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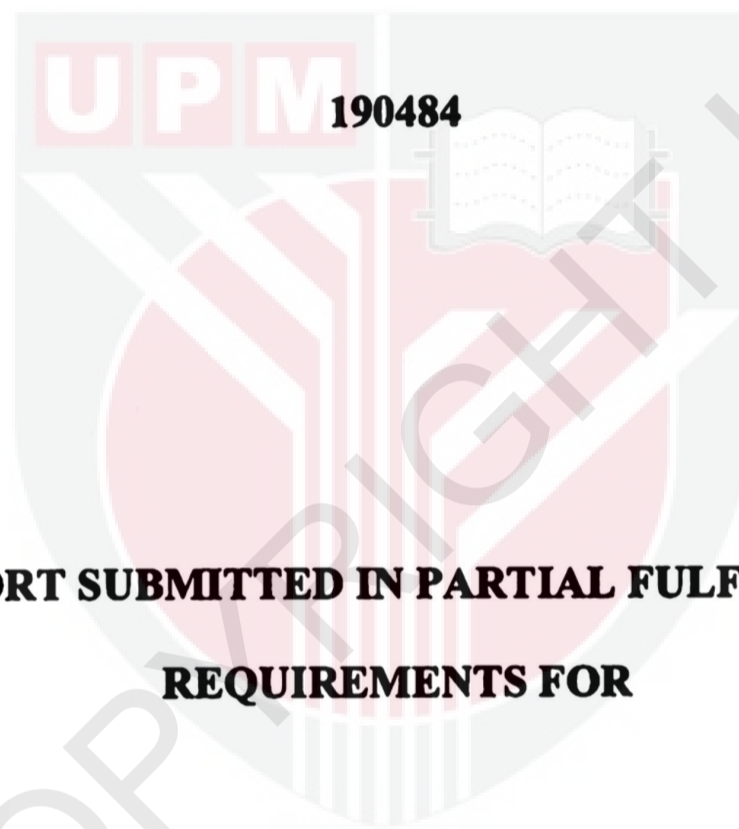
***CHARACTERIZATION OF TENSILE STRENGTH, THERMAL
PROPERTIES, SELF-HEALING AND ANTI-FOGGING PROPERTIES OF
GELATIN BASED FILM FROM TILAPIA FISH SCALES***

NUR HAFIZAH SALIM

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FK 2020 56**

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PROPERTIES, SELF-HEALING AND ANTI-FOGGING PROPERTIES OF
GELATIN BASED FILM FROM TILAPIA FISH SCALES**

NUR HAFIZAH BINTI SALIM



**PROJECT REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE
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Nur Hafizah Binti Salim

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ABSTRACT

Gelatin is a low cost protein that are widely used in pharmaceuticals and food because of its unique functional properties as well as its availability in Malaysia. Red Tilapia's (*Oreochromis spp.*) scale is one of the potential source of gelatin that are taken from marine waste in Malaysia. Gelatin is derived from fibrous protein collagen in animal skin, connective tissue and bone. Regarding the problems that occurred on plastic packaging that has limiting factors in which relatively poor performance under impact loading due to lack of plastic deformation, self-healing materials are getting more attention to reduce fatigue and subsequent probability of failure. Therefore, increase the development of packaging materials that have the ability to self-heal in which can restore the films function. Demand of longer shelf life and higher quality of foods which in need of good mass transfer barriers to moisture, oxygen, carbon dioxide, lipid, flavour and aroma between food components and the surrounding atmosphere led to the finding of packaging materials such as gelatin that can serve good quality of mass transfer barrier that suitable for certain food such as vegetables. Study on the gelatin based films recently found that gelatin have a good properties that allow the diffusion of water vapour and gasses that can be a cause to the food spoilage. Problems regarding synthetic polymers as packaging materials also has led to serious ecological issues in which they takes hundred years to degrade in which contribute to

the development of studies of materials that can degrade in shorter time such as gelatin. Moreover gelatin is extract from marine waste such as Red Tilapia's (*Oreochromis spp.*) scale in which can reduce pollution and encourage recycling of waste. Recent studies has been proof that gelatin exhibit excellent film forming properties incorporate to its gelling properties. Recent studies also stated that gelatin has the self-healing properties due to the swelling or the versatility of the polymer network with the present of water (H₂O). Self-healing materials is said to have antifogging behaviour due to its hydrophilic nature in which it can be the best added value to the films when apply to the foods. Incorporating to the excellent properties of the gelatin itself, the objective of this research study were stacked to characterize the tensile and thermal properties of gelatin based films (with and without glycerol) and study of the self-healing and anti-fogging properties of gelatin based films (with and without glycerol) that is taken from Red Tilapia species (*Oreochromis spp.*). The gelatin films were characterized by its tensile properties, thermo-gravimetric analysis (TGA) which determine its thermal stability and differential scanning calorimetry (DSC) which determine the glass transition temperature and the melting temperature of the films. It shows that incorporating the glycerol towards the gelatin films decrease the tensile strength value to almost 78.3% while increase the flexibility of the films to almost 92.9%, decrease the decomposition temperature to 5.07% at between range of

temperature 455.2°C to 486.6°C which improve thermal stability of the films and decrease the glass transition temperature from 36.5°C to 28.3°C as well as melting temperature of the gelatin based films from 92.5°C to 41.0°C. In order to determine the self-healing of the gelatin based films, the films were damaged by razor blade and drops of water (H₂O) were added. The films stimulate to the water (H₂O) that added and it response by swelling which later on filling the damaged gap between the films. In several minutes, the films healed and restore its function. Meanwhile, anti-fogging behaviour of the gelatin based films were determined from the water vapour and oxygen (O₂) transfer through the films that covered mushrooms in the container. From the study, it can be conclude that gelatin based films that were taken from Red Tilapia species (*Oreochromis spp.*) have the excellent properties which have the ability to self-heal when damaged and exhibit good anti-fogging behaviour.

ABSTRAK

Gelatin ialah protein kos rendah yang banyak digunakan dalam bidang farmasi dan makanan kerana fungsi sifatnya yang unik serta ketersediaannya di Malaysia. Sisik Tilapia Merah (*Oreochromis spp.*) adalah salah satu sumber gelatin yang berpotensi diambil dari sisa laut di Malaysia. Gelatin berasal daripada kolagen protein berserat pada kulit haiwan, tisu penghubung dan tulang. Mengenai masalah yang berlaku pada pembungkusan plastik yang mempunyai faktor pembatas di mana prestasi yang agak buruk kerana kekurangan ubah bentuk plastik, bahan yang mempunyai sifat penyembuhan diri semakin mendapat perhatian untuk mengurangkan kemungkinan kegagalan plastik. Oleh itu, tingkatkan pengembangan bahan pembungkus yang mempunyai kemampuan untuk menyembuhkan diri sendiri yang dapat mengembalikan fungsi filem. Permintaan jangka hayat makanan yang lebih lama dan kualiti makanan yang lebih tinggi yang memerlukan penghalang jisim yang baik untuk kelembapan, oksigen, karbon dioksida, lipid, rasa dan aroma antara komponen makanan dan suasana di sekitar menyebabkan penemuan bahan pembungkus seperti gelatin yang dapat menyumbang kepada kualiti penghalang pemindahan jisim yang sesuai untuk makanan tertentu seperti sayur-sayuran. Kajian mengenai filem berdasarkan gelatin baru-baru ini mendapati bahawa gelatin mempunyai sifat baik yang memungkinkan penyebaran wap air dan gas yang boleh

menjadi penyebab kerosakan makanan. Masalah mengenai polimer sintetik sebagai bahan pembungkusan juga telah menyebabkan masalah ekologi yang serius di mana ianya memerlukan masa ratusan tahun untuk terdegradasi di mana menyumbang kepada pengembangan kajian bahan yang dapat terdegradasi dalam masa yang lebih singkat seperti gelatin. Lebih-lebih lagi gelatin adalah diekstrak daripada sisa laut seperti skala Tilapia Merah (*Oreochromis spp.*) yang dapat mengurangkan pencemaran dan menggalakkan kitar semula sampah. Kajian baru-baru ini membuktikan bahawa agar-agar menunjukkan sifat pembentukan filem yang sangat baik yang diintegrasikan dengan sifat gelnya. Kajian terbaru juga menyatakan bahawa gelatin mempunyai sifat penyembuhan diri kerana pembengkakan atau fleksibiliti rangkaian polimer dengan adanya air.

Bahan penyembuhan diri dikatakan mempunyai sifat *anti-fogging* kerana sifat hidrofiliknya di mana ia boleh menjadi nilai tambah baik untuk filem apabila digunakan pada makanan.

Mengikut sifat gelatin itu sendiri, objektif kajian penyelidikan ini disusun untuk mencirikan sifat fizikal dan haba filem berasaskan gelatin (dengan gliserol dan tanpa gliserol) dan kajian mengenai sifat penyembuhan diri dan *anti-fogging* filem berasaskan gelatin (dengan gliserol dan tanpa gliserol) yang diambil dari spesies Tilapia Merah (*Oreochromis spp.*). Filem gelatin dicirikan oleh sifat tegangannya,

analisis termo-gravimetri (TGA) yang menentukan kestabilan termalnya dan kalorimetri imbasan pembezaan (DSC) yang menentukan suhu peralihan kaca dan suhu lebur filem. Ini menunjukkan bahawa menggabungkan gliserol ke arah gelatin filem menurunkan nilai kekuatan tegangan hingga hampir 78.3% sementara meningkatkan fleksibiliti filem hingga hampir 92.9%, menurunkan suhu penguraian hingga 5.07% di antara julat suhu 455.2°C hingga 486.6°C yang bertambah baik kestabilan haba filem dan menurunkan suhu peralihan kaca dari 36.5°C hingga 28.3°C serta suhu lebur filem berasaskan gelatin dari 92.5°C hingga 41.0°C. Untuk menentukan penyembuhan diri dari filem berasaskan gelatin, filem-filem itu dirosakkan oleh pisau cukur dan setitis air (H₂O) ditambahkan. Filem gelatin bertindak balas dengan air (H₂O) yang telah ditambahkan dan kemudian pembengkakan mengisi jurang yang rosak antara filem. Dalam beberapa minit, filem-filem itu sembuh dan fungsinya filem kembali pulih. Sementara itu, sifat *anti-fogging* filem berasaskan gelatin ditentukan dari pemindahan wap air dan oksigen (O₂) melalui filem yang digunakan untuk menutup cendawan di dalam bekas. Dari kajian tersebut, dapat disimpulkan bahawa filem berdasarkan gelatin yang diambil dari spesies Red Tilapia (*Oreochromis spp.*) Mempunyai sifat yang sangat baik yang mempunyai kemampuan untuk sembuh sendiri ketika rosak dan menunjukkan tingkah laku anti-kabut yang baik.

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CHAPTER 1

INTRODUCTION

1.1 Background

The developing of food industry contribute to an increasing number of new applications of gelatin as the material use to wrap the new food products and packaging products. Generally, gelatin products is use as emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials and micro-encapsulating agents. This products application and technology growth with the trend to replace synthetic agents with more natural ones which is gelatin.

Gelatin is a protein substance that is derived from collagen which is a natural protein present in the tendons, ligaments, and tissues of mammals. However, there is an increasing demand for the alternative sources of gelatin due to the sociocultural

problems and religious reasons that contribute to the selection of fish as a sources to generate gelatin. Fish is good a source of protein because its muscles are made up of high nutritional value proteins that contain high levels of essentials amino acids. There are few types of gelatin sources such as mammalian (pig and cow), marine including fish (warm-water and cold-water) and squid, poultry (chicken, birds, and ducks), and insect sources. Based on this research study, Red Tilapia's (*Oreochromis spp.*) scale is one of the potential source of gelatin that had been used. Figure 1.1 shows the Red Tilapia's (*Oreochromis spp.*) fish.



Figure 1.1: Red Tilapia's (*Oreochromis spp.*) fish

The properties and functionality of gelatin depends on the type of collagen, sources, ages of animal, processes, and etc. These are probably due to the different

contents of amino acids and molecular weight distribution (Hanani, 2015a). These sources are normally generated as a by-product following animal slaughter by the muscle food processing industry (meat, poultry, and seafood). Gelatin are produced by boiling the connective tissues, bones and skins of animals which are usually cows and pigs. In gelatin manufacture, two methods are usually used which are the acid and the alkaline processes to produce type A and type B gelatin respectively (Mariod, Abdalbasit, Fadul & Hadia, 2013).

Gelatin is a high molecular weight polypeptide and an important hydrocolloid uses in a wide range of food products largely because of its gelling and thickening properties. Gelatin have the excellent abilities to form strong, transparent gels and flexible films that are easily digested, soluble in hot water, and capable of forming a positive binding action. Therefore, gelatin can be applied widely in in several industries especially food industries. Moreover, in recent studies, gelatin is being choose as the material in developing the gelatin based-film use in packaging and coating the food products. Furthermore, the selection of gelatin also due to the self-healing properties in which they can recover their structures and function due to the Schiff based linkage reaction.

1.2 Problem Statement

Nowadays, plastic films are a popular choice for the food packaging purpose. The food packaging industry is growing day by day with the new technology to fulfil customer demand in preparing and searching the alternative packaging materials to replace petroleum based plastic film for the food packaging. Most of the materials that are used in food packaging are non-degradable materials which contribute to the environmental problems. The most widely used of plastic packaging is made up of non-degradable materials which is petroleum-based plastics. Polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP) and polystyrene (PS) are the examples of petroleum based-plastics.

The reliance on these synthetic polymers as packaging materials has led to serious ecological problems in which they takes hundred years to degrade. Built for the long haul, these polymers seem inappropriate for applications in which plastics are used for short time periods and then disposed. In contrast, biodegradable polymers disposed in bio-active environments and can be degrade by the enzymatic action of microorganisms such as bacteria, fungi and algae. Their polymer chains may also be broken down by non-enzymatic processes such as chemical hydrolysis. Biodegradable polymers are often derived from plant processing of atmospheric carbon dioxide (CO₂). Bio-degradation converts them to CO₂, methane (CH₄), water (H₂O), biomass,

humic matter, and other natural substances. Biodegradable polymers are thus naturally recycled by biological processes (Gross & Kalra, 2002).

