UNIVERSITI PUTRA MALAYSIA

FORMULATION OF RICE BRAN OIL NANOCOSMECEUTICAL LOTION LOADED WITH GERMINATED BROWN RICE EXTRACT

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By

SITI HAJAR BINTI SAHARUDIN

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

December 2015

DEDICATED TO:

My beloved family:

Abah & Mak, Angah, Amin, Kakak and Adik

For the understanding, encouragement, unconditional love

and support throughout the course of this work.

Thank you for believing in me.

Along loves you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

FORMULATION OF RICE BRAN OIL NANOCOSMECEUTICAL LOTION LOADED WITH GERMINATED BROWN RICE EXTRACT

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SITI HAJAR SAHARUDIN

December 2015

Chairmain: Assoc. Prof. Zalinah Ahmad, PhD

Faculty: Institute of Bioscience

Synthetic active compounds have been widely incorporated in cosmetic products all over the world due to its low price as well as it is easy to earn. Despite its advantages, the use of synthetic compounds in a long time could affect consumers' health. As an alternative, natural active compounds can be used since it is safe and good for health. The aim of this study is to formulate a natural plant based nanoemulsion which consist of rice bran oil (RBO) and germinated brown rice (GBR). RBO is oil that has been extracted from bran layer of rice kernel while GBR is brown rice that has undergone a germination process. Both RBO and GBR consist of compounds that could be a good antioxidant agent and excellent cosmeceutical supplement such as vitamin E, γ-aminobutyric acid (GABA), γ-oryzanol, and phenolic compounds.

Phase diagram was constructed to determine the composition that will be used for nanoemulsion formulation. Three different types of non-ionic surfactants were tested to identify its ability to form stable system. The surfactants were polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monooleate (Tween 80), and polyoxyethylene sorbitan trioleate (Tween 85). These surfactants are widely used in topical products due to their excellent safety, effectiveness levels, and economical cost. The results showed that the system containing Tween 80 has larger isotropic region compared to Tween 20 and Tween 85 system. Thus, Tween 80 was selected as the surfactant for further study.

The composition selected from Tween 80 ternary phase diagram has been modified by adding different concentrations of xanthan gum (0.2%, 0.4%, 0.6%,

0.8%, and 1.0%). The droplet size and pH of the formulations did not affected by the xanthan gum concentration. However, increasing the concentration of xanthan gum has increased the zeta potential, stability and rheological properties of the formulations. Consequently, xanthan gum plays a vital role in improving the physicochemical properties of the formulations.

Three formulations were formulated with different concentrations of GBR extract (0%, 2% and 4%). The droplet size of the formulations decreased when GBR extract concentrations were increased. Meanwhile, increase in GBR extract has increased the zeta potential and stability of the formulations. The pH ranges of the formulations were in the skin's pH range and all formulations have shear thinning behaviour. The study has found that GBR extract has an essential role in enhancing the characteristics of the formulations.

Increase in GBR extract concentration in the formulations has increased the total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of the formulations. All formulations did not exhibit skin irritation potential. Additionally, all formulations possessed low skin lightening efficiency. Formulation without GBR extract did not have anti-inflammatory effect. Whereas formulations with 2% and 4% of GBR extract possesses low anti-inflammatory effect. Besides that, all formulations did not have anti-microbial properties.

Formulation loaded with 4% of GBR extract was better compared to two other formulations in terms of it characteristic and cosmeceutical efficacy. As the conclusion, formulations modified with xanthan gum and loaded with GBR extract have been successfully formulated and characterized. Their potential cosmeceutical efficacies have also been profiled. However, additional *in vitro* and *in vivo* studies are recommended in order to further validate other potential cosmeceutical benefits of the RBO nanoemulsion and GBR extract on human skin.

