UNIVERSITI PUTRA MALAYSIA

PREPARATION AND CHARACTERIZATION OF TILAPIA FISH SCALE GELATIN BASE FILM

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ABSTRACT

The economy of freshwater fish in Malaysia reported to be increasing in aquaculture production. It is about 33% of the fresh water fish are contributed by tilapia. The increase rate of tilapia production, causing by the high demand of tilapia fish, thus contribute to the by-product of the fish. This amount of waste from Tilapia is a useful resource for collagen extraction. By, optimizing yield based on the extraction method and heating temperature and time of fish gelatine extraction. Also, determine whether effects of different extraction temperature and time will affect that mechanical properties of the film. This research was carried out to observe the changes in the properties of Tilapia fish scale films by comparing film at different temperature and extraction time. Furthermore, Glycerol was added to the film as a plasticizer. The film thickness film corporate with glycerol is between 0.14 mm to 0.16 mm and film that purely without addition of glycerol is between 0.10 mm to 0.12 mm. While the tensile strength and elongation at break were studied to determine the mechanical properties of the film. The scanning electron microscopy (SEM) was done to study the morphology of surface film between fish scale gelatine film corporate with glycerol and the pure fish scale gelatine film. While for FTIR the result shown that the amide band A, B, I, II and III were found in both films with or without glycerol. The IR ratios (intensity ratio) of all the collagen extracted from tilapia fish scale were 1.17 for both film (with glycerol and without glycerol). The intensity ratio is used to indicate the triple helical structure of collagen. All of these results prove that the helical structures of the collagen were reserved properly in good conditions. Next, the current study found that the colour do changes from transparency white to yellowish milky white when the extraction method at the highest temperature and longer time. The result shows that, as heating temperature and thermal treatment time was increased the tensile

strength of films increase. Besides, Fourier transform infrared spectroscopy (FTIR) proved that the collagen of these fish were integrated and native. These experiments confirmed that the addition of glycerol as plasticizer influences the mechanical, physical and the amino acid content in the film. In conclusion, these findings suggested that fish waste skin, scale, bone and fin collagen possess the potential to be an alternative collagen source for a variety of uses in many fields.

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Chapter 1

Introduction

1.1 Background of study

Synthetic plastic films have been widely utilized as food packaging materials, due to their good properties such as strength, lightness and transparency. Nevertheless, most all synthetic plastics are non-biodegradable, and their excessive usage has contributed to dangerous environmental problems. Moreover, there is an increasing concern about the likely risk that plasticizer such as phthalate used in plastics can leach out into food products. Researchers have linked phthalates to asthma, attention-deficit hyperactivity disorder, breast cancer, obesity and type II diabetes, low IQ, neurodevelopmental issues, behavioural issues, autism spectrum disorders, altered reproductive development and male fertility issues. Thus, there is an urgent need to find substitute such as edible or biodegradable films that can provide a barrier and mechanical security for food products with a purpose to reduce the role of food plastic packaging materials.

Bio-plastics is a term used for packaging materials derived from renewable resources, and which are considered safe to be used in food applications. In general, compared to

conventional plastics derived from petroleum, bio-based polymers have more diverse stereochemistry and architecture of side chains which enable research scientists a greater number of opportunities to customize the properties of the final packaging material (Liu, 2006). The primary challenge facing the food industry in producing bioplastic packaging, currently, is to match the durability of the packaging with product shelf-life. Whether, by applying the film alone or forming in combination, environmental temperature, relative humidity, presence of active bacterial and spoilage microorganisms, ultraviolet exposure, etc. are the common modes of degradation in food quality and spoilage. These factors that cause deterioration of the food product are also factors that influence the rate of degradation of the bioplastic material, and special care must be taken to develop bioplastic materials which address these concerns.

More importantly, processes must be developed such that innovative developments in the properties of bioplastics materials can be implemented for industrial-scale applications.(Liu, 2006).

Gelatine is a natural water soluble protein characterized by the absence of an appreciable order and the random configuration of polypeptide chains in aqueous solution. It is obtained from the partial hydrolysis of collagen a fibrous protein mainly found in certain parts of vertebrate and invertebrate animals as bones, skins, connective tissues and tendons (Shankar, Jaiswal, & Rhim, 2016) and its structure consists of rigid bar-like molecules that arranged in fibres inter-connected by covalent bonds.

Gelatine also have its own properties associated to surface behaviour such as protective colloid function, emulsion and foam formation and stabilization, adhesion and cohesion and film-forming capacity and properties related to gelling behaviour like gel formation, thickening, texturizing and water binding capacity (Schrieber & Gareis, 2007). Therefore, a

wide number of final applications and uses can be obtained, in food, packaging, pharmaceutical, cosmetic and photographic industries. In particular, gelatine is used to provide gelling, stabilization, texturization and emulsification for bakery, beverages, confectionary and dairy products in food industry (Schrieber & Gareis, 2007)

At the same time, regarding current practice of fish processing generates large amounts of by-products, which can make up to three-quarters of the total weight of a fish. (Regenstein & Zhou, 2007; Rustad, Storrø, & Slizyte, 2011; Shahidi & Venugopal, 1994) Typically, fish processing by-products consist of viscera, heads, trim, skins, scales, and bones, as well as fish that are damaged or unsuitable for human consumption or further processing, and by catch (Rustad, 2003; Rustad et al., 2011). Furthermore, the presence of several components of the fish by product led to fish harmful environmental effects or end up as low value products such as fish meal, silage, and fertilizer (Gómez-Guillén, Hurtado, & Montero, 2002; Rustad et al., 2011; Shahidi & Venugopal, 1994) .

Since protein is the major ingredient in most fish on a dry mass basis, fish processing by-products can be viewed as an alternative raw material for the formulation of high-protein ingredients, particularly for the production of food grade gelatine, due to the large quantities of collagen present in fish skins, scales and bones (Gómez-Guillén et al., 2002; Muyonga, Cole, & Duodu, 2004; Nagai & Suzuki, 2000).

Collagen and gelatine are rich in Fish scales, it have be used in food industry to improve certain properties of the food and it can be used for encapsulation and edible film formation (Karim & Bhat, 2009). Reported that fish gelatine exhibits excellent film-forming property of biopolymers including sarcoplasmic protein, myofibrillar protein, soy protein, whey protein, polysaccharides and lipids (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka,

2006; Muyonga et al., 2004; Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004; Shiku, Hamaguchi, & Tanaka, 2003). Nowadays there are many research and project development of product regarding the fish skin (Regenstein & Zhou, 2007; Souda & Sreejith, 2014; Wangtueai & Noomhorm, 2009). Most previous studies concerning fish gelatine based films were primarily restricted to fish skin. Then, this research on using fish scales will increase the by-product of the fish and simultaneously will increase the value and development in the packaging industry.

Biggest problem in fish gelatine film is that it have poor mechanical properties than some synthetic films such as oriented polypropylene (OPP) and films mammalian gelatine films (Jongjareonrak et al., 2006; Pérez-Mateos, Montero, & Gómez-Guillén, 2009; Shiku et al., 2003), this will qualify them as a good packaging films. Consequently, there are few studies that have been conducted with an objective to improve the carrying out of gelatine films by addition of cross-linking agents such as aldehydes (Bigi, Boanini, Cojazzi, Falini, & Panzavolta, 2001), transglutaminase or 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (Kołodziejska & Piotrowska, 2007). But then by addition of cross-linking agents will be resulting in high cost of some agents thus limiting their practical application. The other alternatives to increase the durability of fish gelatine films are by using physical cross-linking methods, such as γ irradiation, heat treatment, and UV (ultra violet treatment), could thus be utilized as an alternative method to improve the properties of gelatine films from the standpoint of food safety and economy. The effect of heat treatment on the properties of fish gelatine films was only carried out through heating film-forming solution. Furthermore, by increasing the temperature during extraction will lead to improving the water resistance properties of films. Thus increase the mechanical properties. Thus, this study to shown that there is an easy way to improve the strength of the gelatine by thermal.

1.2 Problem statement

There are many alternative types of packaging. Nowadays plastic film packaging has become a concern problem because of the increasing solid waste in the landfill. Thus, by several studies and development film based on gelatine has become quite popular and used in many product packaging. But mostly it being extracted from mammals such as pork and cow. Due to some restriction in religion this has become one of the problem. Furthermore, people are looking for another alternative way. One of it is by using fish by product to product gelatine.

As fish processing industries dispose huge amounts of fish waste. The fish waste industry's annual discard amount is approximately 25% of the total production. Which is about 20 million tonnes in Malaysia (FAO, 2012). Still, since majority of waste from fish processing industries were bones, skin, scales, and fins, which constitute over 70% of fish. And, since fish have large amount of protein in its by product. Thus, fish gelatine is produced.

But at there are few restrictions on fish gelatine where it easily damaged, low strength and low durability. Hence, by using the effect on dehydrated thermal treatment on fish scale gelatine films can improve their resistance and mechanical properties. And will solve the problem regarding fish gelatine poor properties.

1.3 Objective of the study

1. To optimize extraction yield based on different extraction temperature and time of Tilapia fish gelatine extraction.
2. To determine the effects of different extraction temperature and time on mechanical properties and characterization of the film.

Chapter 2

Literature Review

2.1 Packaging material

Plastic is the most common packaging material and, at the same time, one of the most difficult to dispose. The factors common to all plastics are that they are light, strong and cheap to manufacture. It is for these reasons that they are used so much, as an alternative to cardboard glass packaging materials. Almost 10% of our rubbish consists of different types of plastic. They are a problem in landfills as they are bulky, they contaminate degrade slowly. Separated the rest of the waste, they can must be upgraded for the good of everyone.

2.1.1 Synthetic plastic

Consumers nowadays have high demands of plastic for manufacture of many products. Also there are high demand of plastic for packaging products. Thus for effective packaging that adequately protects the product is made possible by plastic. Plastic are made from crude oil, a

non-renewable resource. Even though, plastic can be recycled but the amount of solid waste generated by plastic becomes a problem.

Over last fifty years, the production of plastics has reached large levels. Approximately 200 billion pounds of plastics are produced worldwide every year. The oil that is used as raw material, as well as the oil required for energy, consumes between six and eight percent of the total world oil production. Though this is a small percentage, by time the amount of petroleum used to make plastics does contribute the depletion of fossil fuels. Thus will contribute to current rise in raw material costs.

For a solution, the biodegradable plastics are produced as a solution. This plastic is made from natural sources; the polymers are isolated from raw material. Depending on the material used, different technologies are required to produce plastic. Mostly the technique involves synthesized using chemicals or by fermenting the sugars.

2.1.2 Biodegradable film packaging

Now, there has been an increasing concern of the environmental problem caused by the massive utilization of synthetic non-biodegradable materials especially for packaging. As a consequence, much effort has been made to develop biodegradable or edible films from bio-polymers to produce environmentally friendly packaging as an alternative to synthetic plastic packaging films. Among agricultural macromolecules, protein has been empirically used as packaging materials due to its abundance, biodegradability, nutritive value. In addition, Agra-packaging based on proteins are mostly characterized by remarkable functional properties, due to their heterogeneous specific structure, and nutritive value (CUQ, AYMARD, CUQ, & GUILBERT, 1995; Gerrard, 2002) Protein-based films have impressive oxygen and carbon

dioxide barrier properties in low relative humidity condition compared to synthetic films. Properties of protein-based films depend on several factors such as the source of protein, pH of protein solution, plasticizers, the preparation conditions and substances incorporated into film-working solutions (CUQ et al., 1995; Gerrard, 2002; Jongjareonrak et al., 2006).

As biodegradable film packaging material is naturally composed of polymers that can be ultimately degraded by microorganism through the composting process to produce natural breakdown compound such as carbon dioxide, water, methane and biomass (Nur Hanani, Roos, & Kerry, 2014). Thus by having these characteristics this fish gelatine film is being classified in edible and non-edible biodegradable polymers. Biodegradable materials derived from food components such as polysaccharides, proteins and lipids are edible and have attracted considerable interest in recent years due to their potential powers to replace traditional plastics and act as food contact edible films and/or coatings (Nur Hanani et al., 2014)

2.1.3 Bio based packaging

"Bio based food packaging materials or biopolymers are materials derived from renewable sources. These materials can be used for food applications". (Comstock et al., 2004; Weber et al., 2002). Bio based polymers may be divided into three main categories based on their origin and production: Category 1: Polymers directly extracted/removed from biomass. Examples are polysaccharides such as starch and cellulose, chitosan/chitin and proteins like casein and gluten. Category 2: Polymers produced by classical chemical synthesis using renewable bio based monomers. A good example is polylactic acid(Kandemir et al., 2005), a bio polyester polymerised from lactic acid monomers. Category 3: Polymers produced by microorganisms or genetically modified bacteria. To date, this group of bio based polymers consists mainly of the polyhydroxyalkonoates, but developments with bacterial cellulose are in progress.

2.1.4 Edible film packaging

Edible films are determined as a slight stratum of fabric which can be consumed and provides a barrier to moisture, oxygen and solute movement for the nutrient. The material can be a complete food coating, or can be disposed as a continuous layer between food components (Giannakopoulos & Guilbert, 1986). Edible films can be shaped as food coatings and freestanding films, and have the potential to be used with food as a gas aroma barrier (Kester & Fennema, 1986).

Nevertheless, biodegradable dible films and coatings have received considerable attention in recent years because of their advantages over synthetic films. The primary advantage of edible films over traditional synthetics is that they can be eaten by the packaged products. On that point is no package to dispose even if the films are not consumed, they could yet lead to the decrease of environmental contamination. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials. The films can enhance the organoleptic properties of packaged foods provided they contain various components (flavourings, colourings, sweeteners). The films can be used for individual packaging of small portions of food, particularly products that currently are not individually packaged for practical reasons.

The films can serve as carriers for antimicrobial and antioxidant factors. In a similar application they can also be applied on the surface of food to control the dispersal rate of preservative substances from the surface to the inside of the food. Some other potential possible application for edible films could be their use in multilayer food packaging materials together with non-edible films. In this case, the edible films would be the internal layers in direct contact with food materials. Production of edible films causes less waste and pollution, even so, their

permeability and mechanical properties are mostly poorer than synthetic films (Kester & Fennema, 1986). Therefore, this work will result in producing higher permeability and mechanical properties by using different extraction temperature and time.

