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DEVELOPMENT AND CHARACTERIZATION OF SWIFTLET NEST-BASED FORMULATION IN NANO-COSMECEUTICALS

SITI HUSNAA BINTI MOHD TAIB

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By

SITI HUSNAA BINTI MOHD TAIB

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

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SITI HUSNAA BINTI MOHD TAIB

July 2015

Chair : Siti Salwa Abd Gani, PhD
Faculty : Halal Products Research Institute

Purified swiftlet nest (SN) was used as an active ingredient in the development of nano-cosmeceuticals using homogenizer. The sample preparation method comprises cleaning, drying with freeze dryer and grinding of SN. Then the fine powder of SN was added to the formulation. Prior to addition of SN into the formulation, the compound present in raw SN and purified SN were identified and compared by Fourier Transform Infrared (FTIR) spectroscopy analysis, nitrite test, protein analysis and moisture analysis.

One of the major nutrients in SN is protein. The protein content in purified SN (60.1%) higher than raw SN (57.5%) which is differs significantly at 95% confidence level. This was due to “Freeze-Drying" method that was carried out by low operating temperature. This process leads to minimal damage of the heat-sensitive materials. Hence, protein is able to be preserved and enhanced. The study showed raw SN has low concentration of nitrite (0.25 ppm) whereas purified SN devoid of nitrite. Making frequent water changes during the process of cleaning SN was able to reduce nitrite as nitrite is highly soluble in water. The FTIR results indicated that the main compounds present in the SN samples were carbohydrates and protein. Moisture content of purify SN should be less than 10%. For this study, moisture content of the purified SN (7.45%) was slightly higher from its raw SN (5.62%).

The formulations consisted of oil in water emulsions. In manufacturing cosmetics, formulation and optimization processes are two important issues. In this work, Response surface methodology (RSM) was utilised in order to investigate the influence of the nano-cosmeceuticals composition; purified SN (1–5% w/w), and Tween 80 (3-6% w/w) as well as the preparation method; time of homogenization (10–30 min), on the physicochemical properties of swiftlet nest-based nano-cosmeceuticals. The response variables were particle size and zeta potential which are very important characteristic in nano-cosmeceuticals. Formulation and optimization of three independent variables were carried out to obtain an optimum SN-based nano-cosmeceutical with the lowest particle size and high stability formulation.

After the optimization process, formulation coded as SN25 was selected as the best formulation with the particle size of 136.35 nm and zeta potential of -40.2 mV. This was obtained experimentally and was closer to the predicted value 136.22 nm and -40.07 mV, respectively. Formulation SN25 was determined to be 2.58% SN, 3.99% Tween 80, 90.03% deionized water and 3.4% other ingredients, with a time of homogenization of 17 min. The pH value of optimized formulation was 6.49. The formulation was stable after undergoing thaw cycles test, at room temperature and 45°C for three months.

The rheological property of optimized formulation was investigated using rheometer. The viscosity of the optimized formulation was found to decrease with the increase in the applied shear rate. Thus, these types of formulation could be categorized as pseudoplastic or shear thinning material. The optimized formulation was found to be non-irritating with a Human Irritancy Equivalent (HIE) score below 0.90. Potassium sorbate used as preservative in the formulations successfully prevented microbial growth. The inhibition zone activities of formulation showed inhibitions bacteria *Methicillin Resistant Staphylococcus aureus* (MRSA) and yeast *Candida albicans*.

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

PEMBANGUNAN DAN PENCIRIAN FORMULASI BERASASKAN SARANG BURUNG WALIT DALAM NANO-KOSMESEUTIKAL

Oleh

SITI HUSNAA BINTI MOHD TAIB

July 2015

Pengerusi : Siti Salwa Abd Gani, PhD
Fakulti : Institut Penyelidikan Produk Halal

Sarang burung walit (SN) yang tulen (sudah dibersihkan) telah digunakan sebagai bahan aktif dalam pembangunan nano-kosmeseutikal menggunakan mesin pengsebati. Kaedah penyediaan sampel terdiri daripada pembersihan, pengeringan dengan pengering sejuk beku dan pengisaran SN. Kemudian serbuk halus SN telah ditambah kepada formulasi. Sebelum penambahan SN ke dalam formulasi, sebatian yang hadir dalam SN mentah dan SN yang tulen (dibersihkan) telah dikenal pasti dan dibandingkan dengan analisis spektroskopi Inframerah Transformasi Fourier (FTIR), ujian nitrit, analisis protein dan analisis kelembapan.

