Penicillium cyclopium	36.1	31.6
Candida lypotica	37.8	0.3
Rhizopus niveus	26.2	0.5

Claon and Akoh., 1994 studied the synthesis of esters by transesterifications in an organic solvent. Geraniol was used to react with methyl acetate by five kinds of lipases. The reaction was carried out with geraniol 0.1 M and methyl acetate 0.03 M in 2 ml hexane at 30 °C and 200 rpm for 24 hours. Lipase from *Candida artarctica* gave the highest conversion of 33.44% while those from *Mucor miehei*, *Aspergillus oryzae*, *Candida cylindracea*, and *Pseudomonas sp.* were 20.61, 6.90, 5.22, and 3.89%, respectively.

Langrand et al., 1990 investigated ester syntheses in an organic solvent from 35 combinations of short chain acids and alcohols. They included acetic, propionic, butyric, valeric, and caproic acids, as well as methanol, ethanol, butanol, iso-pentanol, hexanol, citronellol, and geraniol by four kinds of lipases. They found that lipase from *Aspergillus sp.* was more active with short chain acids and alcohol. Lipase from *Mucor miehei* and

Rhizopus arrhizus were more active with long chain acids, while lipase from *Candida rugosa* was more active with propionic and butyric acids as well as butanol, hexanol, and iso-pentanol.

Kamiya et al., 1995, studied the esterification of menthol and fatty acid using lipase from *Candida cylindracea* as a catalyst with varying carbon chain length of acids from C2 to C18. The result showed that the enzyme was more active with long chain fatty acids than with the short ones. Esterifications of different alcohols and carboxylic acids were also studied by Langrand et al., 1990, using with four types of lipases. They disclosed that lipase from *Rhizopus arrhizus* and *Mucor miehei* preferred to react with high carbon chain lengths of acids. In contrast, lipases from *Aspergillus niger* and *Candida rugosa* were active only with carboxylic acid with C3 and C4 (by selection five carboxylic acid which are acetic, propionic, butyric, valeric, and caproic acid).

Claon and Akoh., 1994, studied transesterification of geraniol with five esters in organic solvent. Lipase from *Candida cylindracea* was employed as the catalyst, and showed that the effect of ester on the reaction were the following : isopropenyl acetate showed the highest conversion of 47.31%. The other three groups (methyl acetate, isoamyl acetate, and triacetin) were around 5% conversions, while ethyl acetate groups gave only 0.5% conversion. Similar to the previous work, Yee et al., 1995, studied transesterification with geraniol as one of the substrates. The lipase and ester groups in this study were from *Candida rugosa* and triacyl glycerols : triacetin, tributyrin, tricaproin, and tricaprylin, respectively. From the chain length it indicated that tributyrin gave 96.2% yield, while tricaprylin, triacetin, and tricaproin gave only 29.9%, 22.1%, and 17.6% yield, respectively.

Langrand et al, 1988, studied the two lipases *Candida rugosa* and Porcine pancreas for transesterifications of two alcohols : geraniol and isoamyl alcohol and two esters : ethyl acetate and ethyl butyrate, and found that the two alcohols were 4-fold more active with ethyl butyrate than ethyl acetate.

The results from Lokotsch et al., 1989, for interesterifications of (-)menthol with four different triacyl glycerol groups with the lipase from *Candida cylindracea* are shown in table 2.3.

Table 2.3 Interesterification of (-)menthol with various acyl donors (triacyl glycerol) after 20 hours

Acyl donors	Specific activity (μmol/g cat-hr)
Triacetin	24.2
Tributyrin	26.1
Trilaurin	28.7
Trilinolein	30.4

It could, therefore, be concluded that a particular type of lipase is specific to certain types, and chainlengths of substrates. Thus, a suitable type of lipase is highly dependent on a reaction of interest.

2.2 Effect of reaction conditions

2.2.1 Effect of temperature

Lokotsch et al., 1989, showed the effect of temperature on hydrolysis, esterification, and interesterification using lipase from *Candida cylindracea* as illustrated in figure 2.1 which led to conclusion that even with an identical type of enzyme, optimum temperature varied with types of reactions.