2.2 Physio-chemical Properties of Fish Collagen

Amino acid composition and thermal stability are the major factors that contribute to the physical-chemical properties of collagen. These characteristics vary among fish species. Amino acid composition in collagen plays a significant part in thermal stability. Collagen triple helix consists of repeating sequences of X-YGly, in which X and Y are proline and hydroxyproline, Gly represents glylcines. Presents of amino acids (proline and hydroxyproline) promote the establishment of H bonds between hydroxyproline residues belonging to the X and the Y positions of adjacent chains (Berisio et al., 2004). Thus, a higher amount of 17 amino acids in a collagen would contribute to higher thermal stability, for instance, in the brownstripered snapper skin contains 212 residues per 1000 residues of amino acids, would bear a high denaturation temperature of 31.50 °C (Jongjareonrak et al., 2005) as compared to grass carp skin collagen with amino acid contents of 186 residues/1000 residues shows the denaturation temperature of 24.60 °C (Duan et al., 2009). Also, high amino acid compositions resulted in high denaturation temperatures, although both animals live in different habitats. For example, subtropical fish species such as the Oreochromic niloticus Tilapia (Sujithra et al., 2013) had nearly similar characteristics (amino acid compositions and thermal stability of 32 °C) to mammalian collagen (porcine skin collagen with thermal stability of 37.80 °C). Consequently, fish collagen with similar characteristics to mammalian collagen would show that fish collagen is suitable to be an alternative origin of mammalian collagen in various applicable fields

Therefore, this study attempts to enforce the use of tilapia scales as it possesses similar characteristics of amino acid and thermal stability to mammalian collagen and act as the alternative way in the evolution of film packaging based on protein.

2.3.1 Amino Acid Composition in Tilapia

The amino acid composition between fish and mammalian collagen are slightly different, as amino acid composition affects the collagen molecule structure, while the structure and conformation of the collagen in solutions affect the physical properties of the collagen such as rheological properties (Ramachandran, 1988; Zhang, M. et al., 2010).

As a guide for an approximate amino acid composition of fish waste collagen extracted from fish, Table 2 lists the amino acid composition of acid soluble collagen of red and black tilapia skin (Jamilah & Harvinder, 2002) in comparison with Table 3 calf skin collagen (Duan et al., 2009). Fish collagen has been proven to differ widely in their amino acid composition. In particular, the amount of amino acids which are hydroxyproline and proline vary significantly among fishes. About 34 % of glycine was present in all fish skins and calf skins collagen (Table 2 and 3). Reports from (Jongjareonrak et al., 2006; Liu, 2006; Nagai & Suzuki, 2000) revealed that fish skin had approximately 23 % of glycine as it is the most abundant amino acid in collagen. However, Nile Tilapia skin had 35.6 % of glycine (Zeng et al., 2009) which higher than all fish skins and calf skin. Generally, glycine appears evenly, at every third residue all through most of the collagen molecules, except for the first 10 from the C-terminus and the first 14 amino acids from the N-terminus (Foegeding et al., 1996; Jongjareonrak et al., 2005; Zeng et al., 2009). Low composition of cysteine, and tryptophan, and very low moderate amounts of methionine and tyrosine are found in tilapia skin (Jamilah & Harvinder, 2002) .High quantities of glycine (Gly), and alanine (Ala) are the characteristics of all collagens (Ogawa et

al., 2004), as clearly seen in tilapia skin and calf skin collagen, these two amino acids are in high amounts which is more than 100 residues/1000 residues. Methionine and phenylalanine distribution in calf skin collagen is the lowest amount compared to the other amino acid contents in calf as depicted in table 3. There is no reported evidence to relate these amino acids and denaturation temperatures of collagen (Table 3). While for tilapia skin the lowest amino acid are cysteine where it only contains about 0.2% from total amino acid content. Here, the highest is Glycine where about 44% total amino acid content. The higher amount of amino acid content will be resulting in higher mechanical strength and higher collagen yield.

As reported by (Weng & Wu, 2015) gelatine that being extracted from tilapia scales mainly composed of β-chain and α-chain and their degraded products with amino acid contents of 21.2%. Stated that, Because of the cross linking between β-chain and α-chain which will **change** the formation of film from ionic and hydrogen bond to hydrophobic and covalent bonds. Where, lead to improving the water resistance properties of films. Thus increase the mechanical properties. As known, the cross linking of β-chain and α-chain could be induced by heating (Weng & Wu, 2015)

The amino acid composition of gelatin from black and red tilapia skin (mg/g)

Amino acid	Red tilapia	Black tilapia
Aspartic acid	38.9±4.59	39.9±2.78
Glutamic acid	71.7±1.08	76.9±2.23
Serine	Not detected	Not detected
Glycine	308±12.1	338±55.3
Histidine	Not detected	Not detected
Arginine	29.5±0.84	35.1±1.34
Threonine	134±8.35	155±7.86
Alanine	76.1±5.20	90.3±1.44
Proline	Not detected	0.55±0.07
Tyrosine	5.95±0.78	6.84±0.22
Valine	17.7±1.60	22.8±1.25
Methionine	14.2±2.03	17.8±1.12
Cysteine	1.51±0.14	2.97±1.37
Isoleucine	8.39±0.14	9.40±0.71
Leucine	18.2±1.27	20.3±0.38
Phenylalanine	18.6±0.51	21.8±1.27
Lysine	21.3±2.21	28.0±2.01
Tryptophan	Not detected	Not detected
Total	764	865.41

Values are means ± standard deviations of eight analyses from four replicates.

Figure 2. 1: : The amino acid composition of gelatine from black and red tilapia skin (mg/g)

(Weng & Wu, 2015)

Amino acid (residues/1000 residues)	Calf skin collagen
Aspartic acid	45
Threonine	18
Serine	33
Glutamic acid	75
Glycine	330
Alanine	119
Cysteine	-
Valine	21
Methionine	6
Isoleucine	11
Leucine	23
Tyrosine	3
Phenylalanine	3
Hydrolysine	
Lysine	26
Histidine	5
Arginine	50
Tryptophan	-
Hydroxyproline	94
Proline	121
Imino acid	215

Figure 2. 2: The amino acid composition of gelatine from Calf skin collagen (mg/g)

2.3.1 Thermal Stability

Thermal stability is the ability of molecules to be stable at high temperatures. Molecules with a higher stability have a higher resistance to decompose at high temperatures. For collagen, the thermal stability is determined by its denaturation temperature (Td). The Td is determined by analysing the viscosity of the collagen, as Td is calculated as the temperature when fractional viscosity is 0.5 (Nagai and Suzuki, 2000a; Nagai et al., 2010).

A promising feature of fish collagen is that it has a close Td to mammalian collagen, as a high thermal stability in collagen would boost its applicability for food and pharmaceutical industrial use as it can replace mammalian collagen which are used in food and pharmaceutical

industry (Nakagawa and Tagawa, 2000; Lee et al., 2001; Rodziewicz-Motowidlo et al., 2008; Singh et al., 2011). All fish species and mammalians have different Td which is related to their environment and body temperature (Rigby, 1968; Kittiphattanabawon et al., 2005; Singh et al., 2011). Moreover, it is also suggested to be correlated with their amino acid content, and the complex interactions determined by these amino acids such as cross-linking of the amino acids, and the degree of hydroxylation of proline (Kittiphattanabawon et al., 2005; Rodziewicz-Motowidlo et al., 2008; Singh et al., 2011).

In many studies, tropical fishes were found to have higher Td than coldwater fishes. However, the Td of most fish collagen is lower than mammalians, such as porcine collagen (37 °C).The tilapia fish-scale collagen has a high denaturation temperature at 36 °C compared with that of fish scales in other fish species(Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). While Cold-water fish's skins such as Hake (10 °C) 23 (Ciarlo et al., 1997), Alaska Pollack (16.8 °C) (Kimura and Ohno, 1987), and cod skin (15 °C) (Rigby, 1968) have relatively low Td. For subtropical fishes, Td was 26.5 °C (Japanese sea bass), 25.6 °C (chub mackerel), and 25.0 °C (bullhead shark) (Nagai and Suzuki, 2000a). Nevertheless, Td of tropical fish scales, skins, bones and fins are higher than cold-water fish species because Td is correlated with the environment and body temperature of the fish.

2.3 Fish Waste as Source of Collagen

Every year, over 100 million tonnes of fish is being collected globally with 29.5 % of a chapter is used as fish feed due to its lack of functional properties (Kristinsson and Rasco, 2000; See et al., 2010). Approximately 70 -85 % of a capture becomes fishery processing waste and 30 % of these wastes are bones, fins, scales, skin which contains high levels of collagen, depending on the process used and the types of products (Shahidi, 1994).

In 2010, fishery captures and aquacultures supplied the world with 148 million tonnes of fish, where 128 million tonnes were used as food. Preliminary data showed that in 2011, an increase of 154 million metric tons of fish was retrieved, and 131 million tonnes of these will be accounted for as food (Table 1 and Figure 1). For the past 50 years, there was an average 3.20 % per year increase rate in the period of 1961 - 2009, that was growing faster than the world's population increasing growth rate of 1.7 % per year (Figure 2). Out of the 126 million tonnes for human consumption, Africa had the lowest fish consumption (9.1 million tonnes), West Asia covered two-thirds of the total consumption by 85.4 million tonnes (FAO, 2012).

In year 2010, Malaysia's aquaculture and capture production hit 2 million metric tons compared to 1 million metric tons per year in 1990 as depicted in Figure 3 (FAO, 2013), where 31 % was accounted as inedible fish waste (FAO, 2012). These discards can serve as good unprocessed materials for manufacturing high protein food such as gelatin. Modification of these discards into value-added products to make extra profit has benefits for both economic and waste management of the fish industry (Choi and Regenstein 2000). In Malaysia, 90 % of marine fish are from sea chapter, whereas the remaining are of freshwater and aquaculture fish. Roughly 75 % of the fish supply are for human consumption; on top of that, 25 % is used for fish oils. 30 % of the fish supplies for human consumption are processed into canned food, fish fillets, chips and protein products. Waste products that are to be discarded are produced at the end of the processing and human consumption. Fish processing itself is a foremost contributor to the overall environmental pollution, particularly emitting waste organic odor problems. Several types of attempts have been practiced to resolve this problem of waste discarding which comprise of manufacturing of fish sauce, protein concentrates and hydrolysates protein (Ibrahim et al., 2013)

	2006	2007	2008	2009	2010	2011
			(Million tonnes)			
Production						
Capture						
Inland	9.80	10.00	10.20	10.40	11.20	11.50
Marine	80.20	80.40	79.50	79.20	77.40	78.90
Total capture	90.00	90.30	89.70	89.60	88.60	90.40
Aquaculture						
Inland	31.30	33.40	36.00	38.10	41.70	44.30
Marine	16.00	16.60	16.90	17.60	18.10	19.30
Total aquaculture	47.30	49.90	52.90	55.70	59.90	63.60
Total world fisheries	137.30	140.20	142.60	145.30	148.50	154.00
Utlization						
Human consumption	114.30	117.30	119.70	12360	128.30	130.80
Non-food uses	23.00	23.00	22.90	21.80	20.20	23.20
Population *(billions)*	6.60	6.70	6.70	6.80	6.90	7.00
Per capita food fish supply *(kg)*	17.40	17.60	17.80	18.10	18.60	18.80

Figure 2. 3: World fisheries and aquaculture production and utilization (FAO, 2012)

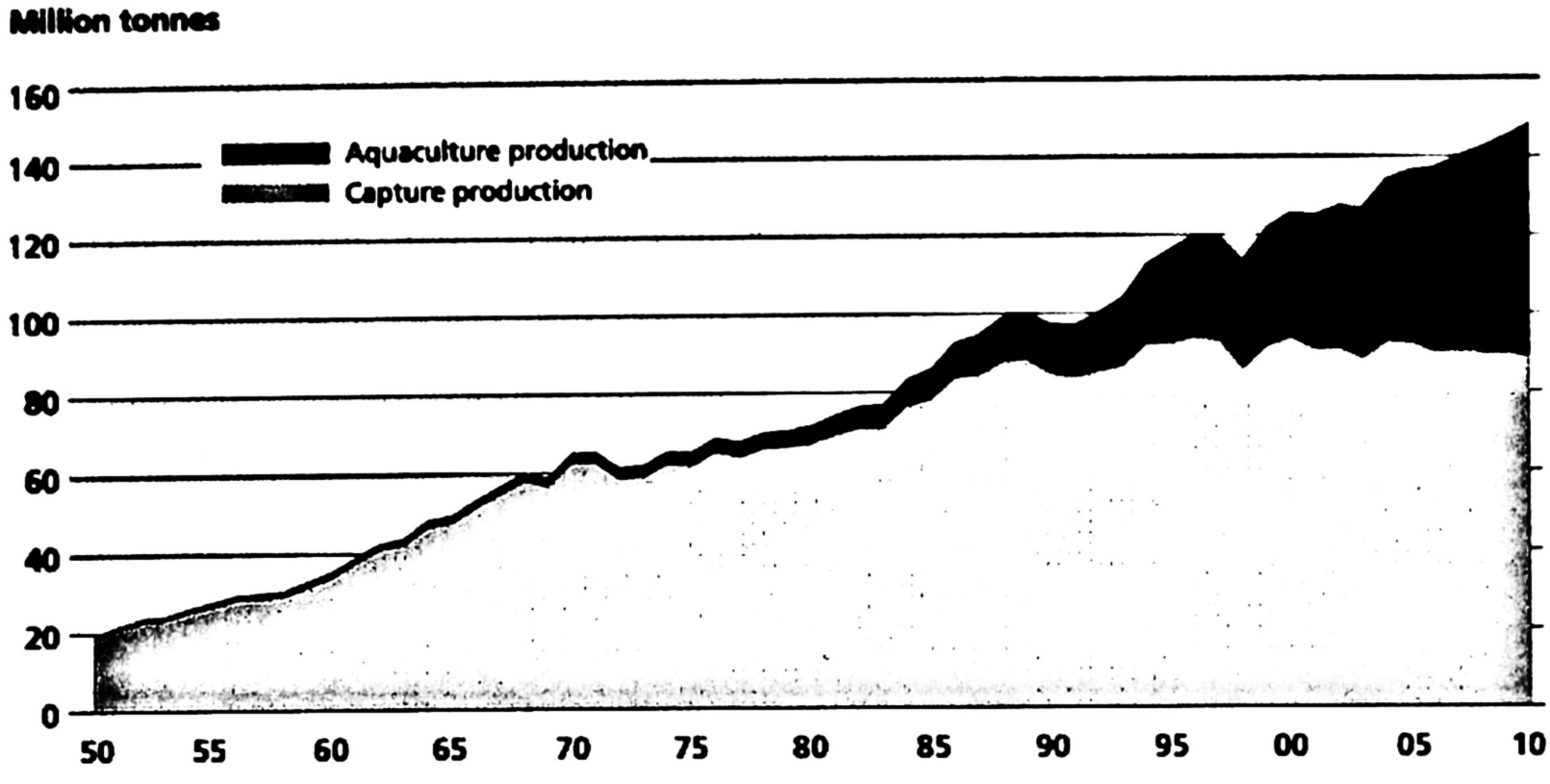

Figure 2. 4: World fisheries and aquaculture capture production (FAO, 2012)

