# THE PRODUCTION OF ANTI AGING CREAM WITH UV PROTECTION FROM CHICKEN FEATHERS

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# DEVELOPMENT OF BIO-MEDICAL & COSMETIC PRODUCTS FROM KERATIN PROTEIN

# THE PRODUCTION OF ANTI AGING CREAM WITH UV PROTECTION FROM CHICKEN FEATHERS

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**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**

**JANUARY 2012**

# ACKNOLEDGEMENT

The development in the making of anti aging creams from chicken feathers gives me such a beautiful memorable moment in life. It began from the toughest moment in order to make the very first step in the beginning, to the brightest moment by the time this project won the gold medal at BioMalaysia 2011.

First and foremost, I would like to express my deepest gratitude to Dr. Arun Gupta, my final year project supervisor for his brilliant ideas and supports. He gives me a lot of guidance from the beginning of this project till the end. I will always remember his willingness to listen the problem and also successful event happen regarding on this research.

My sincere thank to Dr. Kausar Ahmad and Mr. Taufiq from International Islamic University Malaysia (IIUM), Faculty of Pharmacy for all your guidance and support. I also acknowledge all IIUM members which give me full cooperation.

I acknowledge my sincere indebtedness and gratitude to my parents for their love, dream and sacrifice throughout my life. I acknowledge the sincerity of my only younger brother for the support and help to finish this valuable research.

I would like to express special thanks to Ramanan Perumal which give me an ideas to start this new invention of keratin protein. To Mohd Taufik bin Ibrahim and all my friends, I owe you all big thanks for your support. The lecturers and lab engineers, thanks for giving me a lot of guidance and advice to finish the project. Lastly, thanks to all which directly or indirectly involve with this project.

# ABSTRACT

A research entitled the development of biomedical and cosmetic products from keratin protein. Protein builds up most of the outer surface on human body. In this research, chickens feathers will be used as protein sources. Hence, the protein in the chicken feather is extracted and used in the personal care products. The personal care products focused in this research is anti aging cream with ultra violet (UV) protection. The production of anti aging cream is using formulation created by safe and cheap ingredients easily available in Malaysia. The cream is then analyzed with pH meter, micro centrifuge, particle size analysis, rheometer, phase separation and tested with rat skin. All the data collected shows the creams are stable, no phase separations happen to cream and no irritation on the rat skin. In conclusion, there is no harm and safe in the usage of this anti aging cream with UV protection.

# ABSTRAK

Penyelidikan ini dihasilkan untuk mengkaji akan penghasilan produk-produk bio-perubatan dan kosmetik dari keratin protein. Protein ini biasanya terhasil secara semulajadi di lapisan kulit manusia. Dalam penyelidikan ini, bulu ayam dijadikan sebagai sumber protein. Jadi, protein akan diekstrak dari bulu ayam dan digunakan dalam produk-produk penjagaan diri. Penghasilan krim anti penuaan dengan perlindungan UV yang dihasilkan dari bahan-bahan yang murah dan mudah didapati di Malaysia adalah fokus utama dalam penyelidikan ini. Krim ini diuji dengan penganalisis saiz partikel, rheometer, pengasingan fasa yang berlaku pada krim dan juga kesan pengunaan krim ini kepada tikus makmal. Berdasarkan peemerhatian dan keputusan yang diperolehi daripada kajian yang dijalankan, tiada perubahan warna, tiada pengasingan fasa yang berlaku pada krim dan juga tiada kesan seperti kemerah-merahan yang terhasil di lapisan kulit tikus makmal Kesimpulannya, krim anti penuaan ini selamat digunakan.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Chicken feather wastage is made up approximately eleven million pound from the commercial poultry processing plant annually. The disposal process for chicken feather is expensive. It is also can be difficult because the chicken feather is burning up with the incinerator plant, buried in the soils and also recycled as a low quality of poultry foods. These processes mostly give the bad effects to the environment, especially the burning of chicken feather which will release the green house gasses in the air. There are several alternatives invented based on the chicken feather application, but the wastage of chicken feather is still does not change as much as possible because of its low requirement.

