UNIVERSITI PUTRA MALAYSIA

ENZYMATIC PRODUCTION OF FERULOYLATED ACYLGLYCEROLS FROM OLIVE FATTY ACID DISTILLATES AS SUNSCREEN AGENTS

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

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Chairman: Associate Professor Lai Oi Ming, PhD

Faculty: Biotechnology and Biomolecular Sciences

This work was instigated to produce a novel sunscreen agent based on ferulate glycerol esters with the incorporation of olive fatty acid distillate, glycerol and ethyl ferulate by enzymatic processes.

A suitable HPLC detection method was devised for the quantification of the produced novel ferulate glycerol esters. The best separation was found in the combined analytical parameters of mobile phase of 90:10 (ml/ml) acetone: acetonitrile, with additional 1.2% (ml/ml) of acetic acid, running through a reversed phase C8 column (Purospher® STAR RP-8e 5 µm, Merck, Germany) with detection by a UV detector at 350 nm. The peaks retention time was confirmed at 3.14, 3.27, 3.37, 3.96., 4.14, 6.70, 6.95, 7.44 and 7.88 min for ferulate glycerol, ferulic acid, ethyl ferulate, ferulate monoolein, the unidentified peak A, peak B, peak C, peak D and ferulate diolein, respectively.

The effects of reaction parameters such as lipase type, reaction temperature, lipase concentration, molecular sieves content, free fatty acid to ethyl ferulate molar ratio and total glycerol to acyl-donor molar ratio on the production of ferulate glycerol ester were studied. The immobilized lipase Novozym® 435 of *Candida antarctica* was found to be the best performing lipase and it was observed that the reaction parameters of the reaction at 5% (w/w) of Novozym® 435, reaction temperature of 70 ºC, with a total glycerol to fatty acid and ethyl ferulate molar ratio of 1:2:1, in presence of 3% (w/w) molecular sieves had resulted in an optimal yield of 64.19% of ferulate glycerol esters.

The resulted ferulate glycerol ester mixture was partially purified through micro-filtration and neutralization process and this had increased the ferulate glycerol esters content to 70.12% (yield). The partially purified ferulate glycerol esters mixture was analyzed through *in-vitro* analysis for its SPF value, mean critical wavelength and the UVA/UVB protection ratio. The results indicated that the partially purified ferulate glycerol esters mixture at 70.12% (yield) purity provides a protection factor of SPF 10.40 in the UVB region and a moderate protection in the UVA region. A test on 15% (w/w) preparation of the partially purified ferulate glycerol ester confirms that the ferulate glycerol esters mixture is capable of providing a basic protection of SPF 3.94, with good protection in the UVB region and a moderate protection in the UVA region.

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

PROSES TINDAKBALAS BERMANGKIN UNTUK PENGHASILAN ASILGLISERIDA BERFERULIK DARIPADA MINYAK DISTILAT ZAITUN UNTUK DIGUNAKAN SEBAGAI AGEN PERLINDUNG SINARAN UV

Oleh

KONG CHING

Mei 2008

Pengerusi: Profesor Madya Lai Oi Ming, PhD

Fakulti: Bioteknologi dan Sains Biomolekular

Kajian ini bertujuan untuk menghasilkan satu agen penabir suria berasaskan ester-ferulik hasil proses esterifikasi dan gliserolisis bermangkin lipase daripada distilat minyak zaitun, gliserol dan etil-ferulat.

Satu kaedah HPLC telah dibentuk untuk menganalisis bahan ferulat gliserol ester yang dihasilkan. Pemisahan juzuk yang terbaik didapati daripada kombinasi parameter analisis termasuk fasa bergerak pada 90:10 (i/i) aseton: asetonitril, ditambah dengan 1.2% (i/i) asid asetik, yang melalui fasa tidak bergerak C8 kolum fasa berbalik (Purospher® STAR RP-8e 5 μm, Merck, Germany) dan dikesan oleh alat pengesan UV pada 350 nm. Masa retensi setiap juzuk telah dikenalpasti pada 3.14, 3.27, 3.37, 3.96, 4.14, 6.70, 6.95, 7.44 dan 7.88 minit untuk gliserol berferulat, asid ferulik, etil-ferulik, monoolein-berferulik, juzuk A, juzuk B, juzuk C, juzuk D dan diolein berferulik.