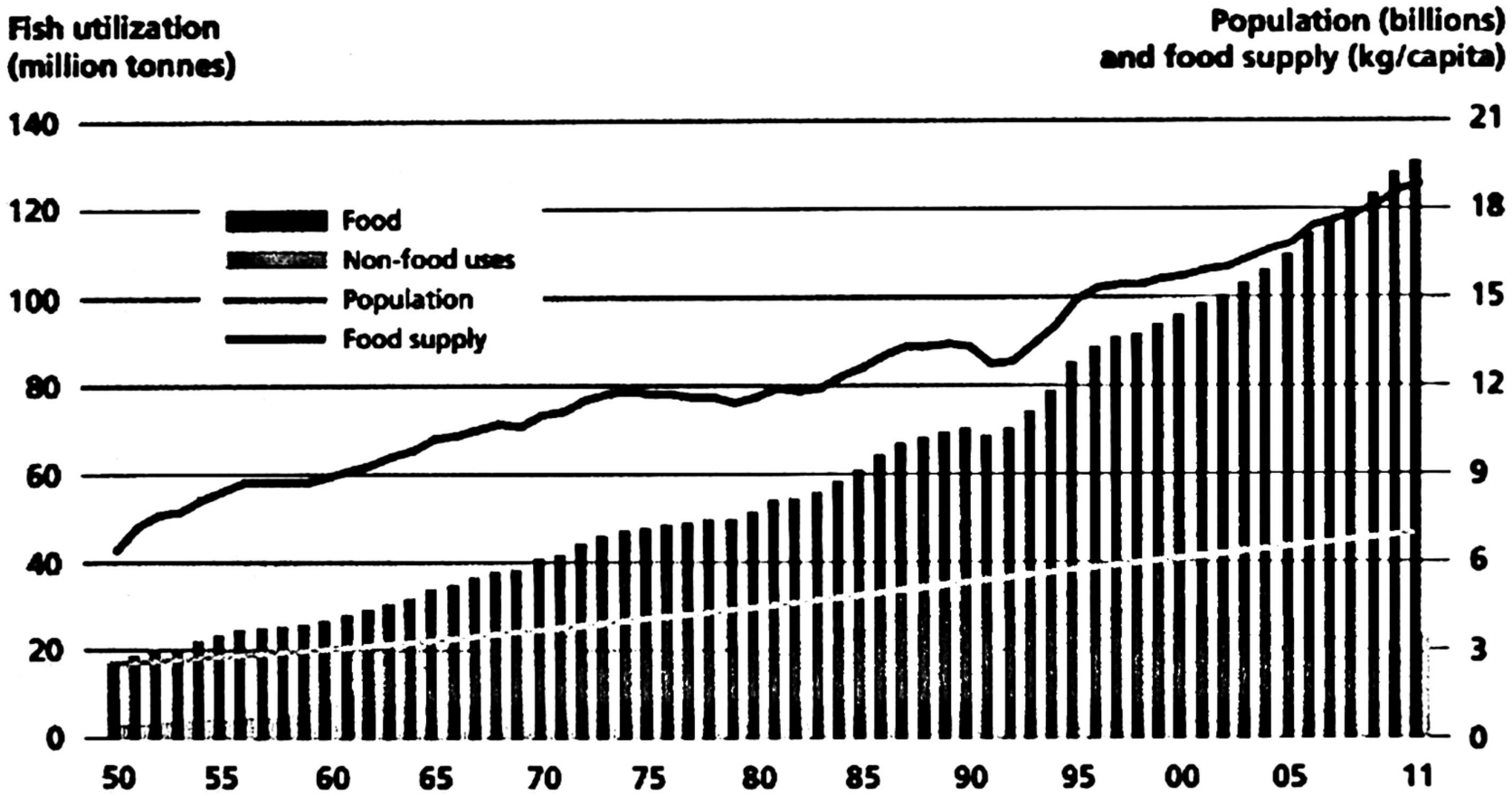

Figure 2. 5: World utilization and supply of fish (FAO, 2012).

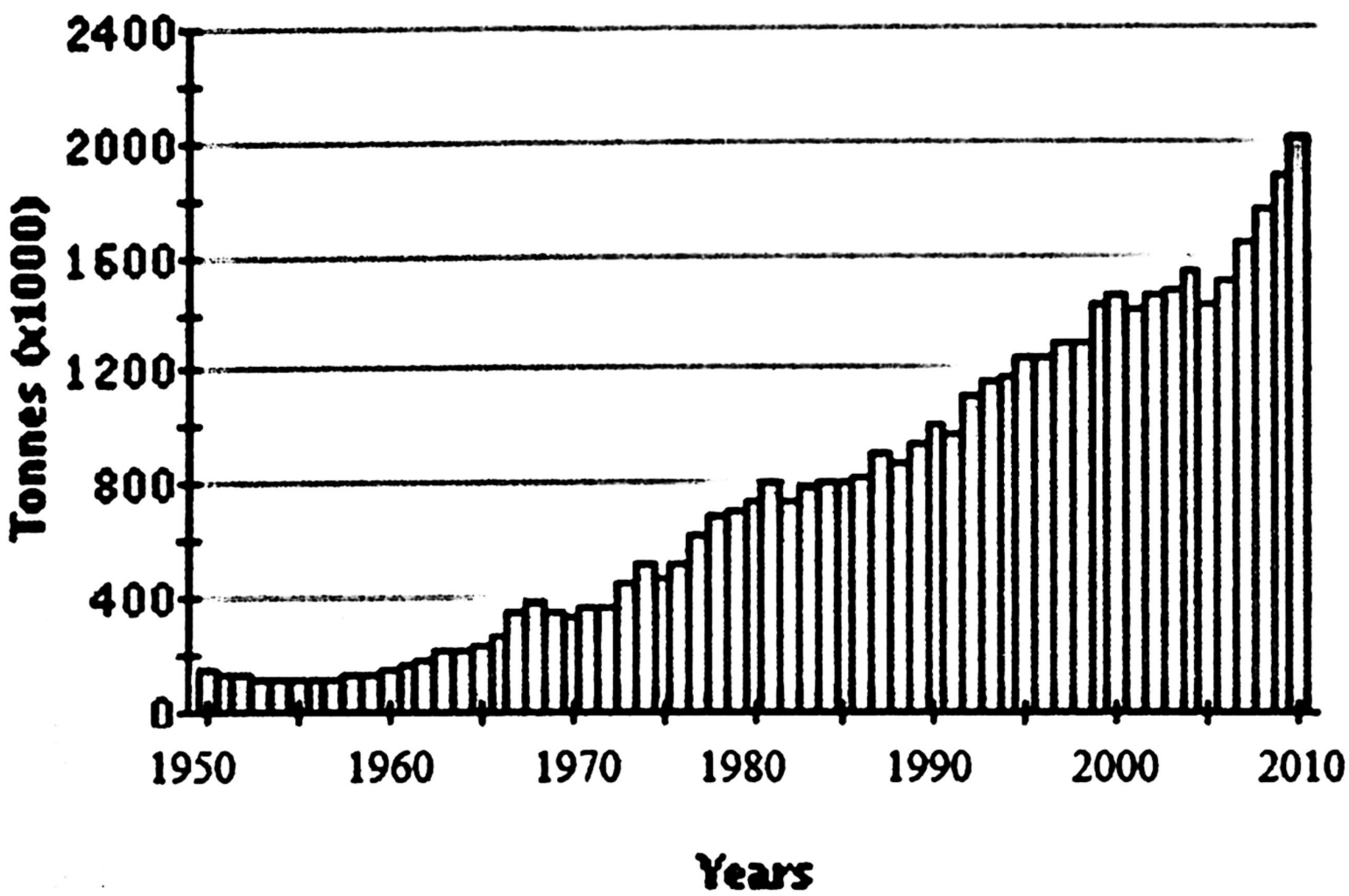

Figure 2. 6: Malaysia's aquaculture and capture production from year 1950 to 2010

2.3.1 Protein Film

As the time goes the native specific states remains same, protein generally exist as either fibrous proteins, which are water insoluble and serve as the main structural materials of animal tissues, or globular proteins, which are soluble in water or aqueous solutions of acids, bases or salts and function widely in living systems (Scope, 1994). Fibrous proteins are fully extended and associated closely with each other in parallel structures, generally through hydrogen bonding, to form fibres. The globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulphide) bonds (Scope, 1994). Generally, proteins must be denatured by heat, acid, base, and/or solvent in order to form the more extended structures that are required for film formation.

Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films is affected by the degree of chain extension and the nature and sequence of amino acid residues.

2.3.2 Gelatine Film

Gelatine is obtained by controlled hydrolysis from the fibrous insoluble protein, collagen, which is widely found in nature as the major constituent of skin, bones and connective tissue, Gelatine is composed of a unique sequence of amino acids. The characteristic features of gelatine are high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of hydrophilic character (Ross, 1987).

Gelatine films could be formed from 20-30% gelatine, 10-30% plasticizer (glycerine or sorbitol) and 40-70% water followed by drying the gelatine gel (Guilbert, 1986). Gelatine is used to encapsulate low moisture or oil phase food ingredients and pharmaceuticals. Such encapsulation provides protection against oxygen and light, as well as defining ingredient amount or drug dosage (Gennadios et al., 1994). In addition, gelatine films have been formed as coatings on meats to reduce oxygen, moisture and oil transport (Gennadios et al., 1994).

2.3.3 Fish Gelatin

Gelatine from marine sources (warm- and cold-water fish skins, bones, and fins) is a possible alternative to bovine gelatine (Kim & Mendis, 2006; Rustad, 2003; Wasswa et al., 2007). One major advantage of marine gelatine sources is that they are not associated with the risk of outbreaks of Bovine Spongiform Encephalopathy. Fish gelatine is acceptable for Islam, and can be used with minimal restrictions in Judaism and Hinduism. Furthermore, fish skin, which is a major by-product of the fish-processing industry, causing waste and pollution, could

provide a valuable source of gelatine (Badii & Howell, 2006). Fish skin contains a large amount of collagen: Nagai and Suzuki (2000) reported that the collagen contents in the fish skin waste of Japanese sea-bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively.

Production of fish gelatine is actually not new as it has been produced since 1960 by acid extraction, although most of it has been used for industrial applications (Norland, 1990). Detailed extraction procedures and characterization of the properties of fish gelatine were described by (Grossman and Bergman (1992)) in a United States patent. Since then, multiple research groups have further investigated the various aspects of fish gelatine. Gelatine has been extracted from skins and bones of various cold-water (e.g., cod, hake, Alaska Pollock, and salmon) and warm-water (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim) fish. While for this study, warm water tilapia (Oreochromis mossambicus) is used in this project.

2.3.4 Collagen

Collagen is the major structural protein in all animal bodies which consists of three polypeptide chains in the triple coil. Collagen are different greatly in terms of their size, tissue distribution and function (Gelse et al., 2003). Furthermore, there are about 28 types of collagen, namely type I to XXVIII (Gordon and Hahn, 2010). Due to their structure and supramolecular organization, they are categorized into fibril-forming collagens (Collagen type I, II, III, V, and XI), basement membrane collagens (Collagen type IV), microfibrillar collagen (Collagen type VI), anchoring collagens (Collagen type VII), hexagonal network-forming collagens (Collagen type VIII and X), FACIT collagens (Collagen type IX, XII, XIV, XIX, XX and XXI), transmembrane collagens (Collagen type XIII and XVII), multiplexins (Collagen type XV, XVI and XVIII) and others (Collagen type XXVIII) (Gelse et al., 2003; Gordon and Hahn, 2010).

2.3.5 Collagen Source

Bovine and porcine skins are the main industrial sources for collagen production. Arising from the 1930s, the first fresh material to be used for the output of collagen was porcine skin, and to this day, the most significant raw material for large-scale industrial productions is still porcine skin (Gomez-Guillen et al., 2011). Porcine skin collagens have been bred to transmit bovine spongiform encephalopathy (BSE), whereas fish collagen has a lower risk of acquiring unknown pathogens such as BSE (Yamauchi, 2002). Similarly, religious sentiments of the Jewish and Islamic believers whom prohibits the consumption of pork, whereas Hindus forbid the consumption of cow products, have caused pessimism and fears among consumers to use mammalian collagen as a source (Karim and Bhat, 2009). Therefore, a set of researches has been done to obtain an alternative source of collagen. Hence, far, fish waste collagen was found to have the most similar characteristics to mammalian collagen. Thus, it is likely to draw the industry's attention as an alternative source of mammalian collagen (Nagai and Suzuki, 2000a; Kim and Mendis, 2006).

Throughout the decade, huge numbers of fish species were investigated as alternatives to the source of collagen. All species studied showed to have different denaturation temperature characteristics. This had raised interest among the research communities in optimizing the extraction conditions and yields, and also characterizing the resultant collagens. These collagens were mainly carried out from fish skin and bone residues. Cod, Atlantic salmon and Alaska Pollock are cold-water species that were searched on their Physio-chemical and operational attributes. Low temperature-water species were reported to receive significantly lower denaturation temperatures (approximately 4-17 °C) as compared to tropical species (approximately 18-29 °C). Examples of sub-tropical species were Nile Perch, red Tilapia,

channel catfish, yellowfin tuna, skate or grass carp (Karim and Bhat, 2009; Gomez-Guillen et al., 2011).

Fish collagen that was proposed as potential constituents in the fabrication of functional food, cosmetics, biomedical and pharmaceutical applications were obtained from trout and hake (Montero and Borderias, 1991), brownstripered snapper (Jongjareonrak et al., 2006), deep-seared fish (Wang, A, et al., 2008), walleye pollock (Yan et al, 2008), and unicorn leather jacket (Ahmad and Benjakul, 2010). Fish wastes consist of 5 % of fish scales (Wang and Regenstein, 2009) are major fish industry residues. Red Tilapia and seabream (Ikoma et al., 2003), Sheepshead and black drum (Ogawa et al., 2004), grass carp (Li et al., 2008) and deep-sea red fish (Wang, A, et al., 2008) were widely studied for extraction of collagen from their scales. Fins that where the waste products of canned tuna processing were suggested as a high - quality source of collagen extracted, but with low yields (Aewsiri et al., 2008).