Fish gelatin that are partially hydrolyse or denatured collagen, has received attention as an alternative of mammalian and avian gelatin. Gelatin can be extracted from the by-products that is rich in collagen such as fish skin, bone, scale, or the skin of some invertebrate, and so on. In recent years, by-products obtained from the fishing industry, such as heads, skin, bones, fins, muscle pieces, scales, viscera and others. These by-products are considered potential sources of exploration rather than being disposed (Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, 2011). The use of gelatin from marine waste such as Red Tilapia's (*Oreochromis spp.*) fish scale can reduce pollution and encourage recycling of waste. Thus, it lead to sustainable packaging concept. Marine waste and agro-industrial by-products are biodegradable polymers obtained from renewable resources that considered as sustainable alternative to petroleum-derived polymers (Etxabide, Uranga, Guerrero, & de la Caba, 2017). However, one of the main drawbacks of fish gelatin is its rheological properties, being less stable than the obtained from mammalian sources. Gelatin from fish does not have such restrictions and additionally, and from the economic view, the use of fish by-products to obtain gelatin could be quite interesting for the fish-processing industry.

Generally there are two main factors that affect gelatin properties which are the extraction process and the characteristic of the initial collagen. The extraction process of the gelatin is very important because it determine the distribution of molecular weight in gelatin. There are other factors that should be considered for gelatin that produce from fish skin, such as the different structure of the triple helical collagen molecules, and also the high susceptibility of collage-nous materials to degradation due to a lower content of non-reducible intra-molecular and inter-molecular cross-links in contrast to the more stable mammalian collagen. The collagen from fish bones, has greater microbiological stability due to an increased incidence of cross-links, when it is compared to the collagen of other tissues such as skin (Alfaro, Balbinot, Weber, Tonial, & Machado-Lunkes, 2014).

Nowadays, self-healing materials are getting more attention to reduce the fatigue and subsequent probability of failure. This is because plastic packaging has limiting factors in which relatively poor performance under impact loading due to lack of plastic deformation which the most important aspects regarding the packaging when getting into transportation. The major concern is focused on the recovery or self-healing properties of the plastic film when it is damage in order to help and maintain the quality of the foods. Moreover, the extra characteristic which is anti-fogging properties of the gelatin also can create attention in which can provide the customer

with clear and good packaging characteristic that can help in product marketing and preserve the good quality of foods. Therefore gelatin based film is introduced to add value to the commercial food packaging such as low density polyethylene (LDPE) to package the food in order to maintain the quality of the foods when there is damage occur to the LDPE. This is because when the layer of the gelatin films is damaged, it still can protect the food due to its ability to self-heal. Other than that, gelatin films is a bio polymers which it is a biodegradable materials that can reduce the environmental impacts and increase the use of waste (skin and scale of the fish).

Recently, the consumers also demand longer shelf life and higher quality of foods, while reducing disposable packaging materials and increasing recyclability. Such demands have caused increased the interest in edible and biodegradable films or materials that potentially are used to extend the shelf life and improve the quality of almost any food system where it can be done by serving as mass transfer barriers to moisture, oxygen, carbon dioxide, lipid, flavour and aroma between food components and the surrounding atmosphere (Al-Hassan & Norziah, 2012a). Gelatin film from fish is said to have lower water vapour permeability (WVP) values and mechanical properties compared to films that made up from bovine or pig gelatin (Alfaro et al., 2014).

1.3 Objectives of the Study

- (1) To prepare and characterize the tensile strength and thermal properties of gelatin based film from tilapia fish scales.
- (2) To study the self-healing and anti-fogging properties of gelatin-based film.

1.4 Scope of study

This research work focused on properties of both gelatin based film without glycerol and gelatin based film that added with glycerol. Red Tilapia's (*Oreochromis spp.*) scale were collected from wet market at Jerantut, Pahang in order to prepare and characterize the tensile strength and thermal properties of gelatin based film from tilapia fish scales. The mechanical properties of the gelatin based films was determined in term of their tensile strength (texture analyzer) followed to ASTM D882-02 (2002) and thermal properties were analysed by using differential scanning calorimetry (DSC) and thermo-gravimetric analysis (TGA). In completion for the second objective, the gelatin based films were damaged by using razor blade and drops of water were added using micro injector to the damage zone of the films in which the damage-healing process of the films were then observed by using biology microscope with objective magnification 4x10. The results were then photographed using camera. Lastly the anti-fogging properties of gelatin based films were observed by putting

mushroom in the containers that were covered by the five different characterized film in the chiller for 24 hours. The containers were then transferred to the laboratory conditions normally at 25°C which the relative humidity of the air is 55%. And the fogging behaviour of the five characterize films were observe and record by using camera.

1.5 Thesis organization

This thesis is particularly consists of five main chapters which each of every chapter focusing on different elements of the thesis that emphasize the characterization of the self-healing and anti-fogging properties of fish gelatin based film for food packaging application. Chapter one is introduction part which consist of background study, problem statement, objectives, and scope of the study. Chapter 2 will cover the literature review that discussing about some previous research and work that are related to food packaging application and information regarding gelatin. In chapter 3, methodology of the extraction of fish gelatin, the preparation of the fish gelatin film and characterization of the self-healing and anti-fogging properties of the fish gelatin based film are reported. Then, chapter four of the thesis will cover the result and discussion and lastly chapter 5 will present the conclusion and the recommendation of this study topic regarding their application in the food packaging system.

The logo of Universiti Putra Malaysia (UPM) is centered in the background. It features a shield with a red and white design, including a book and a torch. The letters 'UPM' are prominently displayed in the top left of the shield.

CHAPTER 2

LITERATURE REVIEW

2.1 Gelatin

Gelatin is important bio-polymer that are derive from animal's collagen where the majority of the sources are from mammals. Gelatin is composed of 50.5% carbon, 6.8% hydrogen 17% nitrogen and 25.2% oxygen. It is basically a type of insoluble protein that are produce by the process of hydrolysis of the collagen that are being extracted from a variety animal sources which are the skin, bones and connective tissue. Collagen is part of the basic structure of the animal body including the tendon, bone, skin, membrane and as well as connective tissue.

The molecules of the collagen are arranged in fibre and linked by covalent bonds. There are three polypeptide chains allocated at the molecules which stabilized by the hydrogen and hydrophobic bonds and are arranged in triple helix structure. Most of the collagen types are classified based on their distribution within the tissue. There are type I, II and III in which type I collagen is distributed as dense connective tissue that can be found in the bones, tendons, skin and blood vessels. Type II collagen consist of the tissue that are derived from vitreous humour and cartilage. Meanwhile type III collagen is found in muscle and loose connective tissue (Hanani, 2015b). Gelatin is generally obtain from the heating of the collagen in the condition which is above the transition temperature of collagen's triple-helix structure. The triple helix structure is a form of three peptide strand with a helix structure. It is a heterogeneous polypeptide consists of the mixture of α chains, β chains and γ chains.

The main attributes that best define the overall commercial quality of gelatin are solubility, composition parameters, transparency, colours, tasteless and odourless. While the most important physical properties that also influence the quality and potential applications are the gel strength ("in terms of bloom value") and viscosity (Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, 2011). Cold water fish gelatin have lower concentrations of amino acid (proline and hydroxyproline) as compared to mammalian gelatin and warm water fish gelatin such as tilapia. The

proline and hydroxyproline contents for mammalian gelatin are approximately 30%, 22-25% for warm-water fish gelatin and approximately 17% for cold water fish gelatin. Figure 2.1 shows the source of gelatin.

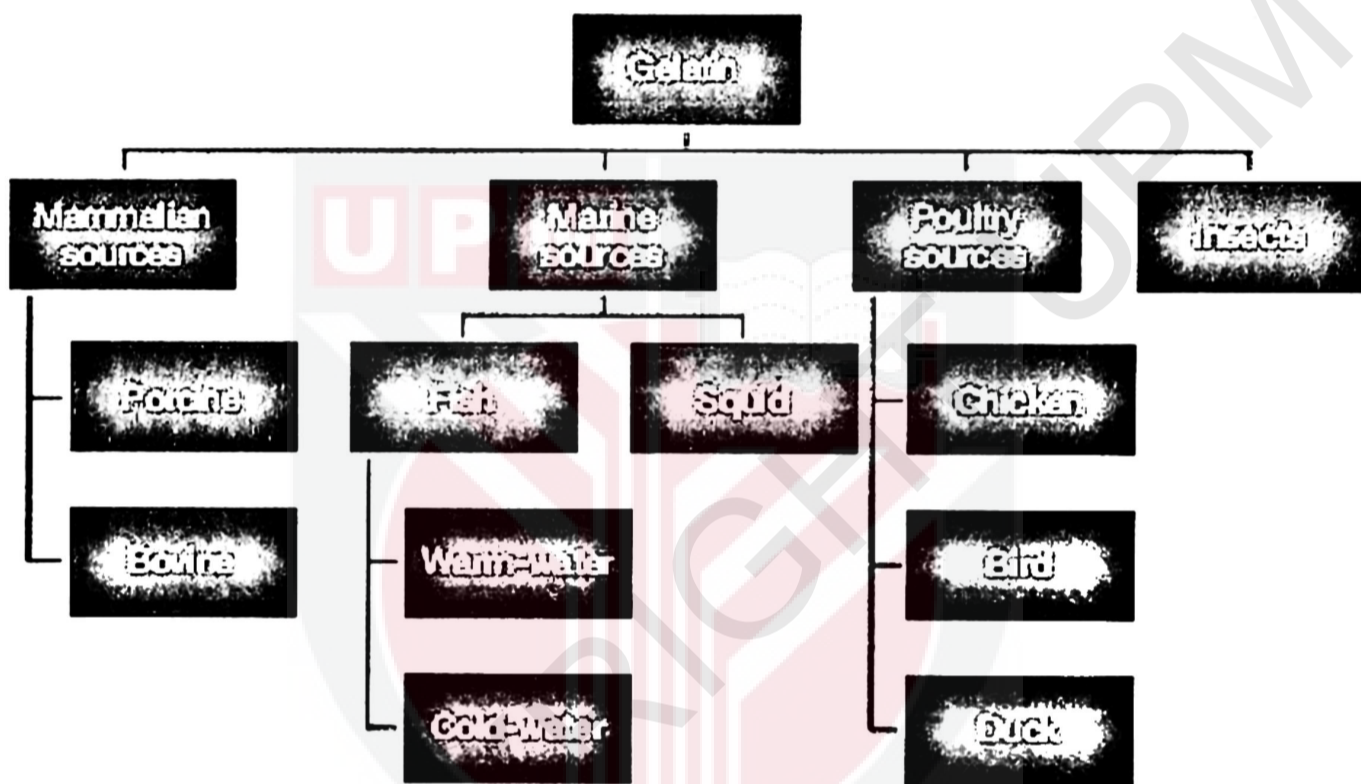


Figure 2.1: Gelatin source

2.2 Mechanical Test

Tensile test is conducted to determine the tensile properties of the gelatin based films such as its tensile strength, Young Modulus and its elongation at break. Tensile is a destructive test process where it measures the force that required to break a composite or plastic specimen and extent to which the specimen stretches or elongates at breaking point. This method allow the determination of material properties to help

designers and quality managers to predict how materials and products behave in their intended applications. Tensile testing is used to guarantee the quality of components, materials and finished products within a wide range industries. Typical applications of tensile testing are included automotive industry beverage industry, construction industry, electrical and electronics industry, medical device industry, packaging industry, paper and board industry, pharmaceuticals industry, plastics, rubber and elastomers industry.

2.2.1 Determination of tensile strength

Based on the study that had been conducted, experiment on specimens of 50 mm long with measuring of the neck was 28 mm and 4 mm wide were carried out at 23°C and 50% relative humidity. Five samples were tested using fixed crosshead speed which was 50 mm/min (El-hefian, Nasef, & Yahaya, 2012). Meanwhile, based on tensile test that had been done on cold water fish gelatin films in other study using an instron 5500R universal testing machine, 0.05 mm thick films were used. Extension rate was set at 10 mm/min and a 100 N load cell was used. The test was conducted according ASTM D6380-00 method (Chiou et al., 2008).

Tensile strength is maximum stress that a material can resist before failure or fracture when stretched. Tensile strength can be determined by the general formula expressed below:

$$\sigma = \frac{F}{A}$$

Where F = force applied to the sample

A = the cross sectional area of the sample

While tensile strain is the measure of sample changes in shape by the length extension of original sample's length. The equation for tensile strain, ϵ is expressed below:

$$\epsilon = \frac{\Delta l}{l}$$

Where Δl = the change in original length of the sample

l = the original length of the sample

2.3 Thermogravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) can measure some properties and such as composition, purity, decomposition reactions, absorbed moisture content and decomposition temperatures. TGA is an ideal analysis to characterize the thermal properties of materials, such as elastomers, thermosets plastics, mineral compounds,

ceramics and also pharmaceuticals and chemical products. Typical applications of TGA include performance of stabilizers, oxidative stability, and plasticizer content of polymers, residual solvent content, and carbon black content, filler content of polymers resins as well as decomposition temperature of the materials.

In TGA analysis, the samples use is heated in a given environment (N_2 , CO_2 , He, etc.) at controlled rate and the change in the weight of the substance is recorded as a function of time or temperature. Then, the temperature is increase at a constant rate for a known initial weight of the substance and the changes in weights are recorded as a function of temperature at different time interval. This plot of the weight change against the temperature is called thermogravimetric curve of thermos-gram and this is the basic principle of TGA itself.

2.3.1 Determination of thermal stability of gelatin based films

From the preparation and thermal properties of gelatin/TGS composite films study, the thermal analysis was carried out by using a computerized differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The heating rate was setup in steps between 0.1 and 99.9°C/min and the measurements were carried out under nitrogen atmosphere which was 30mL/min with the heating rate used was 10 °C/min (Shehap, Mahmoud, Abd El-Kader, & El-Basheer, 2015).

2.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is one of analytical tools used to characterize the physical properties of polymer. DSC can determine the crystallization, melting and mesophormic transition temperatures, entropy changes, glass transition and other effect of changes in heat capacity and latent heat. There are many advantages of DSC compared to other calorimetric techniques regarding heating and cooling rates, including isothermal and temperature-modulated operation. DSC is a tools that is reliable to obtain heat capacity at elevated temperature in a short time. DSC is widely applied in polymer science because of its simplicity and easy used.

2.4.1 Determination of glass transition temperature (T_g) and melting temperature (T_m) of gelatin based films

Differential scanning calorimetry (DSC) analysis on biodegradable films based on blends of gelatin and poly(vinyl alcohol) had been done by using a differential scanning calorimeter DSC TA2010 that have been controlled by a TA5000 system. Samples that weighed 10 mg were placed in aluminium TA pans and were heated at 5°C/min while empty pan was used as reference (Mendieta-Taboada, Sobral, Carvalho, & Habitante, 2008).

