SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

EVALUATION OF FISH WASTE AS STARTING MATERIAL FOR PRODUCING BIODEGRADABLE PROTEIN-BASED THERMOPLASTIC POLYMERS

By

SHERYLYN JOUTI

Supervisor: Dr Ku Marsilla Ku Ishak

Dissertation submitted in partial fulfillment
of the requirements for the degree of Bachelor of Engineering with Honours
(Polymer Engineering)

Universiti Sains Malaysia

JUNE 2017

DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "**Evaluation of Fish waste as Starting Material for Producing Biodegradable Protein-Based Thermoplastic Polymers**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

Name of student: Sherylyn Jouti Signature:

Date: 22 June 2017

Witness by

Supervisor: Dr Ku Marsilla Ku Ishak Signature:

Date: 22 June 2017

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude towards my Almighty Father who has provided me a platform and opportunity to learn something that is completely new to me.

This thesis existence is due to the help, assistance, support and inspiration of several people. Firstly, I would like to express sincere appreciation and gratitude to my FYP supervisor, Dr Ku Marsilla Ku Ishak for her guidance and support throughout the research. Her advice and guidance was the one that push me to work beyond my limits. I will remember all the word of encouragement given to me throughout the research.

Every result of described in the thesis was accomplished with the help and support of my fellow labmates Miss Salwani Shaffie and Miss Siti Amirah bt Alias. They guided me since the very beginning of the research as I am still new in this field. I would like to thank them for their patience in teaching, guiding and supporting me. Miss Amirah joined 1 month later and I admired her for her willingness in learning something new and foreign to her. She was extremely reliable.

I would also like to thank the entire staffs and technician especially Mr Norshahrizol bin Nordin and Mr Mohammad Hassan that willing to spend time in teaching and helping me to familiarise myself with the equipment in the lab. Without them this research could not be completed.

Last but not least, I would like to thank my family and friends who had supported and encourage me. Their moral support was what keeps me going through the tough time while finishing this research.

TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 MATERIALS AND EXPERIMENTAL PROCEDURES

3.1. Material

3.2. Sample Preparation

3.3. Characterization

CHAPTER 4 RESULTS AND DISCUSSIONS

CHAPTER 5 CONCLUSION AND RECOMMENDATION OF FUTURE WORKS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PLA	Polylactic acid
TPS	Thermoplastic starch
TSP	Textured soy protein
Tg	Glass transition temperature
T_D	Decomposition temperature
PEG	Polyethylene glycol
SME	Specific mechanical energy
SS	Sodium Sulphate
SDS	Sodium Dodecyl Sulphate
SEM	Scanning electron microscope
DSC	Differential Scanning Calorimetry
TGA	Thermogravimetric Analysis
FTIR	Fourier Transform Infrared Spectroscopy

PENILAIAN SISA IKAN SEBAGAI BAHAN PERMULAAN UNTUK MENGHASILKAN POLIMER TERMOPLASTIK BERASASKAN PROTEIN TERBIODEGRADASI

ABSTRAK

Objektif utama penyelidikan ini adalah untuk mengkaji kaedah pemprosesan yang sesuai untuk menghasilkan termoplastik protein berasaskan bahan buangan ikan, serta faktor-faktor yang mempengaruhi haba, morfologi, ciri-ciri fizikal dan mekanikal termoplastik protein berasaskan daripada bahan buangan ikan. Dua sampel telah digunakan, isi ikan dan sisa ikan yang kedua-duanya mempunyai kandungan protein yang berbeza. Sampel protein dihasilkan menggunakan extruder berskru kembar, pengadun dalaman dan extruder berskru tunggal. Selepas itu, sifat-sifat mekanikal dan terma sampel protein telah dikenal pasti. Sifat haba daripada termoplastik ikan ditentukan oleh DSC dan TGA dengan mengukur suhu penguraian dan suhu peralihan kaca (T_g). Morfologi permukaan patah daripada termoplastik protein sampel dicirikan menggunakan SEM. FTIR dan ujian tegangan digunakan untuk mengenal pasti sifat-sifat fizikal dan mekanikal diperolehi. Penyelidikan ini telah mendapati bahawa isi ikan mempunyai kekuatan tegangan yang paling tinggi berbanding dengan sisa ikan. Suhu peralihan kaca didapati berkurang apabila kandungan air meningkat. Keputusan TGA menunjukkan bahawa kestabilan haba isi ikan adalah lebih tinggi daripada sisa ikan. Bukti daripada SEM mendedahkan bahawa kaedah pemprosesan yang paling sesuai untuk sisa ikan adalah extruder berskru kembar kerana tunjukkan kehomogenan yang lebih baik daripada pengadun dalaman dan extruder berskru tunggal. Extruder berskru kembar juga menunjukkan menunjukkan sifat-sifat mekanikal dan terma yang lebih baik daripada dua kaedah pemprosesan lain.

EVALUATION OF FISH WASTE AS STARTING MATERIAL FOR PRODUCING BIODEGRADABLE PROTEIN BASED THERMOPLASTIC POLYMERS

ABSTRACT

The main objective of the current work was to investigate the suitable processing methods to produce protein based thermoplastic from fish waste, as well as factors that influenced the thermal, morphology, physical and mechanical properties of the protein based thermoplastic from fish waste. Two samples have been used which is fish fillet and fish waste. The protein samples were produced using twin screw extruder, internal mixer and single screw extruder and the properties were characterized. Thermal properties of the fish thermoplastics were determined using DSC and TGA by measuring the decomposition temperature and glass transition temperature (T_g). Morphology of the fracture surface of the protein thermoplastics samples was characterized using SEM. FTIR and tensile tester were used to identify the physical and mechanical properties obtained. It was found that fish fillet had the highest tensile strength compared to fish waste. The glass transition temperature was reduced as the water content increase. The TGA results show that the thermal stability of the fish fillet is higher that fish waste. Evidence from the SEM revealed that the most suitable processing method for fish waste is twin screw extruder. Twin screw extruder shows a better homogeneity than single screw extruder and internal mixer. Twin screw extruder also show pull out matrix which show a better mechanical properties and thermal properties than the two other processing method.

