STUDIES ON LIPASE-CATALYZED TRANSESTERIFICATION OF TRIGLYCERIDES

by

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Thesis submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the

Indian Institute of Technology Delhi

December, 2006

Dedicated

To

my Father

CERTIFICATE

This is to certify that the thesis entitled "**Studies on Lipase catalyzed Transesterification of Triglycerides**" being submitted by **Mili Prabhakar** to the Indian Institute of Technology, Delhi, for the award of degree of Doctor of Philosophy, has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research report and the results presented in this thesis have not been submitted to any other university or Institute for the award of any other degree or diploma.

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Mr. Bhagwan Singh and Mr. Rana. I am also thankful to Rajkumar, Pitamber bhaiya, Dalveer, Lucky, Sanjay and Suresh bhaiya for their ever- ready help.

I am also thankful to Mr. D. C. Sharma, Textile Engineering Department for the development of SEM images.

Lastly, I take this opportunity to express my deepest gratitude to my parents, brother, parent -in – laws and my husband. My parents and brother always encouraged me to pursue what I wanted. They have given me the strength and confidence to overcome difficulties and accomplish the objective in life. The dreams of my father would have never been fulfilled if I would have not got such a supportive and loving husband. His utmost caring attitude and encouragement helped me to work with great zeal and overcome all the difficulties patiently. Special word of thanks to my parent – in – laws for their affection, constant encouragement and moral support that has helped me in successful completion of the work and writing of the thesis patiently.

Finally and above all, I would like to thank almighty god by whose grace I was able to achieve my goal.

Mili Prabhakar

ABSTRACT

Lipases are a versatile biocatalyst occupying predominant position in the industry. They are capable of catalyzing a range of reactions in non-aqueous environment yielding a variety of products. In recent years, there has been an emphasis on products based on biochemical routes due to stereoselectivity, better product quality and higher degree of purity. The use of lipases in the transesterification of triglycerides is becoming increasingly recognized. Synthesis of products like MLM type structured lipids find application as "nutraceuticals" or "pharmalipids". Monoglycerides particularly of unsaturated fatty acids can be useful in the production of low-fat margarines and for formulating nutraceutical preparations.

In the present studies, MLM type structured lipid was enzymatically synthesized from triolein and caprylic acid. The effect of different parameters-selection of enzyme, bulk reaction environment, relative concentration of the co-substrates, water content, temperature, enzyme concentration and chain length of the acyl donor was found to enhance the synthesis of MLM type lipid. Kinetic studies on acidolysis of triolein with caprylic acid revealed the reaction to follow a pseudo first order reaction kinetics. Mass transfer studies in a packed bed reactor under total recycle demonstrates that the process was external film diffusion controlled and required a minimum linear recycling velocity of 3.54×10^{-5} m/s. The optimized process conditions for triolein-caprylic acid system were further applied to modify apricot kernel (*Prunus armeniaca*) oil that yielded a MLM type lipid containing 66.34% caprylic acid at *sn*-1, 3 position and unsaturated fatty acids at *sn*-2 position.

The synthesis of the other product- monoglycerides was carried out using triolein and glycerol as the substrates. *Humicola lanuginosa* lipase was found to

exhibit alcoholysis activity. Further improvement of the alcoholysis activity was done using two approaches and it was found that inclusion of salts prior to lyophilization to be a good method to increase the alcoholysis activity of Humicola lipase as compared to treatment with PEG. The lyophilized Humicola preparation with 98% KCl content exhibited about 19-fold increase in the specific alcoholysis activity compared to original preparation. The effect of different parameters- bulk environment, relative concentration of co-substrates, water content, enzyme concentration and temperature were found to affect the production of monoglyceride (monoolein). The higher mole ratio of triolein to glycerol favours the formation of monoolein as compared to diolein. The repeated use of the modified lipase was demonstrated through recovering monoolein by cooling the reaction mixture to 10°C and recycling the unreacted mixture with additional triolein and glycerol for a fresh batch.

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