UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF LIPASE CATALYSED SYNTHESIS OF SUGAR ALCOHOL ESTERS USING TAGUCHI METHOD AND NEURAL NETWORK ANALYSIS

SEYEDEH ATENA ADNANI

FS 2011 13

OPTIMIZATION OF LIPASE CATALYSED SYNTHESIS OF SUGAR ALCOHOL ESTERS USING TAGUCHI METHOD AND NEURAL NETWORK ANALYSIS

By

SEYEDEH ATENA ADNANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

March 2011

This thesis is dedicated to my parents, my husband and my dear brother for their love, endless support and encouragement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

OPTIMIZATION OF LIPASE CATALYSED SYNTHESIS OF SUGAR ALCOHOL ESTERS USING TAGUCHI METHOD AND NEURAL NETWORK ANALYSIS

By

SEYEDEH ATENA ADNANI

March 2011

Chairman: Professor Mahiran Basri, PhD

Faculty: Science

Xylitol esters were successfully prepared from reactions of xylitol with free fatty acids (stearic acid, palmitic acid and capric acid), using enzymes as biocatalyst in an organic solvent system. Such a selective enzymatic route obviates the fastidious regime of substrate activation required in chemical synthesis. Preliminary detection and identification of reaction products were facilitated by thin layer chromatography (TLC). Subsequent quantitative studies on main effects of parameters governing the reactions based on conversion of fatty acid were conducted by titration analysis. Enzyme screening showed Novozym 435 to be the most proficient biocatalyst for the reactions. By changing one parameter at a time, the optimum condition was found to be at 60°C and 1:1 molar ratio with 4 g molecular sieve and 0.12 g enzyme in 30 ml solvent in 18 h reaction time. The synthetic reaction was

optimized by Taguchi method based on orthogonal array to evaluate the effect of each parameters and interactive effects of reaction parameters including temperature, time, amount of enzyme, amount of molecular sieve, amount of solvent, and molar ratio of substrates (xylitol: fatty acid). Optimal condition derived from Taguchi method showed that, the method couldreduce reaction time, molecular sieve amount and enzyme amount with high conversion (96%, 92%, 88% for xylitol stearate, xylitol palmitate, and xylitol caprate, respectively) compared to conventional one variable at a time approach.

Artificial Neural Network (ANN) method was also employed for the estimation of esterification yield in enzymatic synthesis of xylitol esters. Various feedforward neural networks were performed using different learning algorithms. The best algorithm was found to be Levenberg–Marquardt (LM) for a network composed of two hidden layers with six and seven neurons in the first and second layers, respectively for xylitol stearate and xylitol palmitate and also seven and five neurons in the first and second layers for xylitol caprate, with hyperbolic tangent sigmoid transfer function. Both models suggested good quality predictions for all independent variables (reaction time, temperature, amount of enzyme, substrate molar ratio, amount of molecular sieve and amount of solvent) in terms of the percentage conversion of xylitol esters. A high coefficient of determination (R^2) (>0.9) and a low mean squared error (MSE) for training and testing data implied the good generalization of the developed models for predicting the reaction conversion.

In order to make the synthesis process more environmentally friendly, the reactions between xylitol and capric acid, caprylic acid, and caproic acid, were also performed in solvent-free system. In this system,similar insolvent system, three methods including one variable at a time, Taguchi method and ANN were used for optimization and prediction of percentage of conversion. By varying one parameter at a time, the suitable temperature was 60°C and reaction time was 29 h (for xylitol caprate and xylitolcaprylate), 18 h (xylitol caproate) with 0.2 g enzyme, 0.14 g molecular sieve (for xylitol caprate and xylitol caprylate) and 0.2 g (for xylitol caproate), molar ratio 0.5 (xylitol: capric acid and caproic acid) and 0.33 (xylitol: caprylic acid). By using Taguchi method and ANN, the actual conversions of esters achieved were 74% (xylitol caprate), 69% (xylitol caprylate), and 60.5% (xylitol caproate) with a small amount of enzyme and molecular sieve, which matched well with the predicted values and indicated good conversion and effectiveness of the reactions without adding organic solvent for synthesis of xylitol esters.

A comparison between one variable at a time, Taguchi method and artificial neural network shows that both Taguchi and ANN can reduce the amount of enzyme, amount of molecular sieve, reaction time and molar ratio in solvent based and solvent free system. So,this optimization method can help to save time and cost of the process. In addition comparison of statistical measures and performances between Taguchi method and ANN shows that ANN was slightly better than Taguchi for data fitting and estimation capabilities.