CHAPTER 1

INTRODUCTION

1.1. Background of Study

Synthetic polymers have been used widely as it possessed high mechanical properties compared to natural polymer. Synthetic polymer is widely used in our daily life such as food packaging, piping, electrical devices, agricultural and in automotive industry (G.O. James, 2002). In agricultural industry, wide range of plastics product has been used as one-off application such as seed trays and mulch film. Once this plastic serves its purpose, it is dispose as waste. This can lead to other environmental problems as these materials are not readily biodegradable such that their resistance to microbial degradation (Vroman, 2009). Disposing it by burning and burying it will pollute the environment. Thus, many researchers have shown interest in developing polymer from the renewable resources. Biopolymers such as starch, cellulose, chitin, polylactic acids, soy protein, collagen, gelatin and many more display chemical and mechanical properties that are suitable for large scale production of bioplastics. Plus, biodegradable polymers can be found in large quantities from the renewable sources.

There are many research conducted for protein materials such as corn, soy and gluten to utilize it as plastic materials (Svenson et al, 2012). However, these researches have not been explored to the great extent. Protein consists of repeating unit similar to synthetic polymer but may contain up to 20 amino acids monomer that form into polypeptide chain. Protein has been used in several applications in the past including adhesives (Rowell, 2012). It has been used way before petroleum based adhesives dominate the market. The example of protein based polymers that have been proven to be useful in replacing the synthetic polymers are soy protein, chitin, silk protein and collagen.

The study of fish waste as a starting material from protein based thermoplastic is still very rare. Using fish waste as a starting material may solve several problem regarding the disposal of the waste. Plus, fish waste promises sustainable sources of raw material and they are less dependent on petroleum products. However, there are some limitations. Materials derived from protein have lower mechanical properties compared to synthetic materials. Protein is sensitive to heat and most of its softening point is above their degradation temperature. Thus, protein matrix modification of fish protein needs to be done to improve the quality of the final product.

The protein matrix modification could be in the form of denaturation or plasticization for ease of processing and was proved to be a good way to improve the performance (Chen and Zhang, 2010). Unfolding of the protein from a structure of native state into an unstructured state with no or little fixed residual structure known as protein denaturation. Disruption of the secondary, tertiary or quaternary structure by the denaturants will introduce new interactions by means hydrogen or ionic bonding. Urea is the most common denaturant for protein. Sometimes, it is accompanied with waters. In order to improve the processability of the protein, plasticizer is added during the thermoplastic processing. Addition of plasticizer will not only ease the processing but also assist in lowering the glass transition temperature (T_g), reduce brittleness and increase flexibility. Common plasticizers for biomaterials are glycerol, polyethylene glycol and sorbitol (Mekonnen et al, 2013). Other than that, water is also often added in order to increase the plasticization effect.

Achieving a flowing protein is the fundamental of thermoplastic processing, where protein molecule chains slide past each other and solidify to form a shape when the temperature is reduced. The most common processing method for biopolymers is extruder. The success of producing protein based materials by using single and twin screw extruders have been proven (Verbeek, 2010). However, twin screw extruder offers more efficient mixing and conveying materials. This is because it did not convey the material through friction forces but through positive displacement. Some research of biopolymer uses internal mixer instead of the conventional extruder (Olatunji, 2016). Multiple advantage offers by the internal mixer makes it suitable to be used in studying the process of the biopolymer. Using internal mixer will ease the operation; reduce the quantity of material used and good result of coupled with a torque rheometer.

1.2. Problem Statement

The main problem of protein is its heat sensitivity. The T_g of protein-based materials is normally higher than its degradation temperature. Thus, addition of additives such as denaturation agents and plasticizer are important in order to be process in typical plastics processing to obtain the final properties of the products.

It was postulated that several challenges will occur such as limitation in processing and low mechanical properties of the material due to low protein content in fish waste. Furthermore, high contain of fat and ash from bones and fins will contribute to the brittleness of the material.

1.3. Research Objective

The purpose of this research is to develop the biodegradable thermoplastic using fish waste as starting material. The objectives of the research include:

I. To investigate the suitable processing methods to produce protein based thermoplastic from fish waste.

II. To identify the effect of water content on the thermal and mechanical properties of the protein based thermoplastic from fish waste.

1.4. Scope of Research Study

This thesis is divided into 5 chapters. In chapter 1, the general introduction of biodegradable polymer is discussed. The potential of protein based thermoplastic was elaborated. It also provides the overview of the research and brief literature survey of the previous and relevant work. The problem statement and the research objectives have also been stated and described in this chapter.

Chapter 2 highlights the detailed of literature review of previous and relevant works about the problems cause by the disposal of synthetic polymers and fish waste, the type of natural polymers, the potential of protein based thermoplastics, the limitations of fish waste thermoplastics, the protein matrix modifications and suitable processing used for protein based thermoplastics.

Chapter 3 listed the raw materials, methods and equipment used in the present work which are twin screw extruder, internal mixer and single screw extruder. The procedure, characterization method and data collection techniques as well as data analysis methods have also been described in details.

