UNIVERSITI PUTRA MALAYSIA

PROCESS DEVELOPMENT FOR HIGHER YIELD PRODUCTION OF DIACYLGLYCEROL OIL VIA PARTIAL HYDROLYSIS

PHUAH ENG TONG

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By

PHUAH ENG TONG

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Chair : Lai Oi-Ming, PhD
Faculty : Institute of Bioscience

The disclosure of diacylglycerol (DAG) oil to replace the conventional edible oils has received increasing interest among researchers and food manufacturers owing to its anti-obesity properties. Distinct processing approaches have been proposed to produce DAG-enriched oil in which enzymatic partial hydrolysis outstands other methods due to its inexpensive raw materials and single-step hydrolytic reaction involved. In present work, single-factor optimization of partial hydrolysis for DAG production from refined, bleached, deodorized palm oil (RBDPO) catalysed by immobilized *Rhizomucor miehei* lipase (Lipozyme RMIM) was carried out in batch system. Effects of four operating parameters namely temperature, enzyme dosage, water content and agitation speed were investigated. Optimum production conditions for palm based-DAG are as follows: temperature = 55^{o}C, enzyme dosage = 10-wt%, water content = 5-wt% and agitation speed = 500 rpm. A DAG yield of 31-wt% was obtained after 6 h of reaction. The partial hydrolysis reaction was found to conform to Ping-Pong Bi-Bi with substrate inhibition mechanism. The optimum operating conditions were then applied to the lab-scale packed bed system.

Packed bed reactor (PBR) is an effective reactor configuration because it enables reusability of the enzyme particles besides enhancing its operational stability. However, mass transfer limitation remains a key challenge in packed bed column system, especially at large scale. A dimensionless mathematical mass transfer model of Colburn factor, J_D, which is a function of Reynolds (Re) and Schmidt (Sc) numbers, was therefore developed to simulate mass transfer phenomena of the reaction mixture in PBR during enzymatic partial hydrolysis reaction. The results revealed that the mass transfer correlation of $J_D=0.92(\mathrm{Re})^{-0.2}$ was able to predict the experimental data accurately. In addition, response surface methodology (RSM) was employed to optimize the process variables namely packed bed height and substrate flow rates on DAG production in PBR. Quadratic models were successfully developed for both DAG and unhydrolyzed triacylglycerol (TAG) with insignificant lack of fit (P>0.05). Optimum conditions for DAG synthesis were evaluated to be 10 cm packed bed height and 3.8 ml/min flow rate with 29-wt% DAG being reported. Immobilized enzyme can be reused up to 10 times without significant loss in enzymatic activity.

The present study also investigated the production efficiency using columns with different length-to-diameter ratios (L/D ratio) to determine the most potential process setup for industrial DAG manufacturing. Practical design issues such as operating temperature, substrate flow rate and reaction time were evaluated with respect to various packed bed column configurations. A column dimension with L/D ratio of two was determined to be the most suitable bed column design for lipase-mediated partial hydrolysis reaction. The optimal reaction temperature, substrate flow rate and residence time for the production of DAG in packed bed column dimension of two were found to be 55^{o}C, 5 ml/min and 5.8 min, respectively. Under these operating conditions, a maximal DAG content of 35-wt% was obtained within the first 2 h. Since scientific knowledge is lacking in the employment of PBR for the production of DAG-enriched oil *via* enzyme-catalysed partial hydrolysis, the findings of the study would facilitate the design of a pilot-scale fixed bed reactor system for lipase-mediated partial hydrolysis to obtain DAG-enriched oil as functional oil without constraints.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN PROSES BAGI PENGHASILAN MINYAK DIASILGLISERIDA YANG TINGGI MELALUI HIDROLISIS SEPARA