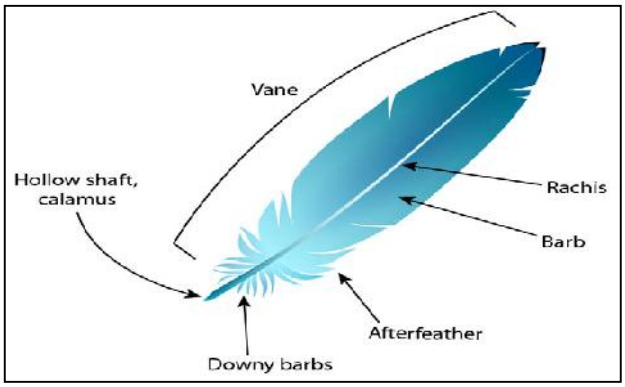


**Figure 1.1:** The anatomy of a chicken feather

Keratin is a natural protein extracted from the chicken feather as shown in Figure 1.1. In the developed country, the usage of keratin in the personal care products is widely used. The personal care product produced from the keratin protein is conditioning shampoo, anti aging cream, facial cleanser and others. There are some differences between the personal care products already produced by the other developed country because the raw materials to extract the protein is sheep wool while in this research, the extraction of keratin protein is from chicken feathers. These two materials will produce two different sequences of amino acids.

## 1.2 Problem Statement

1.2.1 The chicken feather waste is increasing throughout the year and it will cost a lot in the treatment processes.

1.2.2 Large amount of chicken feather available from meat industry creating environmental problems.

1.2.3 High cost of production of keratin protein from sheep wool in Malaysia.

1.2.4 Develop the production of personal care formulation from beta keratin protein produced by the chicken feather because an increasing demand for keratin based products such as anti-aging cream, shampoo.

## 1.3 Research Objectives

1.3.1 To extract the keratin protein from the chicken feather.

1.3.2 To find a suitable method for solubility and later on purify the keratin protein.

1.3.3 Analyze the composition of the keratin protein.

1.3.4 To produce an anti-wrinkle treatment cream from keratin protein produced by chicken feather.

1.3.5 To prepare the formulation of personal care products from keratin protein and analyze the research products.

## 1.4 Scope of Study

1.4.1 Study the production of keratin protein from chicken feather by identified reducing agent.

1.4.2 Study of the purification and analysis of keratin protein composition.

1.4.3 The production of personal care products by using the formulation gathered.

1.4.4 Testing and analyzing the products from the keratin protein.

## 1.5 Rationale and Significance

This research is to be done to develop the new products from the natural protein extracted from chicken feather. Chicken feathers are resistance to degradation and these characteristics contributes to environmental effects. Extraction of natural protein called keratin will be one of a solution in managing the wastage of chicken feathers. Keratin protein has a wide range of use in cosmetic and biomedical products such as conditioning shampoo and anti aging cream. The beta keratin protein will be used to develop new product instead of using alpha keratin protein because the natural protein extracted from chicken feathers is beta keratin protein and the amino acids composition of these alpha and beta keratin are both different.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Feathers

Chicken feather is a material which produced keratin protein. “The structures of the chicken feather consist of beta keratin as its major structural instead of alpha keratin (R.H. Sawyer et al., 2000)”. The keratin protein is used in bio-medical and cosmetic products and this research is about the development of these products with the usage of chicken feather as a keratin source.

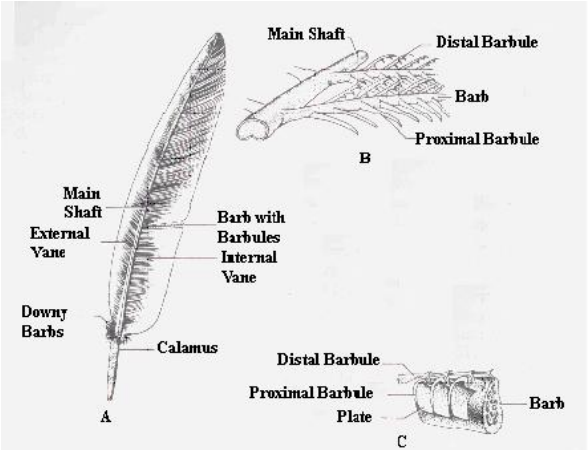


**Figure 2.1** Structure of chicken feather

## 2.2 Protein

Proteins play a big role in the human system daily life which provides the structure to the human body system and also transporting oxygen in the human blood regulating system. Human bodies depending on proteins to perform the life better and protein also have its composition and structure. The differences of each protein classification play its different role in the human body.