Chapter 4 provides the discussion of the experimental results of the research work. The result of tensile test, Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and Scanning electron micrograph (SEM) have been discussed intensively in this chapter.

Chapter 5 presents the significance of the results and the summary of the research study. The recommendations for the future studies in the related research are included in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1. Synthetic Polymer vs Biodegradable Polymer

Synthetic polymers are derived from oil and made by human such as polyethylene, nylon, polyester and epoxy. It has been used in various industries such as food packaging, automotive industry, aerospace industry, computer, agriculture and many other applications (James, 2002). The most common application for synthetic polymer is packaging because it is cheap, easy to process and abundant resources. Packaging is essential in protecting, storage, loading and transporting of the product. This application requires plastic to be strong, leak proof and able to withstand external conditions that the storage or transportation environment will impose (Sajid Alavi et al., 2014). In aerospace industry, some components of aircraft have been replaced with synthetic polymers to reduce weight, cost reduction and improve performance. Though lightweight, synthetic polymers are able to impart directly on the efficiency of the component as well as on that of the complete aircraft (McCarthy et al, 1994). It is also widely used in electrical applications due to their good dielectric properties. Its electrical properties such as dielectric constant, volume resistivity, surface resistivity, dissipation, and power and loss factor are readily controllable (Goosey, 2000). The basic principle for synthetic polymers which is simple structural units for the formation of a 3-dimensional construct also enables it to be applied widely in the medical applications. It is used in biomedicine in drug delivery devices, sutures, implants, clot removal devices and elsewhere (Maitz, 2015).

In agriculture industry, plastic packaging such as seed trays, mulch film, fertilizer bag, and hoop house covers are the most used plastics on the farm (Figure

2.1). In cooler region farmers use plastics to enhance warmth while in hot and dry country, plastic is used to cool soil and plants. For example, plastic film will help to keep the fertilizer from running off the soil when it rains. It also inhibits the growth of weeds and unwanted plant. Recently, it was reported that agricultural plastic film market alone was estimated to be worth USD 5.85 billion in 2012 where it is expected that it will continue to rise to USD 9.66 billion in 2019. Currently, China is the world’s largest consumer (60%) of agriculture plastic films (PRNewswire, 2011).

Among all, Linear Low Density Polyethylene (LLDPE) was the largest consumed material that accounted for more than 55% of the total market in 2012 with global consumption expected to reach 1.470 kilo tons by 2019 (PRNewswire, 2011). This demand product will continue to raise the agricultural film market. Historically, discarded agricultural waste including these plastics are taken to landfill, burned or buried on the farm or local disposal. Only 10 % of plastics are recycled or reuse.

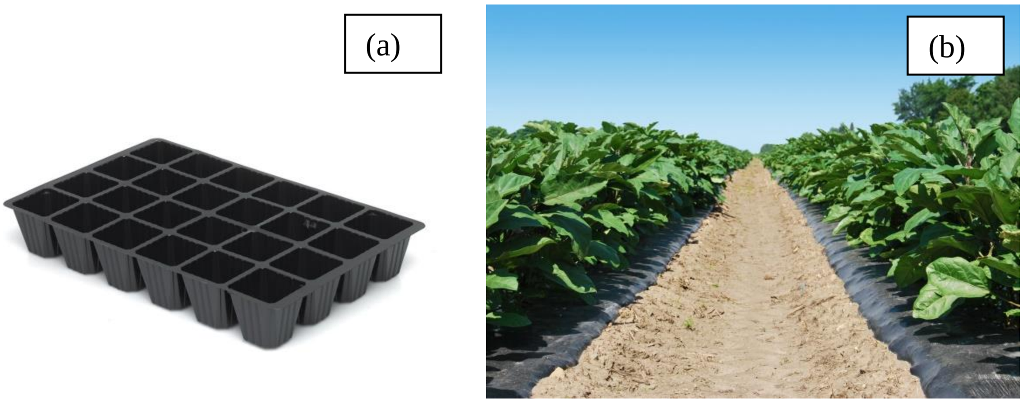


Figure 2.1: Plastic packaging such as seed trays (a) and hoop house covers (b)

Disposing them has become a global issue. These materials are not readily biodegradable as it resists microbial degradation. The materials accumulate the environment and therefore pollute the environment due to its non-degradable properties. According to Vroman et al (2009), most conventional polymers derived from petroleum resources are resistant to degradation. Due to photoinhibitors and stabilizer, polyolefin are resistant to hydrolysis, oxidation and biodegradation. The disposal of petroleum based plastics creates serious issues in large urbanized area of the world. Every year, China generates about 16 million tonnes of plastics waste, India was found to produce 4.5 million tonnes of plastic waste while UK had 1 million tonnes of waste. After several years, the cost of cleaning and recovering the contaminated and dirty area increased as the generation of plastic waste increased. This leads to other problem such as accumulation of plastics waste at the ocean shores that endangered the marine life such as entanglement and indigestion of harmful plastics by marine vertebrates (Thakur et al., 2012).

Therefore, numerous of researchers have shown interest in biodegradable polymer. Biodegradable polymer is a renewable resource that can be found in large quantities. The term biodegradation is defined as fragmentation, reduce in mechanical properties, and sometimes degradation happens through the action of living organisms. (Bastioli Catia, 2005). The market for biodegradable polymer has shown a strong growth thanks to its wide range of applications. Global production of biodegradable polymers has grown since 1990s. In 2005, global capacity of biodegradable plastics was around 360 000 tonnes. (Platt, 2006). Bio-based plastic productions are predicted to increase to 3.45 million MT in 2020 (Sina, 2013).

