UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF ENZYMATIC PRODUCTION OF FATTY HYDRAZIDES AND THEIR APPLICATION AS POLYURETHANE CHAIN EXTENDER

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MASTER OF SCIENCE
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By

TUAN NOOR MAZNEE BINTI TUAN ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for the Degree of Master of Science

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OPTIMIZATION OF ENZYMATIC PRODUCTION OF FATTY HYDRAZIDES AND THEIR APPLICATION AS POLYURETHANE CHAIN EXTENDER

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December 2012

Chair: Nor Azowa Ibrahim, PhD

Faculty: Science

Enzymatic synthesis of fatty hydrazides from palm oil was first carried out using the reaction conditions reported by Mohamad et al. (2008) as for the starting point in optimization work. Fatty hydrazides were prepared by reacting refined, bleached and deodorized (RBD) palm olein with hydrazine monohydrate in the presence of enzyme and *n*-hexane. As for optimization, response surface methodology of central composite design of quadratic model has been applied. The optimum reaction conditions obtained using pH 7 adjusted hydrazine monohydrate were as follows: temperature, 40^{o}C; time, 18 h; percentage of enzyme, 6% and stirring speed, 350 rpm. Study on the effect of pH of hydrazine monohydrate on the formation of fatty hydrazides

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indicated that the reaction was best carried out at pH 12. Further study using the optimum reaction conditions established through response surface methodology and hydrazine monohydrate of pH 12 drastically improved the conversion of the fatty hydrazides from 43.8% (pH 7) to 87.5% (pH 12). Composition of the hydrazides analyzed using GC also increased tremendously from 15.77% to 91.87%.

A bigger scale production of fatty hydrazides (using 1 kg RBD palm olein instead of 100 g) was conducted using the newly established optimum reaction conditions. However, due to the limitation of the reactor, the reaction mixture could only be stirred at the stirring speed of 150 rpm. But interestingly, the yield of fatty hydrazides produced was further increased by 24.4% and the composition of hydrazides shown by GC chromatogram increased by 6% compared to the hydrazides obtained from 100 g RBD palm olein. The GC/MS analysis indicated that the fatty hydrazides prepared contained a mixture of palmityl hydrazide and oleyl hydrazide. ^{1}H and ^{13}C NMR chromatogram further confirmed the component of fatty hydrazides by the presence of proton chemical resonances, ^{1}H NMR, (600 MHz, DMSO-d_6) at δ 8.90 (s, -CONH), 5.32 (m, -CH=CH-), 2.17 (t, *J*=7.8 Hz,-CH_2-CO-), 2.08 (m, -NH_2), 1.98 (m, -CH_2-CH=CH-CH_2-, 4H), 1.46 (m, -$C\underline{H_2}CH_2CO$-), 1.26 (m, -CH_2-, 24H), 0.85 (t, -CH_3) and carbon chemical resonances, ^{13}C NMR, (150 MHz, DMSO-d_6) at δ 171.6 (-CON-) and 129.6 (-CH=CH-).

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These optimized conditions were then applied for synthesis of fatty hydrazides from other triglycerides such as glyceryl trioleate, soybean oil and glyceryl tristearate. Compositions of fatty hydrazides obtained as shown by GC chromatogram were in the range of 81 to 91%. GC/MS analysis also indicated that glyceryl trioleate-based hydrazides or oleyl fatty hydrazides and soybean fatty hydrazides contained a mixture of palmityl hydrazide, oleyl hydrazide and linoleyl hydrazide. Stearyl fatty hydrazides were not analyzed using GC/MS due to the limitation of the oven temperature of the instrument. ^{1}H and ^{13}C NMR chromatogram further confirmed the component of fatty hydrazides by the presence of proton chemical resonances, ^{1}H NMR, (600 MHz, DMSO-d_6) at δ 8.90 (s, -CONH), 5.32 (m, -CH=CH-), 2.17 (t, *J*=7.8 Hz,-CH_2-CO-), 2.08 (m, -NH_2), 1.98 (m, -CH_2-CH=CH-CH_2-, 4H), 1.46 (m, -$C\underline{H_2}CH_2CO$-), 1.26 (m, -CH_2-, 24H), 0.85 (t, -CH_3) and carbon chemical resonances, ^{13}C NMR, (150 MHz, DMSO-d_6) at δ 171.6 (-CON-) and 129.6 (-CH=CH-).

