FORMULATION OF ANTI-AGING CREAM FROM KERATIN PROTEIN

NURHAMIZAH BINTI AHMAD ZAINI

A Thesis submitted in fulfilment of
the requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

ACKNOWLEDGEMENTS

It gives me great pleasure in expressing my gratitude to all those people who have supported me and had their contributions in making this thesis possible. First and foremost, I must acknowledge and thank The Almighty Allah for blessing, protecting and guiding me throughout this period. I could never have accomplished this without the faith I have in the Almighty. This final year project has been a wonderful experience as well as a great challenge for me. But it is of course a wonderful memory to cherish for a life time.

I express my profound sense of reverence to my supervisor, Dr. Arun Gupta for his constant guidance, support, motivation and untiring help during the course of my degree. I will forever grateful for his guidance and willingness to listen to all my problems faced during the projects progress. The freedom he gave me to do any amendments to the project as long as it's yield a progress is the most important and wonderful thing that brought success for this project.

I also owe big thanks to all the lecturers, lab engineers and my friends. Without their guidance, I would never complete this project. Their training, advises and guidance will I apply all the time to gain a success in the field I will involve.

FORMULATION OF ANTI-AGING CREAM FROM KERATIN PROTEIN

ABSTRACT

Ageing is the phase where the body efficiency and metabolic activities decline after reaching maturity stage. The presence of free radical can cause alterations in collagen and make changes to membrane features. A large quantity of chicken feather is available as a waste product in Malaysia. Chicken feathers have high percentage of keratin protein content and can be a suitable protein source. Research was conducted using the keratin protein as the main component for the formulation of anti-aging cream. The difference between the formulations was on the concentration of keratin protein and the chemical that are used. The process involved are first dissolved the chemical in aqueous phase and heated up at 70°C. Then, the other component was mixed and heated up at 70°C. The oily phase was added to the aqueous phase and pre-emulsified. All the mixture was emulsified with homogenizer for the uniform mixing. The analysis by FTIR showed the presence of carboxyl, alcohol and amino groups. Clinical trial was done on rabbit for toxicology test. As conclusion, anti-aging cream can be produced from the keratin protein. The anti-aging cream was undergoing another analysis to check whether it is effectiveness.

FORMULASI KRIM ANTI-PENUAAN DARIPADA PROTEIN KERATIN

ABSTRAK

Penuaan adalah fasa di mana kecekapan badan dan penurunan metabolik aktiviti-aktiviti selepas mencapai peringkat kematangan. Kehadiran radikal bebas boleh menyebabkan perubahan dalam kolagen dan membuat perubahan kepada ciri-ciri membran. Satu kuantiti besar bulu ayam adalah disediakan sebagai produk sisa di Malaysia. Bulu ayam mempunyai peratusan yang tinggi kandungan protein keratin dan boleh menjadi sumber protein yang sesuai. Penyelidikan telah dijalankan menggunakan protein keratin sebagai komponen utama bagi penggubalan krim anti-penuaan. Perbezaan antara rumusan adalah pada kepekatan protein keratin dan bahan kimia yang digunakan. Proses yang terlibat adalah dalam fasa akueus yang dipanaskan sehingga suhu 70°C. Kemudian, komponen lain telah dicampur dan dipanaskan sehingga suhu 70°C. Fasa berminyak telah ditambah kepada fasa akueus dan pra-beremulsi. Semua campuran telah diemulsikan dengan homogenizer untuk pencampuran seragam. Analisis oleh FTIR menunjukkan kehadiran alkohol carboxyl, dan kumpulan amino. Percubaan klinikal telah dilakukan pada arnab untuk ujian toksikologi. Sebagai kesimpulan, anti-penuaan krim boleh dihasilkan daripada protein keratin. Krim anti-penuaan telah menjalani analisis lain untuk memeriksa sama ada ia adalah berkesan.