Oleh

PHUAH ENG TONG

Disember 2015

Pengerusi: Lai Oi-Ming, PhD
Fakulti: Institut Biosains

Penemuan minyak diasilgliserida (DAG) untuk menggantikan minyak makan konvensional telah mendapatkan perhatian di kalangan penyelidik dan pengeluar makanan disebabkan oleh sifat-sifat anti-obesiti minyak tersebut. Pelbagai kaedah pemprosesan telah dicadangkan untuk menghasilkan minyak DAG di mana kaedah hidrolisis separa dengan menggunakan enzim lipase bersetanding dengan cara-cara pemprosesan lain kerana kaedah tersebut melibatkan bahan-bahan mentah yang murah dan memerlukan satu langkah hidrolisis sahaja. Dalam projek ini, kaedah hidrolisis separa dioptimumkan dalam sistem batch bagi menghasilkan DAG daripada minyak kelapa sawit yang telah ditapis, diluntur warna dan dinyahbau (RBDPO) dengan menggunakan lipase tersekat-gerak daripada *Rhizomucor miehei* (Lipozyme RMIM). Kesan empat parameter operasi iaitu suhu, dos enzim, kandungan air dan kelajuan pergolakan telah dikajikan. Keadaan penghasilan DAG yang optimum adalah seperti berikut: suhu = 55^{o}C, dos enzim = 10-wt%, kandungan air = 5-wt% and kelajuan pergolakan = 500 rpm. Sebanyak 31-wt% DAG dapat dihasilkan selepas 6 jam. Tindak balas hidrolisis separa didapati mematuhi mekanisme Ping-Pong Bi-Bi dengan perencatan substrat. Keadaan operasi optimum kemudiannya digunakan dalam reaktor lapisan terpadat berskala makmal.

Reaktor lapisan terpadat (PBR) merupakan reaktor yang efisien kerana reaktor tersebut membolehkan kebolehgunaan enzim lipase selain meningkatkan kestabilan operasinya. Walau bagaimanapun, fenomena pemindahan jisim dalam PBR telah menjadi cabaran utama terutamanya dalam reaktor berskala besar. Oleh itu, model matematik tidak berdimensi bagi pemindahan jisim iaitu faktor Colburn, J_D yang merupakan gabungan nombor Reynolds (Re) dan nombor Schmidt (Sc) telah dicadangkan bagi mensimulasikan pemindahan

jisim dalam sistem PBR semasa reaksi hidrolisis separa berlaku. Model ini telah diperiksa dengan pelbagai nilai n dan keputusan menunjukkan bahawa korelasi pemindahan jisim $J_D=0.92(Re)^{-0.2}$ dapat meramalkan data eksperimen dengan tepat. Di samping itu, kaedah gerak balas permukaan (RSM) telah digunakan untuk mengoptimumkan kedua-dua pembolehubah tidak bersandar iaitu ketinggian lapisan turus dan kadar aliran substrat bagi memaksimumkan hasil DAG dalam sistem PBR. Model kuadratik digunakan untuk mewakili kedua-dua pembolehubah bergerak balas iaitu DAG(y) dan triasilgliserida (TAG) yang tidak bertindak balas ($_{(un)}$TAG) dengan kekurangan penyesuaian yang tidak ketara ($P>0.05$). Keadaan optimum untuk mensintesis DAG adalah 10 cm ketinggian lapisan turus dan 3.8 ml/min kadar aliran substrat dengan 29-wt% DAG dilaporkan. Lipase tersekat-gerak boleh digunakan semula sehingga 10 kali tanpa kehilangan aktiviti enzim yang ketara.

Selain itu, kecekapan penghasilan DAG degan menggunakan ruangan lapisan turus berbeza yang mempunyai nisbah tinggi lapisan turus kepada diameter dalaman ruangan lapisan turus (nisbah L/D) yang berbeza telah dikajikan bagi menentukan ruangan lapisan turus yang paling berpotensi untuk menghasilkan DAG di industri. Isu-isu praktikal seperti suhu operasi, kadar aliran substrat dan masa tindak balas telah dinilaikan bagi setiap konfigurasi ruang padat. Dimensi ruangan dengan L/D nisbah dua merupakan reka bentuk ruang lapisan turus yang paling sesuai untuk reaksi hidrolisis separa yang dimangkinkan olen enzim lipase. Keadaan optimum bagi menghasilkan DAG adalah 55^{o}C suhu operasi, 5.0 ml/min kadar aliran substrat dan 5.8 min masa kediaman dalam PBR. Di bawah keadaan operasi tersebut, sebanyak 35-wt% DAG dapat diperolehi dalam tempoh 2 jam pertama. Oleh kerana pengetahuan saintifik tentang penggunaan sistem PBR untuk menghasilkan minyak DAG melalui hidrolisis separa dengan bantuan enzim masih kurang, hasil penyelidikan ini dipercayai akan memudahkan kerja perekabentuk reaktor berskala besar untuk pemprosesan minyak DAG melalui kaedah hidrolisis separa tanpa kekangan.

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I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of (student's name) on his (her) thesis entitled ("Title of Thesis") 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 (insert the name of relevant degree).

