CHAPTER VII

SUMMARY AND RECOMMENDATIONS

7.1 Summary

Synthesis of ferulate esters in this study was accomplished by using ethyl ferulate as acyl donor and olive oil as acyl acceptor in an organic solvent system. The improvement technique by employing two different specificities of lipases of Novozym 435 and Lipozyme RM IM concurrently as biocatalyst demonstrated a very simple and extremely effective method. Furthermore, this dual lipases system offers environmentally friendly process, milder reaction conditions and safer than chemically-catalysed route. In addition, the larger portion of Lipozyme RM IM compare to the Novozym 435 in an optimum ratio provided positive reduction for the total biocatalyst costs.

Four factors influencing the lipase-catalysed synthesis of ferulate esters were successfully inspected based on the preliminary experiments using a conventional one-variable-at-a-time approach. They were: reaction temperature, incubation time, lipase dosage and mass ratio substrates. Further optimization was applied by statistical approach of RSM to study the interactive effect between those factors in enhancing the percentage conversion of ferulate esters. The correlation between the factors was explained using a mathematical model and a number of set of optimal conditions with more than 90 % conversion were projected from the study. Comparison between predicted and experimental values revealed good correspondence between them demonstrated the validation of the model generated.

The biological activity of the synthesized ferulate esters were effectively investigated using four types of antimicrobial tests, they were: agar well diffusion, minimum inhibitory concentration through MTT assay and spectrophotometric method and also minimum bactericidal concentration. The outcomes suggested that synthesized ferulate esters not totally depend on type of bacteria, either Gram-positive or Gram-negative, but also depend on concentration of the sample, microenvironment of each bacterium and development of resistance (Cloete, 2003). The overall elevated percent inhibition of bacteria towards synthesized ferulate esters can be easily illustrated as follow: *Bacillus subtilis = Klabsiella pneumonide < Escherichia coli < Salmonella typhimurium < Staphylococcus aureus < Staphylococcus epidermidis*.

Additionally, some of the physical features and chemical components of synthesized ferulate esters were well examined through SPF value, peroxide value, saponification value and iodine value. As expected, combination between ethyl ferulate and olive oil exhibited potent characteristics which were believed to develop a multifunctional range of ingredients for pharmaceutical, food and particularly cosmetic industries.

7.2 Recommendations for Further Studies

Findings in this work are directive towards several ideas which needed further investigation, some of which are listed below:

1. Quantification analysis using high-performance liquid chromatography (HPLC) for better visualization of the ferulate esters species synthesized (Yang et al., 2012).
2. Preliminary study on scale-up study to pilot scale via appropriate bioreactor design.
3. Development of a new experimental design to remove excess water and ethanol generated through the process in enhancing products recovery (Sun et al., 2014).
4. Application of this dual lipases system in catalyzing more complex reactions or synthesizing other esters.
5. Exploration of other characteristics of the products to be used as novel antioxidant agent in diverse field such as ABTS (2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)), DPPH (2, 2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power) and ORAC (oxygen radical absorption capacity) assays (Thaipong et al., 2006).
6. Substitute olive oil with abundant low-cost local oil such as coconut oil and palm oil.
7. Molecular modelling simulation studies might likely offer useful suggestions to rationalize the results obtained.