Fish offal from semi-processed products of marinated or salted herring or cold-smoked salmon were researched as a origin of collagen where it was found that smoking did not change a collagen's denaturation temperature and had higher denaturation temperatures compared to collagens from marinated or salted skins (Kolodziejska et al., 2008; Gomez-Guillen et al., 2011).

Collagen from alligator bones which were created as a huge waste in China, Thailand and the southern United States were also examined. These collagens were type I collagens that had nearly similar intrinsic characteristics to those of tropical fish species such as black drum and Sheepshead (Wood et al., 2008; Gomez-Guillen et al., 2011).

Giant red sea cucumber was also thought a possible collagen source for pharmaceutical applications (Liu et al., 2010) where it was also found to be of type I collagen but had very low amino acid content as compared to cold-water fishes. Poultry by-products were also utilized

for the output of collagen-based, high value-products as collagen type I and type III were extracted (Cliche et al., 2003; Gomez-Guillen et al., 2011).

2.4 Tilapia

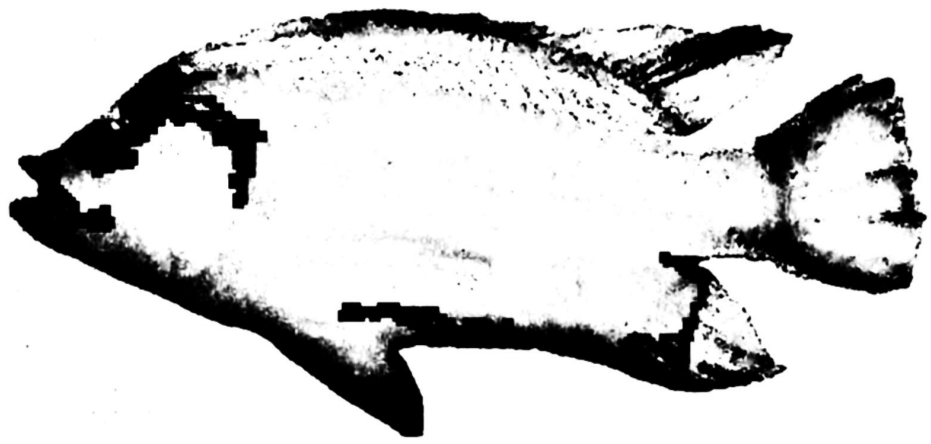

Figure 2. 7:Tilapia (Oreochromis mossambicus)

Kingdom:	Animalia
Class:	Actinopterygii
Order:	Perciformes
Family:	Cichlidae
Tribe:	Tilapiini
Genus:	Tilapia

Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers and lakes and less commonly found living in brackish water. Historically, they have been of major importance in artisanal fishing in Africa, and they are of increasing importance in aquaculture and aquaponics. Tilapia can become a problematic invasive species in new warm-

water habitats such as Australia, whether deliberately or accidentally introduced, but generally not in temperate climates due to their inability to survive in cold water.

The economically of fresh water fish in Malaysia reported to be increase in aquaculture production. And about 33% of the fresh water fish are contributed by tilapia. The increase rate of tilapia production causing by the high demand of tilapia fish thus contribute to the by-product of the fish.

2.4.1 Tilapia fish scale

Warm-blooded fish gelatine has similar properties to the mammalian sources. As the best parts of the fish are the scales and bones that are rich in collagen which can be a source of gelatine. As tilapia (Oreochromis mossambicus) is a warm-blooded fish it is suitable for the extraction. As for the scale, tilapia (Oreochromis mossambicus) has a large surface area scale thus will store large amount of collagen.

2.5 Industrial Extraction Process of Collagen

Collagen in its native insoluble form has to be treated first, only then it can be converted into a form that is appropriate for extraction. This pre-treatment is a step that is completed by heating the collagen in acid or alkali at 45 °C and above (Karim & Bhat, 2009; Pérez-Mateos et al., 2009) . Then the process of chemical pre-treatment will rupture non-covalent bonds, cleaving intra- and intermolecular covalent crosslinks which cause the protein structure to be disordered, therefore creating sufficient swelling and solubility of collagen(Karim & Bhat, 2009; Pérez-Mateos et al., 2009) .

Heat treatment are needed to breaks the hydrogen bonds and covalent bonds, thus will resulting unstable the triple helix and the transition of helix to coil and converts it into soluble gelatine (Gomez-Guillen, Gimenez, Lopez-Caballero, & Montero, 2011; Gómez-Guillén et al., 2002). The properties, preservation of the raw material, pre-treatment, extraction process parameters such as temperature, pH and time, affect the polypeptide chain length and the gelatine's functional properties (Karim & Bhat, 2009).

Production of collagen or gelatine differs among these raw materials used. First, all raw materials are cut, washed and cleaned to eliminate non-collagenous materials. Then, the crude collagenous materials undergo further processes to produce either type A or type B gelatine depending on their source.

Acid treatment will have produced Type A gelatine and is more appropriate for less covalently cross-linked collagens known as young collagen. While Type B gelatine is extracted from alkaline and acid treatment (Karim & Bhat, 2009).

During the extraction process, the raw materials are submerged in warm water, with regulated temperatures, to extract the gelatine. This liquid form is precipitated with NaCl to clean and refine the product which is recovered by filtration. It may also include centrifugation, ultra-filtration and ion exchange to remove the inorganic materials. Then, the liquid is applied to a multi-phase vacuum evaporator to subtract the water content from it, producing a very viscous solution. A high temperature flash sterilization is applied to prevent the possibility of microbial contamination. Subsequently, the gelatine is made into a gel form by chilling in a rotator, mincing and depositing it as a bed onto an open-weave belt that goes through a drying chamber. A filtered dry air stream is flowed into the chamber and through the belt, which dries the gelatine. Following physical and microbiological testing, the gelatine particles are crushed,

milled and mixed together to make trade-quality gelatine for specific applications. The gelatine is also examined for approval, packed, stocked and supplied (Karim & Bhat, 2009).

2.5.1 Types of Extraction

Generally, there are two types of extraction methods widely used to extract collagen from fish skin, scales, bones and fins. These two types of extraction methods are extraction of acid solubilized collagen and extraction of pepsin solubilized collagen. The difference between these extraction methods is that the latter involves the use of porcine pepsin to cleave telopeptide cross-linked regions without breaking the integrity of the triple helix (Zhang et al., 2007). However, the yield, chemical composition and characteristic of the extracted collagen, using these two methods would differ from one another.

2.5.2 Gelatine extraction method

Gelatine was extracted from tilapia scales according to the method of (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009) with some modifications. Scales were soaked in a mixed solution containing 0.05 M NaOH and 25 % alcohol with a scale/solution ratio of 1:4 (w/v), then placed at 10 °C overnight to remove lipids and non-collagenous proteins. Alkaline treated scales were washed with tap water to achieve the neutral pH. Demineralisation of tilapia scales was carried out using 5 volumes of 0.05 M HCl solution for 2 h at room temperature. Then, the demineralised tilapia scales were washed to neutral pH with tap water. After pre-treatment, the swollen scales were soaked in distilled water at 80 °C for 1 h to extract the gelatine. The extract was centrifuged at 15,000×g, 20 °C for 30 min with a refrigerated centrifuge. The supernatant of gelatine extract was freeze-dried and the gelatine powder obtained was stored until use.

2.5.3 Optimization

Optimization studies the effects of various factors on the process of an experiment to determine the optimized conditions for optimal results. An appropriate experimental design is therefore required for complex processes where the targeted response is influenced by many factors (Li and Fang, 2007). The efficiency of the collagen extraction process from fish waste is affected by several process factors such as acid concentration, extraction time, temperature and so on. Thus, a suitable experimental design to analyse multiple factors is required.

In this experiment the rate of thermal treatment by 30 min 1 hour and 2 hours are taken as variable. Also, the temperature is varying by 80°c, 90°c and 100°c. lastly, by adding the plasticizer does it affect the tensile strength and the texture of the film produce later.

2.5.4 Plasticizer

Plasticizer is a vital ingredient of the most formulation. It helps to improve the flexibility of the film and reduces the brittleness of the film. Plasticizer significantly improves the film properties by reducing the glass transition temperature of the polymer. The selection of plasticizer will depend upon its compatibility with the polymer and also the type of solvent employed in the casting of strip. The flow of polymer will get better with the use of plasticizer and enhances the strength of the polymer.

As Gelatine is prepared by the thermal denaturation of collagen, isolated from animal skin, bones and fish skins. Gelatine is a generic term for a mixture of purified protein fractions obtained either by partial acid hydrolysis (type A gelatine) or by partial alkaline hydrolysis (type B gelatine) of animal collagen and/ or may also be a mixture of both. It is readily soluble in water at temperatures above 40°C, forming a viscous solution of random-coiled linear polypeptide chains.

2.5.4.1 Glycerol

Glycerol is one of the plasticizers used in edible film packaging belongs to polyols group. Polyols group are cited as one good plasticizers because of their ability to reduce intermolecular hydrogen bonding while increasing intermolecular spacing. Then glycerol will react by hydrogen bonding due to the presence of hydroxyl groups in the molecule. Thus will have higher thickness and density and will elevated the thermal degradation temperature to higher values and thus, increased the thermal stability of the film. Also, hydroxyl groups in glycerol have strong affinity with water molecules enabling glycerol containing films to easily retain water within their matrix and form hydrogen bond. Hence, glycerol acts as water-holding agent. Plasticizers interfere with polymeric chain association facilitating their slipping and thus enhancing film flexibility. Glycerol decreases the rigidity of the network, producing a less-ordered film structure and increases the ability of polymer chains movement (Kołodziejska & Piotrowska, 2007). This will cause the increase in flexibility of the films.

2.5.5 Rheological Properties

Gelatine contains features that categorize it as a thermo-reversible gel. This is imputable to the fact that the bonding energy is feeble inside fish gelatine (Karim & Bhat, 2009). The main physical properties of gelatine gels are gel strength and gel melting points, which are both dependent on the molecular weight. It is too reliant on the complex interactions that take place due to the amino acid makeup and the ratio between α/β chains in the gelatine (S. M. Chou et al., 2004). The gel strength is directly correlated with the α-chain content in gelatine. And, as the α-chains presence are higher, the greater the strength of the gel.

But then, as there is a presence of peptide that has high ratio with higher or lower molecular weight than a number of α-chains, thus it will decrease the gel strength. And as

gelatin can produce thermally reversible gels. Then the gels produce by fish gelatin will begin to melt at temperature below human body. This called "melt –in-the-mouth" property.

These rheological properties are a function of temperature and the concentration of gelatin, which is present in different species. These properties are developed during the transformation of collagen into gelatin. The process involves the disintegration of the helical structures into random coils (Hill, Ledward, & Mitchell, 1998). When they are cooled down the random coils will transition into helix's however, during this time they are attempting to reform back to their original structure (Karim & Bhat, 2009). This structure will be responsible for the strength and integrity of the gelatin gel.

2.5.6 Extraction of Gelatine

The extraction of gelatine can only be carried out after the collagen has converted into gelatine. The process of conversion occurs on a molecular level. The first step is to apply enough heat that the collagen fibrils will shrink to less than one-third of their original length. This occurs at the critical temperature, which is known as the shrinkage temperature. At the shrinkage temperature, the fibres are disassembled, and the triple helix arrangement of the polypeptide subunits in the collagen molecule will collapse (Foegeding et al., 1996). The collapse is due to the many non-covalent bonds as well as covalent inter and intra-molecular bonds breaking. The heat will disrupt most of the hydrogen bonds that are responsible for stabilizing the collagen structure.

This will result in the helical collagen structure converting into an amorphous state known as gelatine. From the different methods of extraction that are used for the various sources of fish, the extracted gelatine has a molecular weight which varies from 15 to 400 kDa (Nollet et al., 2012). The primary goal of extraction is to be able to extract the highest quality gelatine with the max yield and the desirable physical properties. Therefore, the factors need to be

adjusted according to which property is most desired. The adjustment of pH and other parameters can result in shorter extraction time yet still provide high-quality gelatine with high bloom strength (Johnston-Banks, 1990).

2.5.7 Drying

After extraction, the last process is drying, which by using freeze drying method. Where, freeze-dried gelatine is known for expressing the highest gel strength and foam formation. and by using freeze drying method the dry gelatine can be save for longer period of time.

Chapter 3

Methodology

3.1 Introduction

The process begins with preparation of materials and all the material were mix together before undergoes the casting process, then the film was analysed using texture analyser and all the sample were triplicate and result from the experiment were discussed and present in graph.

3.2 Raw Material

Tilapia fish scales were collected from a local wet market at Jerantut, Pahang. These wastes were immediately washed with tap water and frozen until further used. Generally, Tilapia fish scales samples were thawed at room temperature prior to the extraction procedure. The respective collagen samples from the scales) were extracted according to the extraction conditions. Extraction of the tested parameters by the objective and result that intended. While glycerol as a plasticizer are obtained from supplier.

3.3 Extraction of Scale Collagen

Gelatine was extracted from tilapia scales according to the method of (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009) with some modifications. Scales were soaked in a mined solution containing 0.05 M NaOH and 25 % Methanol with a scale/solution ratio of 1:4 (w/v), then placed at 10 °C overnight to remove lipids and non-collagenous proteins. Alkaline treated scales were washed with tap water to achieve the neutral pH. Demineralisation of tilapia scales was carried out using 5:1 of 0.05 M HCl solution /scale for 2 h at room temperature. This to ensure that at the end of extraction process only protein was extracted. Then, the demineralised tilapia scales were washed to neutral pH with tap water. After pre-treatment, the swollen scales were soaked in distilled water at 80°C, 90°C and 100°C for 30 minutes, 60 minutes and 120 minutes, this to extract the gelatine. The extract was centrifuged at 15,000×*g*, 20 °C for 30 min with a centrifuge (Avanti J-25; Beckman, California, USA). The supernatant of gelatine extract was freeze-dried and the gelatine powder obtained was stored at −18 °C until use.