Since the petroleum era is coming to an end, finding environmentally friendly and sustainable alternatives to petroleum based plastics is an important challenge (Dale, 2003). However, petroleum based polymers gives much better advantage as compared to biodegradable polymers. Therefore, research and development of biodegradable polymer need to be done continuously in order to improve its properties and applications.

2.2 Natural Polymer

Natural polymer is those which are present in, or created by, living organisms. There are two types of natural polymers: those that came from the living organism and those polymerized polymer that is made up of renewable resources. Natural polymers also include polymers that are produced by biological process such as bacteria synthesis or fermentation. Natural polymer can be grouped based on their formation method as addition and condensation polymers just like synthetic polymers. Most natural polymers are condensation polymers which are formed from monomer units combining to form small molecules. There are six types of existing natural polymers depending on their sources. Table 2.1 illustrates the type of natural polymers and their examples. Natural polymers include polysaccharides, polynucleotides, polyisoprenes, proteins, polyesters and lignin (Olatunji, 2016). The best known natural polymers are starch and cellulose (John, 2012).

Table 2.1: The major types of natural polymers (Teegraden, 2004)

Types	**Examples**
Polysaccharides	Cellulose (Wood, cotton, flax, hemp) Amylose, amylopectins (starches [potatoes, corn, tapioca], glycogen, dextrans) gums
Proteins	Egg whites, gelatin, enzymes, muscle, collagen, elastin, silk, wool
Polynucleotides	DNA, RNA
Polyisoprenes	Natural rubber, gutta-percha, chicle
Polyesters	Poly(3-hydroxylbutyrate), cork
Lignins	Binder for cellulose fibers, cell walls

Usually, natural polymers can be process using conventional processing method such as extrusion, internal mixer and compression moulding. However, some cases will limit the applicability of some of the conventional polymer processing techniques to natural polymers. Some of the limitations have been eliminated or reduced by the researchers by modifying the polymer. For example, starch will be mixed with plasticizer in order to form thermoplastic starch. This modification enables starch to be processed by using extrusion (Olatunji, 2016). Table 2.2 shows some of the applications of natural polymers. Most of the applications are packaging and disposable items

Table 2.2: Some applications of natural polymers (Vroman, 2009)

Natural Polymers	Applications
Starch and Polyesters	Collection bags for green waste, agriculture films, disposable items
Starch	Wrapping plastics
PLA	Sanitary products, sport clothes, conditioning and packaging
Cotton seed protein	Agricultural films
Polyester amide	Disposable items, flower containers.

2.2.1 Starch

Starch is totally environmentally friendly as it is purely biodegradable which consists of amylose (poly-α-1,4-D-glucopyranoside), a linear and crystalline polymer and amylopectine (poly-α-1,4-D-glucopyranoside and α-1,6-D-glucopyranoside), a branched and amorphous polymer.

Starch is mainly extracted from wheat, rice, potatoes and corn. Amylose and the branching point contribute to the amorphous region while short branching chains will contribute in crystalline component (Thomas Sabu, 2013). The amount of amylose in starch is different depending on the sources of starch, yielding to different mechanical properties and biodegradability. The elongation and the strength increase as the amount of amylose in the starch increases. The glucoside links start to break at 150°C and the granules will collapse at temperature above 250°C. Retrogradation, a return to a more orderly state, which is partly crystalline, can be observed in starch (Aurel, 1976). For example, reorganization of hydrogen bonds happens at low temperature especially during cooling (Vroman, 2009).

Usually, starch is used as thermoplastics. With presence of specific amount of water, plasticizer and heat, it is plasticized through destructuration then it is extruded (Martin et al., 2001). Commonly used plasticizers are polyols as glycerols (Weber al., 2000). By using polyols, it can induce a recrystallization reaction or known as retrogradation. The extruded starch properties depend on the amount of water and relative humidity (Imam et al., 1995). Thermoplastic starch (TPS) is sensitive to humidity. The thermal properties of TPS can be more influenced by the water content rather than its molecular weight (Van Soest, 1996). Therefore, obtained TPS is almost amorphous. Through retrogradation process, a new crystalline formed induced by the process can remain in the thermoplasticized product. Besides that, the plasticizer content is an important factor in improving the flexibility and elongation of the material. The interactions between starch and plasticizers are weak when the plasticizer content is lower than 10% wt and it causes the material to be fragile and difficult to work with. The flexibility and elongation properties of starch improved when the plasticizer content is higher than 20% wt (Myllarinen, et al., 2002). Starch is widely used in agricultural applications. It is often used as agricultural mulch films. It will degrade into non-toxic products when in contact with soil microorganisms. Besides that, starch is used to control the release of agricultural chemicals (Chandra, 2009). Starch-based polymers also often added as additives to reduce the movement and the consumption of fuel in automotive industry (Vroman, 2009).

2.2.2 Cellulose

Cellulose is the most common organic compound which consist of chemical formula $C_6H_{10}O_5$. Unlike starch, cellulose is a straight chain polymer where no coiling or branching occurs (Visakh et al, 2013). Cellulose consists of linear chains of (1-4)-ß-D-glucopyranosyl units. The linearity of cellulose assists in molecules to arrange in side by side manner. The hydroxyl group in the system will form hydrogen bond with oxygen atoms either on the same or neighboring chain which will hold the chains firmly side by side (Visakh et al, 2013).