Kesan parameter reaksi seperti jenis mangkin lipase, suhu tindak balas, kepekatan mangkin lipase, jumlah penapis molekul, nisbah asid lemak kepada etil ferulat serta nisbah jumlah gliserol kepada penderma-asil terhadap penghasilan bahan ferulat ester telah dikaji. Mangkin lipase Novozym® 435 daripada mikrob *Candida antarctica* merupakan mangkin terbaik untuk tindak balas ini. Selain itu, tindak balas bermangkin lipase dengan 5% (w/w) mangkin lipase Novozym® 435, pada suhu tindak balas 70 ºC, mengikut nisbah gliserol kepada asid lemak dan etil ferulik 1:2:1, ditambah dengan 3% (w/w) penapis molekul telah menghasilkan ester gliserol ferulat yang optimal iaitu 64.19% konversi.

Ester ferulat yang terhasil ditingkatkan tahap ketulenannya melalui proses penapisan-mikro dan proses peneutralan dan jumlah ester gliserol ferulat didapati telah naik kepada 70.12% (hasil). Ester gliserol ferulat separa tulen ini kemudiannya dianalisa dengan kaedah *in vitro* untuk mengetahui nilai SPF, purata gelombang kritikal (mean critical wavelength) serta nisbah perlindungan UVA/UVBnya. Hasil analisis mendapati bahawa ester gliserol ferulat separa tulen ini pada 70.12% (hasil), boleh memberikan perlindungan SPF sebanyak 10.40, dengan perlindungan baik dalam lingkungan cahaya UVB serta perlindungan sederhana dalam lingkungan cahaya UVA. Ujian pada ester ferulat separa tulen dalam 15% (b/b) pula, menunjukkan bahawa ester ferulik ini boleh memberikan perlindungan asas SPF 3.94; memberikan perlindungan baik dalam lingkungan cahaya UVB dan perlindungan cahaya UVA yang sederhana.

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This 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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Date: 11th September 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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KONG CHING

Date:

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LIST OF ABBREVIATIONS

Abs	Absorbance
DAG	Di-acylglycerol
DO	Diolein
EF	Ethyl Ferulate
ELSD	Evaporative Light Scattering Detector
EO	Ethyl Oleate
FA	Ferulic Acid
FDO	Feruloylated Diolein
FE	Ferulate Esters
FFA	Free Fatty Acid
FG	Ferulate Glycerol
FMO	Feruloylated Monoolein
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IPD	Immediate Pigment Darkening
IR	Infra Red
IV	Iodine Value
LOD	Limits of Detection
LOQ	Limits of Quantitation
MAG	Mono-acylglycerol
MED	Minimal Erythema Dose
MO	Monoolein
n.d.	not detected
NaOH	Sodium Hydroxide

OA	Oleic Acid
OFAD	Olive Fatty Acid Distillate
OFE	Oleo-ferulate Esters
PORIM	Palm Oil Research Institute Malaysia
RBDO	Refined, Bleached and Deodorized Olive oil
RPM	Revolution per minute
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
Rt	Retention Time
SD	Standard deviation
SPF	Sun-Protection Factor
SQ	Squalene
TAG	Tri-acylglycerol
TEWL	Transepidermal Water Loss
TO	Triolein
UV	Ultra Violet
v/v	volume / volume
w/w	weight / weight