3.4 Film Preparation

Gelatine powder was dissolved with distilled water at 60 °C for 30 min to obtain the final protein concentration of 2 % (w/v) determined by the Lowry method (Lowry et al. 1951). The solution was added with glycerol as a plasticizer at the concentration of 20 % (w/w) of gelatine. Then film forming solution (FFS) was poured onto a rimmed silicone resin plate (50 × 50 mm) placing on a level surface and dried in an environmental temperature of room for 24 h. Then the resulting films were manually peeled off.

3.5 Calculation of Extracted Yield Collagen

Collagen yield was estimated by measuring the percentage of the weight of collagen extracted from the weight of the fish skins, scales, bones and fins. Collagen yield was expressed as percentage of dry weight of collagen yield based on the freeze dried weight of the fish waste raw materials fish scales. Equation 3.1 was used for the estimation of collagen.

$$Collagen\ (\%) = \frac{Dry\ weight\ of\ collagen\ (g)}{Dry\ weight\ of\ fish\ waste\ (g)}\ x\ 100\ \% \qquad \text{Equation 3.0}$$

3.6 Mechanical Properties – Elongation at break, Tensile Strength & Thickness

The film thickness was measured using a micrometre (Thickness Gauge; Ozaki MFG Co., Tokyo, Japan) to the nearest 0.001 mm at five random locations of the films. Tensile strength (TS) and elongation at break (EAB) were measured using a texture analyser (TMS-PRO; Food Technology Co., USA) with 100 N load cell. The tests were operated according to the method described by (Iwata, Ishizaki, Handa, & Tanaka, 2000) with a slight modification. Two rectangular strips (width 15 mm; length 45 mm) were cut from each gelatine film to measure the mechanical properties. Initial grip separation and mechanical crosshead speed were set at 30 mm and 60 mm/min, respectively. TS (MPa) was calculated by dividing the maximum load (N) necessary to pull the sample film apart by the cross-sectional area (m^2). EAB (%) was calculated by dividing film elongation at the moment of rupture by the initial grip length of sample and multiplied by 100. Five samples of each film type were used for testing.

3.7 Proximate components

The tilapia fish scale gelatine was subjected to proximate analyses including moisture, crude protein and ash contents according to the AOAC methods (AOAC 2005).

3.8 Colour Detection Technique

Tilapia fish scale film was taken for the measurement of colour using lab scan XE spectrocolorimeter in terms of CIE 'L' (lightness and yellowness) 'a' (redness and greenness) and 'b' (blueness), Yi (Yellowness index) and Wi (Whiteness index) following the method of Chantrapornchai et al. (1998). ΔE (colour difference) values were calculated using the following formula:

$$\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{½} \qquad \text{Equation 3.1}$$

3.9 Fourier Transform Infrared Spectroscopy (FTIR)

The sample was sent to Material Characterization Laboratory (MCL) Faculty of Engineering UPM and was examined. All spectra were obtained using a FTIR (Omnic spectra) spectrophotometer (Perkin Elmer, USA). A measurement technique whereby spectra are collected based on measurements of the temporal coherence of a radiative source. A total of 0.5 g film was fixed onto the crystal and knob of the Nicole i50 and placed on the FTIR apparatus. At an interval of 1.0 cm-1, each sample was subjected to 32 scans from 4000 to 400 cm-1 at a resolution of 4.0 cm-1. s.

3.10 Film morphology

Sample was sent to Institute of advance study (ITMA) University Putra Malaysia. Scanning electron microscopy (SEM) of the films was performed with a JEOL JSMP 100 (Japan) electron microscope. Film pieces were mounted on aluminium stubs using a double-sided tape and then coated with a layer of gold (40–50 nm), allowing surface and cross-section visualization. All samples were examined using an accelerating voltage of 5 kV.

Chapter 4

Result and discussion

4.1 Result overview

In this chapter, the film and result obtained from several tests which were mechanical test, colour detection method, Fourier transform method and differential scanning calorimeter was discussed in detailed. Figure 4.1 shows results overview for tests done on the fish gelatine film.

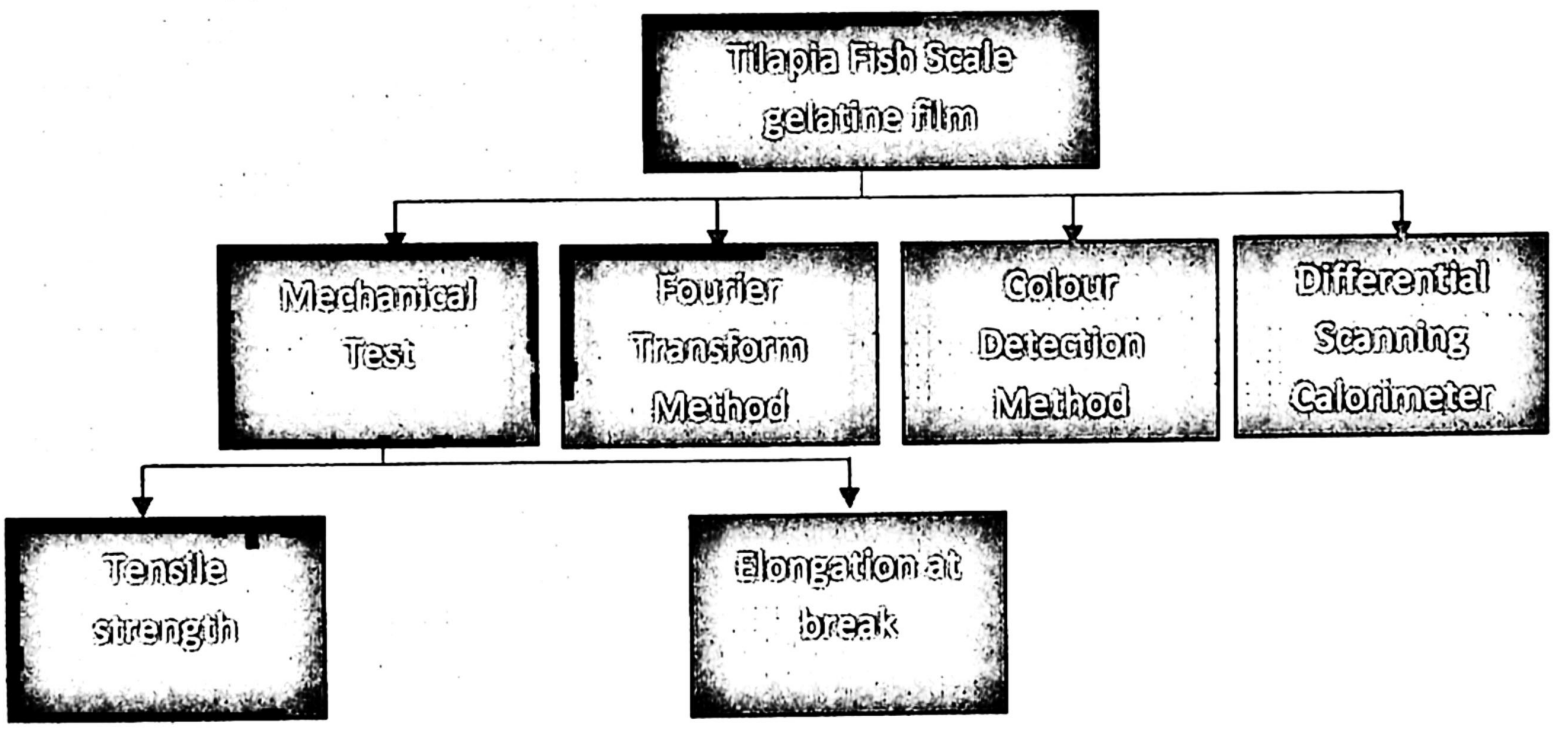

Figure 4. 1:Overview Tests Done On the Tilapia Fish Gelatine Film.

4.2 Extracted collagen yield

A total of 30 experimental runs were assessed and the extracted collagen yield was attained according to the experiment where the tilapia fish scale was undergone different parameter as shown in figure 4.1 above. As a results, the effects of the two factors on the yield of collagen as well as their interactions are calculated using the equation 3.1. Thus, the results of extraction temperature and time on collagen yield from tilapia fish scale waste are shown in table 4.1. High yields are obtained in range 120 minutes for 80 °C, 90°C, and 100°C. with the highest yield obtained exactly at 100 °C for 120 minutes. And as for the lowest yield obtained in range 30 minutes for 80°C.

For tilapia fish scale, the highest yield obtained was 24.07 %. It was higher compared to silver carp scale of 1.45 % (on a wet weight basis) (Fan et al., 2009) scales of bighead carp 2.70% (on a dry weight basis) (Klymus, Richter, Chapman, & Paukert, 2015), but lower to scales of red seabream, Japanese sea bass and sardine which yielded 37.5 %, 41.0 % and 50.9 %, respectively (Nagai & Suzuki, 2000).

Generally, collagen yields are affected by temperature, extraction time for both factors, collagen yield improved with the raise in temperature and time This is due to the fact that collagen is a thermos-instable protein that easily denatures at room temperatures owing to its chemical structure. Generally, fish collagens vary in their amino acid composition, in particular to the amounts of the amino acids (proline and hydroxyproline) between species (Gudmundsson & Hafsteinsson, 1997). A lesser amount of hydroxyproline in a collagen exhibits a lesser thermal stability compared to those with a higher amount of hydroxyproline (Muyonga et al., 2004) .

Table 4. 1 : Shows The Results of Extraction Temperature and Time On Collagen Yield from Tilapia Fish Scale Waste.

Tilapia Fish Scale	Temperature (°C)	Time (Minutes)	Collagen (%)
1	80	30	12.88
2	80 °C	60	15.92
3	80 °C	120	19.52
4	90 °C	30	13.20
5	90 °C	60	16.84
6	90 °C	120	21.25
7	100 °C	30	13.94
8	100 °C	60	17.08
9	100 °C	120	24.07

This is attributed to the assistance of hydroxyproline in the interchain linkage by hydrogen bonds that stabilize the collagen's triple-helix structure. Consequently, manipulating the temperature for a given duration in the collagen extraction process is critical to maintain the collagen's natural structure. Raising the temperature to a definite amount and duration can help disintegrate collagen into gelatine which boosts the yield of fish scale extract.

4.3 Mechanical test

4.3.1 Introduction

This research, results of fish gelatine film with various parameters (different time and temperature) as shown in table 4.1. The pure fish gelatine film without plaasticizer is first being tested as reference. The addition of plasticizer was then tested and compared to the pure tilapia fish scale gelatine film. An increase in temperature and time extraction. According to (Mota, Peres Pinto, & de Lima, 2017) and (Vieira, Da Silva, Dos Santos, & Beppu, 2011)plasticizers as glycerol has imparted to the film flexibility.

4.3.2 Tensile strength

A higher tensile strength is generally preferred for a variety of packaging products. When packaging heavier products a packing tape with a higher tensile strength is preferred to help ensure a better seal.

Tensile play an important role in determining the mechanical properties of edible films (Hebel et al., 2014). Because as these film are used in many food applications no matter small or industry. Tensile strength is used to indicate the film strength. Table 4.2 shows that there was significant effect of different parameter with glycerol and different parameter without glycerol on tensile strength of tilapia fish scale gelatine film. It can see that as the temperature increase and time increase the tensile strength increase.

As shown in table 4.2 result obtained for tensile strength of pure tilapia fish scale gelatine film were between 38.05 to 50.17 MPa. While for tensile strength of pure tilapia fish scale gelatine film with glycerol between 46.61 to 61.50 MPa. This is showing that higher values of tensile

strength compared to the film with no glycerol. While as the higher value of tensile strength correspond to stronger film, as the tensile strength of a film was the maximum stress that a film can withstand being stretched before necking or cracking (Mota et al., 2017). This because the good interaction between fish gelatine and glycerol.

Table 4. 2: Show the effect of thermal treatment of films on tensile strength (TS) of tilapia scale gelatine films

Heating temperature (°C)	**Heating time (minutes) Tensile (MPa)**					
	With glycerol			Without glycerol		
	30 mins	60 mins	120 mins	30 mins	60 mins	120 mins
80 °C	46.61	47.50	48.00	38.05	43.63	43.79
90 °C	46.91	50.06	51.06	42.08	45.61	46.30
100 °C	49.18	55.02	61.50	46.11	49.59	50.17

The higher temperature and time shown the higher tensile strength because of gelatine molecules possibly became more stretched at 100 °C. This most likely favoured the inter-connection of gelatine molecules via hydrogen bonding due to more junction zones during film formation. Thus film network with increased tensile strength was obtained. While looking to the table 4.2, can be conclude that the integrity. molecular weight, hydrogen bonding and covalent bond of protein chains that go through different heat treatment might contribute to the network structured of film obtained, based on the previous study (Shiku et al., 2004).

The tensile strength of gelatine films at 80 °C for 60 minutes without glycerol were 43.79 MPa, which based on the previous study (which extraction done at the same temperature and duration) was higher than that of shark skin gelatine films (tensile strength = 27.29 MPa) (Igel, Heidrich-Meisner, & Glasmachers, 2008)(Limpisophon et al., 2009) and tilapia skin gelatine films (TS = 37.51 MPa) (Weng & Wu, 2015) due to the higher amino acid content (Karim & Bhat, 2009). Furthermore, the tensile strength of tilapia scale gelatine films with glycerol (50.17 MPa) was almost close to that of OPP (orientated polypropylene) films (TS = 50.7 MPa) (Shiku et al., 2004) ,this shown that tilapia fish scale gelatine can be used as an alternative to replace synthetic polymer and for food packaging.

4.3.3 Elongation at break

The elongation of a material refers to the difference of length between an unstretched tensile-strength sample and the breaking point length. High elongation is great for stretch films and help to secure and unitize a load. It is also important for a stretch film to have an adequate tensile strength for the load it is securing. Table 4.3 shows that there was significant effect of different parameter with glycerol and different parameter without glycerol on EAB of tilapia fish scale gelatine film. As can conclude the EAB of films did neither change with heating at 80°C nor 90°C, while decreased by heating at 100 °C.

Regarding from the table 4.3, shown that while using plasticizers the EAB of the film increase. As known, plasticizers are used to reduce brittleness and increase flexibility and strength of the film by reducing intermolecular forces and increasing the mobility of biopolymer chains (Lagarón, López-Rubio, & José Fabra, 2016). While at the high temperature the EAB decrease

causing by lose of the hygroscopic character of the film which help to loosen the interaction between the chain links in the film. Thus will low the flexibility in the polymer structure.