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

FORMULASI LOSYEN NANOKOSMESEUTIKAL MENGGUNAKAN MINYAK DEDAK BERAS DAN DITAMBAH DENGAN EKSTRAK BERAS PERANG CAMBAH

Oleh

SITI HAJAR SAHARUDIN

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Pengerusi: Prof. Madya Zalinah Ahmad, PhD

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Sebatian aktif sintetik telah banyak digunakan di dalam produk kosmetik di seluruh dunia kerana harganya yang rendah dan ia mudah untuk didapati. Disebalik kelebihannya, penggunaan sebatian sintetik dalam jangka masa yang lama boleh menjejaskan kesihatan pengguna. Sebagai alternatif, sebatian aktif semulajadi boleh digunakan kerana ia adalah selamat dan baik untuk kesihatan. Tujuan kajian ini adalah untuk menghasilkan nanoemulsi semulajadi berasaskan tumbuhan yang terdiri daripada minyak dedak beras (RBO) dan beras perang cambah (GBR). RBO adalah minyak yang telah diekstrak daripada lapisan bran pada isirung beras manakala GBR adalah beras perang yang telah melalui proses percambahan. Kedua-dua RBO dan GBR mengandungi sebatian yang boleh menjadi agen antioksidan yang baik dan bahan tambahan kosmeseutikal yang sangat baik contohnya vitamin E, asid γ-aminobutirik (GABA), γ-*oryzanol*, dan sebatian fenolik.

Gambar rajah fasa telah dibina untuk menentukan komposisi yang akan digunakan bagi penghasilan nanoemulsi. Tiga jenis surfaktan bukan ionik telah diuji untuk mengenal pasti keupayaannya membentuk sistem yang stabil. Surfaktan-surfaktan tersebut adalah polioksietilena sorbitan monolaurat (Tween 20), polioksietilena sorbitan monooleat (Tween 80), dan polioksietilena sorbitan trioleat (Tween 85). Surfaktan-surfaktan ini digunakan secara meluas di dalam produk topikal kerana ciri-ciri keselamatannya yang bagus, keberkesanannya, dan harga yang berpatutan. Hasil kajian menunjukkan bahawa sistem yang mengandungi Tween 80 mempunyai kawasan isotropik yang lebih besar berbanding dengan sistem Tween 20 dan Tween 85. Oleh itu, Tween 80 telah dipilih sebagai surfaktan untuk kajian seterusya.

Komposisi yang dipilih daripada gambar rajah fasa Tween 80 telah diubah suai dengan menambah kepekatan gam xantan yang berbeza (0.2%, 0.4%, 0.6%, 0.8%, dan 1.0%). Saiz titisan dan pH formulasi tidak dipengaruhi oleh kepekatan gam xantan. Walau bagaimanapun, peningkatan kepekatan gam xantan telah meningkatkan potensi zeta, kestabilan dan sifat-sifat reologi bagi formulasi. Oleh itu, gam xantan memainkan peranan penting dalam meningkatkan sifat-sifat fizikokimia bagi formulasi.

Tiga rumusan telah dihasilkan dengan kepekatan ekstrak GBR yang berbeza (0%, 2% dan 4%). Saiz titisan formulasi berkurang apabila kepekatan ekstrak GBR meningkat. Sementara itu, peningkatan kepekatan ekstrak GBR telah meningkatkan potensi zeta dan kestabilan formulasi. Julat pH bagi formulasi berada di dalam julat pH kulit dan semua formulasi mempunyai sifat pencairan ricihan. Kajian telah mendapati ekstrak GBR mempunyai peranan penting dalam meningkatkan ciri-ciri formulasi.

Peningkatan kepekatan ekstrak GBR di dalam formulasi telah meningkatkan jumlah kandungan fenolik (TPC) dan kuasa antioksida penurun ferik (FRAP) bagi formulasi. Semua rumusan tidak menunjukkan potensi perengsaan kulit. Disamping itu, semua formulasi memiliki kecekapan pencerahan kulit yang rendah. Formulasi tanpa ekstrak GBR tidak mempunyai kesan anti-radang. Manakala formulasi yang mengandungi 2% dan 4% ekstrak GBR mempunyai kesan anti-radang yang rendah. Selain itu, semua formulasi tidak mempunyai ciri-ciri anti-mikrob.

Formulasi yang ditambah dengan 4% ekstrak GBR adalah lebih baik berbanding dengan dua rumusan lain dari segi ciri-ciri dan efikasi kosmeseutikal. Sebagai kesimpulan, formulasi yang diubah suai dengan gam xantan dan ditambah dengan ekstrak GBR telah berjaya dihasilkan dan diuji ciri-cirinya. Potensi efikasi kosmeseutikalnya juga telah diukur. Walau bagaimanapun, kajian *in vitro* dan *in vivo* tambahan adalah disyorkan untuk mengesahkan lebih lanjut potensi kosmeseutikal lain bagi nanoemulsi RBO dan ekstrak GBR pada kulit manusia.

