PRODUCTION OF NATURAL PROTEIN USING CHICKEN FEATHER.

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of the requirements for the award of the Degree of
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I declare that this thesis entitled “Production of Natural Protein Using Chicken Feather” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

Signature :……

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Date : 29 Nov 2010

I dedicate this thesis to my family without whom none of these would have been worth a challenge…

Wonderful and Amazing parents;
Mr.Perumal Sennasamy & Mdm Muniammah maniam.

Also Sweetest siblings

This is for all of you…

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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. Even though many hard works and scarifies came from me for this project, there are a lot of people without whom this project would not even lifted from the ground let alone completion.

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ABSTRACT

A research was conducted on producing protein from chicken feathers. Protein is an important nutrient needed by our body to maintain body structures and important ingredient for cosmetic products. Chicken feathers have elevated keratin protein content and can be a suitable protein source. The main processes are dissolving chicken feathers and separation of proteins. Reducing agents Potassium cyanide, thioglycolic acid and sodium sulfide used for the dissolving process. Ammonium sulfate is used for the separation process. Once the feathers are dissolved ammonium sulfate solution is added to the solution which will precipitate protein. The precipitated protein is washed with water and dissolved in sodium hydroxide solution to obtain protein solution. Sodium sulfide has the highest efficiency in dissolving chicken feathers since the feathers are dissolved in a very short period of time. After the methods of precipitation, washing and dissolving the protein solution obtained is confirmed as pure protein solution by biuret test. An analysis by the Ftir confirmed the presence of carboxyl acid and amino groups only. Thus the sample obtained is true protein since the presence of functional groups is proven. From this research can be concluded that protein can be produced from chicken feathers. Hopefully chicken feathers will be used as a source of protein production in a bigger scale in the future.

ABSTRAK

Penyelidikan dilakukan untuk menghasilkan protein dari bulu ayam. Protein adalah nutrisi penting yang diperlukan oleh tubuh kita untuk menjaga struktur tubuh dan bahan penting untuk produk kosmetik. Bulu ayam mempunyai kandungan keratin protein tinggi dan boleh menjadi sumber protein yang sesuai. Proses utama adalah melarutkan bulu ayam dan pemisahan protein. Kalium sianida, asid thioglycolic dan sodium sulfida digunakan untuk proses larut. Amonium sulfat digunakan untuk proses pemisahan. Setelah bulu dilarutkan larutan amonium sulfat ditambah ke dalam larutan dimana protein berhasil. Endapkan protein dicuci dengan air dan dilarut dalam larutan natrium hidroksida untuk mendapatkan larutan protein. Natrium sulfida mempunyai kecekapan yang terbaik dalam melarutkan bulu ayam sebab bulu dilarutkan dalam masa yang sangat singkat. Selepas kaedah presipitasi, mencuci dan melarutkan protein yang diperolehi dikukuhkan sebagai larutan protein dengan ujian biuret. Sebuah analisa oleh FTIR mengesahkan ada kumpulan karboksil dan kumpulan amino saja di dalam sampel. Jadi sampel yang diperolehi adalah larutan protein benar kerana terdapat kedua dua kumpulan tersebut. Kesimpulanya protein boleh dihasilkan dari bulu ayam. Semoga bulu ayam akan digunakan sebagai sumber pengeluaran protein dalam skala yang lebih besar di masa mendatang.

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LIST OF ABREVIATIONS

UV-Vis	-	Ultra Violet Visual Spectrometer
FTIR	-	Fourier Transform Infrared Spectrometer
IR	-	Infrared

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
%	-	Percent
g	-	Gram
L	-	Liter
rpm	-	Rotation Per Minutes
M	-	Molarity
ml	-	Milliliter
A	-	Absorbance
I	-	Length
c	-	Concentration
ε	-	Molar Absorptivity
cm^{-1}	-	Reciprocal Centimeter
mg	-	Milligram

CHAPTER 1

INTRODUCTION

1.1 Backround

This research is about extracting natural proteins from chicken feathers by using reducing agents that will decrease the stability of keratin fibers in the solid form found in feathers. These reagents will break down disulphide bonds, hydrogen bonds and salt linkages of the keratin fibers in order to dissolve it into natural protein. Currently there is an increasing interest in the development of materials that are environment friendly, obtained from renewable resources. The main renewable materials 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 are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer are joined together by the peptide bonds between the

carboxyl and amino groups of adjacent amino acid residues. Protein deficiency is a serious cause of ill health and death in developing countries. Protein deficiency plays a part in the disease kwashiorkor. War, famine, overpopulation and other factors can increase rates of malnutrition and protein deficiency. Protein deficiency can lead to reduced intelligence or mental retardation, see deficiency in proteins, fats, carbohydrates. The protein shortage for food and feed oblige us to look for new protein sources, including waste products.

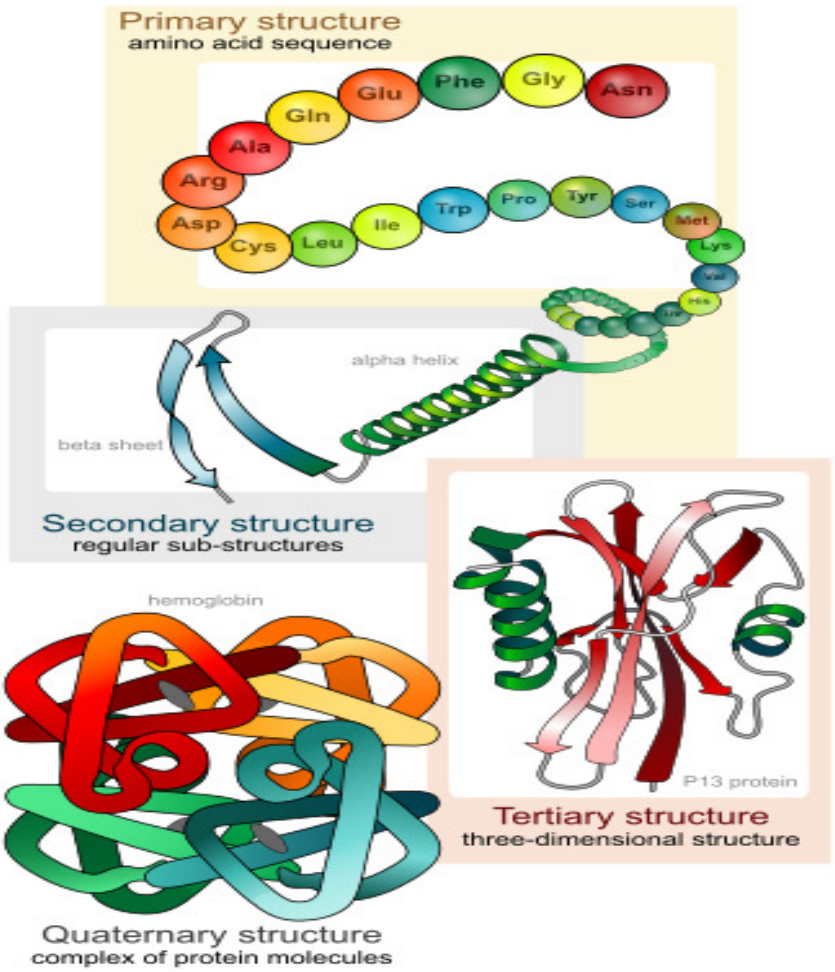


Figure 1.1: The different structures of protein.

Feathers are bio-resource with high protein content (more than 750 g kg-1 crude protein). Poultry slaughterhouses produce large amounts of feathers. Further, burning in special installations is economically ineffective. Uncontrolled disposal of feathers is environmentally unacceptable. The solution of the problem is obligatory since poultry production plays a vital role in the protein supply and also in the agricultural economy for many countries in the world. Five percent of the body weight of poultry is feathers; from a slaughterhouse with a capacity of 50 000 birds daily are produced 2-3 tones dry feathers. The β-keratins in feathers, beaks and claws — and the claws, scales and shells of reptiles — are composed of protein strands hydrogen-bonded into β-pleated sheets, which are then further twisted and cross linked by disulfide bridges into structures even tougher than the α-keratins of mammalian hair, horns and hoof.

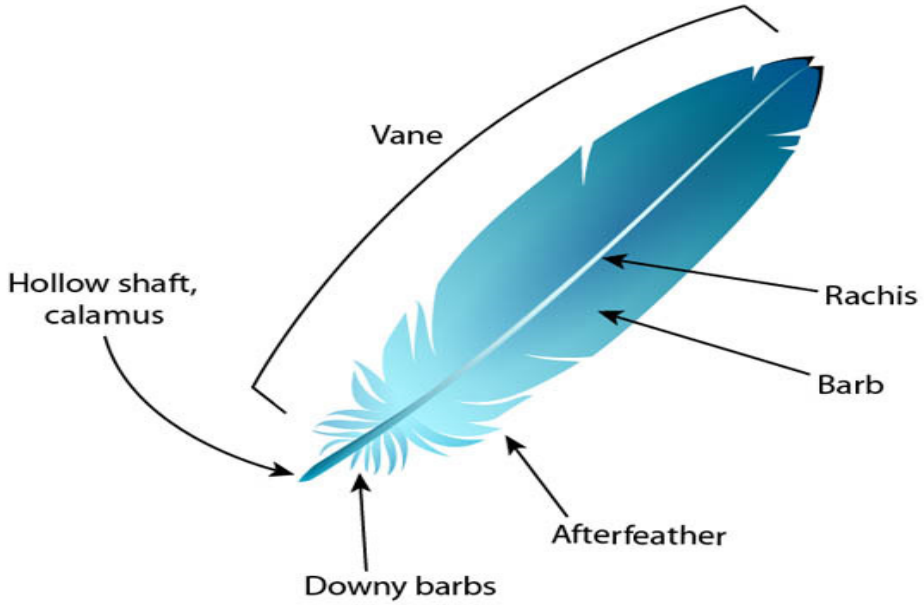


Figure 1.2: Important parts of a feather.

Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan. Over 90% of the dry weight of hair are proteins called keratins, which have a high disulfide content, from the amino acid cysteine. The robustness conferred in part by disulfide linkages is illustrated by the recovery of virtually intact hair from ancient Egyptian tombs. Feathers have similar keratins and are extremely resistant to protein digestive enzymes. Different parts of the hair and feather have different cysteine levels, leading to harder or softer material. Manipulating disulfide bonds in hair is the basis for the permanent wave in hairstyling. The high disulfide content of feathers dictates the high sulfur content of bird eggs. The high disulfide content of hair and feathers contributes to the disagreeable odor that results when they are burned.In chemistry, a disulfide bond is a covalent bond, usually derived by the coupling of two thiol groups. The linkage is also called an SS-bond or disulfide bridge. The overall connectivity is therefore R-S-S-R. The terminology is widely used in biochemistry. Formally the connection is called a persulfide, in analogy to its congener, peroxide (R-O-O-R), but this terminology is obscure. Disulfide bonds are usually formed from the oxidation of sulfhydryl (-SH) groups, especially in biological contexts.

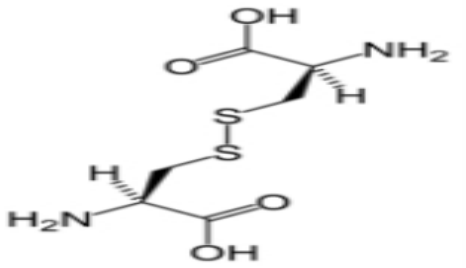


Figure 1.3: Disulfide bond between amino acids Cysteine.

Reductants used in this research for reduction of disulfide groups of feather keratins are thioglycolic acid, potassium cyanide, and sodium sulfide. The reductants act very quickly and without bringing about any other appreciable chemical alteration or damage to the protein yield. Products prepared from the solutions behave as true proteins, and not as products of hydrolysis. Their solutions are precipitated by ordinary protein precipitants such as sulfosalicylic acid, ammonium sulfate and lose this property when digested by trypsin or pepsin.

Thiglycolic acid is the organic compound $HSCH_2CO_2H$. It contains both a thiol (mercaptan) and a carboxylic acid. It is a clear liquid with a strong unpleasant odor. It is simply reduces the disulfide to sulfhydryl groups with no other appreciable chemical change.

Potassium cyanide is an inorganic compound with the formula KCN. This colorless crystalline compound, similar in appearance to sugar, is highly soluble in water. Most KCN is used in gold mining, organic synthesis, and electroplating. Smaller applications include jewelry for chemical gilding and buffing. KCN is highly toxic. The moist solid emits small amounts of hydrogen cyanide due to hydrolysis, which smells like bitter almonds. Not everyone, however, can smell this odor: the ability to do so is a genetic trait. Potassium cyanide reduces feather combined with 0.1N sodium hydroxide.

Sodium sulfide is the name used to refer to the chemical compound Na_2S but more commonly its hydrate $Na_2S{\cdot}9H_2O$. Both are colorless water-soluble salts that give strongly alkaline solutions. When

exposed to moist air, Na_2S and its hydrates emit hydrogen sulfide, which smells much like rotten eggs or flatus. The dissolving action of sodium sulfide has been known for a long time and is used industrially.

Ammonium sulfate,$(NH_4)_2SO_4$, is an inorganic salt with a number of commercial uses. The most common use is as a soil fertilizer. It contains 21% nitrogen as ammonium cations, and 24% sulfur as sulfate anions. In fertilizer the purpose of the sulfate is to reduce the soil pH. It is used to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out.

Copper(II) sulfate is the chemical compound with the formula $CuSO_4$. This salt exists as a series of compounds that differ in their degree of hydration. The anhydrous form is a pale green or gray-white powder, whereas the pentahydrate ($CuSO_4 \cdot 5H_2O$), the most commonly encountered salt, is bright blue. The anhydrous form occurs as a rare mineral known as chalcocyanite. The hydrated copper sulfate occurs in nature as chalcanthite (pentahydrate), and two more rare ones: bonattite (trihydrate) and boothite (heptahydrate). Archaic names for copper(II) sulfate are "blue vitriol" and "bluestone".

Potassium hydroxide is an inorganic compound with the formula KOH. Its common name is caustic potash. Along with sodium hydroxide (NaOH), this colourless solid is a prototypical strong base. It has many industrial and niche applications. Most applications exploit its reactivity toward acids and its corrosive nature. In 2005, an estimated 700,000 to 800,000 tons were produced. Approximately 100 times more NaOH than KOH is produced annually. KOH is noteworthy as the precursor to most soft and liquid soaps as well as numerous potassium-containing chemicals.

Sodium hydroxide (NaOH), also known as lye and caustic soda, is a caustic metallic base. It is used in many industries, mostly as a strong chemical base in the manufacture of pulp and paper, textiles, drinking water, soaps and detergents and as a drain cleaner. Worldwide production in 2004 was approximately 60 million tonnes, while demand was 51 million tonnes. Pure sodium hydroxide is a white solid available in pellets, flakes, granules, and as a 50% saturated solution. It is hygroscopic and readily absorbs water from the air, so it should be stored in an airtight container. It is very soluble in water with liberation of heat. It also dissolves in ethanol and methanol, though it exhibits lower solubility in these solvents than does potassium hydroxide. Molten sodium hydroxide is also a strong base, but the high temperature required limits applications. It is insoluble in ether and other non-polar solvents. A sodium hydroxide solution will leave a yellow stain on fabric and paper.

1.2 Problem Statement

- Determine the best reducing agent that would produce higher amount of protein.
- Establish an extraction method that would have a minimal damage on chicken feather's protein.
- Use suitable protein precipitant for higher protein purification.
- The substance obtained at the end of experiment act as true protein.
- Analysis on the protein obtained.

1.3 Objectives

- Produce natural protein from chicken feather as an alternative source of natural protein.
- Find the keratin reducing (dissolution of chicken feather) efficiency of each reductants by comparing the amount of protein obtained.
- Find a suitable method to purify protein obtained.

1.4 Scope of Project

- Study the solubility of chicken feather keratin under different reductants and obtain natural protein by reducing the keratin in chicken feather. Strength of different reducing agents is to be identified.
- Amount of protein obtained under different reducing agents studied.
- Separation of protein using protein precipitating agent.
- Purification and analysis of the protein obtained

1.5 Rationale And Significance

Protein shortage for food force scientist to look for new protein sources, including waste products. Feathers are bio-resource with high protein content (more than 750 g kg-1 crude protein). Keratin is the main component of feathers, representing nearly 90% of feather weight. Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan. The feathers constitute up to 10% of total chicken weight, reaching more than 7.7×108 kg/year as a by-product of the poultry industry. This excessive material is discarded in several cases, being a material of difficult degradation that may become an environmental problem. These hard keratins of chicken feather which are recognized as a solid wastes generated from poultry processing industry are insoluble and resistant to degradation by common proteolytic enzymes, such as trypsin, pepsin and papain because of their high degree of cross-linking by disulfide bonds, hydrogen bonding and hydrophobic interactions. Keratinous wastes are increasingly accumulating in the environment generated from various industries. To recycle of such wastes, biotechnological alternatives are developing to hydrolyze those materials to soluble into natural proteins. Current commercial production of chicken feather protein involves treatment at elevated temperatures and high pressure, this energy intensive process, results in the loss of some essential amino acids. Natural protein obtained from these feather act as true protein thus have a wide range of usages in fields like cosmetics, food, medicine and biotechnology.

CHAPTER 2

LITERATURE REVIEW

2.1 Feathers.

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 — and the claws, scales and shells of reptiles — 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. Keratin refers to a family of fibrous structural proteins. Keratin is the key structural material making up the outer layer of human skin. It is also the key structural component of hair and nails. Keratin monomers assemble into bundles to form intermediate filaments, which are tough and insoluble and form strong unmineralized tissues found in reptiles, birds, amphibians, and mammals.

The keratin found in feather is called "hard" keratin. This type of keratin does not dissolve in water and is quite resilient. So what is

keratin made from? Keratin is an important, insoluble protein and it is made from eighteen amino acids. The most abundant of these amino acids is cystine which gives hair much of its strength. Keratin filaments are abundant in keratinocytes in the cornified layer of the epidermis; these are cells which have undergone keratinization. Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan.