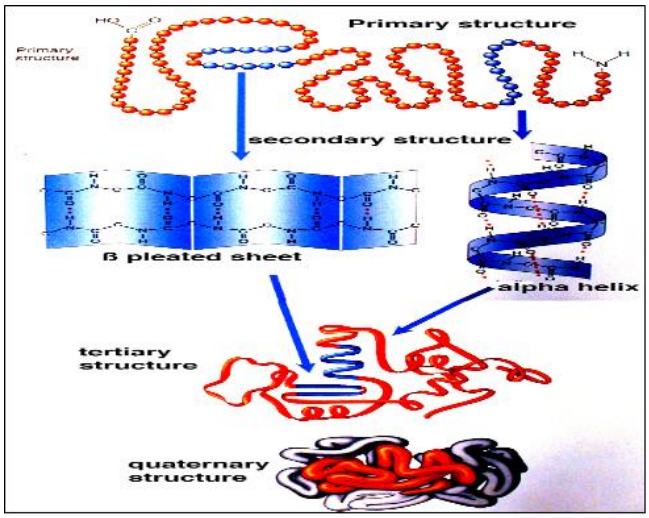


**Figure 2.2** The protein structure

## 2.3 Keratin

"Keratin is a major component of mammalian hair, bird feathers and covered the outermost layer of skin in most animals. The ability to flex in multiple directions without tearing is an important quality of keratin and it is also provides a tough and fibrous matrix in tissues (Sheen and Judy P, 2002)". Keratin proteins have the secondary structure and it is categories in the class of fibrous protein. Fibrous proteins have an elongated shape relatively simple and regular linear structure. The mechanical strength of keratin proteins is high because it is connecting tissues in animals and forming skeletal.

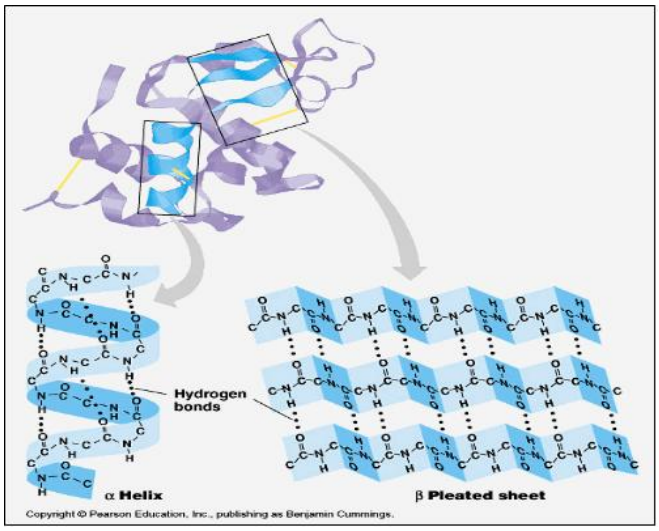


**Figure 2.3** Alpha helix and beta pleated sheet of keratin

There are two types of keratin, which are alpha-keratin and beta-keratin. Alpha-keratins are found in the soft tissues protein fibres of sheep wool, hair and skin. It is twisted together and formed like a rope strand. Alpha-keratins amino acids sequences rich in cysteine and poor in hydroxyproline and proline. Beta-keratins are found in the hard tissues protein fibres such as bird feathers, nails, fish scales and others. The beta-keratins amino acids sequences rich in small uncharged glycine and alanine and poor in cysteine,proline and hydroxyproline.

**Table 2.1** The Amino Acid Composition of Chicken Feathers

| Amino Acid | uM/mg Protein*[1] | % Amino Acid in Protein |
|---|---|---|
| Aspartic Acid | 0.358 | 4.76 |
| Threonine | 0.345 | 4.11 |
| Serine | 1.292 | 13.57 |
| Proline | 0.875 | 1.01 |
| Glutamic Acid | 0.624 | 9.18 |
| Glycine | 1.008 | 7.57 |
| Alanine | 0.411 | 3.66 |
| Valine | 0.618 | 7.24 |
| Cystine | 0.088 | 2.11 |
| Methionine | 0.017 | 0.025 |
| Isoleucine | 0.376 | 4.93 |
| Leucine | 0.570 | 7.48 |
| Tyrosine | 0.102 | 1.85 |
| Phenylalnine | 0.267 | 4.11 |
| Lysine | 0.039 | 0.57 |
| Histidine | 0.001 | 0.016 |
| Arginine | 0.377 | 6.57 |

*Based on sample as 100% protein
[1]Micro mole per milligram of protein

"Keratin is insoluble in water and organic compound. The chemical properties of keratin are weak acids and bases. It is characterized by cystine content in the sequence of keratin amino acids and it can be hydrolysed, reduced and oxidized. High strength of keratin is influenced by the two cysteine molecules by disulphide bonds (Krystyna Wrzesnieska-Tosik, 2007)". "Keratin protein fraction is used in the formulation of anti-wrinkle treatment cream, sulfite hair straightener, conditioning shampoo and other personal care (R.J. Kelly and A.D. Roddick-Lanzilotta, 2003)".