Cellulose is a renewable source that can be found widely but not fully utilized yet. Wood and vegetable fiber mainly consist of cellulose. The cellulose found in wood is present together with lignin, hemicelluloses and small amount of extractives (Krassig, 1993). The primary cell wall of green plants and many forms of algae is cellulose. It is odorless, tasteless and is hydrophilic (Visakh et al, 2013). Neat cellulose can be obtained by delignification by using different processes in the form of pulp. The source and the isolation procedure will affect the cellulose content. Cellulose can also be produced or synthesized by other living organisms. Bacteria, algae and some types of fungi are also responsible in producing cellulose. Bacteria will produce bacterial cellulose under a right condition. It is mainly used as the raw material for beverage and food (Visakh et al, 2013).

Cellulose exists as both amorphous and crystalline structure. Due to its high crystallinity, cellulose is generally insoluble. This ensures stability of cellulose against water and organic solvent. The interest towards cellulose increase as it is eco-friendly, low production cost, and higher specific strength/stiffness compared to their traditional synthetic counterparts (El-Saied et al, 2010). But still, there are some weaknesses of cellulose. This includes in polar and hydrophilic nature and low thermal stability (Akil et al.,2011; Bogoeva-Gaceva et al., 2007; Eichhorn et al., 2010).

The high crystallinity of cellulose may have some drawbacks. Cellulose will not melt when heat is applied on it. Therefore, it is impossible to thermally process cellulose by using conventional processing method. In order to improve its thermoplastic behavior, chemical reaction such as esterification and etherification are carried out (Yu, Long, 2009). But overall cellulose has more advantage compared to its disadvantage. Most of the disadvantages can be overcome by chemical treatment or reaction (Singha, Thakur et al., 2009). Different chemical treatment is done to modify the surface of the cellulose.

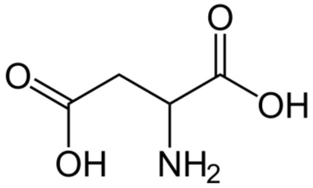
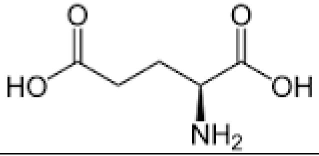
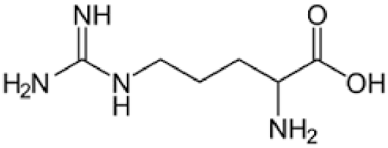
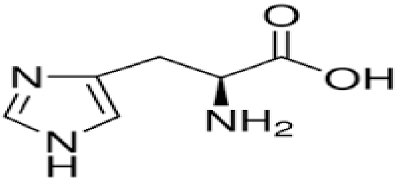
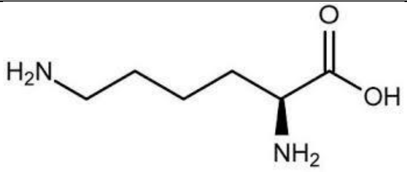
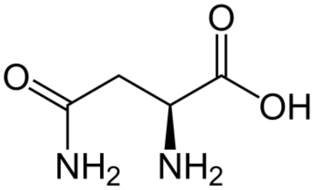
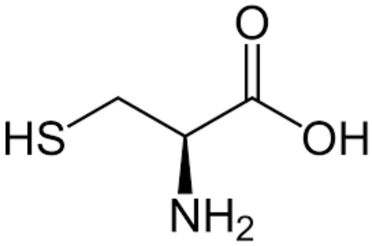
The main applications of cellulose back then are rope, canvas, and sacking made from kenaf. Nowadays, it is used as bioadhesive and mucoadhesive drug delivery systems. These adhesives are drug containing polymeric films. It has the ability to adhere biological membranes after combining with moisture or mucus compounds (Javad, 2013). Recently, cellulose also has been used widely in tissue engineering including injectable tissue scaffolds, bone tissue regeneration, improving bone implant adhesion as well as drug release (Nathan et al, 2017).

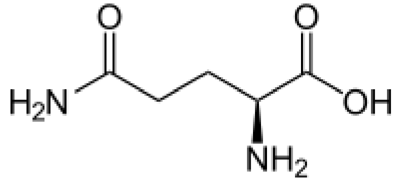
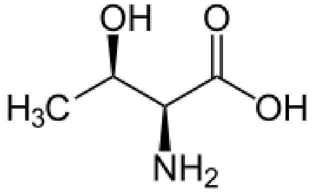
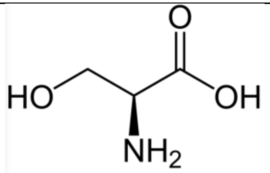
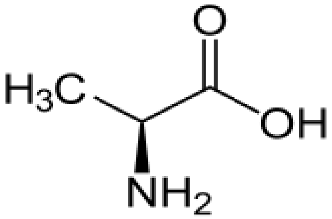
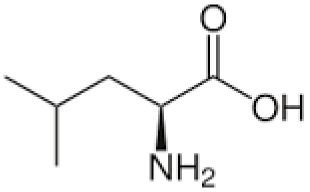
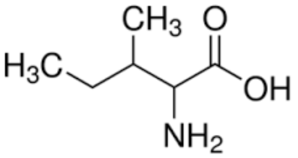
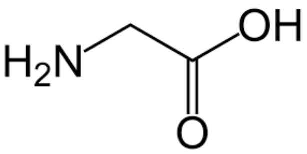
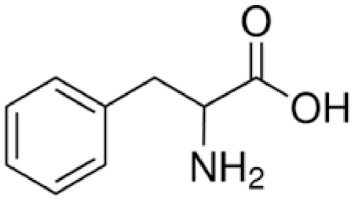
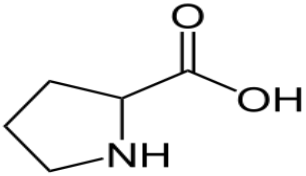
2.3. Protein

Synthetic polymers share some vital characteristics with proteins. Protein consists of repeating unit similar to synthetic polymer but may contain up to 20 different amino acids monomer that for polypeptide chain (Verbeek, 2010)

Proteins are formed by the condensation polymerisation of amino acids. The carbonyl group of one amino acid will join the amino group of another amino and form an amide linkage while eliminating a water molecule. The amide linkage is called peptide bond. Table 2.3 shows the twenty amino acids that are common in all living organisms.