TABLE OF CONTENTS

CHAPTER 3 METHODOLOGY

CHAPTER 4 RESULT AND DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percent
g	Gramme
A	Absorbance
mPas	Milipascal Seconds
cm^{-1}	Recipcrocal centimeter
kg^{-1}	Recipcrocal kilogramme
α	Alpha
β	Beta
°C	Degree celcius

LIST OF ABBREVIATIONS

FTIR	Fourier Transform Infrared Spectroscopy
Wt	Weight
VCO	Virgin coconut oil
SIRIM	Standard Industrial Research Institute of Malaysia
IIUM	International Islamic University Malaysia
IR	Infrared
UV	Ultraviolet
pH	Potential hydrogen
RPM	Rotation per minutes

CHAPTER 1

INTRODUCTION

1.1 Research background

This research is about the production of personal care formulations using the keratin protein from chicken feathers and their use in cosmetics. Feathers are generated in large quantities by poultry processing industries and their accumulation in nature could lead to environmental problems (Mazotto et al. 2010). Currently there is an increasing interest in the development of materials that are environment friendly, obtained from renewable resources. The main renewable resources are obtained from polysaccharides, lipid and proteins.

Proteins are polymers formed by various amino acids capable of promoting-intra and inter-molecular bonds, allowing the resultant materials to have a large variation in their functional properties. Proteins also known as polypeptides that made of amino acids. The amino acids in a polymer joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues.

Several proteins have been investigated in the development of naturally-derived biomaterials, including collagen, albumin, gelatin, fibroin and keratin. Of these, keratin-based materials have shown promise for revolutionizing the biomaterial world due to their intrinsic biocompatibility, biodegradability, mechanical durability and natural abundance (Rouse and Dyke, 2010).

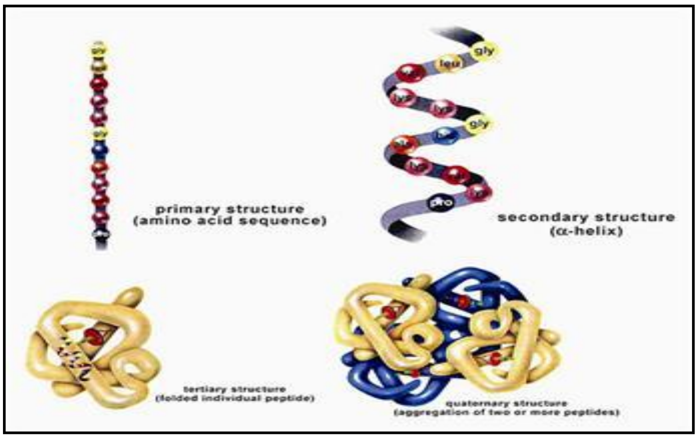

Figure 1.1 The different structures of protein.

(Source: www.G-Biosciences.com)

Feather keratin has been widely studied for use as a bio-based material. It has a characteristic of tough, high modulus, axially oriented composite material of microfibrils within an amorphous matrix, containing β-sheet crystallites and is highly crosslinked by virtue of 7 mol % cycteine. Feather keratin has mechanical strength similar to wool, and is likely the most abundant form of hard keratin in nature. An estimated 5 million tonnes of feathers are produced annually from a reliable production pipeline as a by-product of chicken meat farming, making it a dependable bio-polymer feedstock. Poultry feathers contain about 90% proteins and cheap also renewable source for protein fibers. The secondary structures of the feathers, the barbs are in fibrous form and could be a potential source as protein fibers.

More than 4 billion pounds of chicken feathers are produced in the world every year. There are about 50% of the weights of the feathers is barbs and the other 50% is rachis. Even assuming that 20% of the barbs have lengths greater than 1 inch required for textile applications, about 400 million pounds of barbs will be available as natural protein fibers every year. This means an availability of 8% of the protein fibers consumed in the world every year. Since the two natural protein fibers wool and silk are relatively expensive fibers, using the low cost barbs as protein fibers will make many protein fiber products to be economical and also add high value to the feathers (Reddy and Yang,2007).

Keratin originally referred to the broad category of insoluble proteins that associate as intermediate filaments (Ifs) and from the bulk of cytoplasmic epithelia and epidermal appendageal structures. According to (Rouse & Dyke, 2010) subsequent research of these structural proteins led to the classification of mammalian keratins into two distinct groups that based on their structure, function and regulation. The two groups are hard keratin and soft keratin. Hard keratins means ordered arrays of intermediate filaments that embedded in the matrix of cystine rich proteins and contribute to the though structure of epidermal appendages. While soft keratins usually prefer form of loosely-packed bundles of cytoplasmic intermediate filaments and endow mechanical resilience to epithelial cells. In 2006, Schweizer et.al developed a new consensus nomenclature for hard and soft keratins to accommodate the functional genes and pseudogenes for the full complement of human keratins. This system classifies the 54 functional keratin genes as either epithelial or hair keratins. The characteristics of keratin are insoluble in water, weak acids and bases, as well as in organic solvents. It also has been widely studied for use as a bio-based material because is a tough, high modulus, axially oriented composite material of microfibrils within an amorphous matrix that containing β-sheet crystallites. The amino-acid content of keratin is characterized by a high cysteine content which may change within 2% wt and 18% wt which have a significant amount of hydroxyamino-acids, especially serine (about 15% wt) and a lack of hydroxyproline and hydroxylisine, among other substances. The chemical activity of keratin is connected in a significant degree to the cystine content. The disulphide bond which is formed between two cysteine molecules is responsible for the high strength of keratin and its resistance against the action of proteolitic enzymes. On the other hand, keratin is very reactive, as cystine can easily be reduced, oxidised, and hydrolysed.