A covalent chemical bond between two sulfur atoms which is derived from the two sulfhydryl or thiol groups called disulfide bond. The thiol groups usually from the side chain of amino acid cysteine and it is in the reduced forms. The oxidation reaction is occurs

when two sulfhydryl groups covert to a disulfide linkage, while the reduction reaction occurs when the disulfide bonds is reduced to yields two thiols.

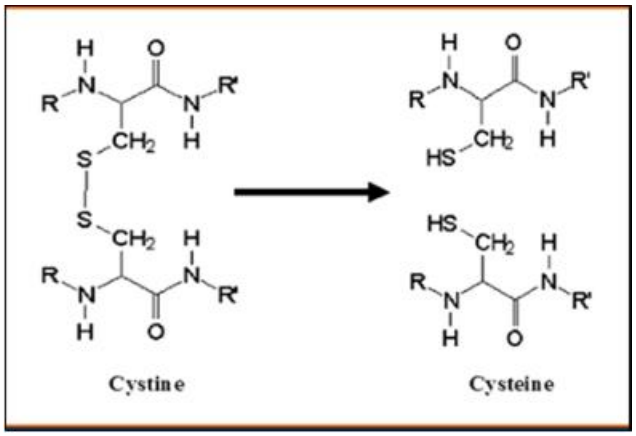


**Figure 2.4** The reduction of disulfide bond

Keratins gene family (KRT) is responsible to provide an instruction for the making of protein called keratin. Human generally has at least 54 functional keratin genes. Each of the keratin genes is divided into two types of keratin which are type I and type II. Type I keratins genes are located in a cluster on chromosome 17 and designed through KRT9 to KRT20. Type II keratins genes are found in a cluster of chromosome 12 and designated through KRT1 to KRT8. Differ tissues have the different combination of keratin protein from type I and type II. These combinations will form a structure called a heterodimer and each of heterodimer make an interaction to form keratin intermediate filament. However, the instruction of genes in the production of keratin also happens to the other mammals.

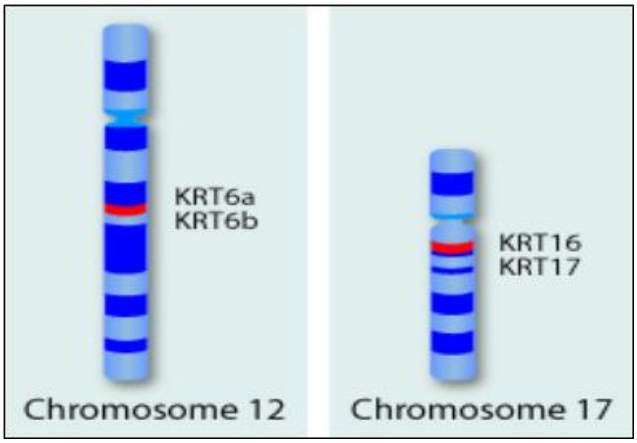


**Figure 2.5** Chromosome of keratin gene family in human being

Biomedical research is solving medical problems by using theories and it can be prove or deny through observation and experimentation. Animals which are having the similar characteristics as human in terms of skin, hair and others can be use to get the best result because if the positive result appear from the test, it does mean that human can use the biomedical products. Animals play a big role in the biomedical research because most of the medical advances in the countries all around the world are dependent on the animal research. Based on the research, the formulation can be use to cure many diseases and can be save millions of life.

## 2.4 Ultra Violet in Anti Aging Cream

Human development makes process of all organs in the body are different in each stage. As human beings grow older, the maximal functioning and reverse capacity is decreasing and this phenomenon called an ageing process (M. Yaar and B.A. Gilchrest, 2007). The appearances of skins for most age changed because of the chronic UV induced damaged labeled as photoageing. Collagen synthesis in skin is inhibited because incomplete degradation of collagen by UV leads it to accumulate as partial fragments in the skin. As the collagen degradation products increased, the less or no collagen will produce.