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Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
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Universiti Putra Malaysia
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Name of Department and/or Faculty
Name of Organisation (University/Institute)
Country
(External Examiner)

____
(Insert name of current Deputy Dean)
(E.g. XXXXX XXXX, 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:

Lai Oi Ming, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Thomas Choong Shean Yaw, PhD
Professor, Ir
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Tan Chin Ping, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Lo Seong Koon, PhD
Biotechnology Research & Development Centre
Wilmar International Ltd. (Shanghai)
(Member)

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

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This is to confirm that:

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Name of Chairman of Supervisory Committee: Professor Dr. Lai Oi Ming

Signature: ______________________________
Name of Member of Supervisory Committee: Professor Dr. Thomas Choong Shean Yaw

Signature: ______________________________
Name of Member of Supervisory Committee: Professor Dr. Tan Chin Ping

Signature: ______________________________
Name of Member of Supervisory Committee: Dr. Lo Seong Koon

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LIST OF ABBREVIATIONS

E_a	Activation energy
[TOMA].[Tf2N]	trioctylmethylammonium bis (trifluoromethylsulphonyl)imide
1(3)-MAG	1-monoacylgycerol or 3-monoacylglycerol
2M2B	2-methyl-2-butanol
2-MAG	2-monoacylglycerol
A x B x C	Enzyme-substrate complex
AAD	Absolute average deviation
Ammoeng 102	Cocosalkyl pentaethoxi methyl ammonium methylsulfate
ANOVA	One-way analysis of variance
AOT	Sodium (bis-2-ethyl-hexyl) sulfosuccinate
BCR	Bubble column reactor
BR	Batch reactor
CA	Caprylic acid
CCRD	Centre composite rotatable design
CO_2	Carbon dioxide
CSTR	Continuous stirred tank reactor
DA	Capric acid
DAG	Diacylglycerol
DGAT	Diacylglycerol acyltransferase
E	Enzyme
E*	Modified enzyme complex
ELSD	Evaporative light scattering detector
EPA	Eicosapentaenoic acid
FBR	Fluidized bed reactor
FDA	Food and Drug Administration
FFA	Free fatty acid
G	Glycerol
GRAS	Generally recognized as safe
H_2O	Water
HPLC	High performance liquid chromatography
IL	Ionic liquid
L/D ratio	Column length-to-inner diameter ratio
LA	Lauric acid
Lipozyme RMIM	*Rhizomucor miehei* lipase
Lipozyme TLIM	*Thermomyces lanuginosus* lipase
LOA	Linoleic acid
MAG	Monoacylglycerol
ME	Methyl esters
MGAT	Monoacylglycerol acyltransferase
MR	Membrane reactor
MTBE	*tert*-butyl methyl ether
MTP	Microsomal triglyceride transfer protein
OA	Oleic acid
PA	Palmitic acid
PBR	Packed bed reactor

PODD	Palm oil deodorized distillate
PUFA	Polyunsaturated fatty acid
RBDPO	Refined, bleached, and deodorized palm oil
Re	Reynolds number
ROFA	Rapeseed oil fatty acids
RSM	Response surface methodology
Sc	Schmidt number
SC-CO_2	Supercritical carbon dioxide
TAG	Triacylglycerol
WHO	World Health Organization
ΔP	Pressure drop

CHAPTER 1

INTRODUCTION

Dietary fats and oils are known to be the key nutrients essential for sustaining life. In addition to providing energy for daily activities, the roles of lipids as fundamental building blocks for healthy cells, carriers for fat soluble vitamins, organ protector and body insulator are well documented. Moreover, the incorporation of dietary fats into food enhances its sensory and textural properties. However, strong evidence demonstrates that increased dietary energy intake especially fat-dense food coupled with inadequate physical activities is the main culprit that leads to the overwhelming incidences of obesity (Golay & Bobbioni, 1997; Astrup, 2005). Recent reports reveal that obesity prevalence rates are rising at an alarming rate and remain to be a critical global epidemic (WHO 2014). Tremendous research studies indicate a strong positive correlation between obesity and adverse health effects such as heart disease, cancer, diabetes mellitus, hypertension besides mental trauma and physical discomfort (Lavie et al. 2009; Artham et al. 2011; Louie et al. 2013). The consequences are potential decline in life expectancy, early retirement, widespread discrimination and increased cost of health care system that burdens the government and economic growth. In United States, obesity-related medical expenses have seen a drastic increase over the years with USD 86 billion of aggregate health care cost being recorded in year 2008 (Finkelstein et al. 2009). A recent published research report also indicates that the prevalence of obesity is rather severe in Malaysia as compared to other Asian countries with 45.3% of its population being overweight and the obese population is forecasted to skyrocket in the next decade (Ng et al. 2014). Although World Health Organization (WHO) advises and limits the dietary fats consumption to 30% of the total calorie intake in order to impart positive effects on human health, the recommended action may sacrifice the mouthfeel quality in fat-based food.