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I certify that a Thesis Examination Committee has met on (30 December 2015) to conduct the final examination of Siti Hajar Binti Saharudin on her thesis entitled “Formulation of Rice Bran Oil Nanocosmeceutical Lotion Loaded With Germinated Brown Rice Extract” in accordance with the Universities and University College 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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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of Supervisory Committee were as follows:

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LISTS OF ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
°C	Degree celcius
EDTA	Ethylene-diamine-tetraacetic acid
$FeCl_3.6H_20$	Iron(III) Chloride Hexahydrate
G	Gram
GAE	Gallic acid equivalent
GABA	Gamma-aminobutyric acid
GBR	Germinated brown rice
H	Homogenous region
HCl	Hydrochloric acid
HIE	Human Irritancy Equivalent
HLB	Hydrophilic Lipophilic Balance
k	Consistency index
L	Isotropic region
M	Multiphase region
mL	Milliliter
Mm	Millimetre
mV	Millivolt
n	Flow behaviour index
Nm	Nanometer
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
Pa	Pascal
Pa s	Pascal second
RBO	Rice bran oil
ROS	Reactive Oxygen Species
TPTZ	2,4,6-Tripyridyl-s-Triazine
Tween 20	Polyoxyethylene sorbitan monolaurate
Tween 80	Polyoxyethylene sorbitan monooleate
Tween 85	Polyoxyethylene sorbitan trioleate
UV	Ultraviolet
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water
w/w	Weight per weight
Ƞ	Viscosity
Ý	Shear rate
ᴛ	Shear stress
ᴛ₀	Yield stress

CHAPTER 1

INTRODUCTION

Nanoemulsions consist of two immiscible liquids stabilized by surfactant and possess droplet sizes ranging from 20 nm to 500 nm (Rezaee et al., 2014). However, Lovelyn and Attama (2011), Shah et al. (2010), and Windhab et al. (2005)stated that nanoemulsions droplet size could range from 50 nm to 1000 nm. Nanoemulsions have increasingly been used in bioactive delivery process for medical and cosmetic applications due to its unique properties. The droplets of nanoemulsion penetrate through the skin surface efficiently due to their small size and thus are suitably employed for delivery of active ingredients to the skin (Sonneville-Aubrun et al., 2004). The tiny droplet size can stand the physical destabilization caused by gravitational separation, flocculation and/or coalescence (Bernardi et al., 2011). In addition, nanoemulsion only requires low surfactant concentration compared to microemulsion (Bouchemal et al., 2004). In general, formation of nanoemulsion is started by construction of ternary phase diagram. Ternary phase diagram is a method used to study the phase behaviour of a system consisting of three substances such as water, oil, and surfactant. It is a way to express droplets interactions on a molecular level. The basic fundamental purpose of phase diagram construction is to mix up the components and monitor the number and nature of the phases resulted from the mixture (Pillai and Shah, 1996).

Cosmeceutical is a hybrid word comes from two words which are cosmetic and pharmaceutical. As the name given, cosmeceutical products have two functions; to beautify the skin and enhance skin's health (Teo et al., 2010). Most cosmetic ranges usually offer to beautify the skin without any health benefit. Recently, cosmeceutical products are arising in demand by consumers and the skin care market has grown rapidly, especially for products that are meant to prevent and treat aging skin (Hyde et al., 2010). Among the products that have been created as cosmeceutical regime include skin moisturizers, perfumes, lipsticks, shampoos, toothpastes, and deodorants.

Rice is one of the most important staple food crops in the world and it is a major source of carbohydrates for over half the world's population (Ohtsubo et al., 2005).There are various types of rice and one of the examples is brown rice. Brown rice is an un-milled rice grain which its husk has been removed. In recent times, the study on germinated rice has become a popular subject due to its advantages and health benefits. Brown rice has also been germinated. Germinated brown rice (GBR) is the result of brown rice that is allowed to germinate by soaking it in the water until the embryo begins to bud. The

nutrients content in brown rice change drastically higher during germination process. GBR is claimed to be rich in nutrients compared to the common white rice (Latifah et al., 2010). It is a good source of γ-oryzanol, tocopherols and tocotrienols, which are considered to be beneficial for health improvement and diseases prevention (Lilitchan et al., 2008). In addition to that, some nutrients are immensely increased in concentration during the germination process of brown rice. These nutrients are γ-aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, magnesium, potassium, zinc, and prolylendopeptidase inhibitor (Kayahara and Tukahara, 2000).