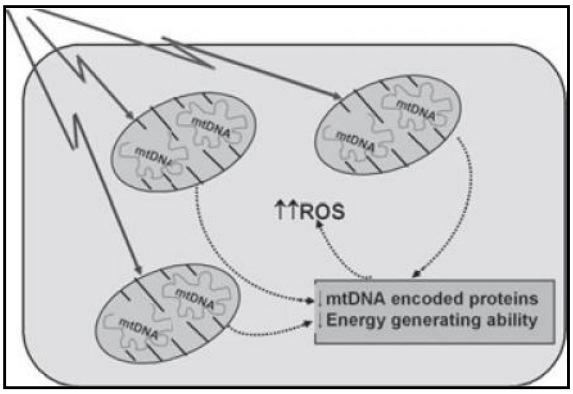


**Figure 2.6** The mechanism of photoageing in skin. *(M. Yaar and B.A. Gilchrest, 2007)*

Figure 2.6 shows the reaction of mitochondria in skin if it is affected by UV. Pro-inflammatory cytokines expression will be induced by reactive oxygen species. Cell ability to generate energy is reduced if the reactive oxygen species is increasing. Large mitochondria's DNA deletions cause proteins containing carbonyl groups in skin to damage.

## 2.5 Keratin in Anti Aging Cream

The invention of anti aging cream with UV protection from chicken feather is a vital part in this research. Keratin provide a quick fix improvement in reducing wrinkles in skin rapidly because it can boost the protein expression which give result to cell growth.

Keratin also has anti-inflammatory property which is suitable for various skin conditions. The skin condition can be acne, eczema, and dermatitis. This can prove that keratin's cream can be applied by person that having sensitive or irritated skin. Antioxidant properties in keratin make the usage of keratin's cream much more applicable because of its ability to neutralize the effect of free radicals which main cause of skin ageing.

A healthier skin having keratin layers of skin that acts as barrier from UV and microorganisms attacks. Moisturizer of the skin can be retaining with the existent of keratin layers since its hold the moisture in skin. A detail of the healthier complexion and problem skin is shown in Figure 2.7.

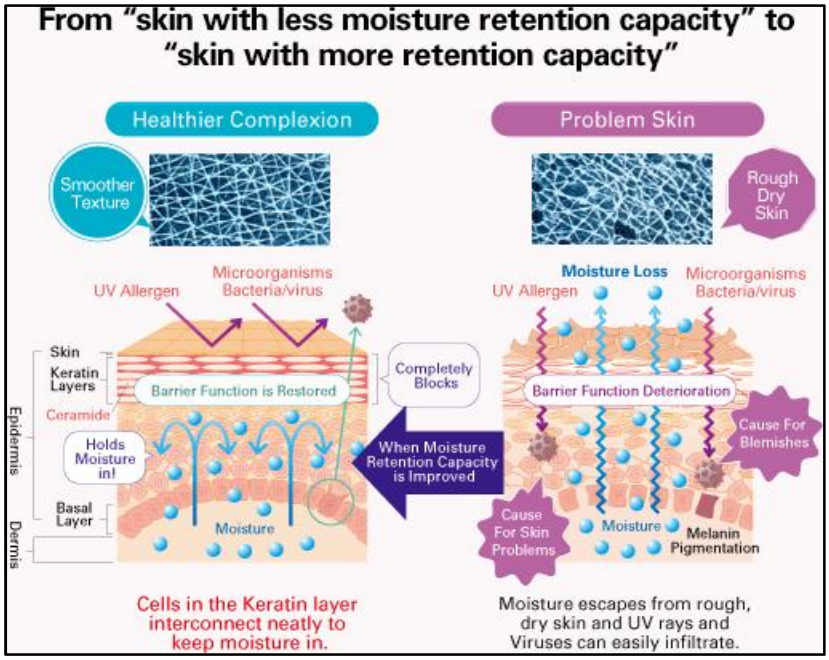


**Figure 2.7** Healthier complexion and problem skin

## 2.6 Oil in Water Cream

Basically, the base cream formulation is divided into two general bases which are oil in water and water in oil. By definition, the main difference to determine either it is oil in water or water in oil is the ability of oil or water droplet to disperse in water and oil. However, the type of emulsifier is determined the type of the cream itself.