Table 2.3: Twenty amino acids that are common in all living organisms

R-Chain	Name	Structure
Acidic amino acids (Ionized polar)	Aspartic acid	
	Glutamic	
Basic amino acids (non-Ionized)	Arginine	
	Histidine	
	Lysine	
Polar Amino Acids	Asparagine	
	Cysteine	

	Glutamine	
	Threonine	
	Serine	
Non Polar amino acids	Alanine	
	Leucine	
	Isoleucine	
	Glycine	
	Phenylalanine	
	Proline	

According to Buxbaum, (2007), the structure of proteins is commonly described in terms of four hierarchical levels of organization: primary, secondary, tertiary, quaternary structures. Figure 2.2 shows the four structures of protein.

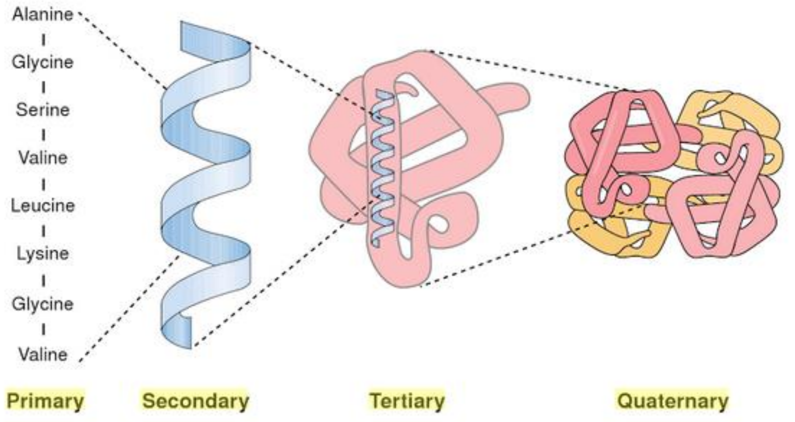
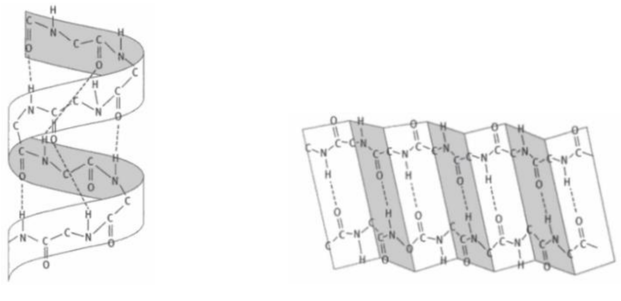


Figure 2.2: The structure of protein (Alissa, 2012)

The primary structure is the linear sequence of amino acids that start with N-terminal and end with C-terminal amino acids. This structure is held together by covalent or peptides bonds. The secondary structures can be described as a highly regular local conformation of amino acids in the protein stabilizes by hydrogen bonding. The two particular common structural motives of the secondary structure are alpha helix and beta sheets. Figure 2.3 below displays the alpha helix and beta sheets structure.



(a) (b)

Figure 2.3: (a) Alpha helix structure and (b) Beta sheet structure (Phillip, 2006)

Meanwhile, the tertiary structure is a three dimensional organization of complete protein chain. The arrangement contains region of ɑ-helix, ß-sheet an random coil which makes the arrangement special (Vaclavik, 2007). Lastly, quaternary structure is the final level of protein structure and consists of more than one polypeptide chain. Each chain is called subunit. The chains can be identical or different where the chain can range from two to more than a dozen. Most common example is two, three and four polypeptides chain which is called dimers, trimers and tetramers respectively (Campbell, 2011).

The properties of the protein are dependent to the molecular structure. The difference between polymer and protein structure are shown in Figure 2.4.

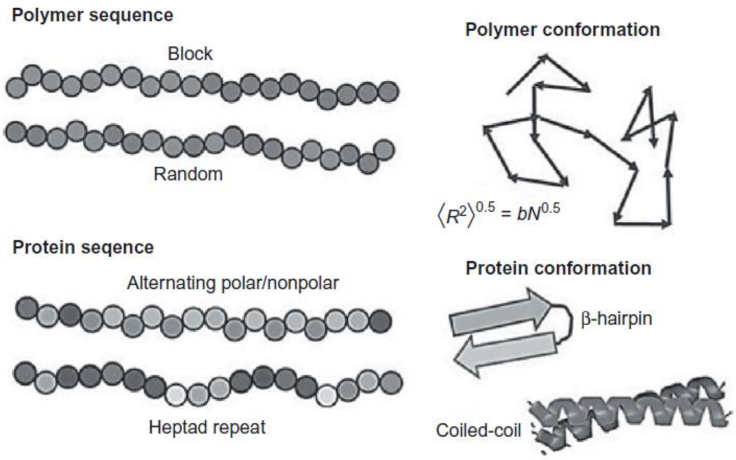


Figure 2.4: Difference between polymer and protein structure, (Olsen 2013).