Salah satu komponen nutrien utama dalam SN adalah protein. Kandungan protein dalam SN tulen (60.1%) lebih tinggi daripada SN mentah (57.5%) yang berbeza dengan ketara pada 95% tahap keyakinan. Ini adalah kerana pengeringan sejuk beku yang telah dijalankan dengan suhu operasi yang rendah. Proses ini membawa kepada kerosakan yang minima bagi bahan-bahan sensitif haba. Oleh itu, protein mampu dikekalkan. Kajian ini menunjukkan SN mentah mengandungi kepekatan nitrit yang rendah (0.25 ppm) manakala SN yang tulen tidak mengandungi nitrit. Pertukaran air dengan kerap semasa proses pembersihan SN boleh membantu mengurangkan nitrit kerana nitrit adalah sangat larut dalam air. Keputusan FTIR menunjukkan bahawa sebatian utama yang hadir dalam sampel SN adalah karbohidrat dan protein. Kandungan lembapan SN yang tulen mestilah kurang daripada 10%. Untuk kajian ini, kandungan lembapan SN yang dibersihkan (7.45%) adalah lebih tinggi sedikit daripada SN mentah (5.62%).

Formulasi terdiri daripada emulsi minyak dalam air. Dalam pembuatan kosmetik, proses formulasi dan pengoptimuman adalah dua perkara penting. Dalam kajian ini, Kaedah Permukaan Respons (RSM) telah digunakan untuk mengkaji kesan komposisi nano-kosmeseutikal; SN ditulen (1-5% w/w), dan Tween 80 (3-6% w/w) serta kaedah penyediaan; masa homogenisasi (10-30 min), ke atas sifat-sifat fizikokimia nano-kosmeseutikal berasaskan sarang burung walit. Pembolehubah tindak balas ialah saiz zarah dan potensi zeta (kestabilan) yang merupakan ciri yang sangat penting dalam nano-kosmeseutikal. Formulasi dan pengoptimuman tiga pembolehubah bebas telah dijalankan untuk mendapatkan tahap optimum nano-kosmeseutikal berasaskan sarang

burung walit dengan saiz zarah yang paling rendah dan kestabilan formulasi yang tinggi.

Selepas proses pengoptimuman, formulasi dikodkan sebagai SN25 telah dipilih sebagai formulasi terbaik dengan saiz zarah sebanyak 136.35 nm dan potensi zeta sebanyak -40.2 mV. Ini diperolehi secara eksperimen dan adalah hampir kepada nilai 136.22 nm dan -40.07 mV, masing-masing. Formulasi SN25 telah ditentukan sebagai 2.58% SN, 3.99% Tween 80, 90.03% air ternyahion dan 3.4% bahan-bahan lain, dengan masa homogenisasi 17 min. Nilai pH formulasi yang optimum adalah 6.49. Formulasi adalah stabil selepas menjalani ujian kitaran mencair, pada suhu bilik dan 45°C selama tiga bulan.

Ciri reologi formulasi yang optimum telah dikaji menggunakan reometer. Kelikatan formulasi yang optimum didapati menurun dengan peningkatan kadar ricih yang digunakan. Oleh itu, jenis formulasi boleh dikategorikan sebagai pseudoplastik atau bahan penipisan ricih. Formulasi yang optimum didapati tidak merengsa dengan nilai Padanan Kerengsaan Manusia (HIE) kurang daripada 0.90. Kalium sorbat yang telah digunakan sebagai pengawet dalam formulasi berjaya menghalang pertumbuhan mikrob. Aktiviti zon perencatan formulasi jelas menghalang bacteria *Methicillin Resistant Staphylococcus aureus* (MRSA) dan yis *Candida albicans*.