| Parameters | Paraffin oil | Coconut oil | Palm kernel oil | Stearic acid | Beeswax | Lanolin |
|---|---|---|---|---|---|---|
| Colour | Colourless | Pale yellow | Yellow | White | Off white | Yellow |
| Odour | Bland | Sweet | Sweet | None | Honey like | Woolly |
| State | Liquid | Liquid | Liquid | Solid | Solid | Semisolid |
| $H_2O$ Solubility | Insoluble | P miscible | P miscible | Insoluble | Insoluble | S miscible |
| SG at 28°C | 0.8548 | 0.8810 | 0.8720 | - | - | - |
| R.I at 40°C | 1.476 | 1.454 | 1.449 | - | - | - |
| Viscosity (cs) | 10.12±0.02 | 50.34±0.04 | 49.82±0.01 | - | - | - |
| Mpt. in°C | - | - | - | 39-60 | 62-70 | 40-44 |
| pH | 7.97±0.01 | 4.00±0.06 | 5.00±0.07 | - | - | - |
| TAV (mg KOH $g^{-1}$) | - | 1.66±0.02 | 0.66±0.02 | 610±0.2 | 24.52±0.03 | 5.86±0.20 |
| FFA (%) | - | 0.84 | 0.34 | 309.4 | 12.35 | 2.95 |
| SV (mg KOH $g^{-1}$) | - | 274.34±0.02 | 249.96±0.06 | 238.00±0.15 | 28.00±0.07 | 88.48±0.02 |
| EV (mg KOH $g^{-1}$) | - | 272.68±0.01 | 249.30±0.05 | 372.00±0.04 | 3.48±0.05 | 82.62±0.18 |
| PV (mg Eq $Kg^{-1}$) | ND | ND | ND | ND | ND | ND |
| IV (mg$I_2$/100 g) | - | $3.5\times10^{-3}$ | $1.37\times10^{-3}$ | - | - | - |

SG = Specific Gravity, RI = Refractive Index, Mpt = Melting point, TAV = Total Acid Value, EV = Ester Value, PV = Peroxide value, P miscible = Partly miscible, S miscible = Slightly miscible, Results are mean±SD of three determinations

**Figure 2.8** Physicochemical analysis of oils and some raw materials ( F.O. Oyedeji and I.E. Okeke, 2010)

| Parameters | Paraffin oil emulsion | Coconut oil emulsion | Palm kernel oil emulsion | VMC |
|---|---|---|---|---|
| Colour | White | Off white | Pale yellow | Pink |
| PH | 6.8±0.06 | 6.37±0.02 | 7.19±0.01 | 7.16±0.07 |
| Conductivity (ms $cm^{-1}$) | 0.12±0.02 | 0.19±0.01 | 0.13±0.01 | 0.01±0.01 |
| Microscopic examination | Even sized globules | Even sized globules | Uneven globules | Even sized globules |
| Dye uptake using water soluble dye | Takes up dye | Takes up dye | Takes up dye | Takes up dye |
| Emulsion type | Oil in water | Oil in water | Oil in water | Oil in water |
| Total acid value (mgKOH $g^{-1}$) | 26.36±0.01 | 48.43±0.03 | 46.59±0.01 | 34.33±0.02 |
| Free fatty acid (%) | 13.28±0.01 | 24.39±0.01 | 23.46±0.07 | 17.29±0.01 |
| Saponification value (mg KOH $g^{-1}$) | 123.20±0.04 | 138.88±0.09 | 155.63±0.02 | 84.00±0.08 |
| Centrifugation (5000 rpm for 1h) | Stable emulsion | Stable emulsion | Emulsion separates into layers | Stable emulsion |
| Cyclical temp variation (25-45 soluble dye°C) | Stable emulsion | Stable emulsion | Emulsion separates into distinct layers | Stable emulsion |
| pH after cyclical variation | 7.00±0.04 | 6.50±0.01 | 6.26±0.01 | 7.35±0.04 |

VMC = Popular Moisturising Cream bought from a Nigerian market used as control, Cyclical temperature variation was carried out 24 h for 3 cycles and then the pH of the emulsion was taken, Results are mean±SD of three determinations

**Figure 2.9** Physicochemical analysis of the emulsions ( F.O. Oyedeji and I.E. Okeke, 2010)

## 2.7 Rheology Test

The measurement of material flow is called rheology test. There are several characteristics can be determined by doing rheology test. There are viscosity and flow curves, oscillation test and creep test. Rheology influences residual stresses, cycle times, and also the void content. Product characterization also employed by rheological measurement.

"The most common needs of rheological tests on pharmaceutical and cosmetics products are to study the fundamental nature of selected system, the quality control of overall processes and different parameters effect for each formulation (Peter Herh et al.,1998)".

## 2.8 Particle Size Test

Size distribution of individual particles in a sample can be analyzed by using particle size analysis. Particle size analysis main features are dispersion of samples into discrete units and particle separation depending on the particle size. Data analyzed by particle size analysis is presented in several ways and usually it is visualized by a cumulative particle size distribution curve. Figure 2.10 shows the different particle shapes will affect the particle size calculation.