- The *α-keratins* in the hair (including wool), horns, nails, claws and hooves of mammals.
- The harder *β-keratins* found in nails and in the scales and claws of reptiles, their shells (chelonians, such as tortoise, turtle, terrapin), and in the feathers, beaks, claws of birds and quills of porcupines. (These keratins are formed primarily in beta sheets. However, beta sheets are also found in α-keratins.)

Amino acids present in feathers:

Cysteine	Aspartic acid
Serine	Alanine
Glutamic acid	Proline
Threonine	Isoleucine
Glycine	Tyrosine
Eucine	Phenylalanine
Valine	Histidine
Arginine	Methionine

Table 2.1: Amino acids found in feather Protein.

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 bothprotein shortage and waste feathers environmental problems can be overcome.

Constituent	Feathers	FPC
Water	495	55
Crude protein	894	95
Fibers	-	6.8
Fat	14.1	13.1
Ash	62.4	87.7
Ca	3.5	3.4
P	1.3	1.1
Na	4.0	14.2
Cl	8.0	22.2

Table 2.2: Chemical composition of feathers and feather protein concentrate (FPC)

2.2 Protein

Protein is a part of every cell in living organism's body, and no other nutrient plays as many different roles in keeping living things alive and healthy. The importance 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. Without it, one would lack the enzymes and hormones needed for metabolism, digestion and other important processes. Natural proteins are proteins purified from natural *sources*.

Highly purified for use in 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.

Figure 2.1: Normal protein sources.

The keratin are the proteins of epidermal and skeletal tissues which are insoluble in the normal protein solvents, neither do digested by trypsin or pepsin and have high cystine content. It is shown in “a study on keratin” journal by David R. Goddard and Leonor Michaelis that chicken feather keratins can be converted to natural protein soluble in alkali or acid and digestible by trypsin and pepsin. This was accomplished by breaking the disulfide bonds of the keratin. The β-keratins of reptiles and birds have β-pleated sheets twisted together, then stabilized and hardened by disulfide bonds. So by breaking these disulfide bonds, strength of the keratin in the chicken feathers can be reduced thus become soluble and converted to natural protein.

Journals on the oxidation of keratins have been published by Lissizin, Stary, and Waldschmidt-Leitz, who used bromine, permanganate, and H_2O_2(hydrogen peroxide) as oxidants. But according to journal of David Goddard these oxidizing agents act very slowly in breaking the disulfide bonds thus slowing down the protein extraction process. But in contrast the reductants are shown to act very quickly and without bringing much of any other chemical alteration. These reducing agents dissolve keratin only at alkaline reaction (pH 10 to 13), but the action is not due to alkali alone. Products prepared from these reactions behave as true proteins, and not as products of hydrolysis. This is proven when these solutions were precipitated by ordinary protein precipitants such as sulfosalicylic acid and lose this property when digested by trypsin or pepsin. Reductants used in this journal are, thioglycolic acid, potassium cyanide, and sodium sulfide.

The chemical processes exhibited by these reagents on simple disulfide compounds such as cystine are formulated as follows by the author.

1) $R\text{-}S\text{-}S\text{-}R + 2HS\text{-}CH_2COOH \rightarrow 2R\text{-}SH + [S\text{-}CH_2COOH]_2$

2) $R\text{-}S\text{-}S\text{-}R + HCN \rightarrow R\text{-}SH + R\text{-}S\text{-}CN$

3) $R\text{-}S\text{-}S\text{-}R + H_2S \rightarrow R\text{-}SH + H_2S_2$

According to the journal these reactions occur only in slightly alkaline solution.

When keratin such as feathers, is treated with thioglycolic acid at a pH of 10 or higher the reaction appears to be identical with that on simpler disulfides. The reaction is simple reduction, no loss of sulfur occurs. The action of cyanide on the other hand is not quite simple. Higher alkalinity(pH 12 to 13) is needed. The natural proteins lose sulfur but the substance obtained behave as true proteins. This gave rise to a question about why higher alkalinity is need for these reaction. According to the journal The cross links between peptide chains in feather keratin are of two kinds, disulfide bonds and bridges formed by the electrostatic attraction of the NHs+ .group of the diamino acids for the COO- group of the dicarboxylic acids. These salt-like bridges will be broken in alkaline solution by removal of a proton from the amino group. It appears that it is necessary to open these links before the disulfide groups may be reduced. So when these higher alkaline solution break the salt linkages it is easier for the reducing agents to react on disulfide bonds and dissolve chicken feather.

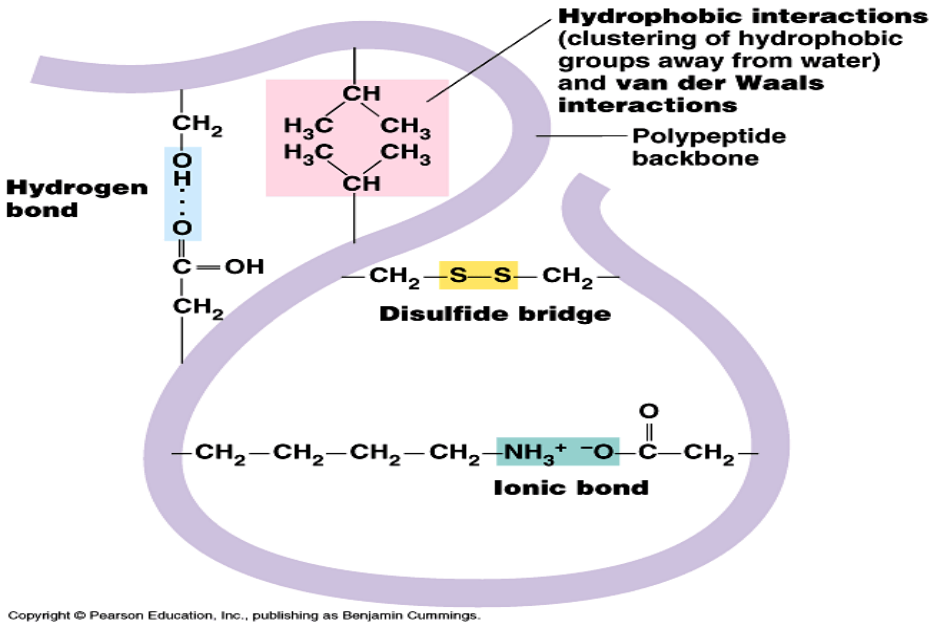


Figure 2.2: Types of bonds commonly found between amino acids.

The dissolving action of sodium sulfide has been known for a long time and is used industrialy. Researchers Kuster and coworkers and Merrill have considered the dissolving of feather keratin as a hydrolysis. But others realized that the action is on the disulfide groups. But the substance obtained are true proteins. Opposed to cyanide preparations these proteins contain more sulfur than the native keratin, though the cystine determinations by the folin and Marenzi method give the same values as feather. It is now known that the action of sodium sulfide on keratins is not identical with its action of sulfide on cystine, and a polysulfide is formed which behaves as cysteine in the Folin and Marenzi procedure. When such a protein is redissolved in weak alkali and

reprecipitated with acid, it undergoes a loss in sulfur, but alwiz such a way that the total sulfur exceeds the cystine sulfur calculated from the cystine determination on the same preparation. In another journal the authors expressed the view that keratins are fibrous proteins whose characteristic properties are essentially determined by the S-S groups of cystine which act as very firmly established cross links connecting the elementary fibers of polypeptide chains. This view was based on the action of certain alkaline reductants which could be shown to reduce disulfides and convert keratin into natural protein soluble in weak alkalies, digestible by true proteases, and in which the sulfur is in the sulfhydryl state. The important role of the disulfide bond had already been emphasized by Speakman and Huist and Astbury on the basis of their chemical and physical studies, including x-ray diffraction patterns of keratins.

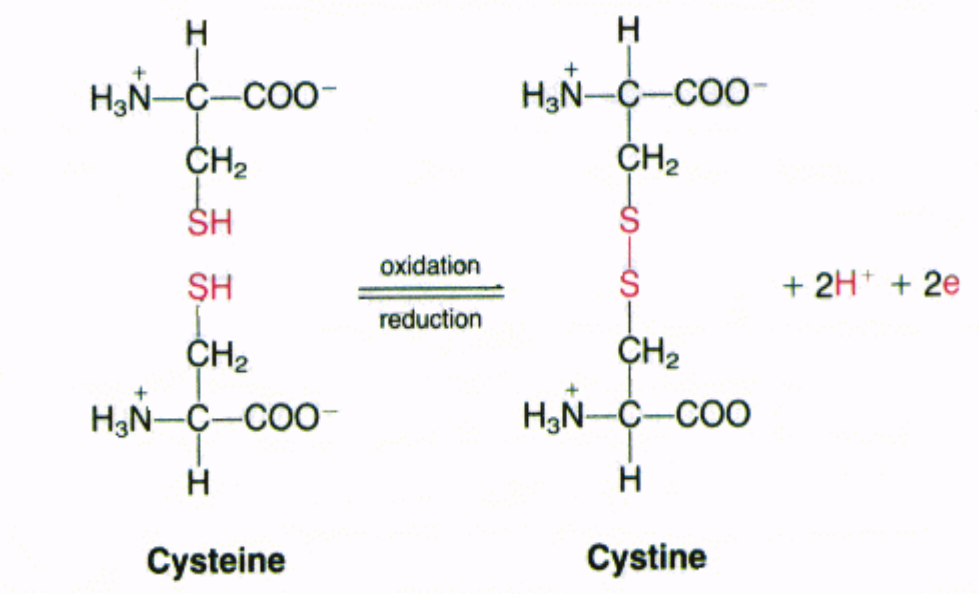


Figure 2.3: Reactions to form and break disulfide bonds.