2.5 Self - healing Properties of Fish Gelatin

Self-healing properties is one of the most impressive properties of biological materials such as gelatin. It is due to the ability to its natural tissue that can heal and regenerate (self-heal) after infliction of physical damage. Gelatin has wide application due its ability to promote cell adhesion and proliferation. The uncontrollable partial renaturation of collagen triple helices of gelatin in aqueous media imposes its cross-linking to maintain the gel structure in the biological structure (Pettignano et al., 2017). Self-healing materials can heal damages when it is stimulated by pH, temperature, humidity or light and most of the self-healing polymer materials are focused on the hydrogels and the ability of the film forming properties of the polymer itself. Nowadays, developing of self-healing materials is being considered in engineering applications. Self-healing materials has become one of the attraction over the decades because of its properties that can increase materials lifetime, improve product safety and reduce replacement costs. Healing of the materials is referring to the recovery properties such as tensile strength, fracture toughness, and surface smoothness (Therriault, Haddad, & Jamroz, 2012). Figure 2.1 shows the chemical structure of gelatin.

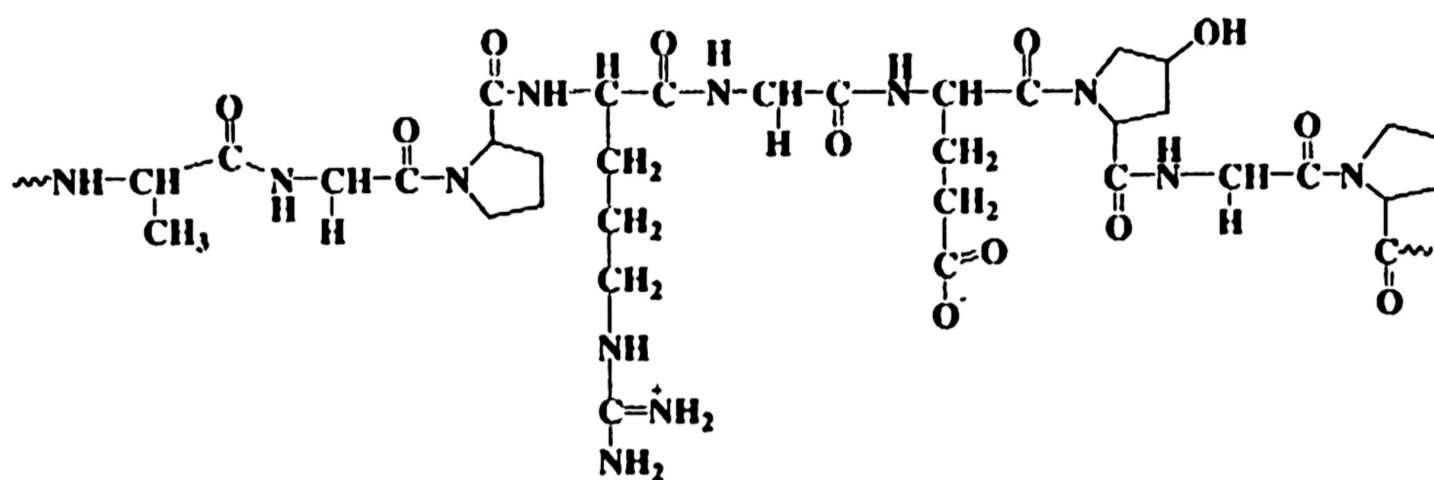


Figure 2.2: Chemical structure of gelatin

2.5.1 Characterization of Self-healing Properties of the Films

Self-healing properties of the gelatin can be characterized by some simple methods inspired by the study of layer by layer assembly of polysaccharides film study that are using acrylamide-modified chitosan (AMCS) and alginate aldehyde (ADA). The self-healing properties of the gelatin film can be characterized by cutting the films with a razor blade until its underlying substrate and caused the gap within the films. Then, water is dropped to the damaged zone of the film and the self-healing of the films can be recorded and observed by using the microscope and FE-SEM. The self-healing properties of the fish gelatin film can be observed after 2 minutes where the new film component will be emerged in the gap due to the swelling of the damaged films and mobility of the polymer chains under the stimulus of the water (Hu et al., 2018). The results show a successful growth of [ADA/AMCS] film on glass substrate was proven

by the scanning electron microscope (SEM) and UV spectrophotometer. The absorbance value at 249 nm (peak of Schiff base linkage) increase with increasing coating cycles. The film not only can self-heal the cuts but also can heal the cuts at the same time repeatedly. These indicates that damaging -healing cycles of the [ADA50/AMCS3]_n films were thickness dependent (Hu et al., 2018)

Other than that, self-healing properties were also being studied by using AMP clay (hybrid clay) and Polyvinylpyrrolidone (PVP). Self-heal ability of the PVP/AMP-clay composite films were done by using sand abrasion tests. The composite film was mounted at an incline of 45 degree and the sea sand with weight of 20g that have the size of 300 to 600 μm in diameter were continuously dropped onto the films at a height of 40cm. Then, the sample was kept under high humidity conditions which is (80% RH) for 48 hours at room temperature. The self-healing properties of the PVP/AMP-clay composite films were observed by the transparency of the samples that were monitored using relative optical transmission (T/T_0) where T_0 is the initial transmission while T is the transmission after the testing was done (England et al.,2016).

Apart from that, gelatin from porcine skin and gelatin from bovine skin were used as the materials for the self-healing study via cross-linking of the gelatin with oxidize alginate. The study were completed by cutting the samples obtained into

several pieces and the pieces were subsequently reconnect by applying gentle pressure and left resting for 7 days. The sample was then observe by optical microscopy and FT-IR analysis. From the study, the results showed better healing at the cut interfaces and higher resistance to stretch (Applications, 2019). In conclusion, there are several method can be used to study about the self-healing properties of characterize films.

2.6 Antifogging Properties of Fish Gelatin Films

Self-healing materials is said to be fog resistant due to its hydrophilic behaviour. Antifogging property of the films was derived from the hydrophilic nature of the materials and water absorbing ability of the films. The fog resistant properties of the gelatin films is due to the water molecules in the condensed droplets is quickly absorbed into the hydrophilic domain of the film by dipole-dipole interaction and hydrogen bond and it is kept in the non-freezing part of the film. While the unabsorbed water droplets could form a thin sheet-like layer on the surface of the smooth film, which then will reduce the light scattered (Hu et al., 2018).

2.6.1 Characterization of antifogging properties of films

From the layer by layer assembly of polysaccharide film study, anti-fogging properties of the gelatin can be characterized by transferring an uncoated glass blank polyethylene (PE) and a double-coated glass with [ADA50/AMCS3]₂ from a -10.0°C refrigerator that were kept for about 4 hours to laboratory conditions at temperature 25.0°C and relative humidity 55.0%. A backdrop covered with the words “WUHAN UNIVERSITY” was placed behind the two glasses to check the fogging behaviour. Camera were used to record the anti-fogging performance of the films. The results show that the uncoated glass were fogged in seconds and words behind the glass become invisible as a result from light scattering of the condensed water droplets while the double coated glass held its transparency and the words behind the glass could be seen clearly (Hu et al.,2018).

Meanwhile based on anti-fogging characterization via clay containing transparent nanocomposite thin films study that use AMP clay (hybrid clay) and Polyvinylpyrrolidone (PVP), the films were deposited onto glass slides and were placed in a refrigerator at 3.0°C for a minimum of 1 hr. Samples were then removed from refrigerator into humid air of laboratory. Then, the deposited films were placed above a pattern surface at room temperature to see it fogging or obscuring of the pattern. The sample then photographed. The result from this study, the coated glass

with PVP and AMP clay held its transparency and the words behind the glass could be seen clearly (England et al.,2016).

2.7 Properties Associated with Gelatin

2.7.1 Chemical and structural properties of fish gelatin

Gel strength, viscosity, gelling and melting points are important to characterize the properties of gelatin. There are many factors that affect these properties such as molecular weight distribution, average molecular weight, concentration of the gelatin solution, gel maturation time, gel maturation temperature and pH. Generally, fish gelatin have lower concentration of amino acids (proline and hydroxyproline) compared to mammalian gelatin. These amino acids function to stabilize the ordered conformation of gel network. The lower content of proline and hydroxyproline will gives a low gel modulus to the gelatin, low gelling and melting temperature of gelatin. Apart from the amino acid composition, the functional properties of gelatin are also influenced by the distribution of the molecular weight, structures and compositions of its subunits (Karim & Bhat, 2009)

2.7.2 Film formation Properties

Film-forming ability of the fish gelatin have been characterize in recent studies that lead to the result where it have good film-forming properties. Moreover, the film that form are transparent, almost colourless, water-soluble and highly extensible films (Alfaro et al., 2014). Fish gelatin is generally different from bovine or pig gelatin in terms of amino acid composition particularly the proline and hydroxyproline contents. Different composition of the amino acid lead to the different water vapour permeability values and mechanical properties.

The characteristic of the fish gelatin film can be improve by adding other proteins, polysaccharides or cross linking agents. These agents help in reducing the molecular forces of the proteins chain and promote the formation of strong covalent bonds in the protein network of the film. Nonelectrolytes such as sugar and glycerol usually will help in increase the viscosity and the gel strength of gelatin. Some research also noting that the addition of the sucrose on various gelatin resulting in the increased of the gel strength. In conclusion, it shows that the gel strength and the viscosity of the gelatin also contribute to the good film forming ability.



CHAPTER 3

METHODOLOGY

3.1 Experimental Design

This chapter include the methodology used for several tests and analysis in this study which are tensile test, thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), self-healing test and fogging test. This study is focussing on the extract gelatin based film in which the gelatin that undergo the chemical pre-treatment and thermal denaturation process in the laboratory. Tensile test, thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), self-healing test and fogging test were mainly analyse and tested on the extract gelatin based films (with and without glycerol). As for the preliminary study of the gelatin based films, commercial gelatin

films (from commercial gelatin powder) were only used to study about the self-healing anti-fogging properties. The results of self-healing and anti-fogging test on the commercial gelatin based films were included in this study.

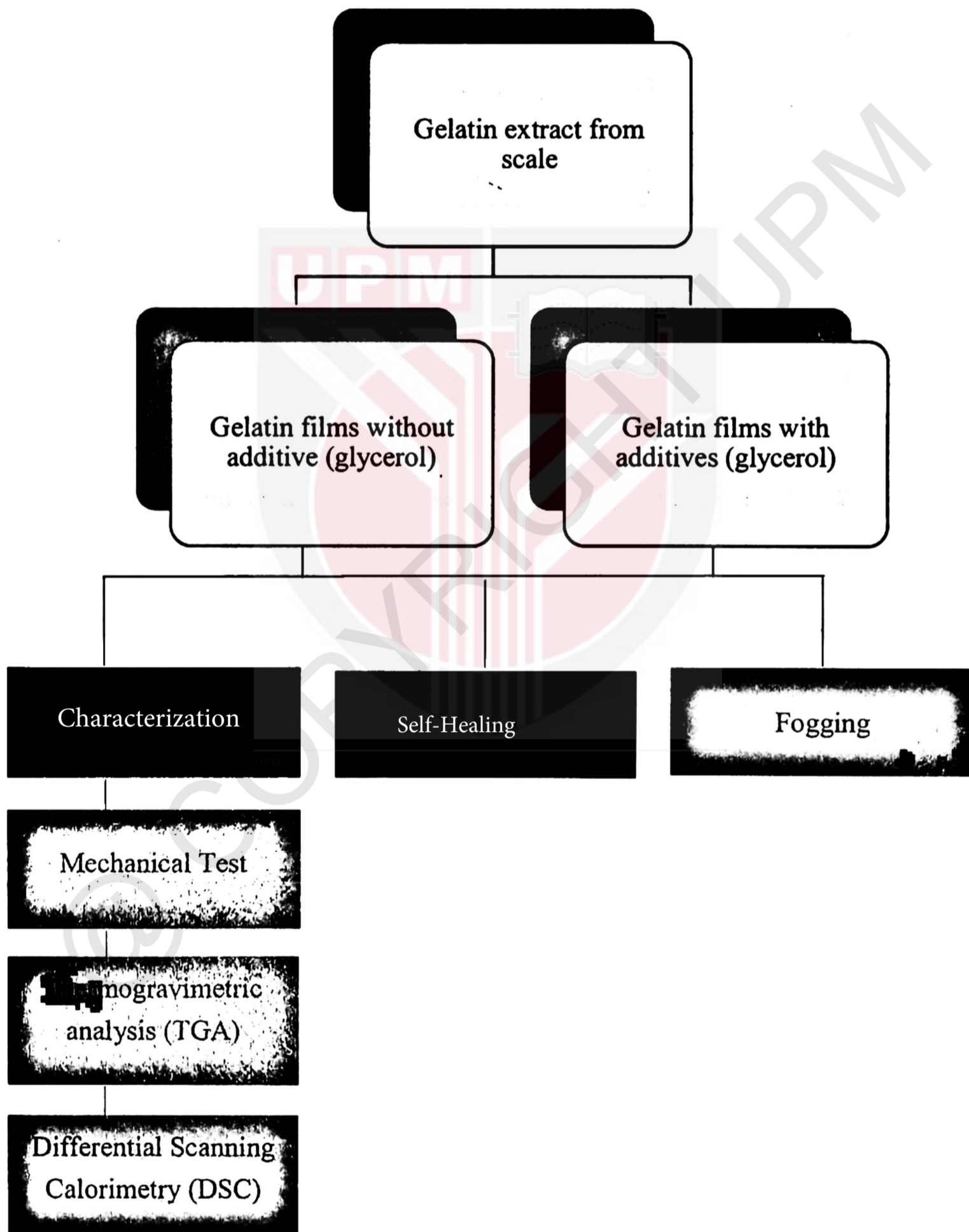


Figure 3.1: Experimental design of the study on gelatin film (scale)