Analysis of product was carried out by Fourier transform-infrared spectroscopy (FT-IR), Gas chromatography-Mass spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR). Some physicochemical properties were also analyzed. The overall results on the physicochemical studies showed that xylitol ester exhibited good physicochemical characteristics. The analyses revealed the suitability of the ester produced in industrial application.

Abstrak ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGOPTIMUMAN SINTESIS ESTER ALKOHOL GULA BERMANGKINKAN LIPASE MENGGUNAKAN KAEDAH TAGUCHI DAN RANGKAIAN SARAF ANALISIS

Oleh

SEYEDEH ATENA ADNANI

Mac 2011

Pengerusi: Profesor Mahiran Basri, PhD

Fakulti: Sains

Ester xylitol telah berjaya disediakan daripada tindakbalas antara xylitol dan asid lemak bebas(asid stearikasid palmitik danacid kaprik), dengan menggunakan enzim sebagai biomangkin dalam sistem pelarut organik.Laluan berenzim terpilih,memudahkan pengaktifan substrat yang diperlukan dalam sintesis kimia.Pengesanan awal dan pengenalan produk tindakbalas tersebut dilakukan dengan kromatografi lapisan tipis (KLT). Kajian kuantitatif seterusnya pada kesan utama parameter yang mengatur tindakbalas berdasarkan penukaran asid lemak telah dilakukan dengan analisis pentitratan. Saringan enzim menunjukkan Novozym 435 menjadi biomangkin yang paling efektif. Dengan menukar satu parameter pada satu masa, keadaan optimum adalah pada 60°C dan nisbah molar 1:1 dengan 4 g penapis molekul dan 0.12 g enzim dalam 30 ml pelarut untuk masa reaksi selama 18 jam. Tindakbalas sintetik telah dioptimakan dengan kaedah Taguchi berdasarkan susunan ortogonal untuk menilai pengaruh setiap

parameter dan kesan interaktif parameter termasuk suhu, masa, jumlah enzim, jumlah penapis molekul, jumlah pelarut, dan nisbah molar substrat (xylitol:asid lemak). Keadaan optimum yang diterbitkan dari kaedah Taguchi menunjukkan bahawa, kaedah itu boleh mengurangkan masa tindakbalas, jumlah penapis molekul dan jumlah enzim dengan hasil yang tinggi (96%, 92%, 88% untuk xylitol stearat, xylitol palmitat, dan xylitol kaprat, masing-masing) berbanding dengan kaedah konvensional satu pembolehubah pada satu masa.

Kaedah Rangkaian Saraf tiruan (RSB) juga digunakan untuk andaian hasil pengesteran dalam sintesis berenzim ester xylitol. Pelbagai rangkaian saraf 'feedforward' dilakukan dengan menggunakan algoritma yang berbeza. Algoritma yang terbaik adalah Levenberg-Marquardt (LM) untuk satu rangkaian terdiri daripada dua lapisan tersembunyi dimana enam dan tujuh neuron di lapisan pertama dan kedua, bagi xylitol stearat dan xylitol palmitat dan juga tujuh dan lima neuron di lapisan pertama dan kedua untuk xylitol kaprat, dengan fungsi pemindahan sigmoid tangen hiperbolik. Kedua-dua model ini menyarankan ramalan berkualiti untuk semua pembolehubah bebas (masa reaksi, suhu, jumlah enzim, nisbah molar substrat, jumlah penapisan molekul dan jumlah pelarut) dalam peratusan penukaran ester xylitol. Satu pekali penentuan yang tinggi (R^2) (> 0.9) dan satu purata kuasa dua (MSE) rendah untuk data latihan dan ujian memberikan generalisasi yang baik dari model yang dibangunkan untuk meramalkan tindakbalas penukaran.