Rice bran oil (RBO) is an oil extracted from bran layer of rice kernel. RBO is used widely as cooking oil in several countries such as Japan, Korea, India, China and Indonesia (Kusum et al., 2011; Ghosh, 2007). Besides serving as a cooking agent, RBO is used as a bioactive supplement due to its high antioxidant value. As it is used both in cooking and as supplements, RBO is claimed to have favourable effects on human health and aesthetic value (da Silva et al., 2006;Danielski et al., 2005). It is rich in antioxidant compounds such as vitamin E and oryzanol which are valuable for cosmetic formulation (Lilitchan et al., 2008). γ-oryzanol is commonly used in sunscreen and anti-ageing products in cosmetic industry(Wu et al., 2013; Juliano et al., 2005). Therefore, it is believed that RBO could protect the skin from UV radiation and improves skin moisture (Vorarat et al., 2010).

Nowadays, people have realized that one of the most effective ingredients of cosmetics is antioxidant, which can interrupt the radical-chain processes, help the skin's natural repair systems, improve cell rejuvenation, and in a long run, may prevent skin-cancer (Guan et al., 2005).Cosmeceutical products that are commercially available not only enhance the appearance of the skin but also improve the health of the skin because of their antioxygenation properties. Synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were widely used in the skin care products.Unfortunately, long term and widespread studies indicate that the super scale use of synthetic antioxidants of cosmetic can result in potential health risks (Williams and Iatropoulos, 1996).As an alternative, natural antioxidants from plants are being used.

1.1 Problem Statement

Over the years, most cosmetics in the market have contained synthetic active ingredients to boost the effectiveness of the desired functions. The synthetic ingredients served as specific purpose mainly for whitening properties, anti-aging, anti-oxidant, and anti-inflammatory. These ingredients were used widely

around the world due to its good stability, longer shelf life, and cheaper than natural ingredients. However, synthetic materials are mostly dangerous for health and pose a threat to consumers if used for a long time for example hydroquinone, paraben, and polycyclic musks (Ocaña-gonzález et al., 2015; Gao and Legido-Quigley, 2011; Hutter et al., 2009).

Natural based cosmetics on the other hand are gentle on the skin than synthetic based cosmetics especially for people with sensitive skin or allergies. Natural based cosmetics are also great for health. RBO and GBR are examples of natural ingredients that come from plants and could be used in cosmetic formulations. In this study, RBO was used as a carrier for the GBR extract. The combination of RBO and GBR extract is expected to increase the antioxidant capacity and cosmeceutical benefits of the formulations.

Currently, there are very few published reports on the addition of GBR extract in cosmeceutical nanoemulsions as active ingredient. The GBR extract may change the properties of the nanoemulsions such as droplet size, zeta potential, and pH. Additional to that, it might affect the stability, rheology, and potential efficacy of the nanoemulsions system.

1.2 Significance of This Research

This study will be a beneficial subject for researchers and manufacturers on the selection of safe materials to be incorporated into cosmeceutical products. Furthermore, this research will study whether each material has an effect on the characteristics of the formulated products. Each material that will be added could possibly alter the stability, characteristics and efficacy of the products.

This study will be an important endeavour in promoting cosmeceutical advantages to consumers as it could enhance health as well as beautify the body part. By understanding the needs of the natural and safe skincare products, consumers will be more attracted to choose natural products over synthetic products.

1.3 Hypothesis of This Research

This study is designed to access the hypothesis that adding GBR extract in formulation will produce a stable nanoemulsion and improve the cosmeceutical efficacies.

1.4 Objectives of This Research

1.4.1 General Objective

This study aims to produce a stable cosmeceutical lotions enriched with germinated brown rice extract which have very small droplets in the nano size range and have good cosmeceutical efficacies.

1.4.2 Specific Objectives

1. To study the phase behaviour of rice bran oil emulsion.
2. To formulate rice bran oil nanoemulsions modified with xanthan gum and characterize the physicochemical properties of the nanoemulsion.
3. To formulate rice bran oil nanoemulsions loaded with germinated brown rice extract and characterize the physicochemical properties of the nanoemulsion.
4. To test the biocompatibility of the nanoemulsions and their potential cosmeceutical efficacy.

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