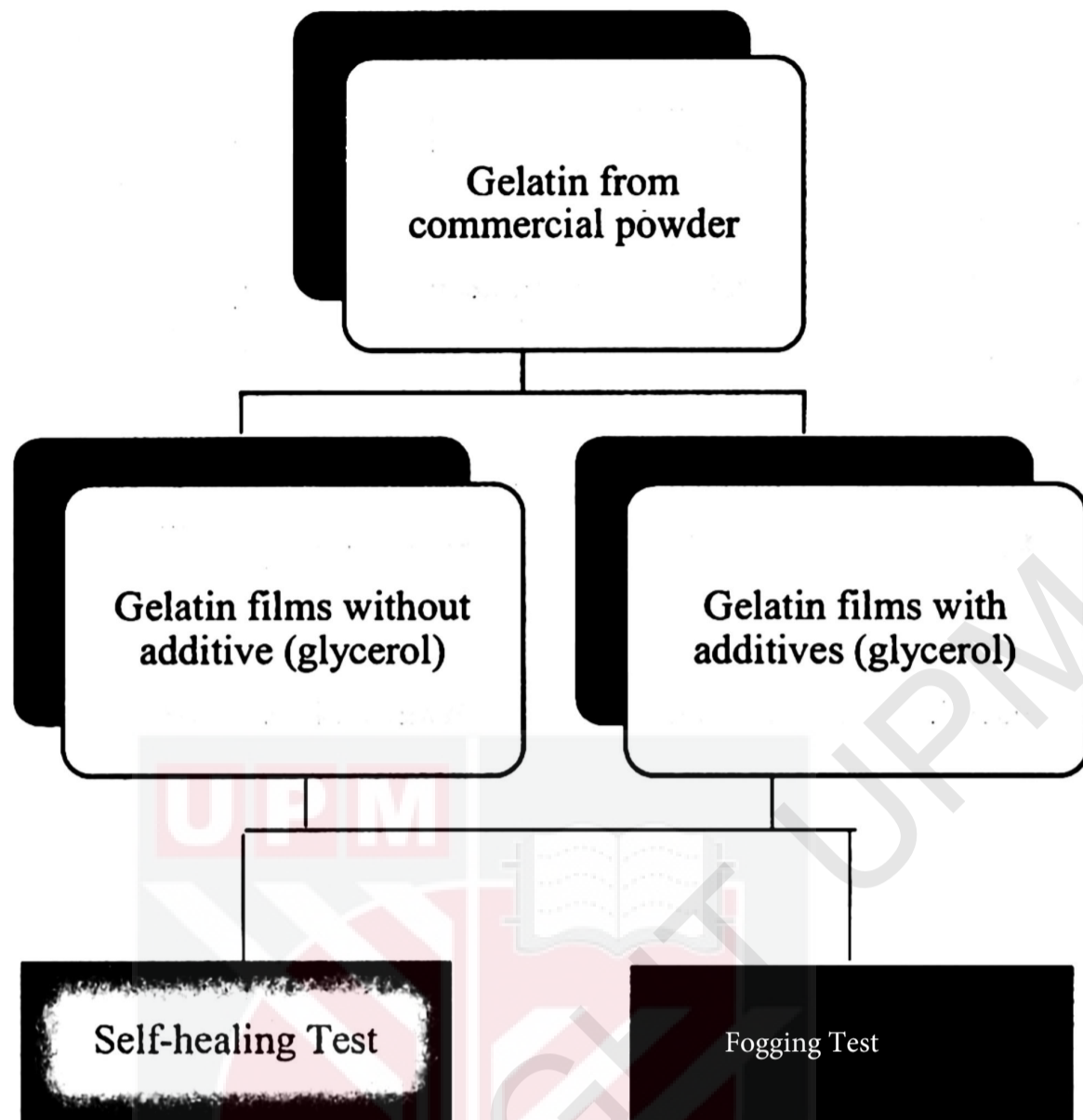


Figure 3.1: Experimental design of preliminary study on gelatin based film from commercial powder

Figure 3.1 and Figure 3.2 show the experimental design study of the gelatin film from gelatin extract (fish scale) and experimental design of preliminary study on gelatin based film from commercial powder respectively. The study on the self-healing and antifogging properties of fish gelatin based films was began with the extraction of the gelation from the tilapia fish scale using the method of chemical pre-treatment and thermal denaturation. The extracted gelatin and commercial gelatin powder was then

prepared as a film after being dissolved in the heated distilled water. Then, the film was peeled after 2 days of drying process. The resulting film was use for the characterization of the self-healing and antifogging properties. During the preparation of the film, the solution of the dissolved gelatin was added with glycerol for the stability of the film network. The self-healing properties of fish gelatin based film were characterized by using four different types of characterized film from bio material based which are the gelatin film and gelatin film that added with glycerol, gelatin film from commercial gelatin powder and gelatin film from commercial gelatin powder that added with glycerol and lastly for the control purpose of the study, the commercial plastic which is low density polyethylene (LLDPE) is use. As for the characterization of the antifogging properties of the film, five different types of characterized films were use which are also the gelatin film and gelatin film that added with glycerol, gelatin film from commercial gelatin powder and gelatin film from commercial gelatin powder that added with glycerol and low density polyethylene (LDPE) film itself for the control purpose.

3.2 Extraction of Fish Gelatin

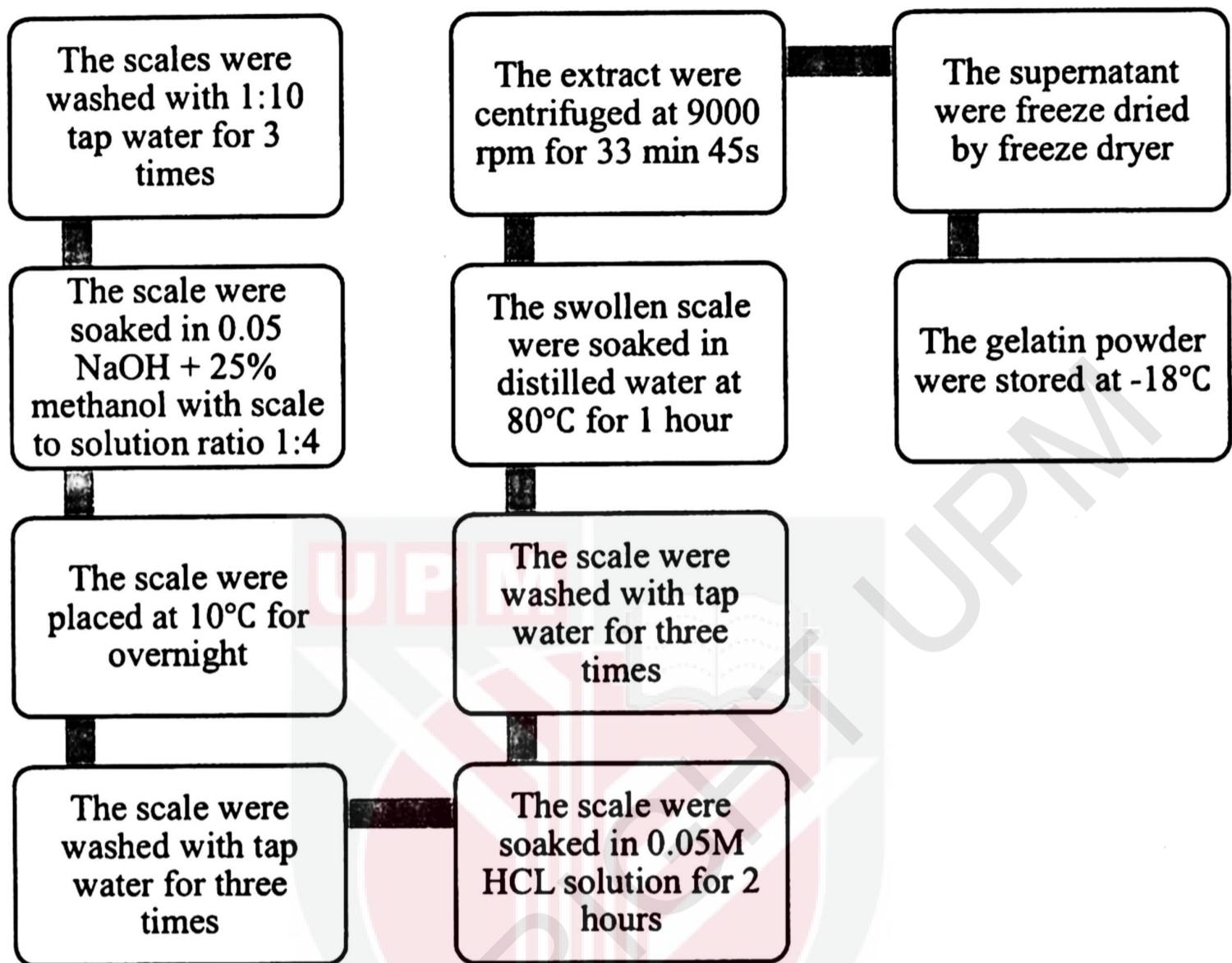


Figure 3.2: Flow chart for chemical pre-treatment and thermal denaturation of fish

gelatin

Figure 3.3 shows the flow chart for chemical pre-treatment and thermal denaturation of fish gelatin from fish scale. The main raw materials for the preparation of the fish gelatin film are tilapia scales. The method use to extract the tilapia fish scale is chemical pre-treatment and thermal denaturation of the fish gelatin. Sodium Hydroxide (NaOH) (R & M chemicals, Ever Gainful Enterprise Sdn. Bhd., Malaysia)

and hydrochloric acid (HCL) (J. T. Baker, Avantor Performance Materials, Inc., Pennsylvania) are the solution use for the chemical pre-treatment and thermal denaturation of the fish gelatin. The method is adapted from the previous study where the fish scale was first washed with 1:10 ratio of tap water for 3 times. Then the fish scale was soak in 0.05 M sodium hydroxide (NaOH) and methanol solution (System, Chemar, Malaysia). The fish scale to solution ratio is 1:4 in which 25% of the solution is methanol and 75% of the solution is sodium hydroxide (NaOH). The fish scale then placed at temperature 10.0°C for overnight. After overnight of soaking with the methanol and sodium hydroxide (NaOH) solution, the fish scale was washed again with tap water for 3 times. Then it is soaked in 0.05 M in hydrochloric acid (HCL) with scale to solution ratio 1:5 for about 2 hours. The fish scale is then washed again with the tap water for 3 times for demineralization purpose. Next, the swollen fish scale was soaked and diluted in distilled water having temperature of 80.0°C for about 1 hour. The extract of the fish scale was then centrifuged (Universal 320, Hettich GmbH & Co., Canada) at 9000 rpm for about 33 minutes and 45 seconds in order to get a clear supernatant fluid. The supernatant was then freeze dried in the freeze dryer for about 2 days and the product was stored and placed at temperature -18.0°C.

3.3 Preparation of Gelatin Based Film

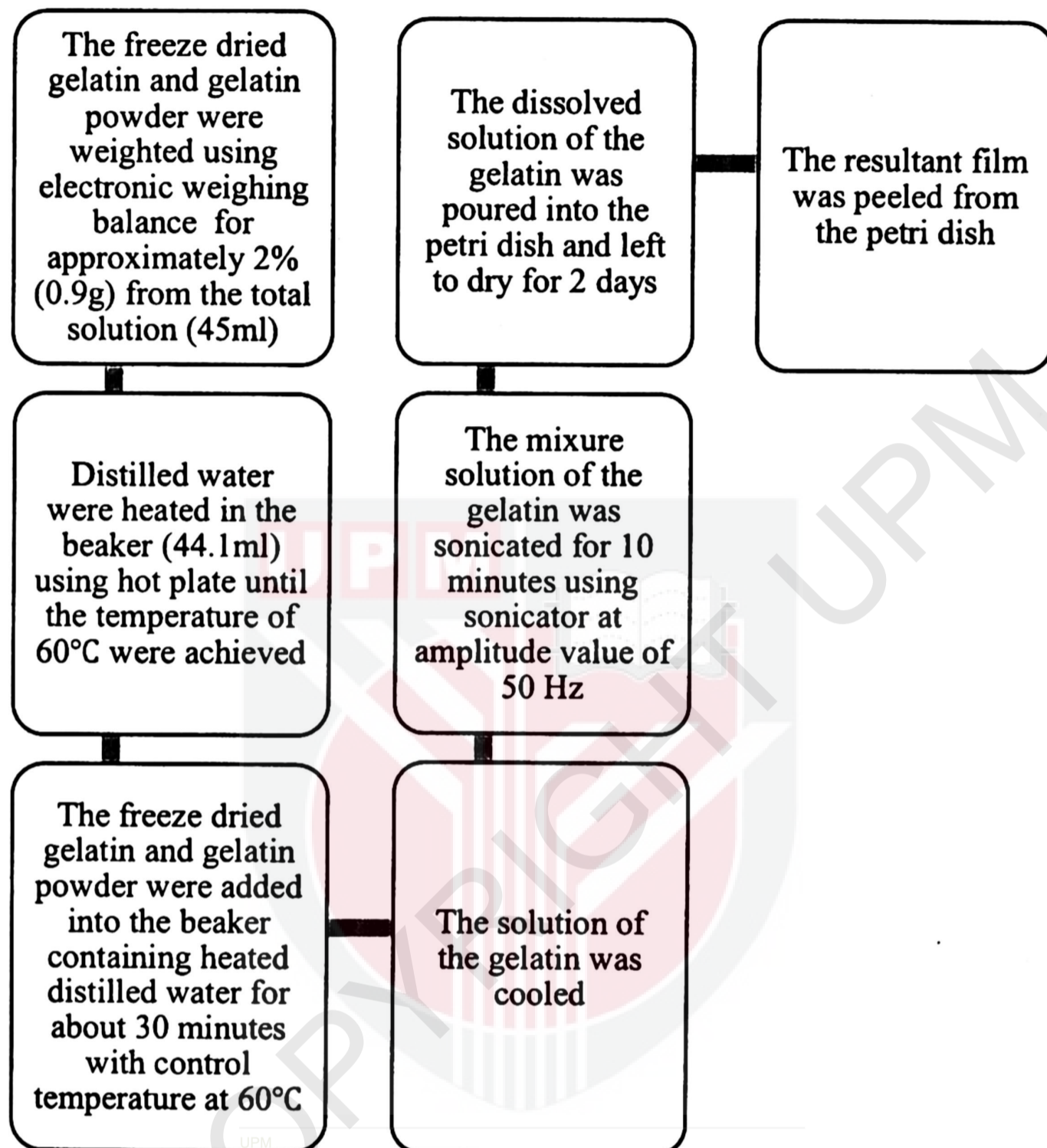


Figure 3.3: Flow chart for the preparation of the fish gelatin based film (without additives)