The results of this study show that, while at higher temperature and higher duration of time with addition of glycerol the tilapia fish scale gelatine film has lower EAB. This, due to water content in the film itself and secondly, due to the interfacial interaction between glycerol and biopolymer matrix. As reported by (Brunnermeier & Pedersen, 2009; Karim & Bhat, 2009; Nagai & Suzuki, 2000; Rustad, 2003), mechanical properties of biofilm is highly dependent on the interfacial interaction between matrix and fillers. Prior studies have noted that glycerol will act as reinforce filler in the protein matrix, then will enhance the strength in tensile. While as for EAB will depend on few factors which is the temperature, extraction time and the addition of plasticizers.

Table 4. 3 : Effect of thermal treatment of films on elongation at break (EAB) of tilapia scale gelatine films.

Heating temperature (°C)	**Heating time (minutes)**					
	With glycerol			Without glycerol		
	30 mins	60 mins	120 mins	30 mins	60 mins	120 mins
80 °C	48.25	49.29	48.13	43.11	42.20	42.00
90 °C	45.38	47.69	45.09	44.02	43.38	47.56
100 °C	46.63	36.13	34.75	48.05	46.63	42.93

4.3.4 Thickness

As for the thickness, there are slightly change with the thickness of the tilapia fish scale gelatine film with and without glycerol. As the table 4.4 shown the thickness the tilapia fish scale gelatine film.

As shown in the table 4.4 where there are slightly change in the thickness of each film but as average the thickness of the film corporate with glycerol is between 0.14 mm to 0.16 mm. This study confirms that while using plasticizers it will increase the mobility of biopolymer chains (Gomez-Guillen et al., 2011; Payne & Veis, 1988; Vieira et al., 2011). Thus this changing due to low molecular weight, will increases the free volume of the film. While for the thickness of tilapia fish scale gelatine film that purely without addition of glycerol is between 0.10 mm to 0.12 mm. This shown that when the film incorporated with glycerol as plasticizer it will fill up the component in the film thus will thicken the film. Based from (Kołodziejska & Piotrowska, 2007; Rezaei, Motamedzadegan, Rezaei, & Motamedzadegan, 2015) the thickness of the film will be based on the drying rate of the film and the gelatine content in the film forming solution. These, two very factor will also affect the thickness of the film.

Table 4. 4: Effect of thermal treatment of films on the thickness of tilapia scale gelatine films.

Heating temperature (°C)	Heating time (minutes)					
	With glycerol			Without glycerol		
	30 mins	60 mins	120 mins	30 mins	60 mins	120 mins
80 °C	0.14 mm	0.15 mm	0.15mm	0.11mm	0.10 mm	0.11 mm
90 °C	0.15 mm	0.15 mm	0.15 mm	0.12mm	0.12 mm	0.11 mm
100 °C	0.15 mm	0.15 mm	0.16 mm	0.13mm	0.11 mm	0.12 mm

4.4 Fourier Transform Infrared Spectroscopy (FTIR)

The structure of collagen from fish wastes were analysed using FTIR. Amide bands A, B, I, II and III were revealed in the tilapia fish scale. The Amide A, B, I, II and III bands were identified and listed in Table 4.5. The wavelength is compared between two different condition of the film which is with plasticizer (glycerol) and without plasticizers (glycerol).

Table 4. 5:Amide Band Wavenumbers for Tilapia Fish Scale with Glycerol and Without Glycerol.

Amide Band	Film Without Glycerol	Film With Glycerol
A	3272.93	3277.77
B	2925.58	2935.68
I	1629.01	1628.54
II	1539.25	1545.22
III	1227.38	1238.89

The IR ratios for amide III bands and the peak at 1450 cm^{-1} region were calculated for tilapia fish scale and are depicted in Table 4.6.

Table 4. 6: The IR ratios for amide III bands and the peak at 1450 cm-1

Collagen	Amide III (cm^{-1})	1450 Peak (cm^{-1})	Ratio
Tilapia fish scale film without glycerol	1227.38	1451.79	1.17
Tilapia fish scale film with glycerol	1238.89	1451.21	1.17

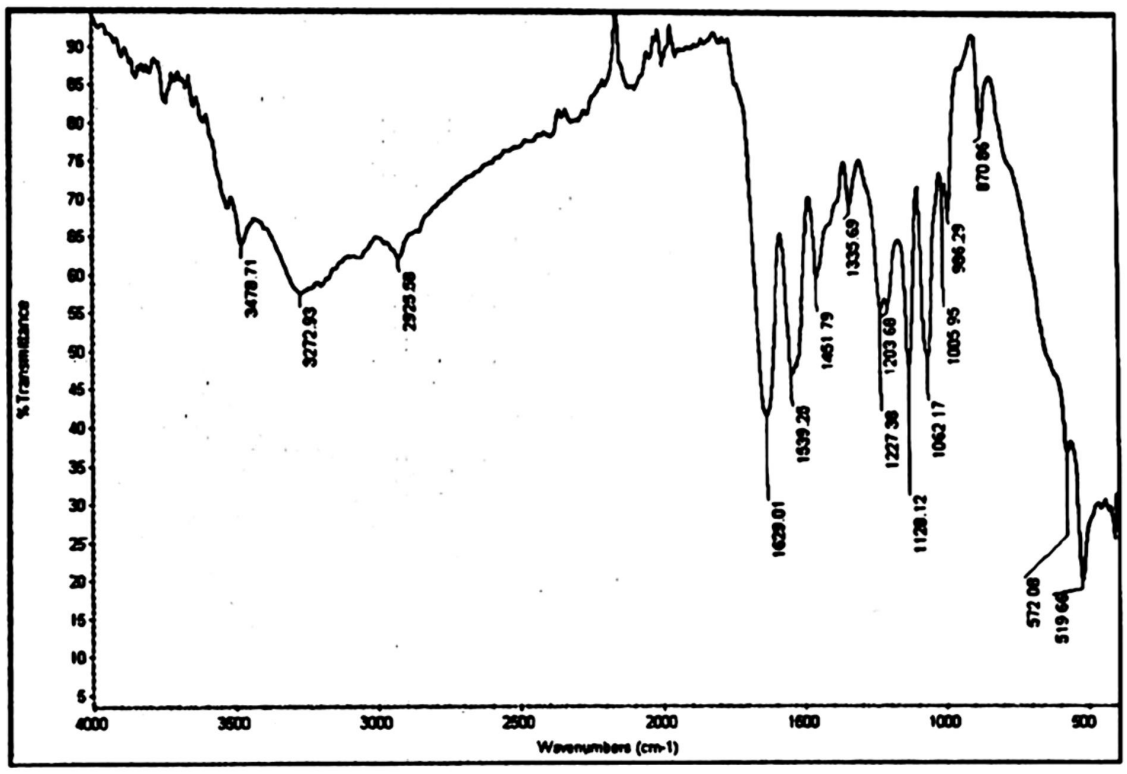

Figure 4. 2:Shows The FTIR Spectra Tilapia Fish Scale without Plasticizer (Glycerol).

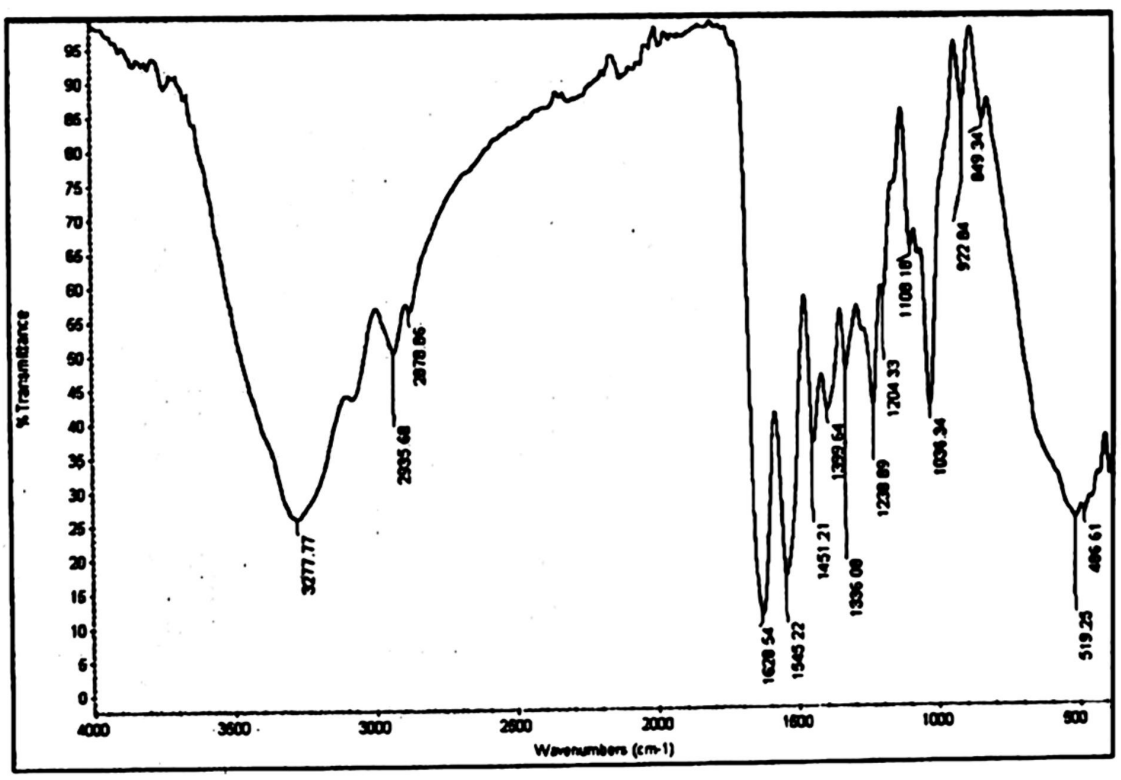

Figure 4. 3Shows The FTIR Spectra Tilapia Fish Scale with Plasticizer (Glycerol).

All the FTIR spectra showed the characteristic peaks of Amide I, II, III and Amide A and B from both data. Which whether with glycerol or without glycerol there will be present of Amide I, II, III and Amide A and B. The Amide A band in the samples were mainly associated with N-H stretching vibrations, as a free N-H stretching vibration appeared in the range of 3400-3440 cm^{-1}(Klymus et al., 2015; Nagai & Suzuki, 2000). And from the table 4.5 shown that both tilapia fish scale gelatine with or without glycerol have the presence of amide band A.

The Amide I band is mostly associated with the stretching vibrations of the carbonyl group all along the polypeptide backbone (Ikoma et al., 2003; Klymus et al., 2015; Nagai & Suzuki, 2000; Wang et al., 2014). The Amide I band is the major structure of collagen. As Amide band is a very sensitive marker for the peptide secondary structure (Bigi et al., 2001; Nagai & Suzuki, 2000; Surewicz, Mantsch, & Chapman, 1993; Wang et al., 2014).

Whereas, N-H bending vibrations can be determine by the presence of amide II bands (Ikoma et al., 2003; Nagai & Suzuki, 2000; Pérez-Mateos et al., 2009). It was reported that the presence of hydrogen bonds and presence of collagen if the amide band II is at lower frequency of 1540 cm^{-1}, it confirms the collagen has in it (Duan, Zhang, Du, Yao, & Konno, 2009; Nagai & Suzuki, 2000; Pérez-Mateos et al., 2009; Wang et al., 2014). And as the table 4.5 shows that there are presence of amide band II at 1539.29 cm^{-1} (without glycerol and 1545.22 cm^{-1} with glycerol, which shown that without glycerol there are presence of collagen which is at frequency below 1540 cm^{-1}. While with glycerol at higher level of frequency due to the presence of glycerol itself. Results from the present study indicated there are N-H bending vibrations which believe to be the N-H bending of the collagen peptide backbone.

Amide III bands indicate C-H stretching (Nagai & Suzuki, 2000; Payne & Veis, 1988; Pérez-Mateos et al., 2009). As the result in table 4.5 shown that amide band III at frequency 227.38 cm-1 (without glycerol) and at 1238.89 cm-1 with glycerol. Compared to other studies, the Amide III bands were also nearly the same as the skin of surf smelt is 1235 cm-1 (Nagai et al., 2010), and the skin of striped catfish has a Amide III band at 1242 cm-1 (Singh et al., 2011). Besides, these collagens also had similar Amide band ranges with bovine collagen (Kirubanandan and Sehgal, 2010). Which indicates the presence of collagen in Tilapia fish scale extraction.

The IR ratios of all the collagen extracted from tilapia fish scale were 1.17 for both film (with glycerol and without glycerol). All of these results prove that the helical structures of the collagen were reserved properly in good conditions. The intensity ratio is used to indicate the triple helical structure of collagen. An IR ratio of just about 1 indicates the occurrence of helical structures (N. N. Singh et al., 2013). The results revealed that the triple helical structures are similar, as shown by the similar absorption ratio between the peak heights of Amide III and 1450 cm-1 (1.17 for tilapia fish scale) . The skin of striped catfish also had a similar intensity ratio of 1.17 (Nagai & Suzuki, 2000; P. Singh, Benjakul, Maqsood, & Kishimura, 2011). The results also indicated that the extraction condition used in the present study were able to preserve the native conformation of the collagen. The native conformation is important for future application of the collagen.

The Amide B band is correlated to the asymmetrical stretch of CH_2 (Muyonga et al., 2004; P. Singh et al., 2011). The tilapia fish scale film, illustrated a strong C-H stretching vibration at around 2925.58 and 2935.68 cm^{-1} for tilapia fish scale gelatine film with glycerol and without glycerol respectively. In comparison, the Amide B band was found 2926 cm-1 for the skins of

striped catfish (P. Singh et al., 2011)and at 2964 cm-1 for skins of surf smelt (Nagai & Suzuki, 2000) reveal to be quite similar, which implies that their structures are nearly the same.

4.5 Colour Detection Technique

Instrumental colour measurements of the freeze dried gelatine powders are shown in Table 4.8. there are no major change regarding the colour of the film itself. Film with lower temperature and times showed higher l*-value (lightness) but lower a*-value (redness/greenness) and b*-value(yellowness/blueness). This maybe because off the rate of heating and the temperature when extraction take place. Such changes in film colour were most likely attributed to the collagen itself when heated to certain temperature.as commercial gelatine is not colourless in solution but has a colour varying from a very pale yellow to dark amber.