In the past proteins often used in preparing adhesives before the petroleum based adhesives started to dominate the market. Bond glue was used often in carpentry and blood was added as an additive for plywood glue mixes. Several of the applications by using protein based polymers are shown in Table 2.4 Protein based polymers includes soy protein, casein, silk protein and collagen.

Table 2.4: Several applications of protein based polymers

No	Sources of proteins	Applications	References
1	Soy Protein	Edible films and sheets	Swain et al., 2004; Gennadios et al., 1996
2	Casein	Microencapsulating agents and active compounds in pharmaceutical applications	Elzoghby et al., 2011
3	Soy protein	Wound dressing and bionanocomposites	Tian, 2012
4	Collagen	Carrier system for delivery of drug, protein and gene.	Khan Ruby and Khan Maitz, 2013
5	Silk Protein	Film and foam	Hardy,2010

2.3.1. Animal Protein

Hernandez-Izquierdo and Krochta (2008) stated that the source of protein can be divided into two which is animal proteins and plant proteins. The common animal proteins are collagen, casein, whey protein, and keratin.

Collagen can be found in mammals and constitutes 30 percent of total protein. It is present in animal skins and hides, blood vessels, tendons, and ligaments, and it is insoluble. Each peptide chain of collagen consists of tripeptide sequence. This tripeptide contains common residue glycinelike gly-X-Y, where X is often the common residue proline and Y is the uncommon residue hydroxyproline. The long chain of the polypeptide will form triply stranded helices and arrange themselves in groups to form fibrils to give strength to bones and allow them to flex under stress. It is widely used in tissue engineering as it has good biocompatibility, biodegradability, and minimal immunological reactions compared to synthetic materials.

Gelatin is protein obtained from denaturation of collagen. Gelatin consists of 19 amino acids. The triple helices of collagen are disrupted and partially hydrolyzed. This will leave chains that are largely disordered and unorganized. Gelatin can be found from hog and cattle skin as well as animal bones. The mechanical and barrier properties depend on the characteristics of the gelation. The characteristics of gelatin are influence by amino acid composition and the molecular distribution (Vroman, 2009). It has excellent adhesive properties and is widely used in bookmaking. It has other industrial applications such as a stabilizer-binder in photographic emulsions (Stevens, 2002). Other than adhesive properties, gelatin also has a good film forming abilities.

Casein contain in milk. Casein is mainly found in skimmed cow's milk. It is manufactured from skim milk by separating casein from whey (Lowery and Antonio, 2012). There are four subunits exist in casein which is alpha s1-casein, alpha s2-casein, beta-casein and kappa-casein. It makes up 38%, 10%, 26% and 13% of casein composition respectively. The ability of casein to form films is influence by the four protein fractions. It is used as labeling adhesive in the bottling industries due to its excellent rheological properties, and in binders, protective coatings, leather finishes and other products.

Whey protein is defined as the remains in the milk serum after coagulation of the casein (Morr and Ha, 1993). Whey is mainly found in cheese manufacturing. It is a byproduct of the cheese-making process. Technically, Whey is the soluble fraction of milk that separated from the casein curd during the manufacturing. Whey protein, separated from its whey, has several applications that are important food industry such as body and bulking agent (Stevens, 2002).

2.3.2. Plant Proteins

Plant protein is equally important commercially. Plant contains protein that will provide structure and biological activity (Hernandez-Izquierdo and Krochta, 2008). Plant proteins are widely available biomaterials and can be made into fibers, films, hydrogels and nanoparticles for biomaterial applications. Example of plant material includes soy protein, zein from corn (maize), wheat gluten, potato proteins and pea proteins. Plant proteins have lower molecular weight and higher net charges (Reddy and Yang, 2008). Compared to animal proteins, most plant proteins have higher moisture sensitivity and lower mechanical strength. However, proteins can be utilized owing to their abundance, low cost, and less potential to be immunogenic compared to animal protein for applications that do not require exposure to high moisture or mechanical strength (Reddy and Yang, 2011).

Soy protein, as the main by-product of the processing of agricultural commodities, is an abundant natural polymer. It has been used as an ingredient in a variety of foods for its functional properties. The functional properties include emulsification and texturing. The health benefits have boosted the popularity of soy protein. There are different categories of soy protein: soy protein isolate, soy protein concentrate and textured soy protein. Soy protein isolate is the most fined form of soy protein and it contains about 90 percent proteins. Soy bean without the water soluble carbohydrate is soy protein concentrate which contains 70 percent of protein. Textured soy protein, also known as TSP, is made upon soy protein concentrate by giving it some texture. The application of soy protein is limited due to its poor water resistance, low adhesive strength, and poor corrosion resistance (Wang et al, 2010). Soy protein is widely used as edible and environmental friendly biodegradable films. The film produce by using soy protein is flexible, smooth, and transparent compared to other plant protein films (Olatunji, 2015).

Wheat gluten is produced from flour. Mixing of wheat flour and water will result in wheat gluten. Wheat gluten protein is made from a collection of different proteins. Gluten protein can be classified into groups based on their solubility in aqueous alcohol. Wheat proteins that are soluble in alcohol are gliadins and glutenins are the insoluble fractions. Gliadins are responsible for viscosity and extensibility while glutenin responsible for the strength, cohesive and elasticity of gluten (Tarte, 2009). Wheat gluten is brittle with the absence of plasticizer. The effect of water, glycerol, sorbital on the glass transition temperature of wheat gluten was studied. Glycerol and sorbitol has higher molecular weights and lower evaporation than water, so their accessibility to various zones is limited. Thus, the plasticizing effect of glycerol and sorbital is lower than water. Incorporating gluten and glycerol will produce a malleable phase (Vroman, 2009). Wheat gluten is an excellent film forming agent. Films prepared form glutenin is stronger than those from gliadins or whole wheat gluten (Thaku, et al, 2017).