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

Siti Salwa Abd Gani, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Amin Ismail, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohamad Zaki Ab Rahman, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

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Name of Chairman of Supervisory Committee: Dr. Siti Salwa Abd Gani

Signature: _____
Name of Member of Supervisory Committee: Prof. Dr. Amin Ismail

Signature: _____
Name of Member of Supervisory Committee: Prof. Dr. Mahiran Basri

Signature: _____
Name of Member of Supervisory Committee: Assoc. Prof. Dr. Mohamad Zaki Ab Rahman

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Central Composite Design
CDC	Centers for Disease Control and Prevention, USA
cm	centimetre
FTIR	Fourier Transform Infrared
HIE	Human irritancy equivalent
ICD	irritant contact dermatitis
min	minute
mL	mililiter
MRSA	Methicillin Resistant Staphylococcus Aureus
nm	nano-meter
mV	millivolt
OECD	Organisation for Economic Cooperation and Development
o/w	Oil in water
Pa	Pascal
Pa.s	Pascal second
PIT	Phase Inversion Temperature
PPO	1,2-dipalmitoyl-3-oleoyl-rac-glycerol
PRE	formulation with addition of preservative
rpm	revolution per minute
RSM	Response Surface Methodology
SA	Sialic acid
SN	Swiftlet Nest
SN25	optimized formulation
Tween 80	Polyoxyethylene (20) sorbitan mono-oleate
w/o	Water in oil
w/w	weight/weight

CHAPTER 1

INTRODUCTION

The term cosmetic, which is used on a daily basis, only places emphasis on outward appearance. Currently, medical cosmetic products which are termed dermaceuticals or cosmeceuticals are in high demand. Furthermore, cosmeceuticals are committed to enhancing and maintaining the long-term function of the skin. Nano-cosmeceuticals are the strongest generation of cosmetic products which use nano-sized systems for the delivery of active ingredients to the skin cells for better penetration. They are subjected to particular manufacturing techniques and contain lower concentrations of surfactant as compared to classical skin care cosmetics (Harwansh *et al.*, 2011).

Recently, research about flora and fauna has been undertaken which might prove beneficial to the human body. Some of them are being used as active ingredients in new cosmetics. One of the fauna that was found to have a good value is swiftlet nest (SN). Several species of swiftlet produce edible nests that are consumed by humans worldwide, as a delicacy or as a medicinal food. There have been a number of studies conducted on the advantages of SN in cosmetics, food and medicine (Set, 2012; Norhayati *et al*., 2010). The main component of the SN is glycoprotein. The existence of glycoprotein is capable of promoting cell division, and it has demonstrated the presence of an epidermal growth factor-like protein (Kong *et al*., 1987; Ng *et al*., 1986). SN has good effect on the skin and becomes attractions to ladies due to its properties of making the skin delicate and grows radiant. Besides, some people believe that a pregnant woman who consumes SN would have a baby with fine and smooth complexion. Although the importance of the SN has provided a high potential in new nano-cosmeceuticals area, research in this area is still limited as it is commonly consumed, not being applied to the skin. Hence, encourage us to develop new formulation of SN-based nano-cosmeceuticals with smaller molecules which are formulated to penetrate deep into the skin.

Formulation and optimization processes are two important issues in the manufacturing of cosmetics. The process of optimizing parameters not only increases the utility of the technologist, but also the quality of the product as well. Since, the lack of any model to optimize the use of the SN in nano-cosmeceuticals formulation which led this study to find an optimal formulation of SN-based nano-cosmeceuticals with nano particle size using response surface methodology (RSM). The RSM appears to be an effective and simple technique of analyzing, improving and optimizing of structures over practicable domain of parameter settings (Shokuhfar *et al*., 2008). RSM is the most popular optimization method used in recent years (Chellamboli and Perumalsamy, 2014; Ryad *et al*., 2010; Baş and Boyacı, 2007). Several studies have been investigated based on the application of the RSM in industrial processes. This method is a set of mathematical and statistical techniques in which a response of interest is influenced by several variables and the aim is to optimize this response by determining the correlation between the response and independent variables (Raissi and Farsani, 2009).

The benefits of SN in nano-cosmeceuticals were investigated. The use of local natural resources in nano-cosmeceuticals can be established. Furthermore, the usage of SN as nano-cosmeceuticals can be developed where it can open up new areas of nano-biotechnology exploitation, which leads to the necessity to extract and process this SN.

General objective of this study is to develop new formulation of nano-cosmeceuticals containing SN using RSM. The specific objectives are:

1 To evaluate the characteristics of SN by nitrite test, Fourier Transform-Infrared (FTIR), protein analysis and moisture analysis.
2 To develop new formulation and optimize the formulation of SN-based nano-cosmeceuticals using RSM.
3 To characterize the properties of formulation with respect to particle size, zeta potential, pH measurement, stability study and rheology study.
4 To assess the safety such as antimicrobial and irritancy test of the optimized formulation.

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