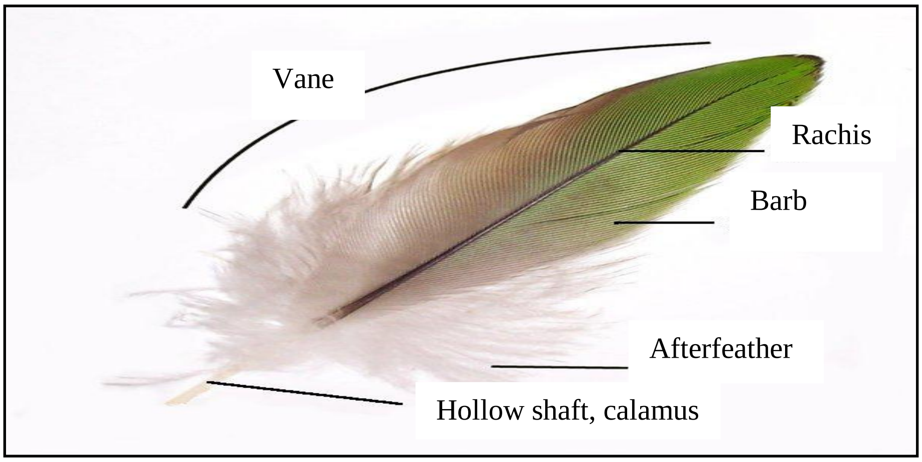

Figure 1.2 Important parts of a feather

(Source: http://commons.wikimedia.org/wiki/File:Parts_of_feather.jpg)

Furthermore, skin which covers the entirety of human and other animal bodies is affected by several changes including wrinkle formation, hardening, spot formation, loss of color, reduced elasticity and the like as a result of external factors such as sunlight, dryness, oxidation, environment stress as well as aging. Actually, skin is largely composed of two layers which are the epidermis and the dermis. A thin, fine layer known as the basement membrane is found between the epidermis and dermis. Metabolism of the epidermis is dependant on a supply of blood and factors produced by dermal cells through the basement membrane. Growth and differentiation of the skin epidermis is regulated by the basement membrane and dermis. The communication between the dermis and dermis are via basement membrane was plays an important role in regulating the function of the skin epidermis. Anti-aging cream is formulated to increase the elasticity of skin after aging.

1.2 Problem Statement

i. Increasing demand for keratin based product such as anti-aging cream.
ii. High cost of production of keratin protein from sheep wool.
iii. To use the chicken feather for better purpose.
iv. High demand for natural product based anti-aging cream.
v. High cost of the existing cream.

1.3 Research Objectives

The objectives of this research listed as below:

i. Formulation of anti-aging cream from keratin protein produced by chicken feathers.
ii. To use more natural chemicals or less toxic chemicals.

iii. To prepare the anti-aging cream by using different concentration of keratin protein and analyze the research product obtained.

1.4 Scope of Study

This study has several scopes in order to achieve the above mentioned objectives which are the production of anti-aging cream by using different concentration of keratin protein and modifying the existing formulation. This study conducted to find the effect of keratin protein on the properties of cream. Then, the research product obtained will be testing by using toxicology test; centrifuge testing, viscosity testing and Fourier transform infrared spectroscopy.