Finally, potential application of fatty hydrazides as a chain extender in rigid polyurethane foam was investigated. The reactivity of foaming process for foam and its thermal conductivity value increased with the presence of fatty hydrazides. However, the closed cell content of the foam seemed to decrease, therefore, the rigid polyurethane foam containing fatty hydrazides exhibits slightly inferior insulation property compared to the foam without fatty hydrazides. Based on these observations, fatty hydrazides might be more useful to be incorporated in formulation for flexible foam.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai mematuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN PENGHASILAN BERENZIM HIDRAZIDA LEMAK DAN PENGGUNAANNYA SEBAGAI PEMANJANG RANTAI POLIURETANA

Oleh

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Sintesis hidrazida daripada minyak sawit menggunakan enzim pada mulanya dijalankan mengikut keadaan tindakbalas yang telah dilaporkan oleh Mohamad et al. (2008). Hidrazida dihasilkan melalui tindakbalas di antara minyak sawit olein dan hidrazin dengan kehadiran enzim di dalam pelarut heksana. Pengoptimuman sintesis berenzim hidrazida daripada minyak sawit menggunakan perisian kaedah rangsangan permukaan, rekabentuk komposit sentral dan model kuadratik telah diaplikasikan. Keadaan tindakbalas optimum yang diperolehi melalui kaedah ini menggunakan hidrazin monohidrat pada pH 7 ialah seperti berikut: suhu, 40^oC; masa, 18 jam; kelajuan pengacau, 350 rpm dan enzim, 6%. Kajian

kesan pH hidrazin monohidrat ke atas pembentukan hidrazida menunjukkan tindakbalas adalah terbaik dijalankan pada pH 12. Kajian lanjut menggunakan keadaan-keadaan tindakbalas optimum yang diperolehi melalui kaedah rangsangan permukaan dan hidrazin monohidrat pH 12 telah secara drastik meningkatkan penukaran hidrazida daripada 43.8% (pH hidrazin 7) kepada 87.5% (pH hidrazin 12). Komposisi hidrazida yang dianalisa menggunakan kromatografi gas juga meningkat secara mendadak daripada 15.77% kepada 91.87%.

Penghasilan hidrazida pada skala lebih besar (minyak sawit olein sebanyak 1 kg telah digunakan berbanding 100 g yang digunakan sebelum ini) dengan menggunakan keadaan tindakbalas yang lebih optimum iaitu suhu, 40^{o}C; masa, 18 jam; kelajuan pengacau, 350 rpm, enzim, 6% dan pH hidrazin, 12 telah dijalankan. Walau bagaimana pun, disebabkan oleh keterbatasan reaktor tindakbalas 10 L yang digunakan, campuran tindakbalas hanya boleh dikacau dengan kelajuan 150 rpm sahaja. Menariknya, hasil hidrazida yang diperolehi telah meningkat sebanyak 24.4% dan komposisi hidrazida berdasarkan kromatogram kromatografi gas telah meningkat sebanyak 6% berbanding hidrazida yang dihasilkan menggunakan 100 g minyak sawit olein. Analisa melalui GC/MS menunjukkan hidrazida yang dihasilkan terdiri daripada campuran palmitil hidrazida dan oleil hidrazida. Komponen kimia hidrazida telah disahkan melalui analisa proton dan karbon-13 NMR dengan kehadiran resonan kimia proton ^{1}H NMR, (600 MHz, DMSO-d_6) pada δ 8.90 (s, -CONH), 5.32 (m, -CH=CH-), 2.17 (t, *J*=7.8 Hz,-CH_2-CO-), 2.08 (m, -NH_2), 1.98 (m, -CH_2-CH=CH-CH_2-, 4H), 1.46 (m, -C$\underline{H_2}$$CH_2$CO-), 1.26 (m, -

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CH_2-, 24H), 0.85 (t, -CH_3) dan resonan kimia karbon, ^{13}C NMR, (150 MHz, DMSO-d_6) pada δ 171.6 (-CON-) dan 129.6 (-CH=CH-).