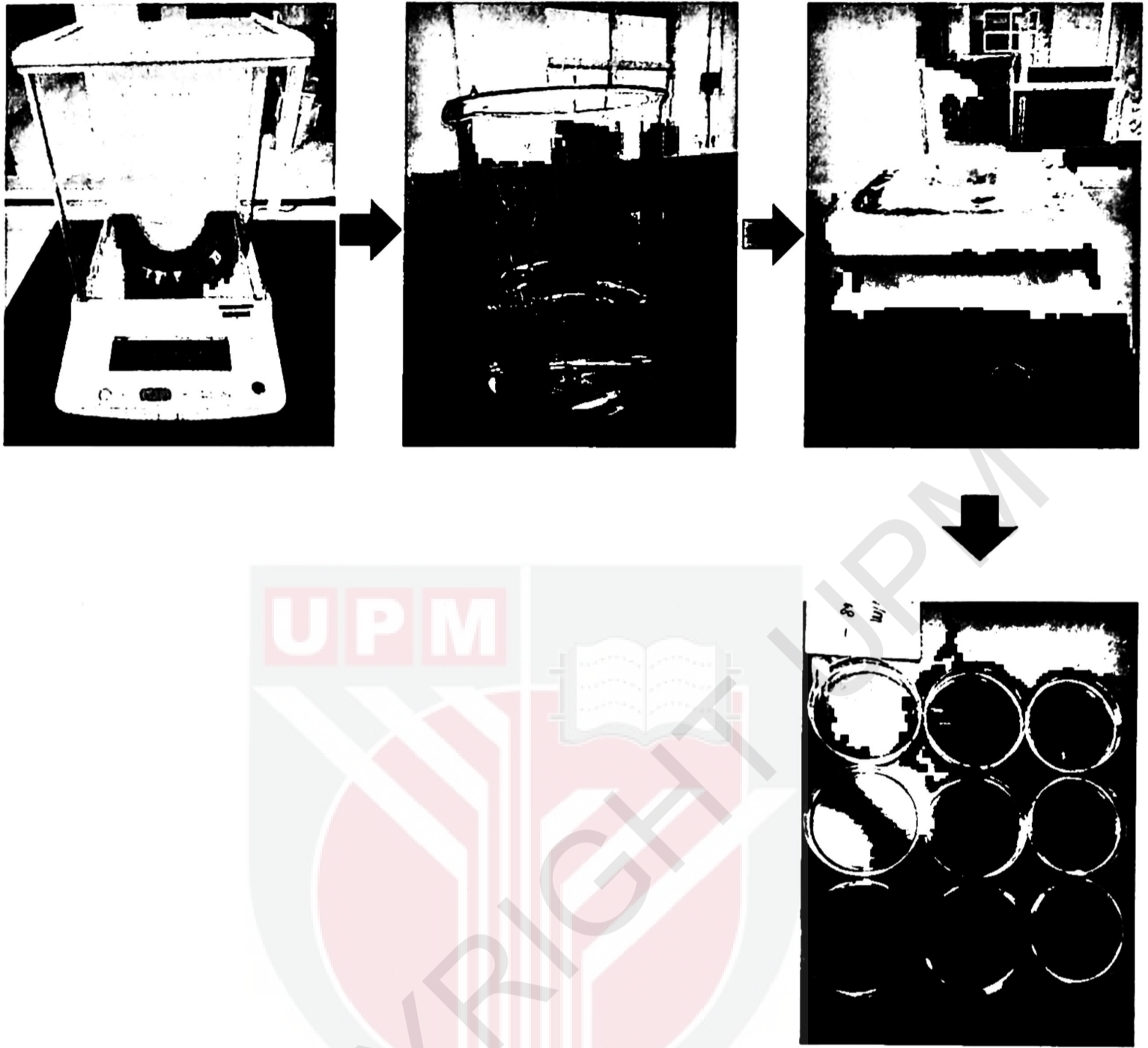


Figure 3.4: Flow process of preparation of the fish gelatin based film (without additives)

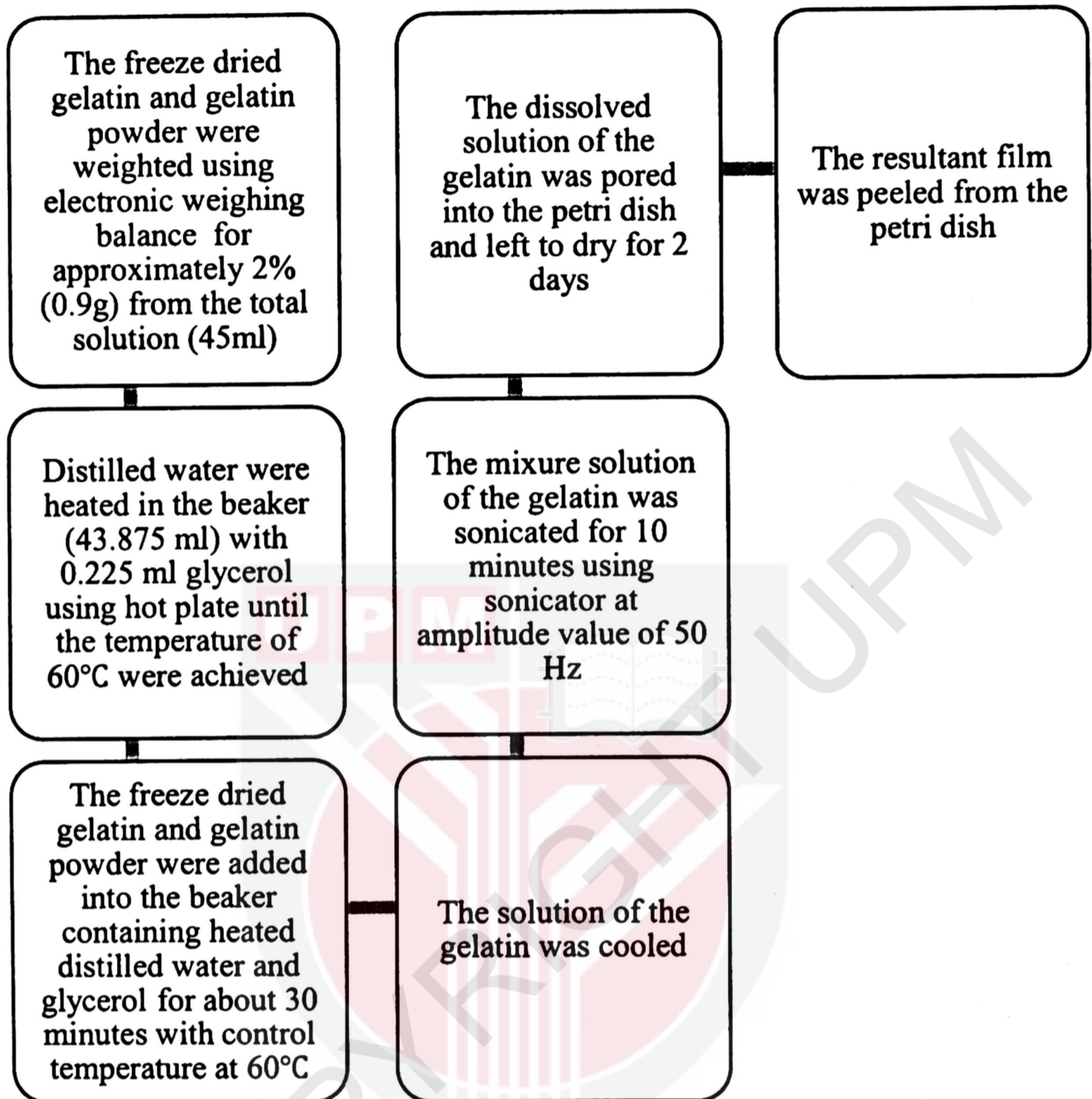


Figure 3.5: Flow chart for the preparation of the fish gelatin based film (with additives)

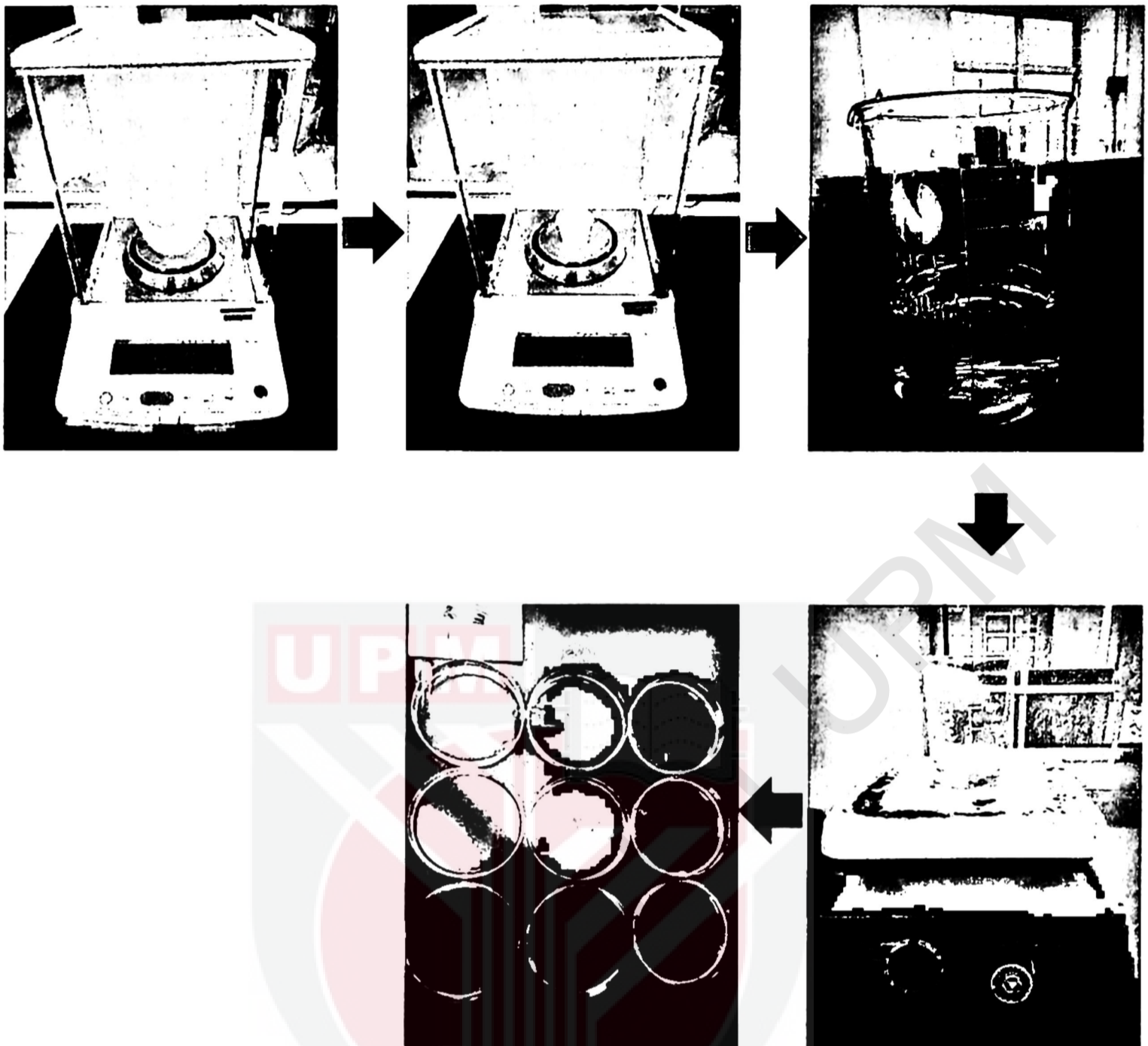


Figure 3.6: Flow process of preparation of the fish gelatin based film (with additives)

Figure 3.4 and 3.6 shows the flow chart for the preparation of the fish gelatin based film without additives and with additives respectively while Figure 3.5 and 3.7 shows the picture of flow process of preparation of the fish gelatin based film (without additives). As for the preparation of the fish gelatin based film, the freeze dried gelatin and gelatin powder were dissolved in distilled water that have been heated at

temperature 60.0°C. The weight of freeze dried gelatin and gelatin powder used is approximately 2% of the total solution that will be prepared. While for the gelatin film that added with glycerol (Sigma Aldrich, Sigma Aldrich (M) Sdn. Bhd., Malaysia) as the additive which referring to Figure 3.6 and 3.7, the weight of the glycerol added is approximately 25% of the weight of the freeze dried gelatin and gelatin powder used. The freeze dried gelatin was then dissolved in the heated distilled water and stirred using magnetic stirrer for about 30 minutes in order to get the smooth solution with the freeze dried gelatin that had totally dissolved in the solution. After that, the solution will be cooled for several minutes and further sonication process. This method use high frequency sound waves to cause cavitation within the fluid (solution) and to remove air bubble. This sonication process use the amplitude value of 50 Hz for 10 minutes process using sonicator ultrasonic liquid processor (Q500, Sonica, USA). Then, the solution that have been sonicated was poured and cast into the petri dish with 14 cm internal diameter and left to dry for about 2 days. In order to control the film thickness, the quantity of the dissolved solution poured into a petri dish was always 45 ml. Next, the resultant film was peeled from the petri dish. The film was stored in a sealed plastic film with 57% of humidity at a temperature of 25.0°C.

3.4 Tensile Properties Test

The triplicates sample strips were cut into characterized size (100mm X 15mm). The thickness of the sample was then measured using a Digital Vernier Caliper (Model MIT-500-196-20, Japan) that can be referred in Figure 3.8. The average values that have been determine were then used to calculate the cross-sectional area of each samples. The tensile strength of the gelatin based films were analysed using Texture Analyzer (Ta xt2, sTable micro system, UK) based on Figure 3.9 below with a load of 5 kN followed to ASTM D882-02 (2002). Utility tape was used to mount the end of each of the specimen in order to prevent slippage of films during testing. Force (N) and deformation (mm) were recorded with cross-sectional test specimens.

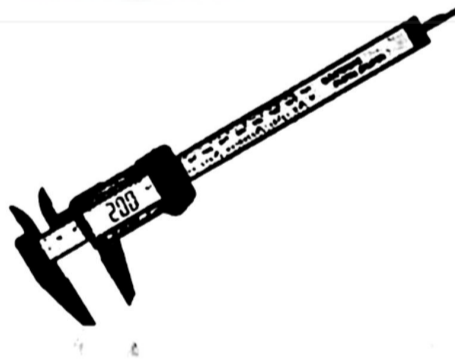


Figure 3.7: Digital Vernier Caliper (Model MIT-500-196-20, Japan)

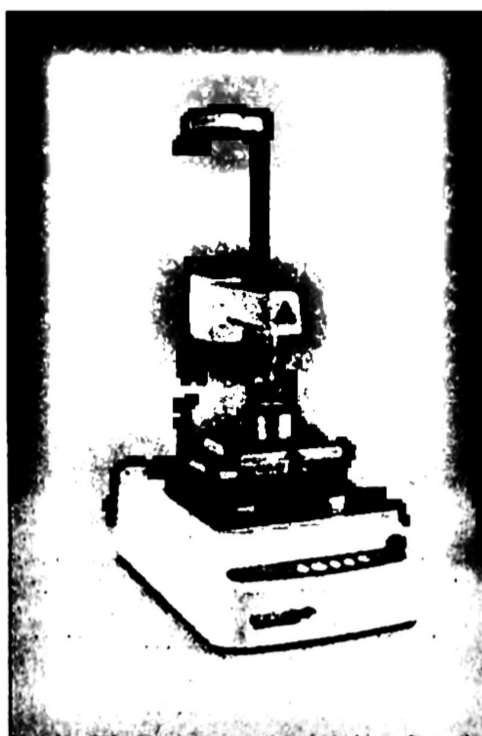


Figure 3.8: Texture Analyzer (Ta xt2, sTable micro system, UK)

3.5 Thermo-gravimetric Analysis (TGA)

By referring to Figure 3.10, thermo-gravimetric test were performed by using Mettler Toledo thermal analyser (TA Instruments, New Castle, PA, USA). This analysis were carried out at temperature ranging from 25.0°C to 800.0°C with a heating rate of 10.0°C/min under nitrogen gas. Film sample of 5-15 mg was placed and the decomposition temperature as well as weight loss profiles were obtained.

