THE USE OF NEW DUAL LIPASES SYSTEM IN THE SYNTHESIS OF FERULATE ESTERS: OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

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AUTHOR DECLARATION

بسم الله الرحمن الرحيم

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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APPROVAL

The thesis entitled "**The Use of New Dual Lipases System in the Synthesis of Ferulate Esters: Optimization by Response Surface Methodology**" by **Nurul Jannah binti Abd Rahman** submitted to the Faculty of Science and Technology, Universiti Sains Islam Malaysia and was accepted as fulfilment of the requirements for the degree of Master of Science.

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ABSTRAK

Sistem dwi-lipase yang baru dan mudah telah diperoleh dalam memperolehi ester ferulik yang tinggi hasil tindakbalas transesterifikasi antara ferulik etil dan minyak zaitun. Pengesanan dan pengesahan produk-produk yang terhasil dimudahkan oleh spektroskopi inframerah (FTIR). Pemeriksaan enzim menunjukkan 1: 9 w/w Novozym 435-Lipozyme RM IM merupakan nisbah yang terbaik. Dengan mengubah satu parameter pada suatu masa, suhu setinggi 60 °C, masa inkubasi selama 12 jam, dos lipase sebanyak 80 mg dan nisbah ferulik etil:minyak zaitun sebanyak 1: 2 merupakan parameter yang optimum dan sesuai dalam tindakbalas tersebut. Selanjutnya, kaedah permukaan respon (RSM) berdasarkan 4-faktor-5-peringkat rekabentuk pusat komposit (CCD) digunakan bagi mengkaji kesan interaktif antara parameter yang terlibat. Model yang terbaik adalah polinomial kuadratik di mana masa inkubasi menunjukkan kesan yang sangat ketara berbanding tiga parameter yang lain. Hasil setinggi 94.03 % telah dicapai dalam kondisi optimum, menghampiri nilai ramalan 94.83 %. Kajian biologi ester ferulik ditentukan berdasarkan ciri-ciri antimikrob terhadap Gram-positif dan Gram-negatif bakteria. Penyaringan awal melalui kaedah serapan-agar diaplikasikan diikuti oleh kepekatan rencatan minimum (MIC) dan kepekatan bakterisidal minimum (MBC). Hasil kajian membuktikan ester ferulik mempunyai ciri-ciri antibakteria yang sederhana terhadap Gram-positif dan Gram-negatif bakteria. Sifat fizikokimia telah ditentukan melalui nilai faktor perlindungan matahari (SPF), nilai peroksida, nilai saponifikasi dan nilai iodin.

ABSTRACT

A novel and simple dual lipases system was developed in attaining high performance of ferulate esters by transesterification reaction between ethyl ferulate and olive oil. Detection and identification of reaction products were facilitated by Fourier Transform Infrared (FTIR). Enzyme screening revealed 1: 9 w/w of Novozym 435-Lipozyme RM IM to be the most efficient lipases ratio for the reaction synthesis. By varying one-parameter-at-a-time, 60 °C of reaction temperature, 12 hrs of incubation time, 80 mg of lipase dosage and 1: 2 of ethyl ferulate: olive oil ratio were shown to be the optimal and suitable reaction conditions. Response surface methodology (RSM) based on four-factor-five-level central composite design (CCD) was further used to study the interactive effects among the parameters. The best fitting model was quadratic polynomial where incubation time exhibited most significant effect over the other three parameters. A high percent conversion of 94.03 % was achieved under the optimum conditions, which compared well with the maximum predicted value of 94.83 %. Biological study of ferulate esters was measured based on their antimicrobial properties against both Gram-positive and Gram-negative bacteria. Screening by agar diffusion test was employed as preliminary features followed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The results proved that the ferulate esters possess moderate antibacterial inhibition against Gram-positive and Gram-negative bacteria. Their physicochemical properties were also well examined through sun protection factor (SPF) value, peroxidase value, saponification value and iodine value.

ملخص

جديد انزيم الليباسيس المزدوج البسيط الذي يوثر بشكل فعال علي تحقيق اداء عالي لسترات الفيروليت بواسطة الاستره بين الايثيل فيروليت وزيت الزيتون. الكشف والتعرف علي نتائج التفاعل كان بواسطة الاشعه التحت الحمراء. فحص الانزيم بين النسبه الوزنيه الوزنيه 1:9 من نوفوزيم 435-ليبوزيم ارام اي ام لتكون اكثر نسبه فعاله للبياسيس لاجل تخليق التفاعل بواسطة تغيير احد البارميترات عند الزمن ودرجة الحراره 60 و الحفظ 12 ساعه , 80 مليجرام من جرعة الليباسيس و ونسبة 1:4 من ايثيل فيروليت الي زيت الزيتون وكانت هذه النسبة ملائمه لظروف التفاعل. منهجية استجابة السطح علي اساس تصميم مركب مركزي كعامل رابع بمستوي خامس استخدم ايضا علي دراسة التاثيرات التفاعليه بين البارمترات . كان افضل نموذج متعدد الحدود من الدرجه الثانيه حيث فترة الحفظ اظهرت اثر كبير علي البارميترات الثلاثه الاخري .نسبه عاليه حوالي 94.3% تما تحويلها تحت ظروف ملائمه والتي قورنت بشكل جيد مع الحد الاقصي للقيمه المتوقعه 94.83% وقد تمت الدراسه البيولوجيه لسترات الفيروليت وكانت مقاسه علي اساس خواصها المضاده للميكروبات ضد كلا من الجراثيم الموجبه والجراثيم السالبه للبكتيريا الفحص كان بواسطة اختبار الانتشار كان يتبع بواسطة اقل تركيز للتثبيط واقل تركيز مبيد للجراثيم . النتائج اوضحت هذا استرات الفيروليت تمتلك تثبيط معتدل للبكتيريا ضد الجراثيم الموجبه بدلا من الجراثيم السالبه. الخواص الفيزيائيه لها كانت ايضا جيده من خلال عامل الحمايه من الشمس وقيمة البيروكسيدات , ورقم التصبن وقيمة اليود

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS

%	Percentage
Abs	Absorbance
ABTS	2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
B	Blank
°C	Degree Celsius
CCD	Central composite design
CF	Correction factor
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EC	Enzyme Commission
EE	Erythemal effect spectrum
EF	Ethyl ferulate
FA	Ferulic acid
FRAP	Ferric reducing antioxidant power
FT-IR	Fourier Transform Infrared
g	Gram
hrs	Hours
HATR	Horizontal attenuated total reflectance
HPLC	High-performance liquid chromatography
I	Solar intensity spectrum
IR	Infrared
MBC	Minimum bactericidal concentration
mEq	Milliequivalents
mg	Miligram
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration

mL	Mililiter
mm	Milimeter
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
N	Normality
nm	Nanometer
OD	Optimal density
ORAC	Oxygen radical absorption capacity
PRESS	Prediction error sum of squares
P-value	Probability value
R^2	Determination of coefficient
RM IM	Immobilized *Rhizomucor miehei*
ROS	Reactive oxygen species
rpm	Round per minute
RSM	Response Surface Methodology
SPF	Sun protection value
TAG	Triacylglycerol
UV	Ultraviolet
w	Weight