With heightened health consciousness, the disclosure of functional diacylglycerol (DAG)-oil has therefore drawn increasing attention of researchers and food manufacturers to replace the conventional edible oil or triacylglycerol (TAG) oil. Previous literatures clearly pointed out that DAG-enriched oil is capable of inhibiting the accumulation of visceral fat and suppressing the blood serum TAG besides increasing the rate of β-oxidation of fatty acids which translates into potent anti-obesity properties (Flickinger & Matsuo, 2003; Teramoto et al., 2004). Apart from that, DAG with exposing hydrophilic group within the molecular structure, exhibits excellent emulsifying capability and has been widely used as emulsifier together with monoacylglycerol (MAG) in food, cosmetic and pharmaceutical products (Shimada & Ohashi, 2003; Masui et al., 2001; Nakajima, 2004). Foreseeing the increasing demand for DAG-oil, Kao Corporation (Japan) began commercializing the functional edible oil under product name of “Healthy Econa Cooking Oil” in early 1999. Sales of this functional edible oil accounts for 80%

of premium oil which constitute around 14% of the total Japanese edible oil market worth ¥10 billion (Sakaguchi, 2001).

Strategy for DAG-oil production includes both chemical and enzyme-assisted approaches, in which the latter exhibits several advantages over chemical method namely, reduced energy consumption, improved selectivity and yield. Enzymatic partial hydrolysis reaction outperforms other methods because of the low cost reactants and single hydrolytic step involved (Lai et al. 2006; Cheong et al. 2007; Lo et al, 2008). Malaysia, being one of the largest palm oil producer and exporter, contributes nearly 17.7 million tonnes, accounting for 11% of the global fats and oils production and becomes a dominant player in the palm oil trade with 44% of the market share (MPOC 2014). To broaden the commercial use and functionality of palm oil in order to stay competitive in edible oils and fats market, production of DAG-enriched oil from conventional palm oil *via* lipase-catalysed partial hydrolysis is indeed a necessity.

To date, literature on the kinetic study of partial hydrolysis reaction and production of palm-based DAG is limited. As such, various operating parameters namely temperature, agitation speed, water content and enzyme load for the production of palm-based DAG in batch stirred reactor system were investigated in present work. In addition, this work aimed to develop a kinetic model to describe the reaction mechanism of partial hydrolysis as well as to provide information on the optimum processing conditions. Analysis of reaction kinetics has great potential because the mathematical model generated are capable of simulating the complex reaction under different conditions and thereby improving the reaction conditions (Fedosov et al. 2013). Although batch stirred reactor could be used to produce DAG *via* enzyme-catalysed partial hydrolysis, abrasion of the matrix particles under mechanical stirring force should be paid attention. The optimum conditions determined were then applied to packed bed reactor (PBR) system owing to its higher reaction rate and enhanced stability of particulate catalysts in PBR system (Phuah et al. 2015). Two important operating variables namely packed bed height and substrate flow rate were evaluated and optimized by response surface methodology (RSM). The application of RSM as a statistical techniques based on the fit of a polynomial model to the experimental data enables evaluation of the effects of multiple operating parameters, alone or in combination, on response variables and prediction of reaction performance accurately (Xu et al. 1998).

Although PBR is a preferred bioreactor configuration, dominance of external mass transfer resistance during enzymatic reaction remains to be major hindrance for fixed bed system especially at large scale. External mass transport limitation exists when the rate of diffusional transport of substrate through the external film of the enzyme particles is the rate determining step which is caused by low substrate flow rate (Chew et al. 2008; McCabe et al. 2005; Kasaini & Mbaya 2009; Murty et al. 2005). Therefore, the mass transfer phenomena in PBR were investigated and represented with external mass transfer model. The development of the mathematical model would enable evaluation of mass transfer coefficients in fixed bed system under different operating conditions. The effects of different bed column designs with distinct column length-to-inner diameter ratios (L/D ratio) were also studied as the column dimensions determine both linear fluid flow rate and external mass transfer coefficient even with constant residence time and substrate flow rate, thereby affecting the efficiency of the packed bed system. In summary, the objectives of this study were as follows:

1. To optimize the reaction parameters for the production of DAG-oil in batch reactor and to evaluate its kinetic mechanism.

2. To optimize the operating parameters on the production yield of DAG in packed bed reactor.

3. To develop a mass transfer model to predict the reactor performance and to simulate the partial hydrolysis reaction in packed bed system.

4. To evaluate the effects of different bed column designs on the production efficiency of DAG.

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