The current study found that the colour do changes from transparency white to yellowish milky white when the extraction method at the highest temperature and longer time. By the experiment conducted shown that there are slightly different colour film based on the extraction temperature and time. Figure 4.4 and figure 4.5 shown the colour different based on the different temperature. In figure 4.4 the yellowish is 100 °C (B) while the other one is 80°C (A). while in figure 4.5 the transparent one is 80 °C (C) while the other one is 90°C(D).

There can be no doubt that the colour attribute of gelatine has practical significance, in that some 60% of world production is consumed by the confectionery industry (Siebert, 1992).

Table 4. 7:Colour Measurement of Tilapia Fish Scale Film

Gelatin		a	l	b	ΔE
80 °C	30 mins	3.7	72	23.7	75.69
	60 mins	4	71.1	25.4	75.6
	120 mins	4	72	25.3	76.42
90 °C	30 mins	4	69.9	27.2	75.11
	60 mins	4.1	71.2	25.4	75.71
	120 mins	4	71.1	25.3	75.57
100 °C	30 mins	4.2	70	27	75.74
	60 mins	4.2	69.5	27.9	75.01
	120 mins	6.2	65.5	36.9	75.43

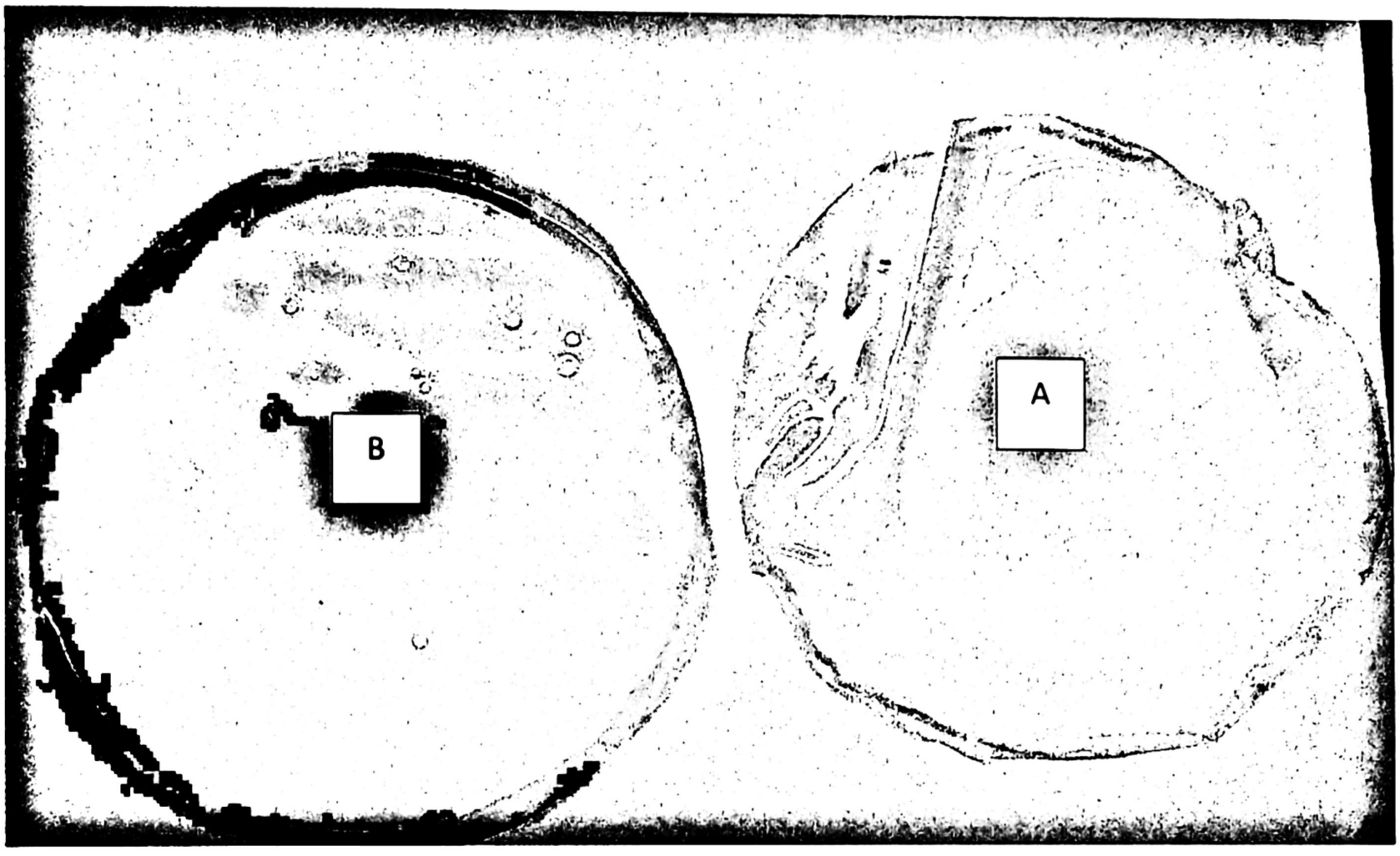

Figure 4. 4 : shown the different film at 80 °C (A) and 100 °C (B)

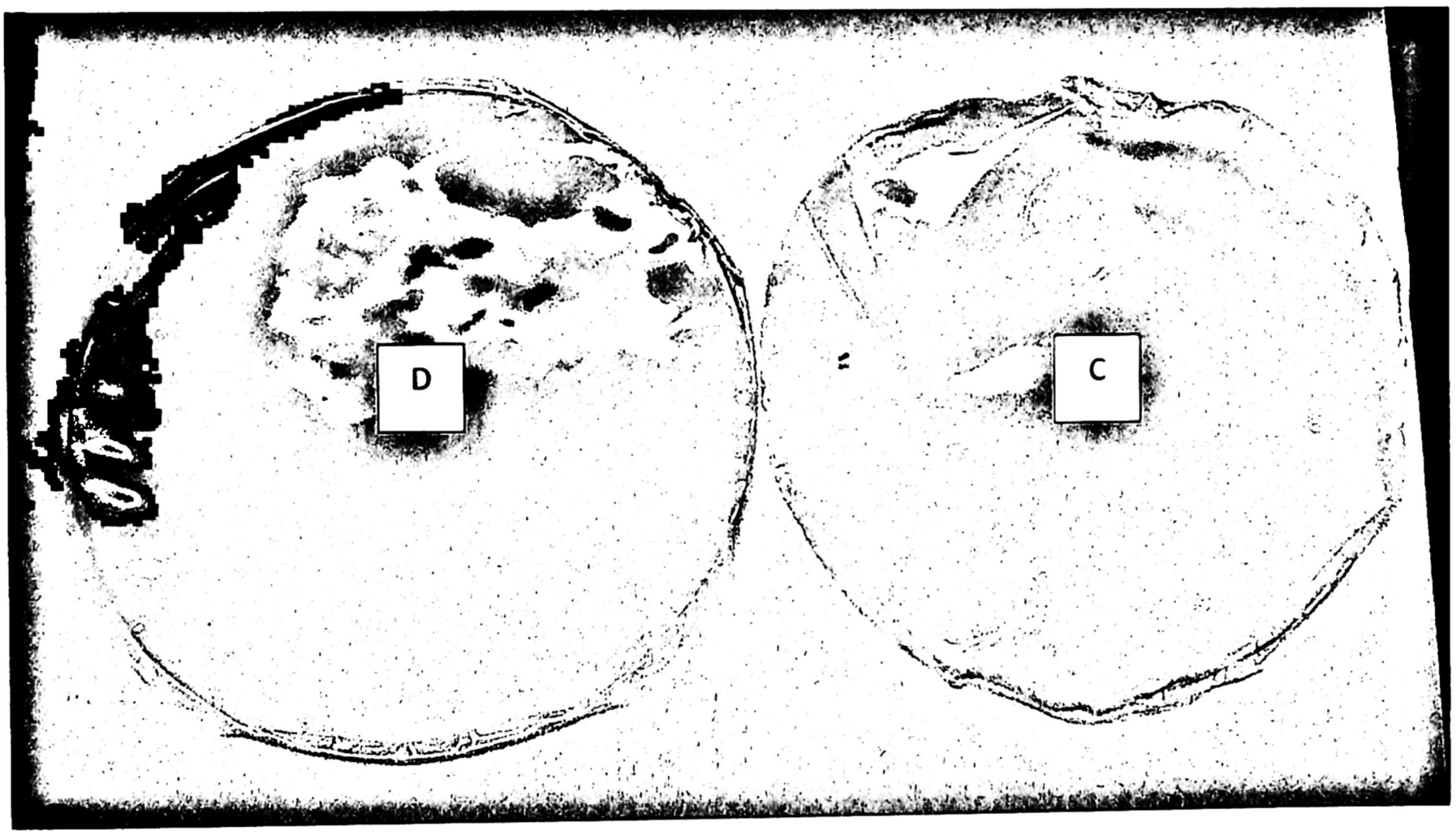

Figure 4. 5: Shown The Different Film At 80 °C (C) And 90°C (D).

4.1 Film morphology

Gelatine films are brittle by nature, large quantities of water vapour pass through them, and they have low glass transition temperatures and low melting points. Glycerol alone improves the mechanical properties of gelatine films. The mutual effects of glycerol as plasticizers result in films with suitable mechanical properties, water vapour permeability and glass transition temperature, and raise their melting points. Fish-skin gelatine films containing glycerol at 2% exhibited the best properties because of their low water vapour permeability, suitable mechanical properties, appropriate colour, heat-sealing capability. This was shown in figure below.

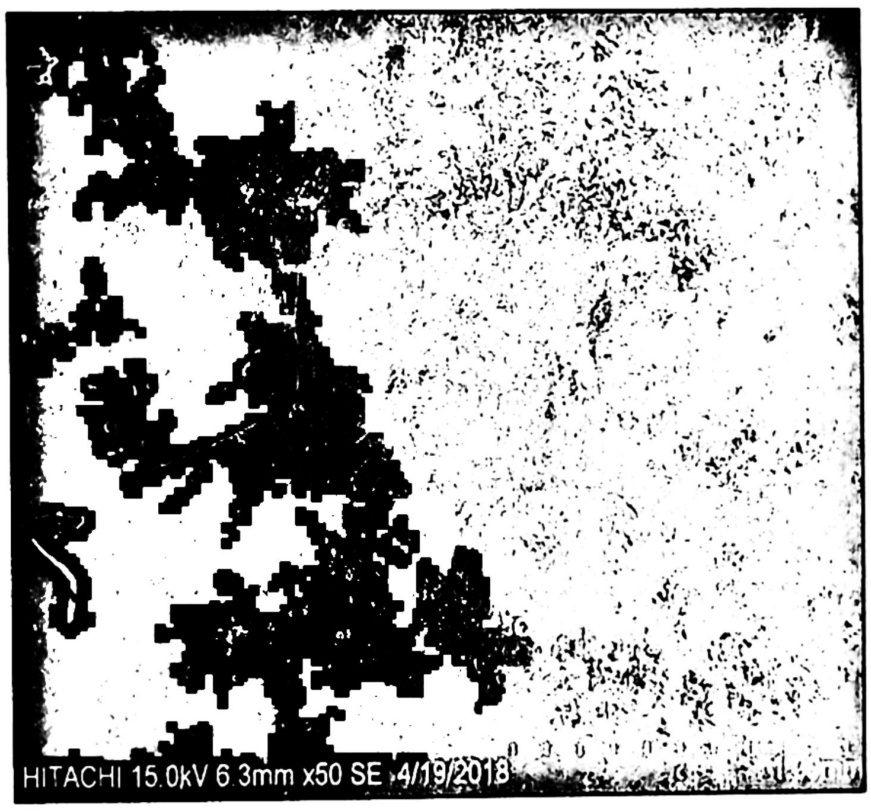

Figure 4. 6: Scanning Electron Microscopy Images Of Surface Tilapia fish scale gelatine film Without Glycerol at 1.00 mm.

Figure 4. 7 : Scanning Electron Microscopy Images of Surface Tilapia fish scale gelatine film Without Glycerol at 50.00 μm.

Figure 4. 8: Scanning Electron Microscopy Images of Surface Tilapia fish scale gelatine film Without Glycerol at 10.00 μm.

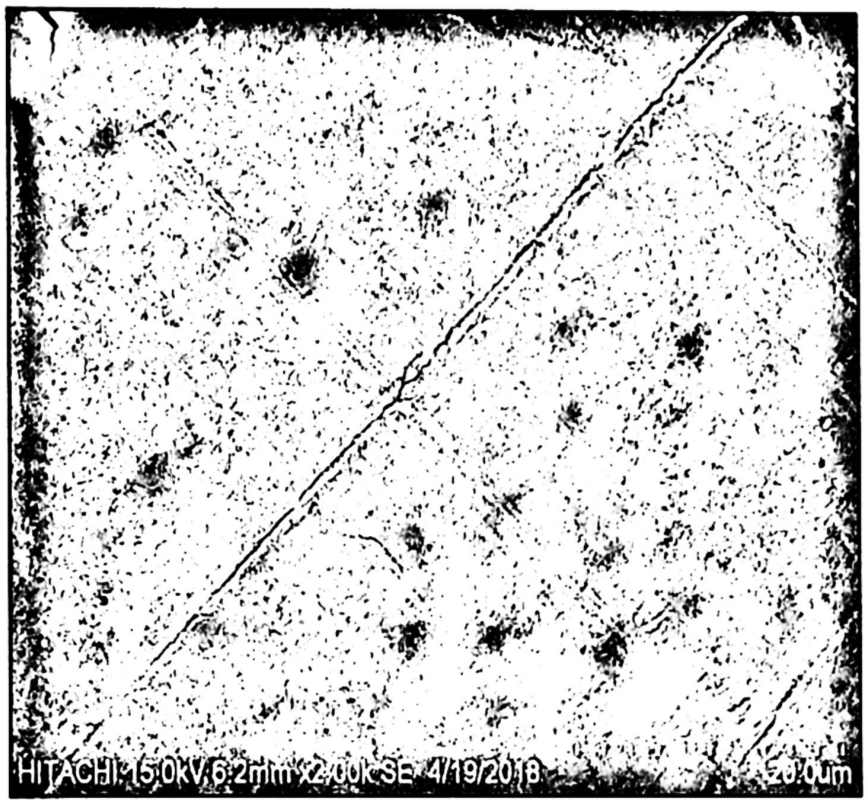

Figure 4. 9: Scanning Electron Microscopy Images of Surface Tilapia fish scale gelatine film with plasticizer at 20.00 μm.