Since keratins have a very high percentage of disulfide sulfur (10 to 15 per cent cystine) and may readily be reduced to sulfhydryl proteins by alkaline thioglycolate, realise that this reduced protein might be a useful material for study as an example of a sulfhydryl protein. We were particularly interested in studying the properties of derived proteins formed by substitution in the sulfhydryl group by reaction with organic halogen compounds according to the following scheme :

A-SH + R-X --+ A-S-R + HX

This reaction occurs with great ease at neutral to mildly alkaline reaction, and has already been applied to proteins for different reasons by Mirsky, Anson, Goddard and Schubert. Although organic halogen compounds can react with amino groups according to Michaelis and Schubert, it will be shown that it has been possible under certain conditions to substitute completely. The sulfhydryl groups of the protein of reduced feather keratin, without substituting amino groups.

While the journal “Molecular Size, Shape and Aggregation of Soluble Feather Keratin” says that another method is Feather keratins were extracted from chicken feathers with an aqueous solution of urea and 2-mercaptoethanol. The keratin solution obtained was dialyzed to remove the reagents. Upon dialysis, extensive protein aggregation occurred. To obtain stable solutions or dispersions in water, cysteine residues were modified prior to dialysis with iodoacetamide, iodoacetic acid, or

bromosuccinic acid, thereby blocking free thiol groups and introducing hydrophilic groups. For the development of biodegradable materials with good mechanical properties from these biopolymers, disulfide bonds between the keratin molecules are needed. Therefore, cysteine residues were only partially modified by using different reagent/cysteine molar ratios. The reaction rate constants of iodoacetate with glutathione and 2-mercaptoethanol were successfully used to predict the degree of modification of keratin cysteine. It was shown that, for carboxymethylated keratin, fewer aggregates were formed for higher degrees of cysteine modification, while more protein was present as oligomers. Aggregates and oligomers were stabilized through intermolecular disulfide bonds.

The journal "Soluble Derivatives of Feather Keratin" they have described about the pretreatment of the feathers before the extraction process begins, which is very important in some methods of extraction. This is because according to the journal some of the protein extraction process from chicken feather using certain reagents will not give the expected result if there is no pretreatment on the feathers. However not all the methods of protein extraction needs pretreatment on the feathers used, a rinse in the hot water will be the only pretreatment needed. In this journal the feathers were washed in a hot dilute detergent solution at neutral pH, and rinsed in warm tap water and then distilled water.

Any pulpy protein in the calamus was squeezed out and the feathers were air-dried. They were then extracted with several changes of light petroleum until a small portion of the solvent gave no residue when allowed to evaporate on a watch-glass. This was followed by two rinses with 95% (v/v) ethanol, one with distilled water and two more with 95% ethanol, after which the feathers were allowed to air-dry. The method used was Reduction and alkylation. In order to determine the optimum

conditions for extraction the effects of temperature, time, pH and concentration of reducing agent were studied for feather keratin.

The reducing agent used was sodium mercaptoacetate with a liquor: feather ratio of 100: 1 (v/w), and the protein concentrations were obtained by determination of nitrogen by the Kjeldahl method. The mercaptoacetic acid was redistilled under reduced pressure to eliminate thiol esters. Below pH 9-5 less than 1% of the protein was extracted at 250 and there was a rapid increase in the amount of protein extracted between pH 9-5 and 10-5. The amount extracted decreased at mercaptoacetate concentrations above 0-2M. For studies on the effect of time and temperature on the extraction process, the conditions used were 0-IM-mercaptoacetate and pH 11-0, at temperatures of 20, 250 and 400. The effect of temperature on the rate of extraction was similar to that found for the extraction of wool proteins.

2.3 Ammonium Sulfate Precipitation

Ammonium sulfate precipitation is a method used industrially to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out. Ammonium sulfate is commonly used as its solubility is so high that salt solutions with high ionic strength are allowed.

The solubility of proteins varies according to the ionic strength of the solution, and hence according to the salt concentration. Two distinct effects are observed: at low salt concentrations, the solubility of the protein increases with increasing salt concentration an effect termed salting in. As the salt concentration (ionic strength) is increased further, the solubility of the protein begins to decrease. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution (salting out).

Since proteins differ markedly in their solubility at high ionic strength, salting-out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate, as it is very water soluble, forms two ions high in the Hofmeister series, and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution). In the preliminary test, the ammonium sulfate concentration is increased stepwise, and the precipitated protein is recovered at each stage. This is

usually done by adding solid ammonium sulfate, but calculating how much

ammonium sulfate to add to a solution at one concentration to achieve a desired higher concentration is tricky, since addition of ammonium sulfate significantly increases the volume of the solution. The amount to add can be determined either from published nomograms or by using an online calculator. Each protein precipitate is dissolved individually in fresh buffer and assayed for total protein content and amount of desired protein.

The aim is to find the ammonium sulfate concentration which will precipitate the maximum proportion of undesired protein, whilst leaving most of the desired protein still in solution or vice versa.

The precipitated protein is then removed by centrifugation and then the ammonium sulfate concentration is increased to a value that will precipitate most of the protein of interest whilst leaving the maximum amount of protein contaminants still in solution. The precipitated protein of interest is recovered by centrifugation and dissolved in fresh buffer for the next stage of purification.

This technique is useful to quickly remove large amounts of contaminant proteins, as a first step in many purification schemes. It is also often employed during the later stages of purification to concentrate protein from dilute solution following procedures such as gel filtration.

The journal “A study of keratin” defines that all the keratin proteins are insoluble in water and resemble casein or denatured proteins. The dry proteins can best be dissolved in weak alkalies by grinding in a mortar with Na2C03 or NaHC03. In dilute NaOH they dissolve readily, but lose sulfur. When the filtrate of wool dissolved in NazS is dialyzed

directly for several days, the protein remains in solution, but it is precipitated by a trace of acetic acid. Among the dry proteins, the cyanide preparation dissolves most readily.

2.4 Biuret Test

The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper(II) ion forms a violet-colored complex in an alkaline solution. Several variants on the test have been developed. The Biuret reaction can be used to assay the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law. In spite of its name, the reagent does not in fact contain biuret ($(H_2N\text{-}CO\text{-})_2NH$). The test is so named because it also gives a positive reaction to the peptide bonds in the biuret molecule.

An aqueous sample is treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper(II) sulfate. If the solution turns purple, protein is present. 5–160 mg/mL can be determined. Cu^+ is a strong reducing agent which can react for example with Mo(VI) in Folin-Ciocalteu's reagent to form molybdenum blue. In this way, proteins can be detected in concentrations between 0.005 and 2 mg/mL. Molybdenum blue in turn can bind certain organic dyes (malachite green, Auramin O), resulting in further amplification of the of the signal.

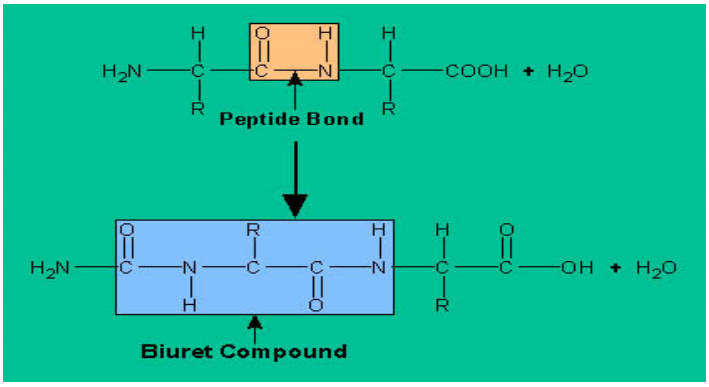


Figure 2.4: The Biuret compound in the Biuret solution.