Bagi menjadikan proses sintesis itu lebih mesra persekitaran, tindakbalas antara xylitol dan asid kaprik, asid kaprilik, dan asid kaproik telah dilakukan dalam sistem bebas pelarut. Dalam sistem ini, serupa dalam sistem pelarut, tiga kaedah termasuk satu pembolehubah pada satu masa, kaedah Taguchi dan RSB digunakan untuk pengoptimuman dan ramalan peratusan penukaran. Dengan mempelbagaikan satu parameter pada masa, suhu yang sesuai ialah 60°C dan masa tindak balas 29 jam (bagi xylitol kaprat dan xylitol kaprilat), 18 jam (xylitol kaproat) dengan enzim 0.2 g, 0.14 g penapis molekul (untuk xylitol kaprat dan xylitol kaprilat) dan 0.2 g (untuk xylitol kaproat), nisbah molar 0.5 (xylitol:asid kaprik dan asid kaproik) dan 0.33 (xylitol:asid kaprilik). Dengan menggunakan kaedah Taguchi dan RSB, penukaran sebenarnya semua ester adalah 74% (xylitol kaprat), 69% (xylitol kaprilat), dan 60.5% (xylitol kaproat) dengan jumlah minimum kuantiti enzim dan penapis molekul, di mana didapati setanding dengan nilai ramalan dan menunjukkan penukaran yang baik dan keberkesanan tindakbalas tanpa menambah pelarut organik untuk sintesis ester xylitol.

Perbandingan di antara satu pembolehubah pado satu masa kaeda Taguchi dan RSB menunjukkan bahawa kedua-dua kaedah Taguchi dan RSB boleh mengurangkan juumlah enzim dan penapis molekul dan masa tindakabalas dan nibah molar, di dalam pelarut dan system bebas pelarut. Olehitu, kaedah pengoptimuman ini boleh membantu menjimatkan masa dan proses perbelanjaan. Tambahan pula, perbezaan pengenal pastian statistik dan prestasi diantara kaedah Taguchi dan RSB menunjukkan bahawa RSB lebih baik berbanding Taguchi bagi penyesuaian data dan kemampuan

penganggaran.Analisis produk dilakukan dengan spektroskopi Fourier Transform-Inframerah (FT-IR), kromatografi gas-spektrometri jisim (GC-MS), dan Resonan Magnetik Nuklear (NMR). Beberapa sifat fisikokimia juga telah dianalisis. Keputusan keseluruhan analisis ini menunjukkan bahawa ester xylitol menunjukkan sifat fisikokimia yang baik. Analisis menunjukkan bahawa ester yang dihasilkan sesuai dalam aplikasi industri.

ACKNOWLEDGEMENTS

Praise and gratitude be to ALLAH, almighty, without whose gracious help it would have been impossible to accomplish this work.

I would like to express my sincere gratitude to my supervisor Prof. Dr. Mahiran Basri for her invaluable advice, continuous support, patience and encouragement through my PhD study. I would also like to thank my supervisory committee members Prof. Dato. Dr Abu Bakar Salleh, Prof. Dr. Mohd Basyaruddin Abdul Rahman, Prof. Dr. Raja Noor Zaliha Raja Abdul Rahman and all the members in Enzyme and Microbial Technology Research Group for their support and lots of great advice and suggestion during the course of study.

Special thank youis due to my best friend, Dr.Naz Chaibakhsh for her help and precious advice and suggestions throughout the duration of my study. Thanks are extended to my friends, Malahat, Lim, Hanim,Dr.Rosa, Dr.Siti Salwa,Uswatun and all my lab-mates in Lab 401. Thank you for making my stay during this research a bearable one with many sweet memories. In addition, I would like to thank staff of Department of Chemistry, Faculty of Science for their help and cooperation during my experimental work.

I am eternally grateful to my dear parents for their ever present love and support, without which I would never have succeeded in my academic endeavours. Love and thanks to my husband for his support, understanding, care and encouragement, he who is my pillar and who alwaysstrengthens me in my happiness and sorrow. To my beloved brother, Arash and sister, Arezoo.I would like to express my deepest affection for their never ending love and support.

I certify that a Thesis Examination Committee has met on 18 March 2011 to conduct the final examination of Seyedeh Atena Adnani on her thesis entitled "**Optimization of Lipase Catalyzed Synthesis of Sugar Alcohol Esters Using Selected Chemometrics Techniques**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy degree.

Members of the Thesis Examination Committee were as follows:

Asmah Hj. Yahya, PhD
Associate Professor
Centre of Foundation Studies for Agricultural Science
Universiti Putra Malaysia
(Chairman)

Mohd Aspolla Hj. Md. Sukari, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Anuar Kassim, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

K. B. Ramachandran, PhD
Professor
Department of Biotechnology, Indian Institute of Technology
Chennai, India
(External Examiner)

BUJANG BIN KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Abu Bakar Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd Basyaruddin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia
Date:18March 2011

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SEYEDEH ATENA ADNANI

Date: 18 March 2011

TABLE OF CONTENTS

LIST OF TABLES