Keadaan-keadaan tindakbalas optimum ini kemudian telah digunakan untuk menghasilkan hidrazida-hidrazida daripada sumber trigliserida yang lain seperti gliseril trioleat, minyak kacang soya dan gliseril tristearat. Komposisi hidrazida-hidrazida yang diperolehi berdasarkan analisa kromatografi gas adalah dalam lingkungan 81 hingga 91%. Analisa GC/MS menunjukkan hidrazida berasaskan gliseril trioleat dan minyak kacang soya terdiri daripada campuran palmitil hidrazida, oleil hidrazida dan linoleil hidrazida. Hidrazida berasaskan gliseril tristearat tidak dianalisa menggunakan GC/MS disebabkan oleh keterbatasan suhu oven peralatan tersebut. Komponen kimia hidrazida telah disahkan melalui analisa proton dan karbon-13 NMR dengan kehadiran resonan kimia proton, ^{1}H NMR, (600 MHz, DMSO-d_6) pada δ 8.90 (s, -CONH), 5.32 (m, -CH=CH-), 2.17 (t, *J*=7.8 Hz,-CH_2-CO-), 2.08 (m, -NH_2), 1.98 (m, -CH_2-CH=CH-CH_2-, 4H), 1.46 (m, -$C\underline{H_2}CH_2CO$-), 1.26 (m, -CH_2-, 24H), 0.85 (t, -CH_3) dan resonan kimia karbon, ^{13}C NMR, (150 MHz, DMSO-d_6) pada δ 171.6 (-CON-) dan 129.6 (-CH=CH-).

Akhirnya, potensi penggunaan hidrazida sebagai pemanjang rantai dalam formulasi busa poliuretana tegar telah dikaji. Profil kereaktifan busa semasa pembusaan dan nilai kekonduksian terma busa telah meningkat dengan kehadiran hidrazida. Walau bagaimana pun, kandungan sel tertutup busa didapati berkurangan, oleh itu, busa poliuretana tegar yang mengandungi

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hidrazida kurang bersifat penebat berbanding busa poliuretana tegar tanpa kehadiran hidrazida. Berdasarkan pemerhatian ini, hidrazida mungkin lebih sesuai digunakan di dalam formulasi busa poliuretana fleksibel berbanding busa poliuretana tegar.

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I certify that a Thesis Examination Committee has met on 27 December 2012 to conduct the final examination of Tuan Noor Maznee Tuan Ismail on her thesis entitled “Optimization of Enzymatic Production of Fatty Hydrazides and Their Application as Polyurethane Chain Extender” 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 Master of Science.

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been dully 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.

TUAN NOOR MAZNEE TUAN ISMAIL

Date: 27 December 2012

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AOCS	American Oil Chemists Society
ATR	attenuated total reflectance
BSTFA	*N,O*-bis(trimethylsily)trifluoroacetamide
CCD	central composite design
DAG	diacylglycerol
DBTDL	dibutyltindilaurate
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
FID	flame ionization detector
FTIR	Fourier transform infrared
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
HCl	hydrochloric acid
H_2O	water
LSD	least significant difference
MAG	monoacylglycerol

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NMR	nuclear magnetic resonance
PU	polyurethane
RBD	refined, bleached and deodorized
RML	*Rhizomucor miehei* lipase
RSM	response surface methodology
TAN	total acid number
TGA	thermogravimetry analysis
2D	two dimension
3D	three dimension
w/w	weight by weight

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