2.5 Infrared Properties Of Protein

FTIR spectroscopy provides information about the secondary structure content of proteins, unlike X-ray crystallography and NMR spectroscopy which provide information about the tertiary structure. FTIR spectroscopy works by shining infrared radiation on a sample and seeing which wavelengths of radiation in the infrared region of the spectrum are absorbed by the sample. Each compound has a characteristic set of absorption bands in its infrared spectrum. Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the Amide I band leads to stretching vibrations of the C=O bond of the amide, absorption associated with the Amide II band leads primarily to bending vibrations of the N—H bond. Because

both the C=O and the N—H bonds are involved in the hydrogen bonding that takes

place between the different elements of secondary structure, the locations of both the Amide I and Amide II bands are sensitive to the secondary structure content of a protein. Studies

with proteins of known structure have been used to correlate systematically the shape of the Amide I band to secondary structure content

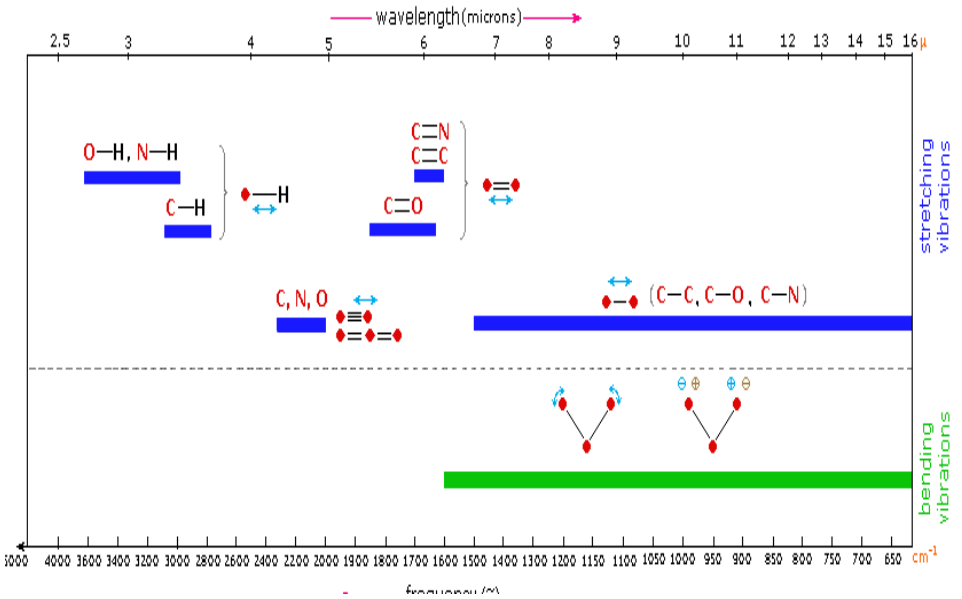


Figure 2.5: Wave lengths of different bonds and functional groups.

The N–H stretches of amines are in the region 3300-3000 cm^{-1}. These bands are weaker and sharper than those of the alcohol O–H stretches which appear in the same region. In primary amines (RNH_2), there are two bands in this region, the asymmetrical N–H stretch and the symmetrical N–H stretch. Secondary amines (R_2NH) show only a single weak band in the 3300-3000 cm^{-1} region, since they have only one N–H bond. Tertiary amines (R_3N) do not show any band in this region since they do not have an N–H bond.(A shoulder band usually appears on the lower wavenumber side in primary and secondary liquid amines arising from the overtone of the N–H bending band: this can confuse interpretation).The N–H bending vibration of primary amines is observed in the region 1650-1580 cm^{-1}. Usually, secondary amines do not show a band in this region and tertiary amines never show a band in this region. (This band can be very sharp and close enough to the carbonyl region to cause students to interpret it as a carbonyl band.)

Another band attributed to amines is observed in the region 910-665 cm^{-1}. This strong, broad band is due to N–H wag and observed only for primary and secondary amines.The C–N stretching vibration of aliphatic amines is observed as medium or weak bands in the region 1250-1020 cm^{-1}. In aromatic amines, the band is usually strong and in the region 1335-1250 cm^{-1}.

2.6 Wavelengths In Protein Summarized

- N–H stretch 3400-3250 cm^{-1}
  - 1° amine: two bands from 3400-3300 and 3330-3250 cm^{-1}
  - 2° amine: one band from 3350-3310 cm^{-1}
  - 3° amine: no bands in this region
- N–H bend (primary amines only) from 1650-1580 cm^{-1}
- C–N stretch (aromatic amines) from 1335-1250 cm^{-1}
- C–N stretch (aliphatic amines) from 1250–1020 cm^{-1}
- N–H wag (primary and secondary amines only) from 910-665 cm^{-1}
- C=O stretch (primary amines) from 1630- 1695 cm^{-1}

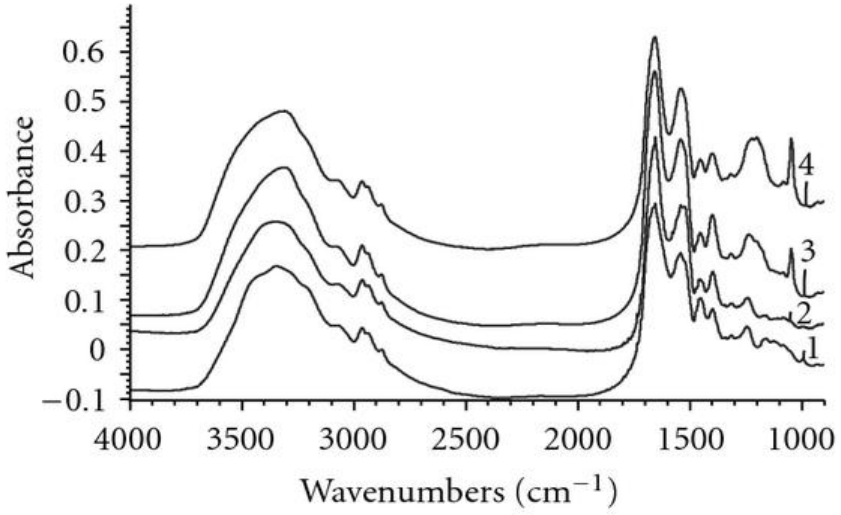


Figure 2.6: Standard curve of protein obtained for different concentrations.

CHAPTER 3

RESEARCH METHODOLOGY/DESIGN

3.1 Introduction

In this chapter detailed methods to produce protein from chicken feathers will be discussed. Also introduction about equipment and apparatuses used will be given. There are three major steps to obtain protein from chicken feathers, dissolving, precipitating and purification. The research was done repeatedly using three different dissolving agents. The product obtained was first tested with biuret test and the solution was analyzed with uv-vis. The final product obtained also analyzed with Fourier transform infrared spectrometer and compared with standard graph.

3.2 Material And Apparatus Used.

3.2.1 Material And Reagents Used:

a) Chicken feather

b) Ether

c) Thiglycolic acid

d) Potassium cyanide

e) Sodium sulfide

f) Sodium hydroxide

g) Ammonium sulfate

h) Potassium hydroxide

i) Copper sulfate.

3.2.2 Apparatus Used:

a) Beaker

b) Volumetric flask

c) Conical flask

d) Quartz cuvette

e) Centrifuge tube

f) Thermometer

g) pH meter

h) Magnetic stirrer

3.2.3 Equipments used.

a) Centrifuge

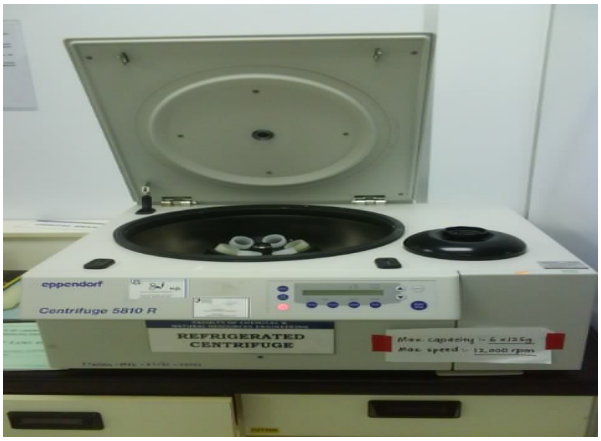


Figure 3.1: Centrifuge

A centrifuge generally driven by an electric motor that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration causes more dense substances to separate out along the radial direction, the bottom of the tube. Centrifuge is very important in this research since it is very efficient in separating solids and liquids.

b) Uv-Visual

Figure 3.2: UV-Vis

Ultraviolet-visible spectroscopy refers to absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this research uv-vis was used to calculate the absorbance of the biuret test solution which is proportional to the number of peptide bond present in the solution.

C) Fourier Transform Infrared Spectroscopy (Ftir).

Figure 3.3: FTIR

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. FTIR technique has made dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared) and opened up new applications of infrared spectroscopy. This equipment played a very important role in this research since it do confirm the functional groups present in the product and enables us identify the projects clearly and also make sure no foreign objects present in the product.

3.3 DETAILED PROCESS OF THE RESEARCH.

3.3.1 Pretreatment Of The Feathers.

1. Chicken feathers are collected from chicken processing plants.

2. The feathers are soaked in ether and left overnight

3. The feathers are then washed with soap water and dried under sunlight.

4. The dried feathers are then blended and kept carefully in sealed plastic bag.

3.3.2 Dissolving Of Chicken Feathers

1. 2L of 0.5M sodium sulfide solution is prepared in a 2L conical flask.

2. 50g of the blended chicken feathers are weighed and added to the sodium sulfide solution.

3. The solution is maintained at 30 °C, pH range: 10-13 and stirred for 6 hours.

4. The solution is then filtered and centrifuged at 10000rpm for 5 minutes.

5. The supernatant liquid carefully collected then filtered using filter paper and made sure it is particle free.

3.3.3 Preparation Of Ammonium Sulfate Solution

1. 700g of ammonium sulfate is dissolved in 1L deionized water.

2. The solution is stirred until all the ammonium sulfate particles are dissolved.

3. The solution is then filtered and made sure no particles present in the solution.

3.3.4 Protein Precipitation

1. The feather filtrate solution collected earlier placed in a beaker and stirred.

2. Ammonium sulfate solution is added slowly (preferably drop wise).

(The ratio of feather filtrate solution and ammonium sulfate solution added is 1:1)

3. The solution is then centrifuged at 10000rpm for 5minutes and the solids particles are carefully collected.

4. The supernatant liquids are collected separately and step 2 and 3 are repeated with it.

3.3.5 Protein Purification

1. The solid particles collected are added into 100ml deionized water and stirred (washing).

2. The solution is then centrifuged at 10000rpm for 5minutes and the solids are gathered carefully.

3. The collected solid particles are then dissolved in 100ml of 2M sodium hydroxide solution.

4. The solution is then centrifuged again at 100000rpm for 5 minutes and all the liquids are collected carefully and stored while the solids are discarded.