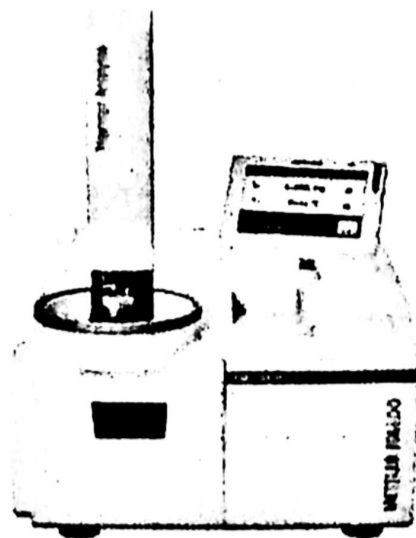


Figure 3.9: Mettler Toledo thermal analyser (TA Instruments, New Castle, PA, USA)

3.6 Differential Scanning Calorimetry (DSC)

Glass transition of different characterized gelatin based films were determined by using differential scanning calorimetry (Universal V3-9A TA Instrument, New Castle, PA, USA) equipment that shows in Figure 3.11. Samples of weighed 5mg were placed in an aluminium sample pan which was immediately sealed. Empty sample fan was used as reference during this analysis. Films samples were then heated from 30.0°C to 195.0°C at a rate of 10.0°C/min. DSC cell was then flush by nitrogen gas at a flowrate of 50 mL/min in order to maintain an inert environment. The transition temperature of gelatin based films were then obtained from the thermos-gram results. The weight loss as function of temperature was present as thermal-gravimetric analysis (TGA) curve.

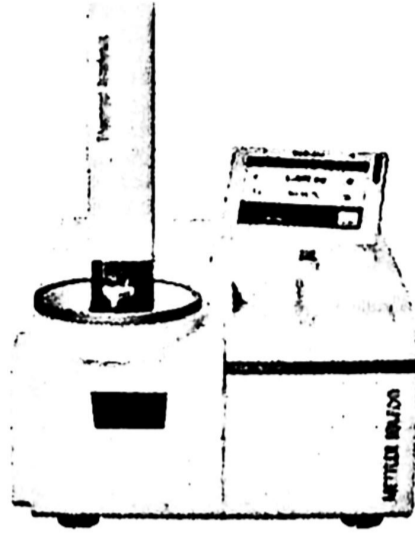


Figure 3.10: Differential Scanning Calorimetry (Universal V3-9A TA Instrument, New Castle, PA, USA)

3.7 Characterization of the self-healing properties

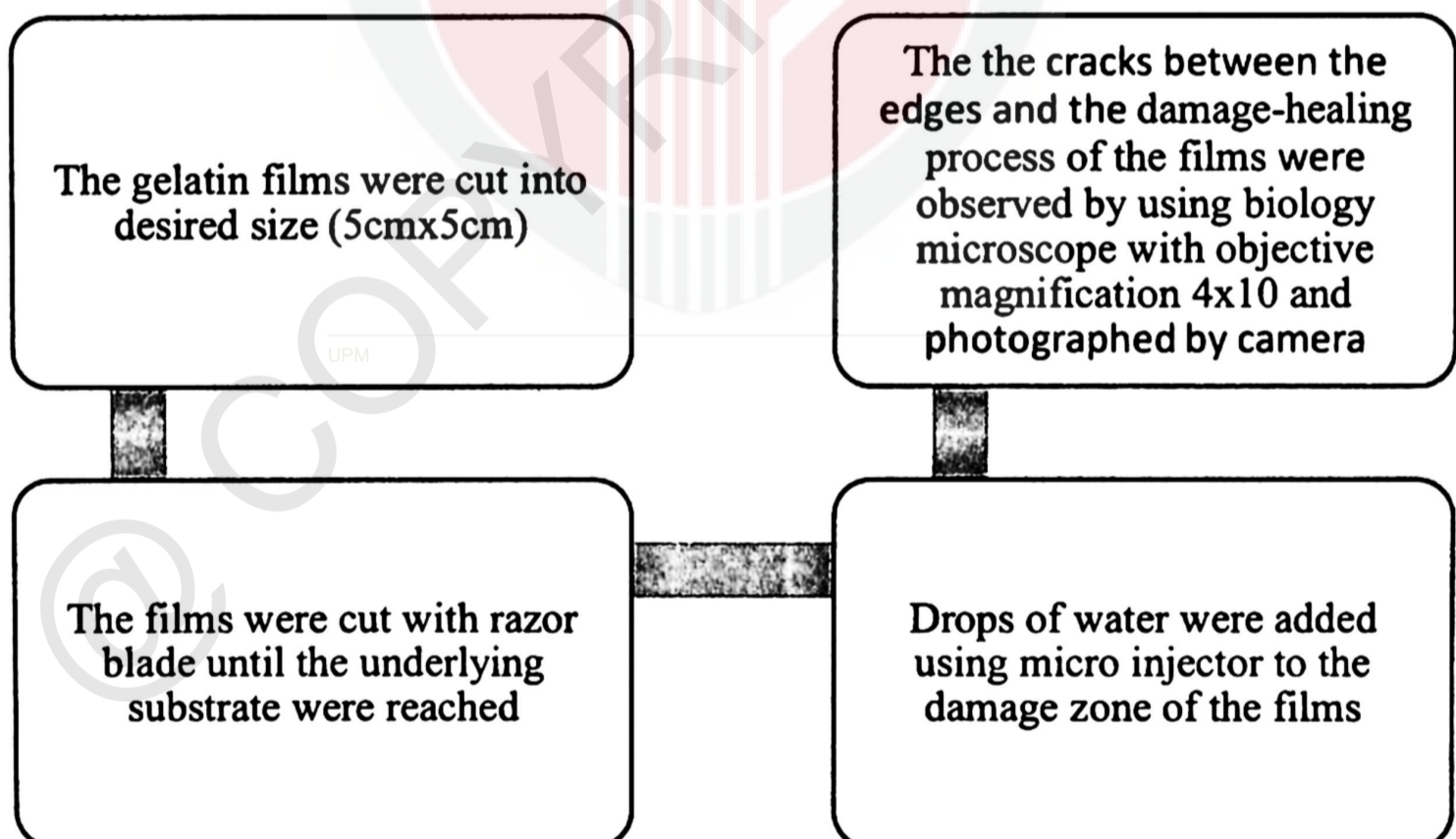


Figure 3.11: Flow chart of the characterization of self-healing properties of the films

Figure 3.12 shows the flow chart of the characterization of self-healing properties of the films. The characterization of the self-healing properties of the fish gelatin based film is based on the observation method where the film was cut using a razor blade until its underlying substrate was reached. The damaged film was then observed and recorded by using biological microscope (CX43, Olympus, Malaysia) with objective magnification of 4x10 as referred in Figure 3.13. Then, drops of water was added to the damaged zone of the film by using syringe. The damage-healing process of the film were observed and recorded at the different time interval (2,4, 6, 8, 10 minutes, 1 hour, 24 hours) using the biological microscope in order to observe the development of the self-healing from time to time.

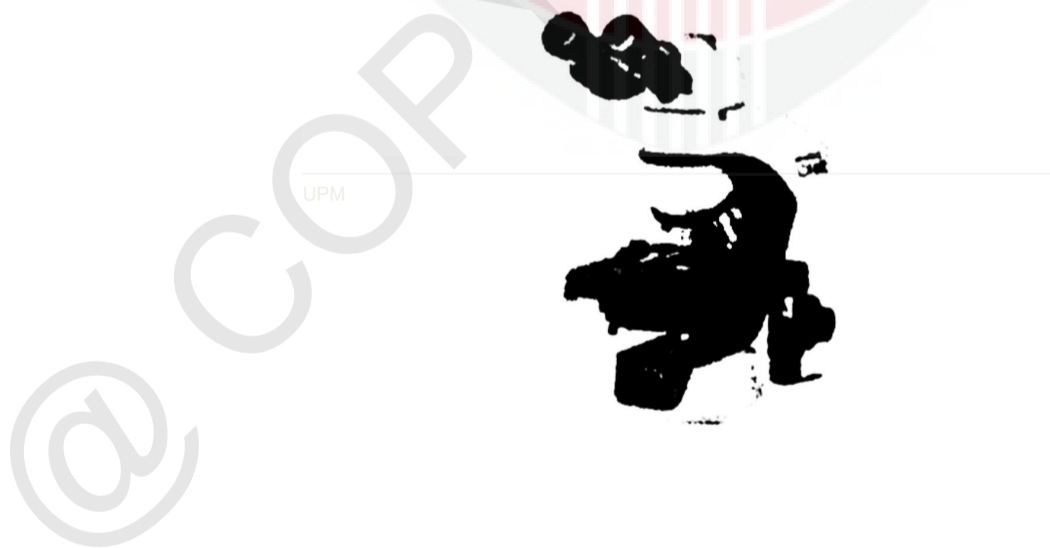


Figure 3.12: Biological Microscope (CX43, Olympus, Malaysia)

3.8 Characterization of antifogging properties

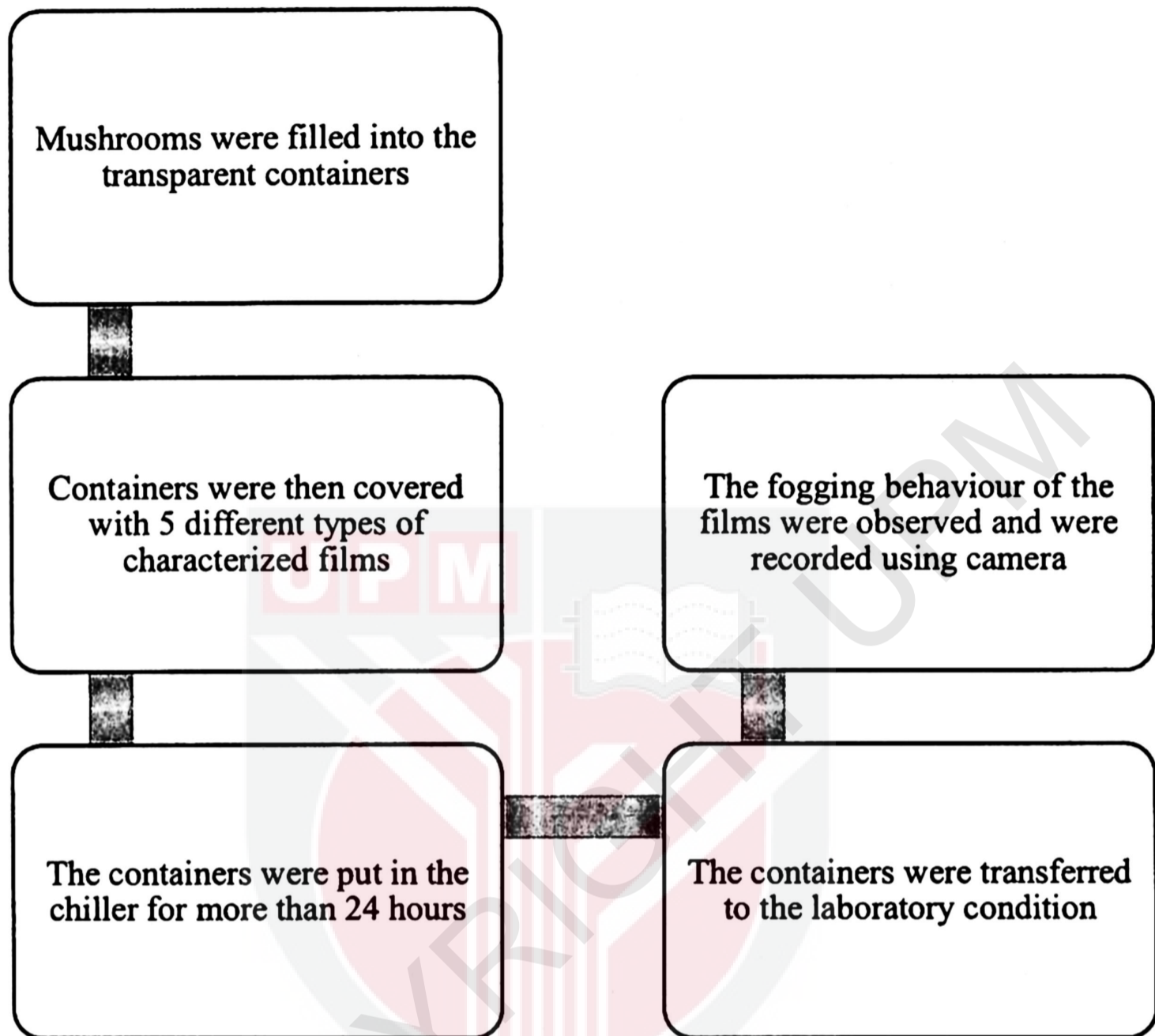


Figure 3.13: Flow chart for the characterization of fogging behaviour of the films

Figure 3.14 shows the flow chart for the characterization of fogging behaviour of the films. Anti-fogging properties of fish gelatin based film was observed by putting mushroom in the containers that were covered by the five different characterized film in the chiller for 24 hours as referred in Figure 3.15 respectively. After that, the containers were transferred to the laboratory conditions normally at 25.0°C which the

relative humidity of the air is 55%. The fogging behaviour of the five characterize films were observe and record by using camera and shown in Figure 3.16.