2.4. Protein Based Thermoplastics

Extensive marine aquaculture involves of farming of finfish or shellfish in a natural habitat. However, great concern over the management and protection of environment has risen upon the development of aquaculture. FAO (2002) stated that 74% of the fish was used for direct human consumption while another 26% will be thrown away. This creates an environmental pollution.

Protein based thermoplastic from the fish waste may be a possible for biodegradable polymer. According to Svenson (2012), most study in the biopolymer has utilized terrestrial protein sources such as starch and cellulose but marine resources have not been fully explored. There are several marine sources that promise high potential starting material for biotechnological application such as the preparation of biopolymers.

Recently, thermoplastic polymers prepared from waste blood have emerged as a promising bioplastic material. The additives such as SDS, SS and urea have been used as the denaturants. The plasticizer like glycerol is used to ease the processing. Water was also added to assists in plasticization of the protein. It has been successfully process by using twin screw extruder. (Verbeek, 2011). The uncomplicated methods used in the preparation of the blood-based polymers can be practice to other proteineous starting materials. There is current study in attempting to use fish meal as the starting material of bioplastic. Cuq et al (1997) have successfully used myofibrillar protein in preparing glassy translucent materials by thermomoulding and plasticizing by combining sorbitol and sucrose. The research aim is to produce edible coatings. Several other studies of fish proteins, both purified skin gelatin and myofibrillar preparation have been used in preparing polymeric film via casting techniques.

So far there is limited study in focused on fish waste which is an abundant marine source with low nutritional and economic value. Svenson (2012) also stated that fish meal from farmed Norwegian salmon is employed as model system in evaluating whether the waste product from the fishing and aquaculture industry can be converted into biomaterials. The studies involve characterization of fish meal, influence of chemical additives and processing techniques.

Protein based materials may exhibit good barrier properties against oxygen, lipids and aroma, but it is water sensitive. Water sensitivity in the form of poor barrier properties against moisture and high moisture absorption contributes in major limitation in the protein based plastic industry. Soy protein sheets submerged in water for 20 hours absorb a huge amount of water which is 180 percent of their initial weight (Zheng et al, 2003). This is due to the presence of hydrophilic protein functional groups, such as $-NH_2$, -NH, -OH, -SH. This can clearly show that protein-based materials have a high tendency to be washed or solubilized in extreme humid environments or when immersed in water. This will eventually give dramatic effects on the mechanical properties of protein biomaterials. Protein is sensitive to heat and most of its softening point is above their degradation temperature. While most synthetic plastics have a glass transition temperature below 100 °C, proteins with less than 5% water show glass transition temperatures close to or above their decomposition temperatures (Tolstoguzov, 1993).

Thus, protein matrix modification is essential. Modification of protein matrix for a better performance could be in the form of denaturation and destructurizing native proteins, plasticization for ease of processability and blending with other polymer.

2.4.1. Protein Denaturation

Protein denaturation is a process of unfolding protein from a structured native state into an unstructured through external stress or compound such as addition of acid or base, inorganic salt, organic solvent, radiation or heat. Denaturation of protein can lead to wide range of properties such as from loss of solubility to protein aggregation.

In most cases, denaturation occurs during processing. Denaturation can be done both by denaturants (chemical denaturation) and temperature. Yue et. al. (2012), state that protein denaturation induced by denaturant can disrupt its secondary, tertiary or quaternary structures, and expose the amino acids side chains. This will therefore introduce new interaction by means of hydrogen and ionic bonding. Most commonly used denaturation agent is urea. Urea often used with some water. Urea can stabilize the globular protein by forming strong hydrogen bonds with water molecules that surround the protein (Luescher et al, 1974).

Generally, the denaturation temperature of proteins may differ due to the protein source, additives and processing conditions. New interaction between chains is introduced by unfolding the protein structure, therefore changing the mechanical properties of the protein matrix. Denaturation can be described as a purely thermodynamic phenomenon which depends on temperature. However, thermodynamic phenomena in a material protein denaturation can only happen if the structure changes. Chain mobility can happen only if the denaturation temperature (T_d) is higher than the glass transition temperature (T_g). As proteins consist of over 20 amino acid monomer units, and therefore their chain structure is quite heterogeneous. The rigidity of main chain and side chain as well as the presence of plasticizer will influence the T_g. Plasticizers are low molecular weight substances that are mixed in the polymer to increase the free volume and mobility of the polymer. This will results in a lower T_g. (Leontine, 2000).

Sochova (1997) combined the principles of denaturation and T_g, who states that the heat capacity jump at T_g and denaturation of globular proteins are important to increase the conformation and mobility of the globules. Thus, in order to denature a protein, the system should be sufficiently above both the T_d and T_g. It can be achieved by increasing the water content, allowing the process to happen at low temperature. Increasing temperature can also be done at lower content of water. Lastly, addition of denaturant in order to obtain a lower T_d. Mixing process include the dispersion or solubilizing of additives such as plasticizer and denaturant with the protein powder. Temperature and shear are often use in this process. During this process, molecular interactions are disrupted, sulphur bridges may be broken and structure may changes. Wellner M et al proved that the hydration of v-gliadins initially resulted in the formation of b-sheets which disappeared upon subsequent increase in water content of the sample. Secondary structure of wheat gluten is changed upon heating to 80°C in the presence of water (Wellner et al, 1994). In the second stage, molecules will rearrange. In the case of extruded samples, the shearing conditions promote alignment of the molecules, leading to new interactions (Camire, 1991).