5. The precipitating, washing and dissolving steps are repeated 3 times.

3.3.6 Biuret Test

1. 1% copper sulphate solution and 1% potassium hydroxide solution are prepared.

2. The 5ml of the solution collected is mixed with potassium hydroxide solution with 1:1 ratio.

3. Three drops of Copper sulphate solution is added to the mixture solution.

4. Changes in the solution observed and recorded.

5. The solution is analysed under uv-vis to obtain its absorbance.

3.3.7 Analysis Of The Sample.

1. The solution collected in purification steps analyzed in ftir and its wavelength graph obtained and compared with standard.

2. The solution fully precipitated using ammonium sulfate, the solids are separated weighed and its weight recorded.

***The whole process repeated with 0.5M thioglycolate solution and 0.5M potassium cyanide solution replacing sodium sulfide solution. Both thioglycolate and potassium cyanide solutions are prepared with 0.1N sodium hydroxide.**

CHAPTER 4

RESULT AND DISCUSSION

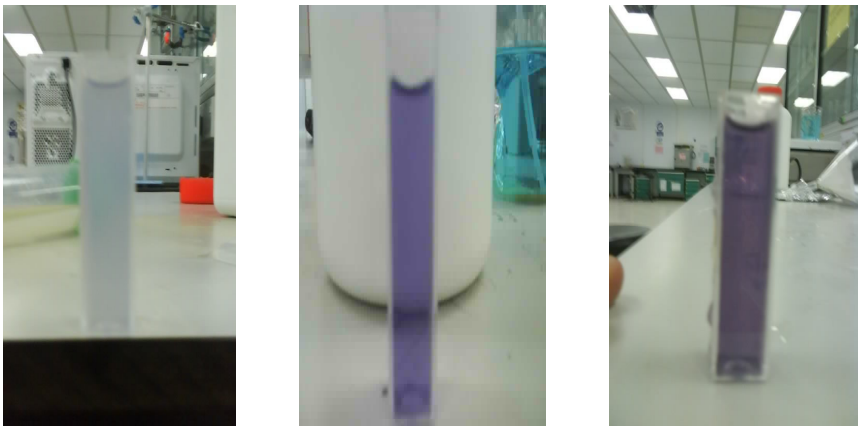
4.1 Introduction

The objective of this research is to produce protein from chicken feather and at the same time find the best reducing agent to dissolve the chicken feather. Usually oxidizing agents such as hydrogen peroxide and bromine are used to oxidize feather keratins to obtain protein. But oxidizing agents damage the protein contents and also act very slowly. But reducing agents act faster and do no damage to the protein content of the feather since reducing agents are specific to the disulfide bond found between amino acids in keratin. In other words reducing agents only reduce the disulfide bonds formed by the amino acid cystein in keratin thus dissolve the keratin. The process also much more simpler than oxidizing agent dissolving the feathers.

4.2 Absorbance Of Biuret Test Solutions

PROTEIN SAMPLES	ABSORBANCE
A (Thioglycolate solution)	0.326
B (Potassium cyanide solution)	0.742
C (Sodium sulfide solution)	1.435

Table 4.1: Absorbance values of the samples.



Sample A Sample B Sample C

Figure 4.1: Visible differences of biuret solutions of all three samples.

4.3 Mass Of Protein Obtained

PROTEIN SAMPLES	MASS (g)
A (Thioglycolate solution)	26.5
B (Potassium cyanide solution)	14.8
C (Sodium sulfide solution)	4.4

Table 4.2: Mass of Protein obtained for different samples.

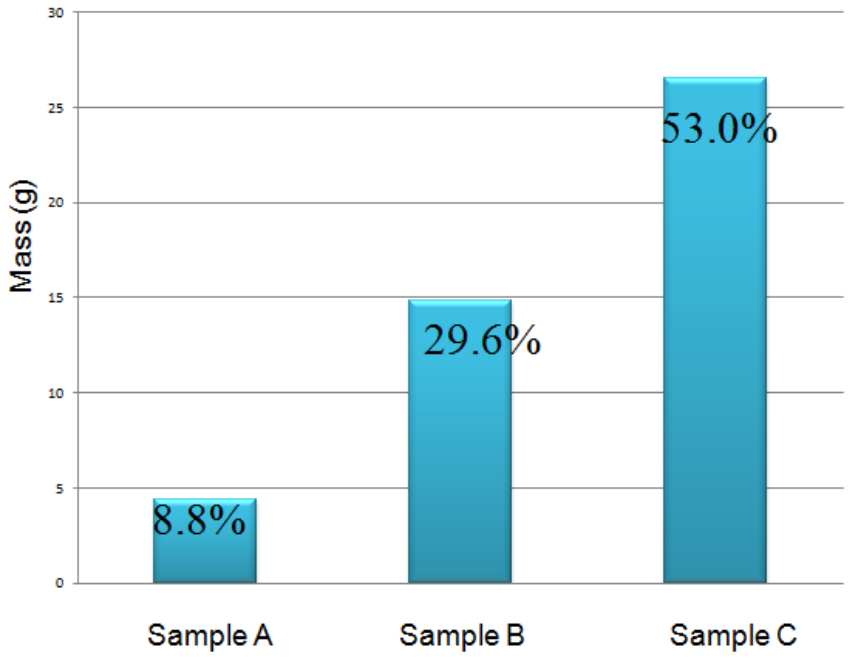
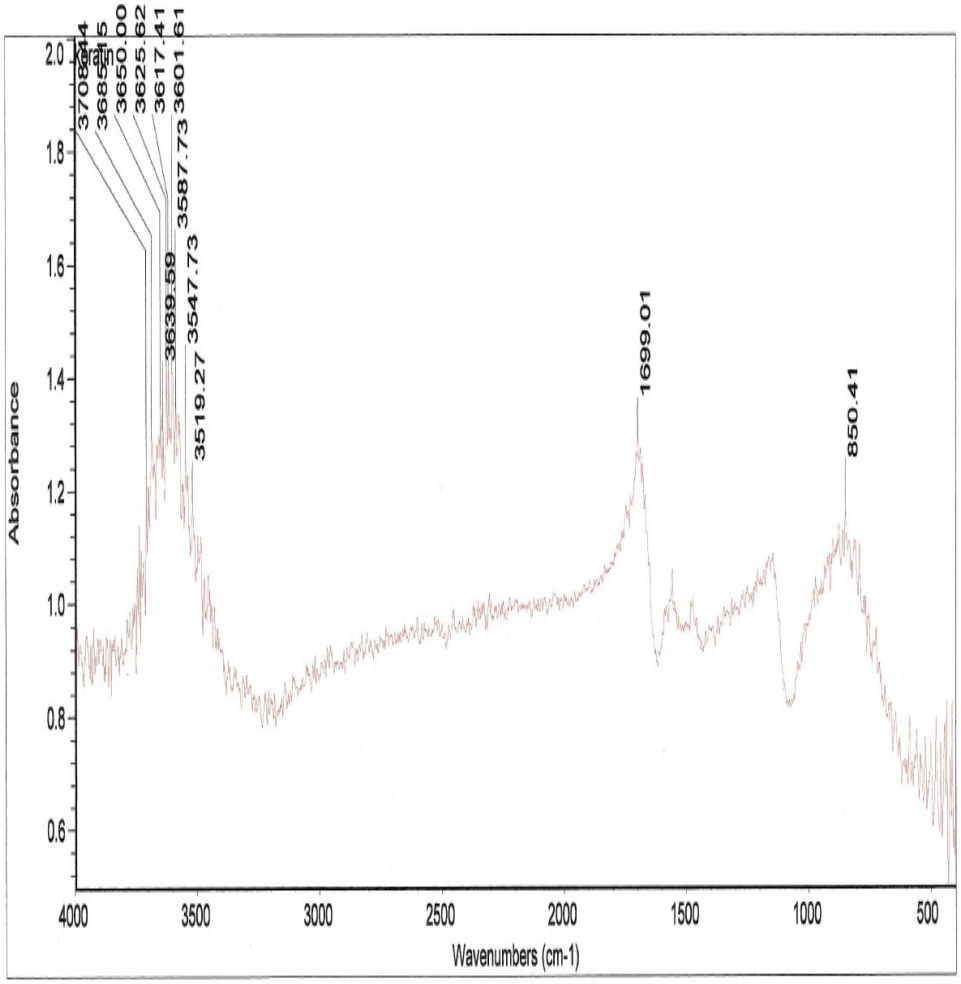


Figure 4.2: Chart showing differences of mass of the different samples.

4.4 Fourier Transform Infrared Spectroscopy Results



Fri Oct 29 09:51:52 2010 (GMT+08:00)
FIND PEAKS:
Spectrum: keratin
Region: 4000.00 400.00
Absolute threshold: 1.154
Sensitivity: 41

Figure 4.3: FTIR result obtained for the final product.