CHAPTER I

INTRODUCTION

By nature, the sun is the primary source of energy. Energy from the sun is emitted to earth in the form of rays, including the infra-red and also the ultraviolet radiation. Sufficiently filtered by the ozone layer, infra-red radiation is responsible in providing heat, whereas the ultraviolet radiation helps to trigger vitamin D synthesis in the skin. However, due to the continuous depletion of the ozone layer, excessive amount of UV radiation has penetrated the atmosphere, and prolonged exposures to the sun increases the chances of sun-burn, photo-aging and cancer. According to Cancer Research UK, some 75,000 new skin cancer cases are reported each year in the United Kingdom and out of these, malignant melanoma accounts for more than 7,000 new diagnoses (Cancer Research UK, 2006). The Centers for Disease Control and Prevention (CDC) reported that in 2002, about 50,000 new cases of skin cancer was diagnosed in the United States alone. In fact, the United States Environmental Protection Agency (EPA) had warned that ozone depletions will lead to 150 million new cases of skin cancer in USA alone by the year 2075 (US EPA, 1987).

To have adequate protection from the effects of the sun, consumers are advised to wear long sleeved clothing, hats and also long pants. Apart from proper clothing selection, consumers are recommended to add sunscreens in the ‘should have list’ for sun-protections. There are two types of sunscreens. Physical sunscreening agents works as a protective shield that scatters the path of UV light from reaching our skin, and the chemical / organic sunscreening agents absorbs the UV light through photochemical excitation and then releases it as a long wavelength radiation.

Sunscreen agents are typically formulated into lotions and creams as products. However, since the 1980s, consumer demand for more effective products that substantively beautify the appearance has resulted in increased basic science research and product development in cosmetic industry. This has eventually kick-started an increasing market for sunscreen agents. The use of sunscreen agents includes not only products such as lotions and shampoos, but also cosmetics that now carry SPF (Sun Protection Factor) values, including lipsticks, foundations and mascara. In early 2001, the sales of sunscreen active ingredients in the US and Western Europe was projected by Kline & Co. to be US$100 million each and will grow at about 4% annually through 2006. Organic sunscreens, was estimated to take account of 80% of the market as they are generally cheaper to produce and easier to formulate (Reisch 2001). In 2004, reports by market research company Freedonia Group Incorporation; stated that the United States demand for cosmetic and toiletry chemicals is expected to be near $7 billion by 2008 (Freedonia Group Incorporation, 2003).

In this work, feruloylated acylglycerols; a novel sunscreen agent was produced, through enzymatic reactions of ethyl ferulate and free fatty acids from olive deodorizer distillates using of Novozym® 435 lipases. Deodorizer distillates of olive oil were used as the source of free fatty acid due to its high oleic acid and squalene content. It also contains a substantial amount of phenolic compounds. Ethyl ferulate was chosen as the ferulate moiety donor, as it is a good antioxidant agent that is lipophilic and is able to melt at the common optimal reaction temperature of biocatalysts. This natural topical ingredient can help protect skin against free

radical-generated damage induced by UV light. Such natural agents may offer protection against photoaging, hyperpigmentation, and skin cancers.

In this work, the ferulic acid portion provides the UVA/UVB protection while the fatty acid portion improves substantivity. The use of biocatalysts in this reaction is both environmental-friendly and cheaper as it does not use solvents. The sunscreen agent produced from this method will be of natural origin, unlike the synthetic sunscreen agents that are currently flooding the market. In fact, the demand for natural personal care product in 2004 was reported to worth US$5 billion and skin care was the leading segment of the market at US$3 billion (Soapwire, 2005). It was speculated by Packaged Facts, a market research publisher, that by 2009, the natural personal care market will climb to US$7.9 billion. This new developed sunscreen agent has a wide application potential; as a sunscreen lotion, as natural personal care product and even as a cosmetic product.

In this study, the effects of various parameters on the yield of feruloylated acylglycerols (ferulate glycerol esters) were evaluated. The ferulate glycerol esters were then partially purified and its SPF (Sunscreen Protection Factor) value was tested through *in-vitro* analysis. The objectives of this study were:

1. to develop a suitable HPLC detection method for the quantification of the produced ferulate glycerol esters.
2. to study the effects of various reaction parameters on the yield of ferulate glycerol esters.

3. to purify the ferulate glycerol esters through suitable partial purification processes
4. to determine SPF value of the final ferulate glycerol esters mixture.