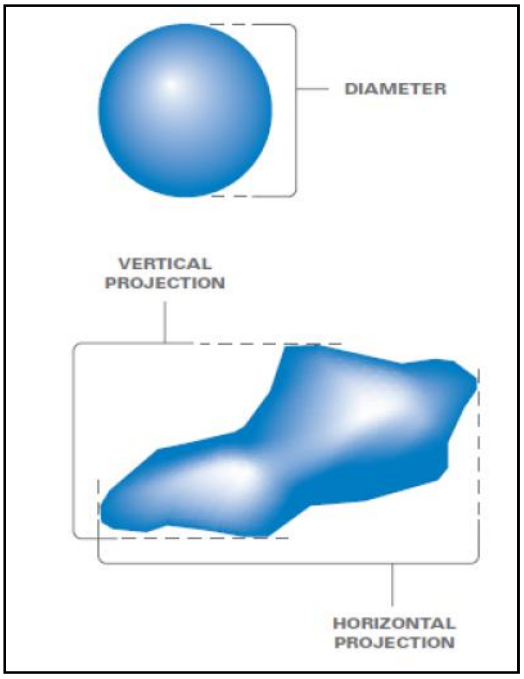


**Figure 2.10** Difference of particle shape in the formulation

# CHAPTER 3

# RESEARCH METODOLOGY

## 3.1 Introduction

The research methodology is details methods in the production of personal care products. The personal care product produced from this research is anti aging cream. Anti aging creams are formulated and keratin is used as active ingredients. The formulation is followed with the testing procedure. In this research, there are seven test is done. These are color, phase separation, particle size analysis, rheology and toxicity test.

## 3.2 Materials and Ingredients

Formulation of anti aging cream in this research is divided into two. In these two different formulations, some of the chemicals are difference. The chemicals used in this research as shown in Table 3.1. The production of anti aging cream is based on weight percentage of 100% for each 100g formulation.

## 3.3 Methods

**Table 3.1:** Composition percentage of each material in Anti aging Cream

| Formulation / Materials | A2 | A3 | A4, F1 | A5,F2 |
|---|---|---|---|---|
| Cetostearyl Alcohol | 3.0 | 3.0 | 12.5 | 12.5 |
| Palm Oil | - | - | 20.0 | 20.0 |
| Isopropyl Palmitate | 6.0 | 6.0 | - | - |
| Sunflower Seed Oil | 6.0 | 6.0 | - | - |
| Glycerin | 3.0 | 3.0 | 12.0 | 12.0 |
| UV Protection Agent | 3.0 | 3.0 | 5.0 | 5.0 |
| Lecithin | 1.0 | 1.0 | - | - |
| Keratin | 1.0 | 2.0 | 1.0 | 2.0 |

### 3.3.1 Research Methodology of Extraction of Protein

**Feathers Treatment**

1. Soak the chicken feather in ether for 24 hours.
2. Wash the feathers with soap water and dry the wet feathers under the sunlight.
3. Collect all the dried feathers and blend the feather. Keep the blend feathers in the sealed plastic bag carefully.

**Dissolving of Chicken Feather**

1. Prepare the sodium sulfide solution in the conical flask.
2. Weight the blend feathers and add in into the sodium sulfide solution.
3. Stir the solution for 6 hours and maintain the condition of solution at 30°C and pH range of 10 to 13.
4. Filter the solution and centrifuge the solution at 10000 rpm for 5 minutes.
5. Filter the solution to get the supernatant liquid.
6. Place the supernatant liquid in a beaker and stir the solution.

**Protein Precipitation**

1. Add an ammonium sulfate solution into the solution and centrifuge the solution at 10000 rpm for 5 minutes.
2. Filter the solution to get supernatant liquid and solid particles.
3. Repeat step 1 and 2.

**Protein Purification**

1. Pour the deionized water into the solid particles and stir the solution
2. Centrifuge the solution at 10000 rpm for 5 minutes and filter the solution to get supernatant liquid and solid particles.
3. Use sodium hydroxide solution to dissolve the solid particles.
4. Centrifuge again the solution at 10000 rpm at 5 minutes. Collect the liquid and discard the solids.
5. Repeat steps 1 to 4 for three times.