4.5 Discussion

Chicken feathers dissolved fully in sodium sulfide solution. Feathers in potassium cyanide and thiglycolate solution are only partially dissolved. Around 45% feathers in potassium cyanide solution and 70% feathers in thioglycolate solution can be filtered out after 6 hours of reaction. This concludes that sodium sulfide reduces chicken feather efficiently than the other two reducing agents.

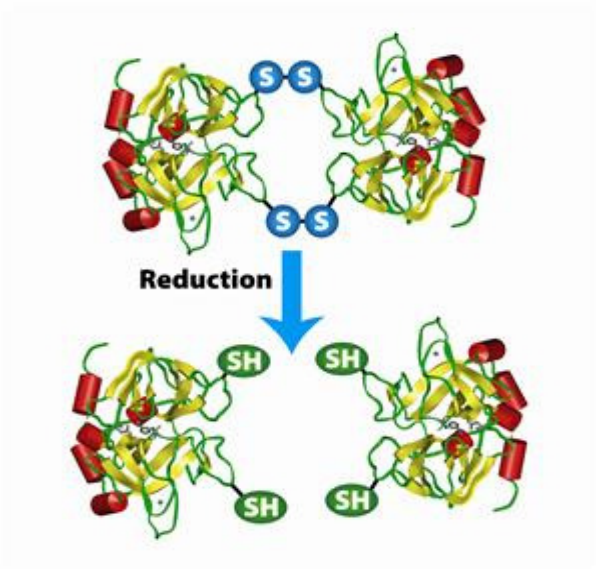


Figure 4.5: Reduction of disulfide bonds.

The presence of protein is confirmed by biuret test. The solution turned purple after reagent is added and this is only possible if peptide bonds are present in it. The more peptide bonds in it the higher the intensity of the purple color. Also the difference between the purple colors of the different solutions is visible seen too. This is in accordance with

absorbance of biuret solution and amount of protein obtained in the end of the research. The higher the amount of chicken feather dissolved the higher the protein obtained.

According to beer lambert law:

A= εIc

Absorbance is proportional to concentration of a solution. So the increasing absorbance also shows protein concentration highest in sodium sulfide reacted solution and lowest in the thiglycolic acid reacted solution.

The dissolving rate of feathers in thioglycolate solution and potassium cyanide solution is low because the the reaction will be highest only if the solution is highly alkaline with pH of the solution is in the range 10 to 13. This is because in the alkaline state the proton will be removed from the the amino group and the ionic bond formed by electrostatic attraction of the NHs+ group of the diamino acids and the COO- group of the dicarboxylic acids can be broken. Due to some reasons these ionic bond must be broken first to reduce the disulfide bonds of the keratin and dissolve the feathers.

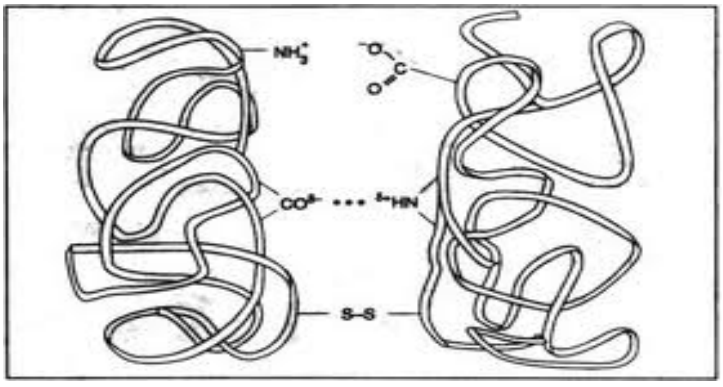


Figure 4.6: The three important bonds need to be broken to dissolve feathers.

The sodium sulfide solution is readily alkaline not like thiglycolate solution or potassium cyanide solution in which sodium hydroxide has to be added to make it alkaline with the ph 10 to 13. This explains why even though both solutions are reducing agents they cannot reduce the disulfide bonds. Because without alkaline state proton cannot be removed thus cannot break the ionic bond. So sodium hydroxide plays a important role in dissolving feathers. So in order to maximize the dissolving ability of potassium cyanide and thioglycolate solution the exact amount of sodium hydroxide to be added must be figured out.

The total mass of the protein obtained for each of the method in the end of the research also shows that 53%, 29.6% and 8.8% of feathers are converted to protein for the solutions of the reducing agents sodium sulfide, potassium cyanide and thioglycolic acid respectively. This is in accordance with the result of the UV-Vis analysis.

The protein sample also analyzed using Fourier transform infrared spectroscopy. The graph obtained matched the standard graph of protein solution. The wavelength obtained from the infrared spectroscopy confirmed C-N bond, N-H bond, and C=O bond are in the sample thus the presence of carboxyl group and amino groups the two groups that will only present in amino acids are undeniable. Therefore the sample confirmed true protein.

The Fourier transform infrared spectroscopy also detects the presence of primary and secondary amines in the protein sample obtained. The shift of the primary protein peak more to the wavelength 1700cm^{-1} than wavelength 1600cm^{-1} gives the assumption that amino acids such as Cysteine, Glutamine, Argine, Asparagine, Glycine, Alanine, Phenilalanine, and Theronine have the high chances to be in the sample protein obtained. Since most of these amino acids are non essential amino acids, the protein from chicken feathers can be used for cosmetic products majorly and also to make medicines. These amino acids also can be used for patients whose body having diseases that cannot form these amino acids on its own.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The research is done to find a alternative source for natural protein. Since chicken feathers are consists of 90% crude protein and pose environmental woes due to its time consuming decomposition, it is an ideal material to obtain natural protein. The chicken feathers are first dissolved using reducing agents and protein precipitated out from the solution using ammonium sulfate. In this research three different reducing agents, thiglycolic acid, potassium cyanide and sodium sulfide are used to dissolve the chicken feathers. The dissolving ability of all three agents are compared and are found that sodium sulfide has the highest efficiency since it does dissolved all the feathers completely in a very short period of time. While thioglycolic acid has the least efficiency in dissolving the feathers. It is also found that the dissolving ability of thiglycolic acid and potassium cyanide mainly depends on the pH of the solution which is

controlled by the amount of sodium hydroxide added to the solution. Ammonium sulfate as expected precipitated protein and at the same time used to purify the protein as the steps repeated few times. The presence of the protein was confirmed first by the biuret test where the reagent changed to purple color in the presence of peptide bonds. A further analysis by the Fourier transform infrared spectroscopy confirmed the presence of amino and carboxyl groups only in the sample, the two groups present together only in amino acids. Thus the product obtained at the end of the research confirmed true protein without the presence of any foreign materials.

5.2 Recommendation.

The efficiency of thioglycolic acid and potassium cyanide solutions depends on the amount of sodium hydroxide added to it which controls the pH of the solution. In order to increase the protein yield of the two solutions the correct amount of sodium hydroxide to be added to must be figured out. The chicken feather must be blended as fine as can since it will speed up the dissolving process. Even though stirrer is used to stir the solution during the dissolving process, the feathers in the solution make the solution to thick to be stirred in order to overcome this mixer can be used to stir the solution. The protein precipitates must be gathered carefully to minimize the protein lost since the amount is too small. In the uv-vis analysis the quartz cuvette should be used instead of normal plastic cuvette since for protein sample cuvette made up from quartz will yield a precise answer.

The protein sample should kept under room temperature to minimize it degradation and advisably in liquid state and closed container, since solid state undergoes degradation faster. The Fourier transform infrared spectroscopy will yield a precise graph if the protein sample is in solid state since some of the liquid samples contain very low protein concentration.

The detailed studies such as toxicity, amino acid composition and chemical composition on the protein sample obtained will lead to the greater understanding of the protein obtained.

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APPENDIX A

Produce Natural Protein From Chicken Feather

Methods With Pictures (Sodium Sulfide Method.)

Feather dissolving Process.

Sodium sulfide solution

Blended chicken feather

Blended feather in Sodium sulfide solution

The feather filtrate solution (after manual filteration)

The feather filtrate solution after centrifuged.

The solution is filtered as an extra precaution

Ammonium sulfate solution

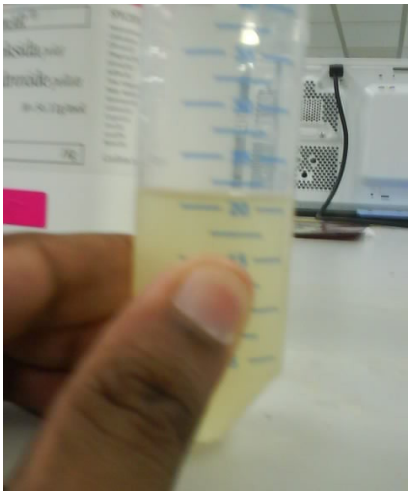
Precipitates formed once Ammonium sulfate added to the solution

The precipitates are then centrifuged.

The precipitates are then collected.

Protein solution obtained after purification process.