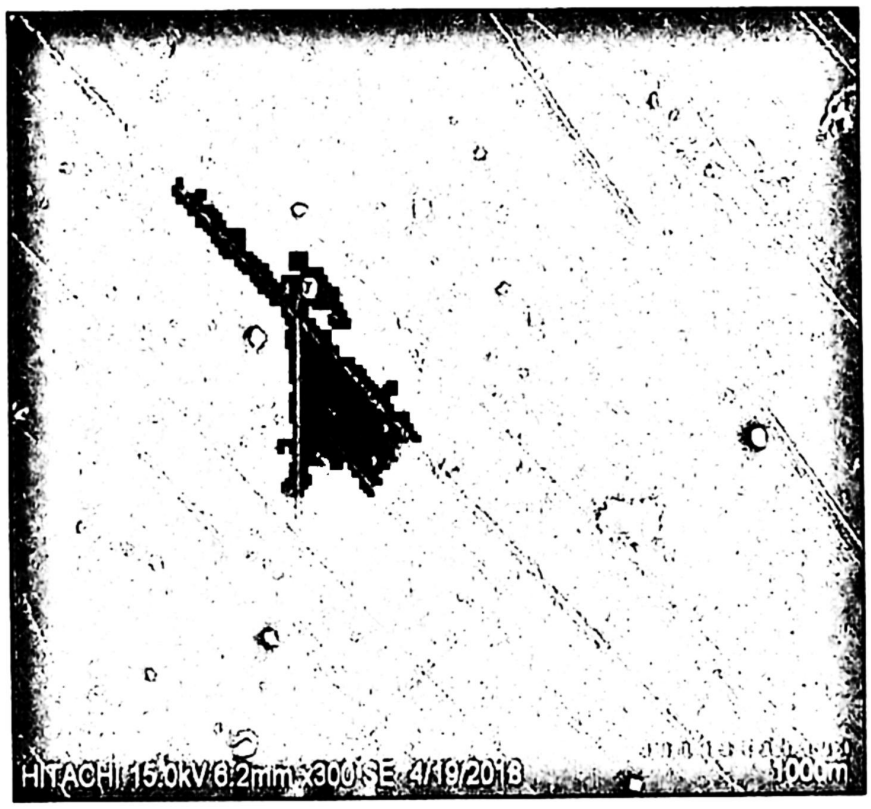

Figure 4. 10: Scanning Electron Microscopy Images of Surface Tilapia fish scale gelatine film with plasticizer at 100.00 μm.

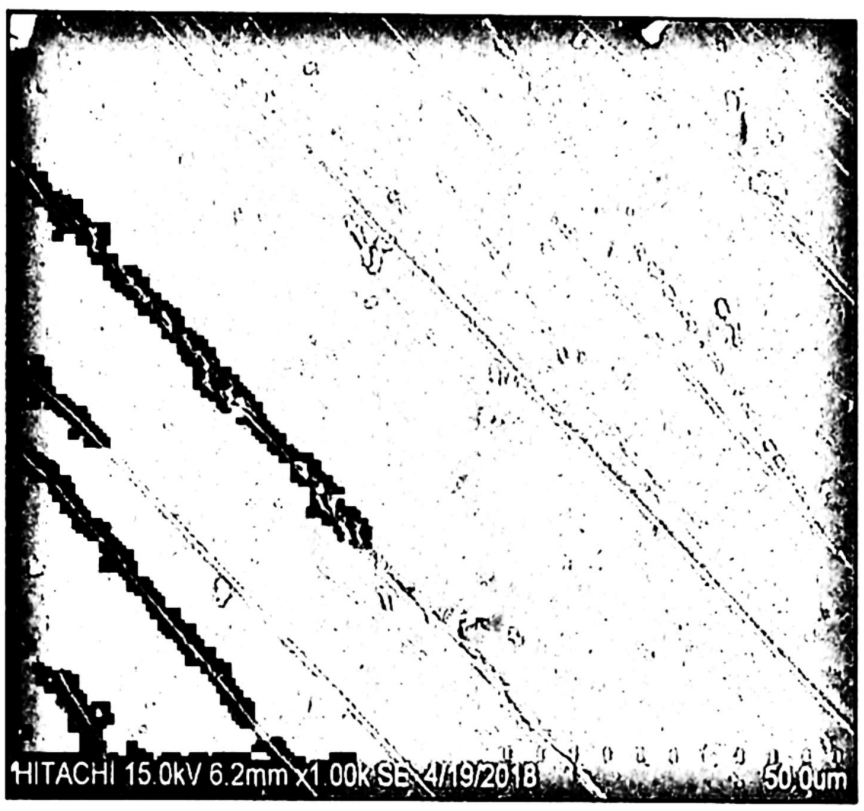

Figure 4. 11: Scanning Electron Microscopy Images of Surface Tilapia fish scale gelatine film with plasticizer at 50.00 μm.

Scanning electron microscopy micrograph of the surface of tilapia fish scale gelatine shown that film with and without of glycerol as plasticizers are illustrated in figure 4.6, 4.7 and 4.8. The addition of glycerol resulting the change of colour from transparent to yellowish white colour, which shown at figure 4.4 and 4.5. The film was magnified using Scanning electron microscopy (SEM) in order to clearly observe the morphology of tilapia fish scale gelatine film incorporated with glycerol.

From the figure 4.4, Scanning electron microscopy (SEM) images demonstrate that the surface of tilapia fish scale gelatine film changes with addition of glycerol. Figure 4.6 shows the film when magnified with 1.00 mm while Figure 4.7 shows the film when magnified with 100 μm and figure 4.8 shows the film magnified by 50 μm. Overall the film seems even but as the figure 4.7 and 4.8 shows that large quantities of water vapour pass through them where structure like flakes lights are shown (Rezaei et al., 2015). This conclude that the film with no additional

plasticizers (glycerol) are brittle by nature because of the surface that allow water vapour to pass through them.

While as a studies reported by (Weng & Wu, 2015) that film has smooth surface because of glycerol dispersed in the film forming solution evenly due to their stability. And as shown in figure 4.9, 4.10 and 4.11 the additional plasticizer does not damage the formation of gelatine film structure. And as (Arfat, Benjakul, Prodpran, & Osako, 2014), the film smoother also best properties because of their low water vapour permeability, suitable mechanical properties, appropriate colour, heat-sealing capability.

And as in figure 4.9 shown that the un even surface. This is due to not uniform distributed throughout the tilapia fish scale gelatine film. The noticeable uneven surface might due to different arrangement of protein molecule during film formation (Arfat et al., 2014). These findings suggest that glycerol dispersed in the film forming solution evenly and smoothen the surface of the tilapia fish scale gelatine film.

Chapter 5

Conclusion

5.1 Conclusion

In this study, Collagen was successfully extracted from the market fish waste with promising yields from tilapia fish scale Collagen and as the parameters on how the extracted process takes place. The yields obtained from tilapia fish scales were range between 24.07 % to 12.88%. the percentage of collagen extracted depend on the thermal treatment temperature and the duration (time of heating) extraction.

As known that, biodegradable film from tilapia fish scale gelatine and plasticizer (glycerol) was developed with further parameters based on temperature and time of extraction. Mechanical test, scanning microscopy test, colour test, FTIR and DSC to investigate the properties of fish gelatine.

Based on result analysis, it can be concluded that the properties of tensile strength and elongation affected by the addition of plasticizer (glycerol). As shown the addition of glycerol with

increasing temperature and time to when extraction of collagen and gelatine will make the film increase in tensile strength at range 46.61 to 61.50 MPa, while the elongation at break decrease as the temperature and heating time increase between 48.25% to 34.75 %. While without plasticizer the tensile strength ranges between 38.05 MPa to 50.17 MPa and the elongation at break range between 42 % to 48.05. With this result, believe that the fish gelatine films prepared from tilapia scales are a potential alternative to replace synthetic polymer films and may be applied as novel food packaging materials.

FTIR analysis run on these fish collagens showed that the extracted collagen was in integrated and were in their native forms. All these fish waste had normal amounts of collagen as amide band found in the film shown the same amount of wavelength found in other collagen. Which amide band A found at 3272.93 cm^{-1} (without glycerol) and 3277.77 cm^{-1} (with glycerol). As for amide band B the wavelength found at 2925.58 cm^{-1} (without glycerol) and 2935.68 cm^{-1} (with glycerol). Another important finding was amide band I where the wavelength found at 1629.01 cm^{-1} (without glycerol) and 1628.54 cm^{-1} (with glycerol). While for amide band II found that the wavelength are at 1539.25 cm^{-1} (without glycerol) and 1545.22 cm^{-1} (with glycerol). The most interesting finding was that amide band III that can be calculated for IR ratio gain the value 1.17 for both film (with or without glycerol) where the amide band III found at 1227.38 cm^{-1} (without glycerol) and 1238.89 cm^{-1} (with glycerol).

SEM showed that the film without glycerol as plasticizer are brittle by nature. While the film with glycerol show that the film has smooth surface because of glycerol dispersed in the film forming solution evenly due to their stability.

In conclusion, these results prove that collagen of tilapia fish scale have the capability to be an alternative source of collagen for many uses in numerous fields.

5.2 Recommendation

For further studies, there are several suggestions. It is recommended that The viability of bones from the same fish (tilapia), for gelatine extraction should also be investigated. Next, the viscos-elastic and gelling properties such as viscosity and gel strength of tilapia gelatine should also be investigated. It is further recommended that the effect of the age of the fish from which scales are sourced, storage conditions and also extraction conditions on those properties be studied. While, the addition additives are added to fish gelatine to improve the properties of the film. As for the odour maybe natural additive can be used to remove the odour or make the film odourless. Regarding, the kinetics of the adsorption of methylene blue dye onto Polyvinyl alcohol-gelatine blend films should be investigated. The effect of parameters such as pH, temperature and salinity on the adsorption should also be established. While, casting maybe a uniform or bigger casting plate should be used to make the film more uniform in thickness. Furthermore, as for the sticky properties of the fish gelatine, by using additive like oil can improve the properties. Furthermore, water permeability test can be done to make sure the measurement of water vapour through the film. This is important because when there is higher amount of permeate water vapour will led to deterioration of the product.

Next, suggestion is that the incorporation of aldehydes or glutaraldehydes may be possible in fish waste collagen to further improve fish collagen's thermal stability, decrease collagen solubility in water and improve their mechanical properties, for extended use in the photographic, OOD, pharmaceutical industry and many more. Due to current technologies and biochemical

advancements, cross-linking of collagen with aldehydes is being utilized to diversify the usage of collagen.

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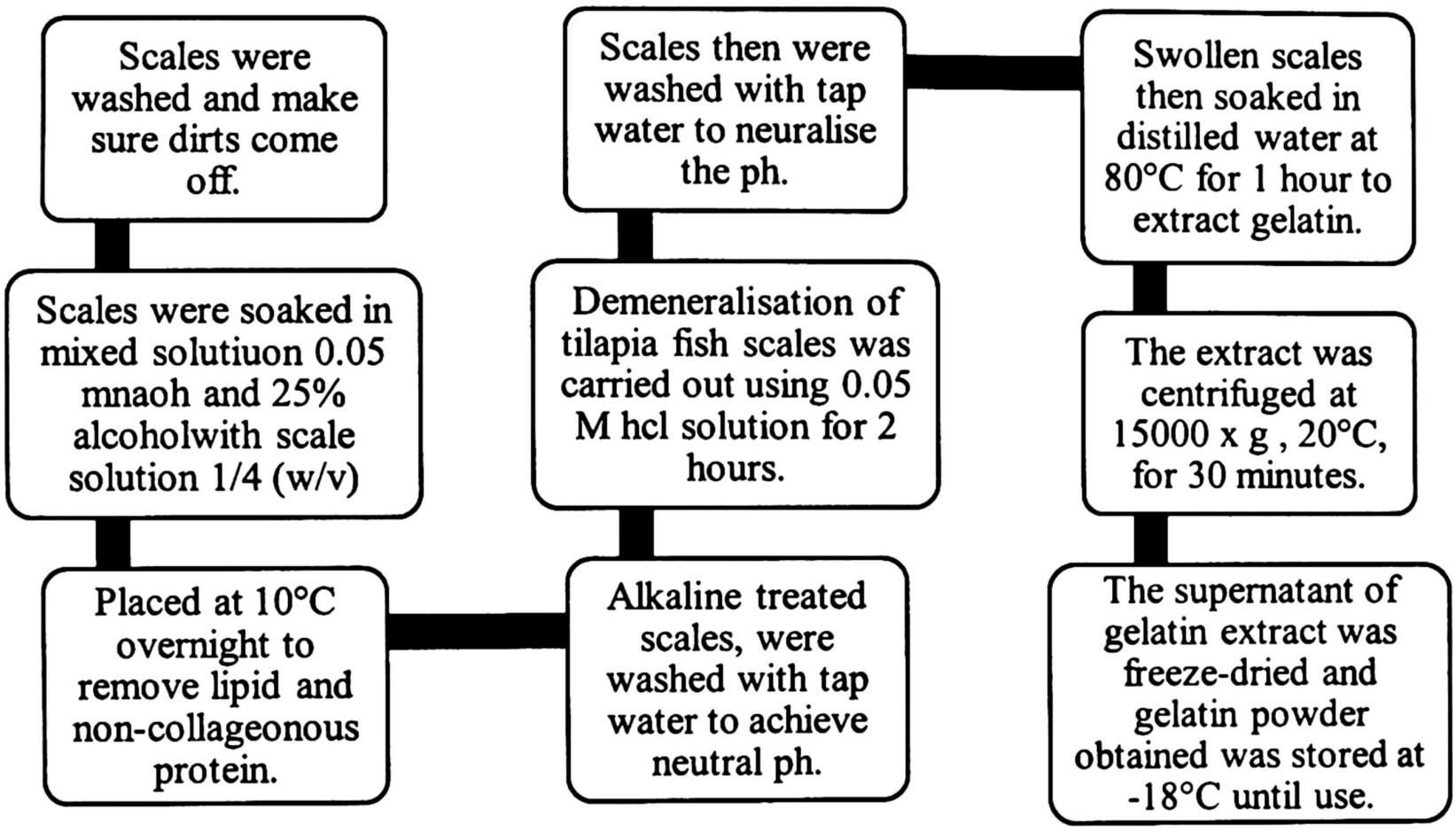
Appendices

Appendix A

Extraction method of scale collagen's flow chart (Limpisophon et al., 2009)



Appendix B

Preparation of gelatin film (LOWRY, ROSEBROUGH, FARR, & RANDALL, 1951)