1.5 Significance of Proposed Study

Chicken feathers are easy to find in Malaysia. in order to reduce the environmental problem, keratin protein can be extracted from chicken feathers to formulate the new product. This study will provide better understanding for the production of personal care products from keratin protein as active pharmaceutical ingredients (API) and hence analyze the research product obtained by doing the testing. This product is formulated from a safer chemical that can protect the human skin. The product must be safe to use for human being and not harmful to their skin. The testing should be done properly so that the product will be used for human skin and prevent their skin from aging. This study also will develop a new product that gives benefit to environment and society.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The review of literature of this research consists of five sections that are related to the research. The first section is introduction of protein, the second section is about the feathers, the third section is keratin protein in feathers, the forth is introduction of skin and the last is formulation of anti-aging.

2.2 Protein

In living organism's body, protein acts as important role in keeping living things alive and healthy. The function of protein for the growth and repair of muscles, bones, skin, tendons, ligaments, hair, eyes and other tissues is proven since a very long time. One would lack the enzymes and hormones needed for metabolism, digestion and other important processes if their lack of protein. Natural proteins purified from natural sources. It is highly purified for use in a molecular biology and immunology researches. Natural proteins quickly were considered useful ingredients for creating a suitable environment for healthy skin and hair because of their ability to bind water with the horny layer of skin and its annexes. Most protein derivatives that are used for cosmetic purposes are obtained from simple proteins, whereas conjugated proteins are used far less frequently.

Kelly and Reddick-Lanzilotta, 2006 stated that proteins and their derivatives are used in a wide range of personal care of formulations, including those intended for use on the hair, skin and nails. As a component of personal care formulations, proteins perform many functions, including conditioning, film forming, as a humectant and an emollient. Most protein that common used is proteins hydrolysed in order to impart sufficient solubility to facilitate inclusion in a formulation. This in particularly the case with keratin proteins, which are inherently insoluble due to the crosslinks associated with the characteristically high degree of cysteine present in the protein.

Kelly and Reddick-Lanzilotta, 2006 stated that disclose the use of hydrolysed proteins and their derivatives, particularly those with high sulfur content, in formulations to protect hair from the insults of environmental and chemical damage. A combination of hydrolysed proteins and a polyamino cationic agent is used to prepare the desired formulations. (Bore et.al, 1990) stated that S-sulphocysteine keratin peptide produced by enzymatic hydrolysis for use as an auxiliary in the dyeing of wool and hair. Enzymatic digestion is used to prepare low molecular weight peptides and achieved the desired solubility.

Figure 2.1 Normal protein sources.

(Source: http://www.bodycare-tips.com)

2.3 Feather

Feathers are among the most complex integumentary appendages found in vertebrates and are formed in tiny follicles in the epidermis, or outer skin layer, that produce keratin proteins. The β- keratins in feathers, beaks and claws are composed of protein strands hydrogen-bonded into β-pleated sheets, which are then further twisted and crosslinked by disulfide bridges into structures even tougher than the α-keratins of mammalian hair, horns and hoof. Mukesh Kumar et al, 2012 show amino acids present in feathers. (Table 2.1)

Table 2.1 Amino acids found in feather protein

Amino Acid	Feather
Alanine	58.9
Arginine	67.7
Cysteine	44.7
Glutamic acid	101.5
Glycine	76.5
Histidine	14.1
Isoleucine	49.1
Valine	74.1
Leucine	84.2
Lys	22.1
Phenylalanine	52.1
Proline	90.5
Serine	114.2
Threonine	48.7
Tyrosine	24.2

(Source: Mukesh Kumar, et.al, 2012)

These feathers are consisting of crude proteins mainly. Researches has been done all over the world to make use these protein content which is a wonderful idea since at the same time both protein shortage and waste feathers environmental problems can be overcome.

Table 2.2 Chemical composition of feathers

Constituent	Feathers(g kg^{-1} to dry mass)
Water	494
Crude protein	890
Fibers	-
Fat	14.3
Ash	62.1
Calcium	3.4
Phosphorus	1.1
Sodium	4.6
Chloride	7.6

(Source: Mukesh Kumar, et.al, 2012)

Table 2.3 Chemically reactive groups in wool and feathers

Reactive group	Keratin-Wool	Keratin-Feather
Free carboxyl	58-66	27-44
Amide	79-98	78
Carboxyl plus amide	137-164	105-122
Phenolic hydroxyl	22-36	11-12
Aliphatic hydroxyl	124-148	134-174
Amino	20-24	7-12
Aromatic	27-43	13-14
Half-disulfide	92-114	57-68
Oxidizable	525-650	309-376
*Content as gram equivalents per 10^5 g of keratin		

(Source: Rivalcoba.V.S.,et al, 2010)