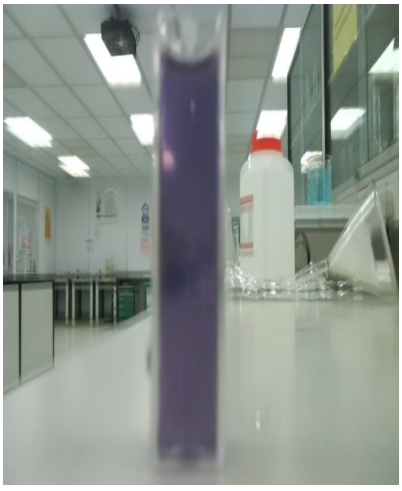
Biuret Test



The Precipitate dissolved solution Mixed with potassium hydroxide Solution.

Three drops of copper sulfate added to the mixed solution.

The Mixed solution turns purple Confirming protein presence.



Clear view of the purple color

APPENDIX B

Material Safety Data Sheets Of Chemicals Used.

Thioglycolic Acid

General:

Synonyms: mercaptoacetic acid, 2-thioglycolic acid, thiovanic acid,
Molecular formula: $C_2H_4O_2S$
CAS No: 68-11-1
EC No:

Physical data:

Appearance: colourless liquid with a strong and unpleasant odour
Melting point: -16.5 C
Boiling point: 120 C at 20 mm Hg
Vapour density:
Vapour pressure:
Density (g cm^{-3}): 1.35
Flash point: 112 C
Explosion limits:
Autoignition temperature:
Water solubility: substantial

Stability:

Readily oxidized by air. Combustible. Incompatible with oxidizing agents.

Toxicology:

Harmful if swallowed, inhaled or absorbed through the skin.

Personal protection:

Safety glasses, adequate ventilation.

Potassium Cyanide

General:

Synonyms: hydrocyanic acid potassium salt
Molecular formula: KCN
CAS No: 151-50-8
EC No: 205-792-3

Physical data:

Appearance: white granular powder or crystals
Melting point: 634 C
Boiling point:
Vapour density: 2.2
Vapour pressure:
Specific gravity: 1.52
Flash point:
Explosion limits:
Autoignition temperature:

Stability:

Stable. Incompatible with a variety of materials, including acids, iodine, peroxides, permanganates, alkaloids, chloral hydrate, metallic salts. Light and moisture sensitive. Contact with acid generates extremely toxic HCN gas.

Toxicology:

Very toxic. May be fatal if inhaled, swallowed or absorbed through skin. Note low LD values below. Extremely destructive of mucous membranes. Causes burns.

Personal protection:

Gloves, safety glasses and good ventilation. Do not use in the open laboratory. Ensure all containers are properly labelled. Keep cyanide poison kit on hand.

Sodium Sulfide

General:

Synonyms: disodium sulphide, sodium sulfide, disodium sulfide, sodium monosulfide
Molecular formula: Na_2S
CAS No: 1313-82-2
EC No: 215-211-5
Annex I Index No: 016-009-00-8

Physical data:

Appearance: crystals of varied colour, with a repulsive odour
Melting point:
Boiling point:
Vapour density:
Vapour pressure:
Specific gravity: 1.86
Flash point:
Explosion limits:
Autoignition temperature:

Stability:

Spontaneously flammable. Incompatible with acids, metals, oxidizing agents. Contact with acid liberates toxic gas.
Fine dust/air mixtures are explosive. Hygroscopic.

Toxicology:

Toxic if swallowed (stomach acids can liberate very toxic hydrogen sulfide gas). Corrosive - causes burns.

Environmental information:

Harmful in the environment - very toxic to aquatic organisms.

Personal protection:

Safety glasses, gloves. Effective ventilation.

Ammonium sulfate

General:

Synonyms: actamaster, ammonium sulphate, diammonium sulfate, diammonium sulphate, dolamin, mascagnite, sulfuric acid diammonium salt
Molecular formula: $(NH_4)_2SO_4$
CAS No: 7783-20-2
EC No: 231-984-1

Physical data:

Appearance: white crystals, granules or powder
Melting point: ca. 280 C (decomposes)
Boiling point:
Vapour density:
Vapour pressure:
Density (g cm^{-3}): 1.77
Flash point:
Explosion limits:
Autoignition temperature:
Water solubility: appreciable

Stability:

Stable. Contact with strong oxidizers may cause fire or explosion. Incompatible with strong bases.

Toxicology:

Not hazardous according to Directive 67/548/EEC.

Transport information:

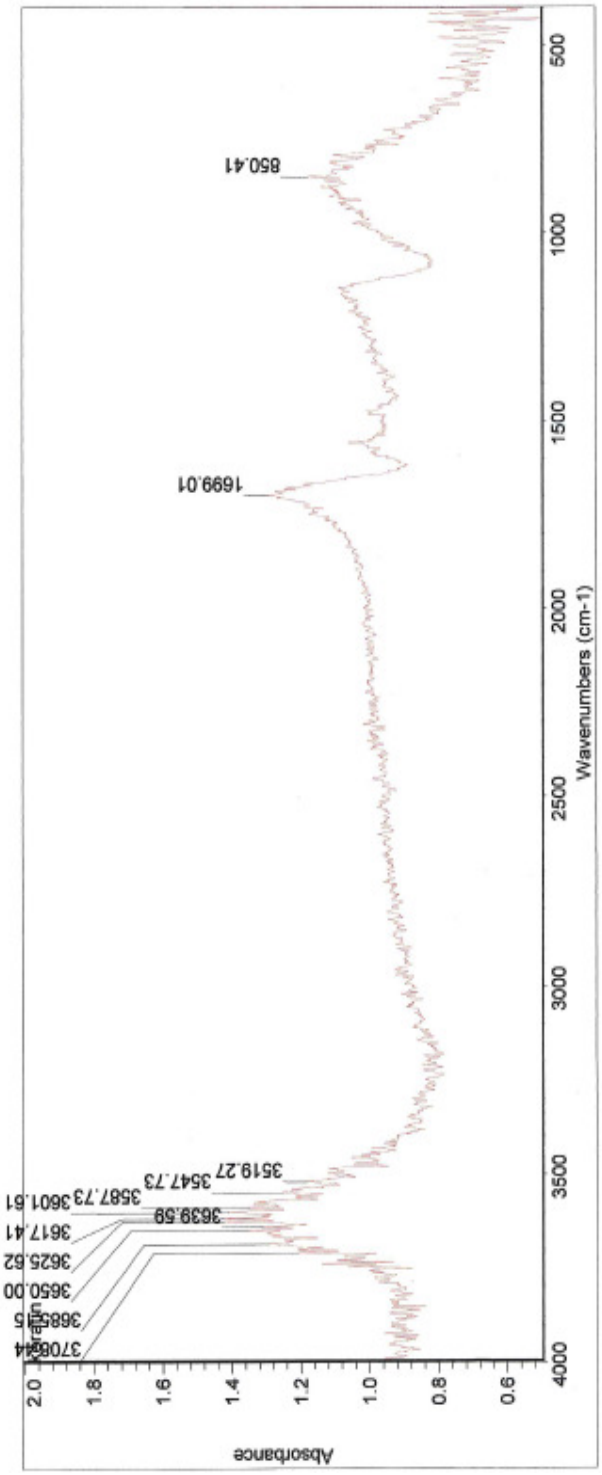
Non-hazardous for air, sea and road freight.

Personal protection:

Minimize exposure.

APPENDIX C

FTIR Result.



Fri Oct 29 09:51:52 2010 (GMT+08:00)
FIND PEAKS:
Spectrum: keratin
Region: 4000.00 400.00
Absolute threshold: 1.154
Sensitivity: 41
Peak list:

Position:	850.41	Intensity:	1.181
Position:	1699.01	Intensity:	1.291
Position:	3519.27	Intensity:	1.178
Position:	3547.73	Intensity:	1.256
Position:	3587.73	Intensity:	1.358
Position:	3601.61	Intensity:	1.419
Position:	3617.41	Intensity:	1.435

Simplified Table of Main IR Frequencies

Wave number, cm^{-1}	Functional Group	Peak Description
3300 – 3600	O-H (alcohol)	Strong and broad
2500 – 3000 can reach	O-H (carboxylic acids)	Very broad (over ~ 500 cm^{-1}), often looks like distorted baseline, above 3000 cm^{-1}.
3200 – 3500	N-H	Doublet in case of NH_2 group of a primary amine or amide
3300	$\equiv$ C—H terminal alkyne	Usually sharp and strong
3000 - 3100	= C—H alkene or arene	Often weak, overlaps with CH alkane absorption
2800 – 3000	C-H (sp^3 carbon)	Strong, broad and multi-banded
2250 - 2220	C$\equiv$N	Medium intensity
2100 - 2260	C$\equiv$C alkyne	Medium intensity for terminal alkynes, very weak for internal
1680 – 1820	C=O (amides, ketones, aldehydes carboxylic acid, esters)	Very strong; lower frequency for amides and when C=O is conjugated
1600 – 1650	C=C alkene, aromatic ring	Check to see if you have C-H unsaturated >3000 cm^{-1} (if not, it's completely substituted)
~ 1600	$-NH_2$ (bending) 1° amines and amides	Only if you have corresponding N-H peak at 3200-3500 cm^{-1} (this peak may be mistaken for C=C otherwise)
1200	Ar-O	Strong (look for =C-H & C=C first)
1050-1150	C-O	
690 and 750	phenyl group	Strong (look for =C-H & C=C first)