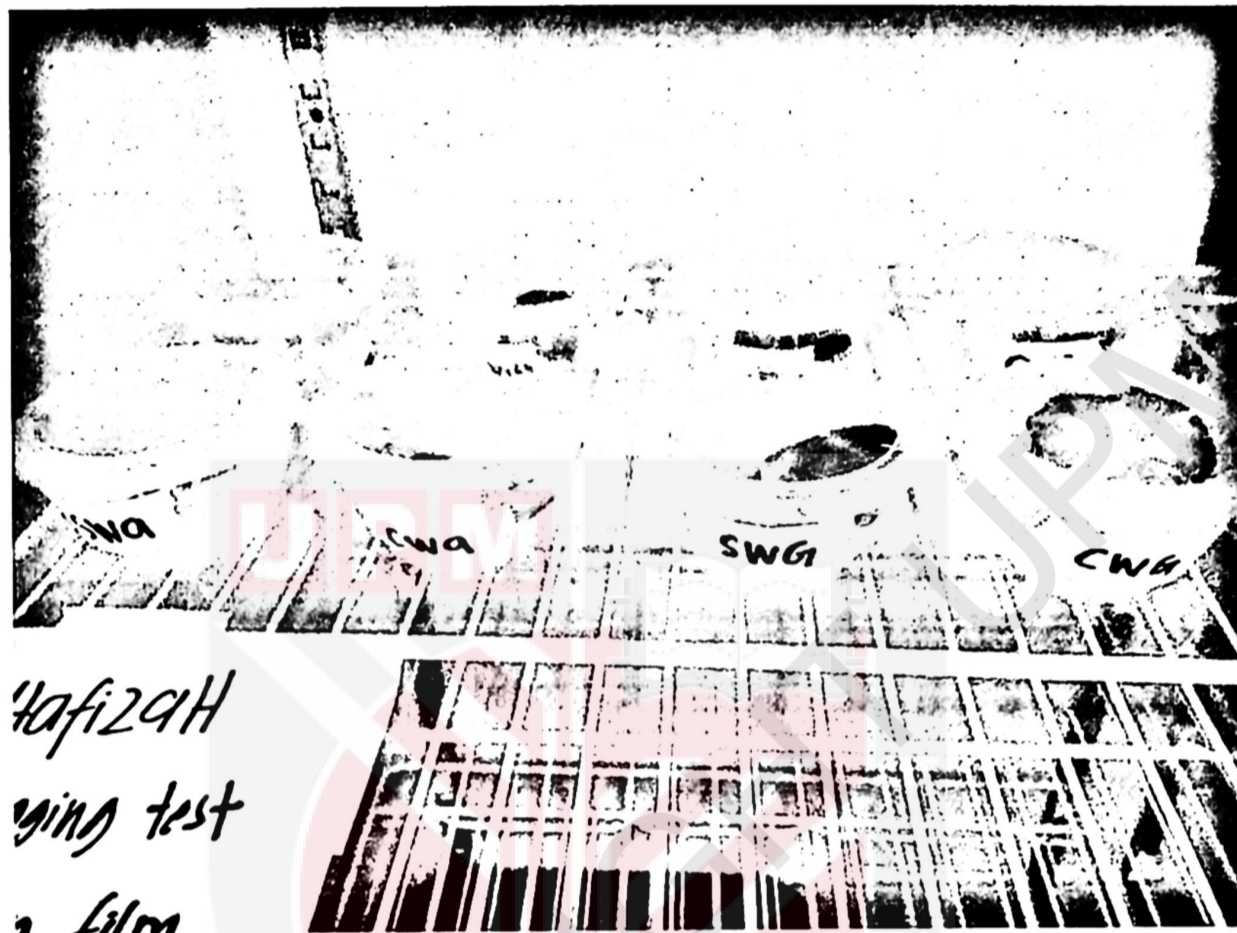


Figure 3.14: Process of fogging test that take place in the chiller



Figure 3.15: Condition of the films after fogging test

The logo of Universiti Putra Malaysia (UPM) is visible as a watermark in the top left corner of the page. It consists of the letters 'UPM' in a stylized font within a red-bordered box.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result Overview

This chapter include the discussion of result and observation obtained from several tests and analysis which are mechanical test, thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), self-healing test and fogging test.

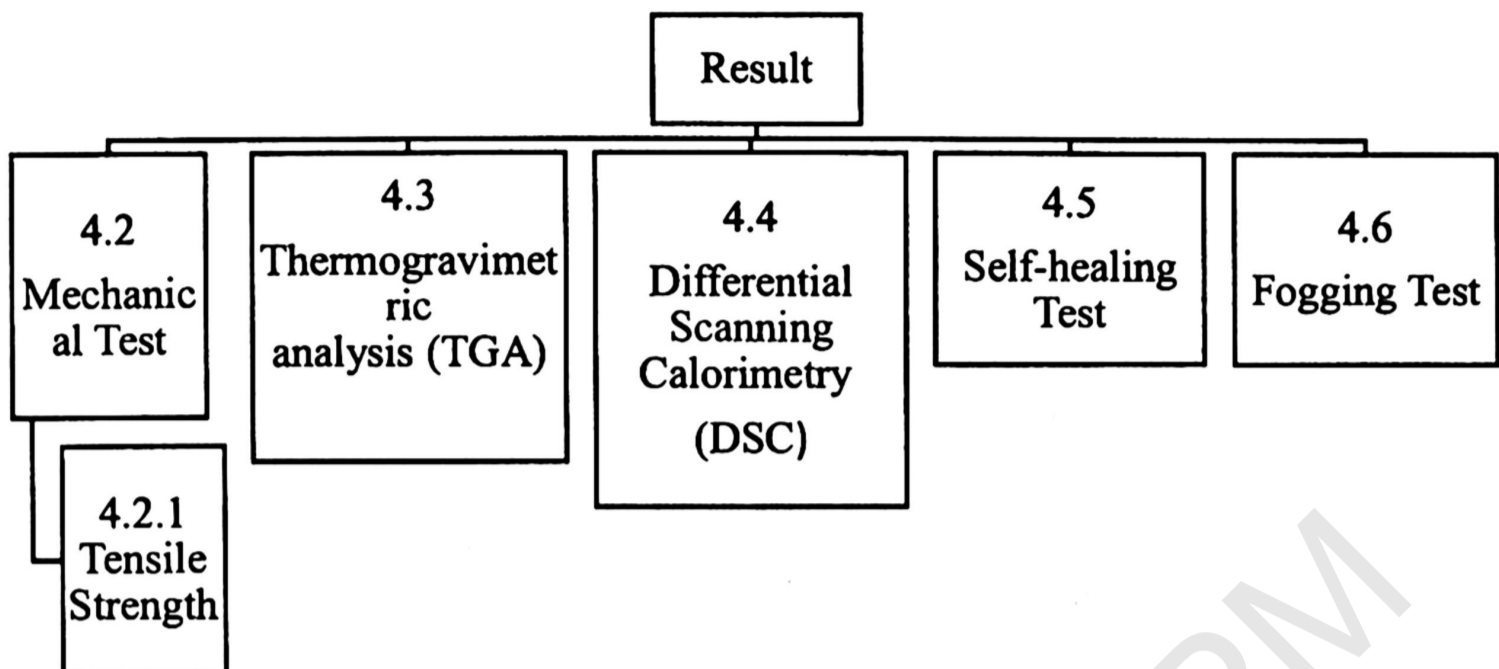


Figure 4.1: Result Overview for tests done on the gelatin based films

4.2 Mechanical Test

Figure 4.2 showed that the stress-strain curve of gelatin film. The result shows gelatin based films have a viscoelastic properties in which its exhibit both viscous and elastic behaviour when undergo deformation. From Figure 4.2, it showed that the stress was gradually increased and it was proportional to the strain when the elastic deformation occurred for the gelatin film without glycerol. The fracture occurred as the gelatin based films were pulled further in which the stress was increased. The tensile strength of the gelatin based films was determined at the stress value in which the fracture started to occur. Based on the results shown, the tensile strength and

elongation at break (EAB) values of the gelatin film without glycerol was determined to be approximately 69.04 MPa and 3.3% respectively meanwhile. The strain value of the gelatin film without glycerol is much lower than that of gelatin films that added with glycerol. Therefore, the gelatin film without glycerol appeared to be more brittle as compared to gelatin film that added with plasticizer (glycerol). Meanwhile, gelatin film with the addition of glycerol had tensile strength and EAB value at 14.98 MPa and 95.5% respectively. Results show significant effect on the addition of plasticizer (glycerol) towards the tensile strength of the gelatin based film. The results showed that gelatin based film that added with glycerol had a lower tensile strength and higher flexibility as compared to the gelatin based film without glycerol that high tensile strength and lower flexibility. Recent studies from (Rivero, García, & Pinotti, 2010) also clearly stated that the addition of glycerol into the commercial gelatin films result increased in the strain value and decreased in stress at break compared to gelatin films without addition of glycerol although the material kept its rigid character. Glycerol contributes to plasticization effect that expand the mobility of polymer chains. It have been used to control the brittleness of the films that give results into stretchable and flexible films due to effect on increasing of polymer chain's mobility (Al-Hassan & Norziah, 2012a). Recent study also stated that the addition of plasticizers can decrease the structural brittleness of gelatin films by reducing the intermolecular forces,

increase the versatility of polymeric chains, and improving the film's stability (Mendieta-Taboada et al., 2008). However, based on the previous study it is stated that increasing the concentration of glycerol at constant polysaccharides-protein composition would reduce the mechanical properties of the materials. Recent study also had stated that glycerol can be both plasticizer and anti-plasticizer depending on their concentration (Liang et al., 2015). In a nut shell, the distribution and density of intermolecular and intra-molecular interaction gave a significant effect to the mechanical properties of the gelatin films in which it is depending on the arrangements and orientation of polymer chains in the network itself (Al-Hassan & Norziah, 2012a). From the perspective of plasticizer's type, glycerol can give more plasticization effect as compared to sorbitol when used at the same mass content in protein, polysaccharides and protein polysaccharides based films. It is also being reported that lower molecular weight of the plasticizer such glycerol has more plasticization affect than the higher molecule ones like sorbitol when used at the same mass basis where the number of moles of glycerol incorporated in the films would be higher than the sorbitol ones. The molecular structures of the plasticizers were used in contribution of reducing the molecular forces of protein chains and therefore reduce hydrophilic characteristic or promote the formation of the strong covalent bonds in protein network of the films.

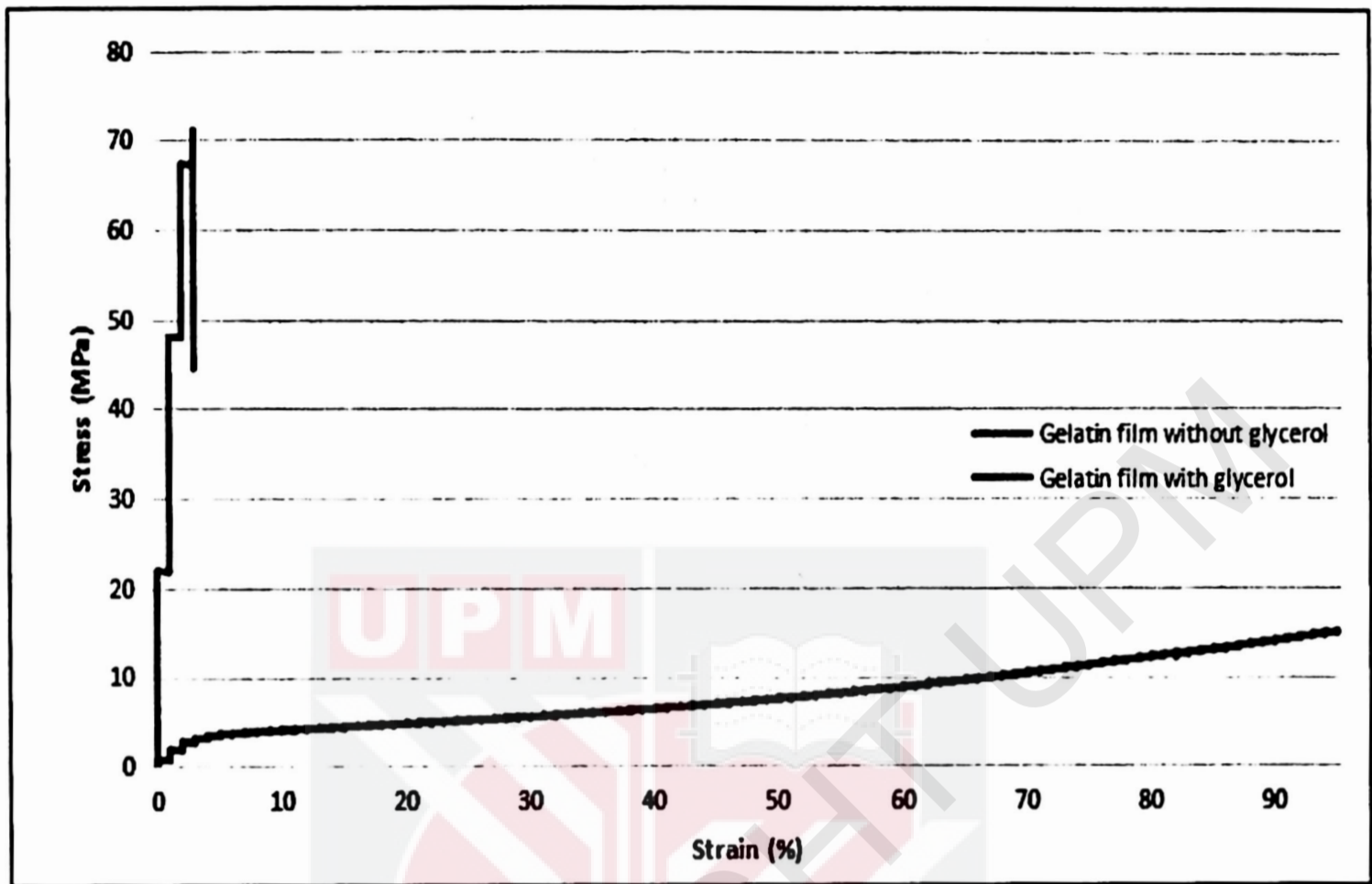


Figure 4.2: Stress-Strain Curve of Gelatin Based Films

4.3 Thermo-gravimetric Analysis (TGA)

Table 4.1 shows the results of thermo-gravimetric Analysis (TGA) for gelatin based film with and without glycerol. TGA curves were analysed as percentage weight loss as a function of temperature in the range of the ambient temperature which is 25.0°C and up to 800.0°C. The decomposition stages and the decomposition temperature were given in the Table 4.1 and the comparison between gelatin based film without glycerol and gelatin film with glycerol is shown in the Figure 4.3 below. Three weight losses which the degradation patterns were observed in the TGA curved

of gelatin based films. The first degradation of gelatin film without glycerol starts from 215.5°C continues to 455.2°C and 654.4°C in a form of 8.2% loss in the weight due to volatilization. The weight loss of water is probably due to the hydrogen bonding in gelatin and water molecules. The second weight loss begins at about 455.2°C and the corresponding 51.1% weight loss is attributed to the decomposition of gelatin main chains. This was probably due to the loss of ammonia and the thermal degradation of gelatin molecule (Shehap et al., 2015). Meanwhile from previous study (Shehap et al., 2015) had stated that the first degradation of commercial gelatin film without glycerol starts from 50.0-150.0°C with mass loss of 9.90% which may be due to the moisture vaporization, meanwhile second degradation which occurs within the range of 220-320.0°C with a mass loss (61.24%) which may be due to loss of ammonia and the thermal degradation of gelatin molecule (Shehap et al., 2015). The difference value of decomposition temperature and incorporation loss weight were probably because of different types of gelatin that used in the referred study that composed of different value of glycine (27%), proline and hydroxyproline (25%) with average molecular weight \approx 100.000 gm/mol while the gelatin that used in this study composed of glycine (20.79 g/100 g) and hydroxyproline. (14.38 g/ 100 g). The first degradation of gelatin film that added with glycerol starts from 164.3°C and the degradation continue to 277.4°C and then 486.6°C. 3.86% loss in the weight for the first degradation

of gelatin film that added with glycerol was also due to moisture vaporization. Second weight loss observed which is 10.72% contributed to the decomposition of gelatin. Based on the results shown in the Table, gelatin film without glycerol has higher weight loss than gelatin film that added with glycerol. It shows that gelatin film that added with glycerol has good thermal stability as compared to original gelatin based film. It can be concluded that the first decomposition step of the gelatin based films may be due to dehydration processed or volatilization of the small molecules while the second step in TGA analysis covered melting temperature of gelatin as physical transition and the thermal degradation processed in which attribute to the loss of ammonia, water dehydration, and imidization (Shehap et al., 2015). Thus, as referring to the decomposition temperature and the incorporation of the weight loss, it was possible to say that gelatin film that added with glycerol was more stable than gelatin film without glycerol. Therefore in general, the addition of the glycerol as additive and plasticizer increase the thermal stability of the gelatin based films in which it is clearly shown in the Figure 4.3, at temperature of 455.2°C for gelatin film without glycerol and at 486.6°C for gelatin film that added with glycerol the incorporation weight loss were 51.08% and 46.01% respectively.