### 3.3.2 Research Methodology of Anti Aging Cream Formulation A2 and A3

**Solution A**

1. 3.0 g of glycerin is weighted and put in the 250ml beaker. The glycerin is then put in the 70°C water bath. Stir the solution for 2 minutes.
2. After 2 minutes, 3.0g of cetostearyl alcohol is pour into the glycerin solution. The mixture is stirred for 5 minutes until all the solid particles dissolve.
3. Isopropyl palmitate and sunflower seed oil are then put into the solution. Stir the solution for a minute until all the mixture mix well.

**Solution B**

4. Lecithin is dissolve in the warm distilled water.

**The mixture**

5. Then the solution B is pour into the solution A.
6. Solution is stirred for 2 minute. Then, put UV protection agent, preservative and penetration enhancer into the solution.
7. The mixture solution is then transferred to the magnetic stirrer hotplate to continuously stirred and cool it down until the room temperature.
8. After the solution reached the room temperature, the API and perfume are put into the solution. Continuously stirred the solution for 3 minutes.
9. The solution is then stirred with the homogenizer for 5 minutes with 14500 rpm.

### 3.3.3 Research Methodology of Anti-Aging Cream Formulation A4, A5, F1 & F2.

**Solution A**

1. 12.5 g of glycerin is weighted and put in the 250ml beaker. The glycerin is then put in the 70°C water bath. Stir the solution for 2 minutes.
2. After 2 minutes, 12.5g of cetostearyl alcohol is pour into the glycerin solution. The mixture is stirred for 5 minutes until all the solid particles dissolve.
3. The palm oil is then put into the solution. Stir the solution for 2 minutes until all the mixture mix well.

**Solution B**

4. The citric acid is dissolve in the distilled water.

**The mixture**

5. Then the solution is pour into the solution A.
6. Triton is weighted and heats the solution for 1 minute, pour triton solution into the mixture of solution A and B. Then, put UV protection agent and penetration enhancer into the solution.
7. The mixture solution is then transferred to the magnetic stirrer hotplate to continuously stirred and cool it down until the room temperature.
8. After the solution reached the room temperature, the API is put into the solution. Continuously stirred the solution for 3 minutes.
9. The solution is then stirred with the homogenizer for 5 minutes with 14500 rpm.

## 3.4 Characterization of Anti Aging Cream

### 3.4.1 Color

The cream samples are placed at the room temperature for 28 days. After 28 days, it will be observed either the color is changing or the same as the beginning.

### 3.4.2 Phase Separation

All samples are observed of its stability. It has been left at room temperature for 28 days at room temperature. After 28 days, the creams are observed if there are phase separation occurred.

### 3.4.3 Centrifuge Test

The centrifuge test is done by using micro centrifuge. The micro centrifuge is adjusted to several rpm for five minutes. Appearance of the anti aging cream once it is centrifuged is observed. Volume of oil separated is recorded and its percentage is calculated to identify the most stable cream base.

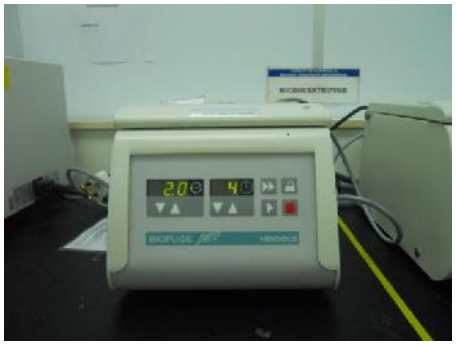

**Figure 3.1** Micro Centrifuge

### 3.4.4 pH Test

The pH of the anti aging cream is analyzed using pH meter. The pH value for each cream formulated is recorded.

**Figure 3.2** pH meter

### 3.4.5 Particle size test

Laser Particle Size Analyzer (BT-9300H) is used to analyze the particle size test for the cream produced. Figure 3.1 and 3.2 shows the particle size analyzer with circulating and dispersing system respectively. Both of these two equipments used in order to identify the particle size of formulated creams.


**Figure 3.3** Particle size analyzer

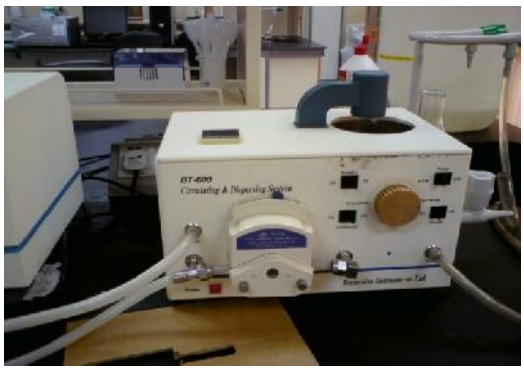

**Figure 3.4** circulating and dispersing system