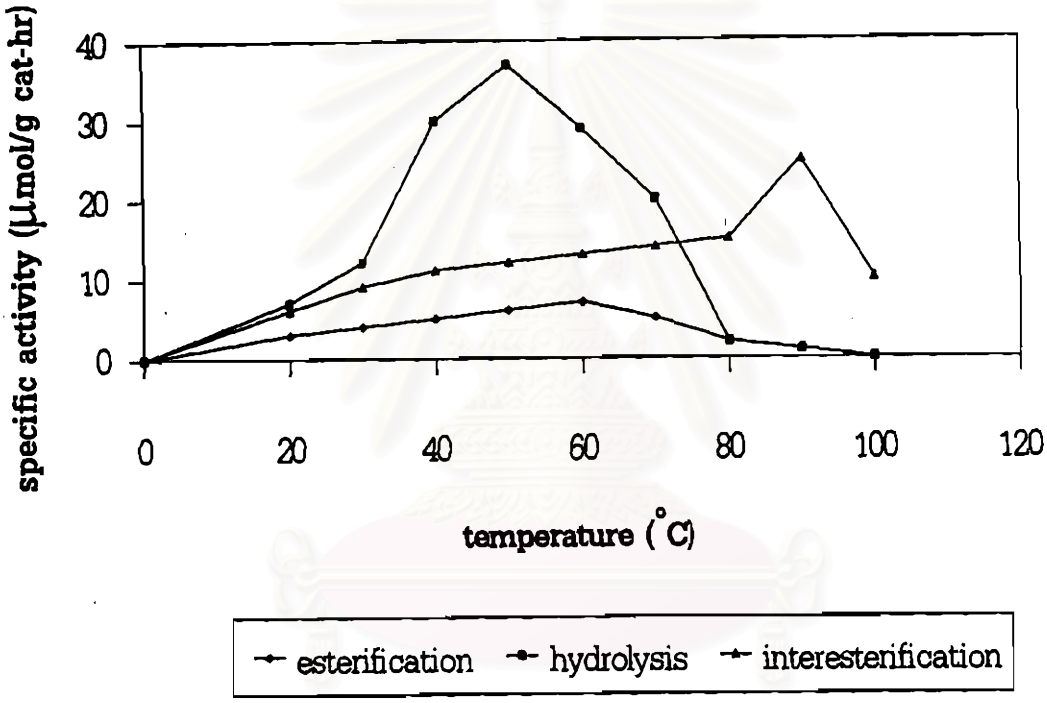

Figure 2.1 Effect of temperature on the activity of lipase from *Candida cylindracea* lipase for the following reactions :

- hydrolysis of (±)menthol
- esterification of (-)menthol and acetic acid
- interesterification of (-)menthol and triacetin

Goto et al., 1994 also studied the suitable temperature of esterification by lipase from *Pseudomonas sp.*. Maximum initial rate was obtained in the temperature range of 20-30 °C using iso-octane as a solvent. Kamiya et al., 1995, indicated that the suitable temperature for esterification by coated-lipase from *Candida cylindracea* was 35 °C.

2.2.2 Effect of solvents

Kamiya et al., 1995, studied the effect of organic solvents on initial esterification rates of (-)menthol and lauric acid using lipase from *Candida cylindracea* as the catalyst. This effect is shown in figure 2.2 which led to a conclusion that activity of the reaction varied with type of organic solvent. It is noticed that higher log P (iso-octane of 4.5) solvent gave higher activity (log P equal 4.0 and 3.5 for heptane and n-hexane, respectively). While cyclohexane (log P 3.2) was a better solvent than heptane and n-hexane even with lower log P value, this might be due to the difference in molecular structure of cyclohexane from others.

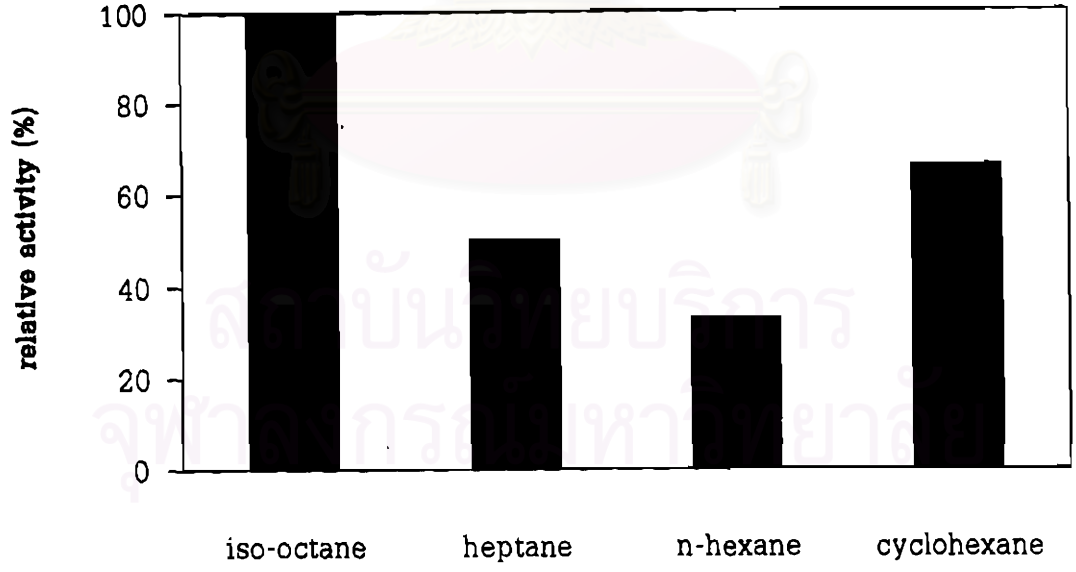

Figure 2.2 Relative activity of esterifications of (-)menthol and lauric acid with various organic solvents

The work by Goto et al., 1994, used coated lipase from *Pseudomonas sp.* as the catalyst for esterification of benzyl alcohol and lauric acid. The effects of ten different organic solvents are shown in figure 2.3. These results, again, could lead to the conclusion that the more hydrophobic the solvent the higher the activity found.

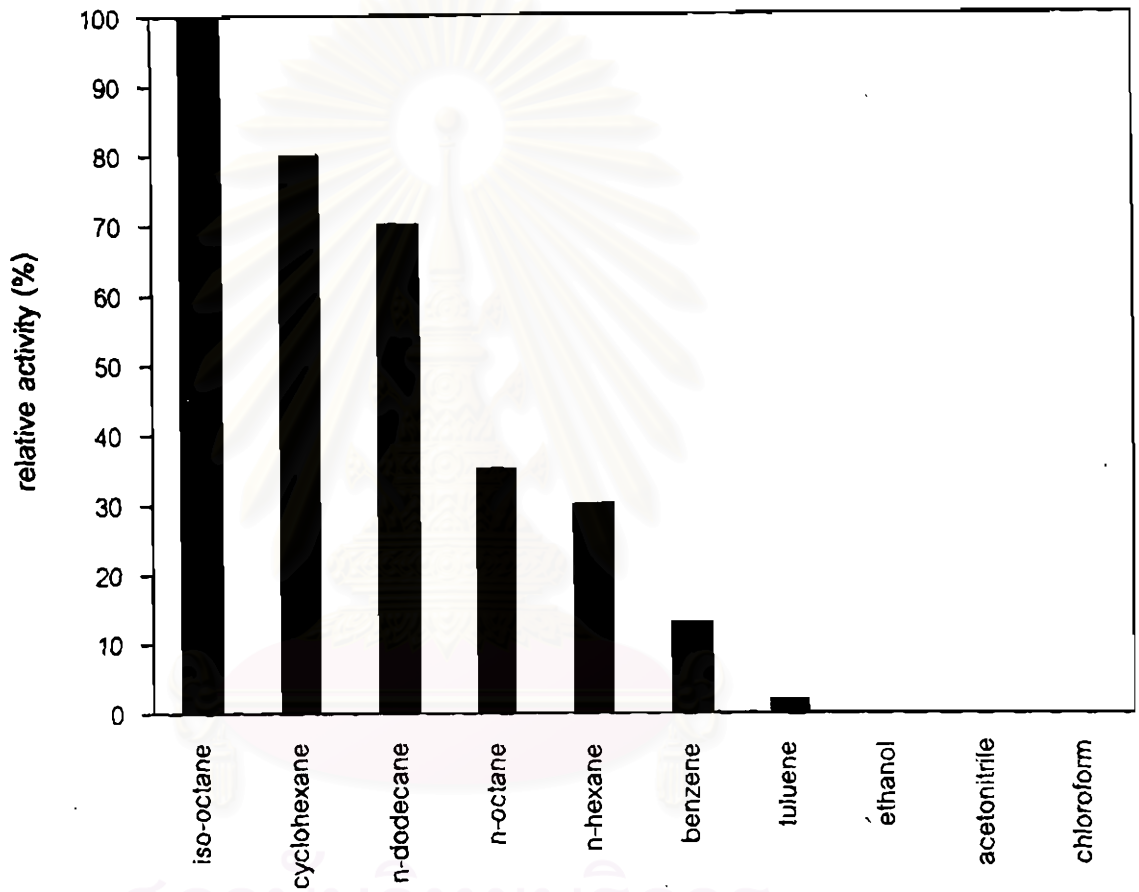

Figure 2.3 Relative activity of the esterifications with ten kinds of organic solvents

Goto et al., 1995, used coated-lipase similar to the previous work for the interesterification of tripalmitin with oleic acid using lipase from *Mucor javanicus* to study the effects of four organic solvents which were iso-octane, n-hexane, toluene, and

chloroform on percent conversion. The iso-octane and n-hexane presented 5-fold percent conversion to that from toluene whereas chloroform almost had no reaction compared to the other organic solvents.