Table 4. 1: Decomposition temperature of gelatin based films original gelatin film and plasticized with glycerol

Sample	TD (°C)	Weight loss (%)	TD (°C)	Weight loss (%)	TD (°C)	Weight loss (%)
Gelatin film without glycerol	215.6	8.20	455.2	51.1	654.4	6.99
Gelatin film added with glycerol	164.3	3.86	277.4	10.72	486.6	46.01

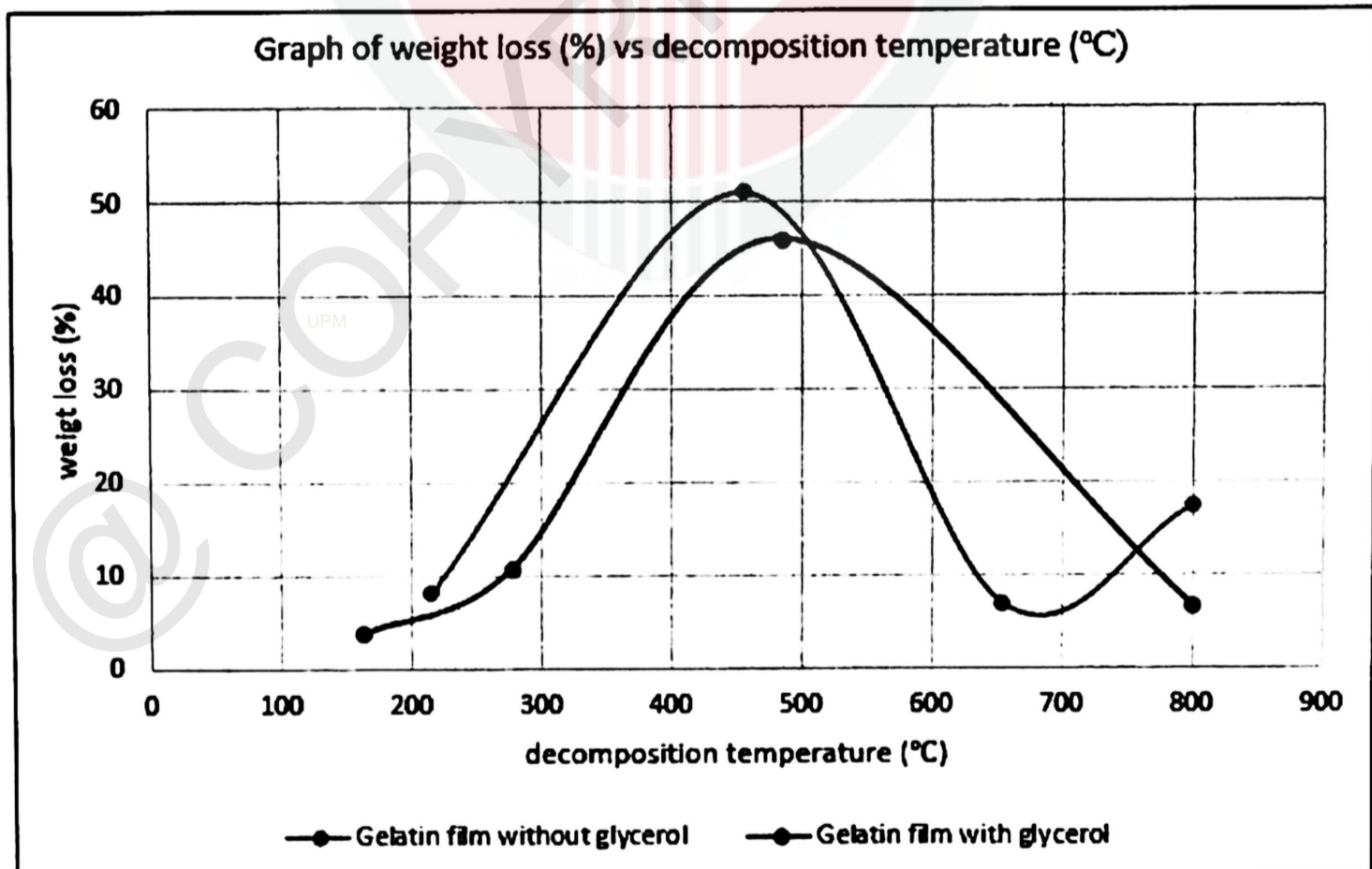


Figure 4.3: Weight loss (%) vs decomposition temperature (°C) of gelatin based films

4.4 Differential Scanning Calorimetry (DSC)

Based on the results of Differential Scanning Calorimetry (DSC) analysis that have been conducted, it was showed that gelatin film without glycerol had high T_g value as compared to gelatin based film that added with glycerol. Referring to Table 4.2, gelatin film without glycerol had a T_g value of 36.5°C and T_m value of 92.5°C. Incorporating plasticizer (0.02 of glycerol) into the gelatin films significantly decrease T_g and T_m values of the films in which it gave values of 28.3°C and 41.0°C respectively. Glycerol that were added to the film makes it more hydrophilic and can retain higher moisture compared to the gelatin film without glycerol when conditioned at the same temperature and humidity (RH%). It is probably because of the hygroscopic character of the glycerol that tends to provide additional water into the gelatin film matrix (Al-Hassan & Norziah, 2012b). Shehap et al. (2015) studied also shows that the T_g and T_m value of commercial gelatin based film without glycerol were approximately at 54.7°C and 86.2°C respectively. The small difference value of T_g and T_m were because of different types of gelatin that used in the referred study were composed of glycine (27%), proline and hydroxyproline (25%) with average molecular weight \approx 100.000 gm/mol while the gelatin that used in this study composed of glycine (20.79 g/100 g) and hydroxyproline (14.38 g/ 100 g).

At temperature around 52.7°C, an endothermic event was observed that indicates time-dependent thermal relaxation in the gelatin film without glycerol. The changes in heat flow was assigned from glass to rubber transition T_g that relates to the amorphous part of the films (Shehap et al., 2015). Then, the glass transition followed by endothermic peak which present the melting of triple-helix crystalline structure. The transition was associated with melting and dissociation of the film (original). Therefore it can be concluded that, plasticizers (glycerol) reduce T_g values due to the ability of the glycerol to penetrate between the polymer chains in order to weaken the interaction between proteins.

Table 4. 2: Thermal characterization of gelatin based films without glycerol and plasticized with glycerol

Sample	T_g (°C)	T_m (°C)
Gelatin film without glycerol	36.5	92.5
Gelatin film added with glycerol	28.3	41.0

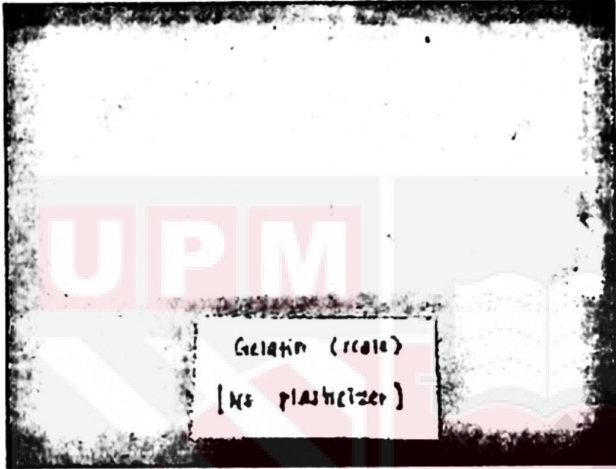



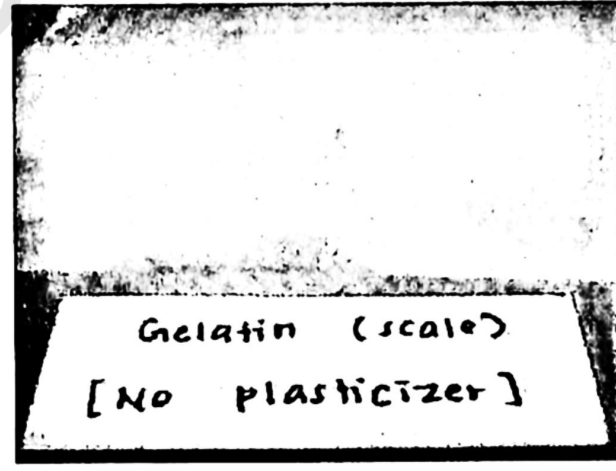
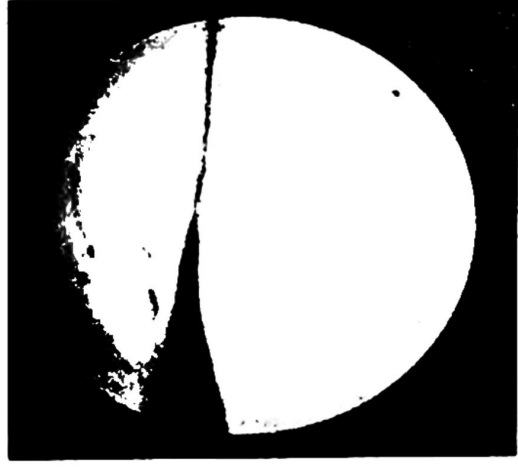
4.5 Self-healing Test

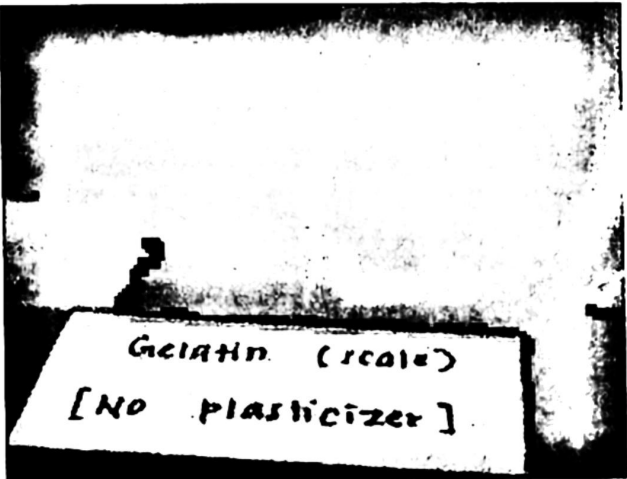
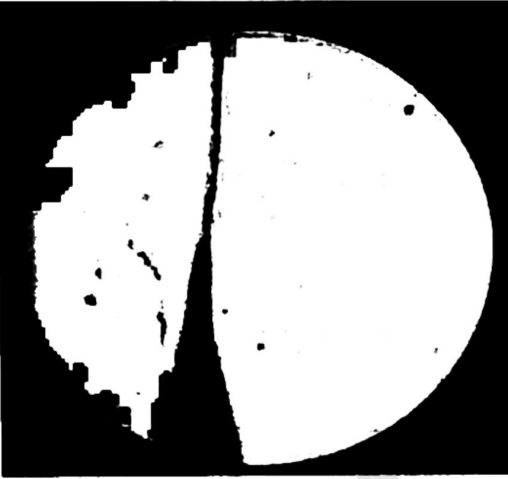
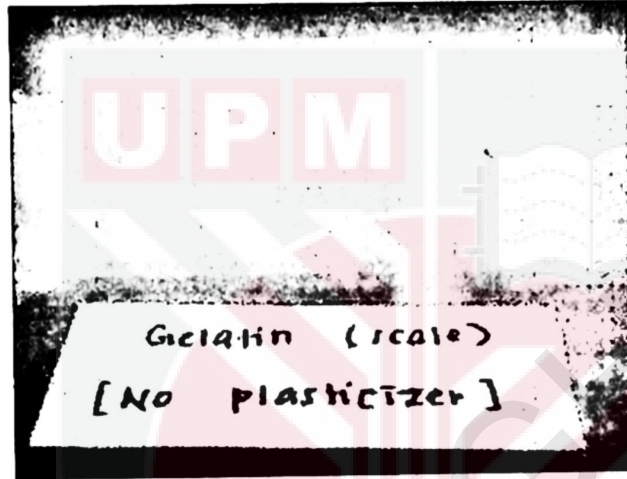

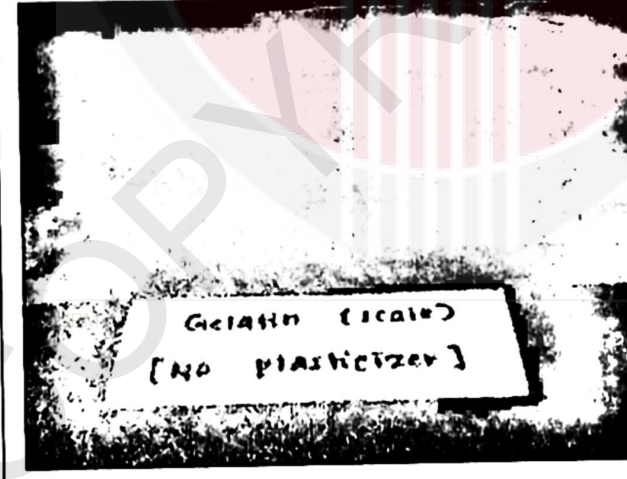

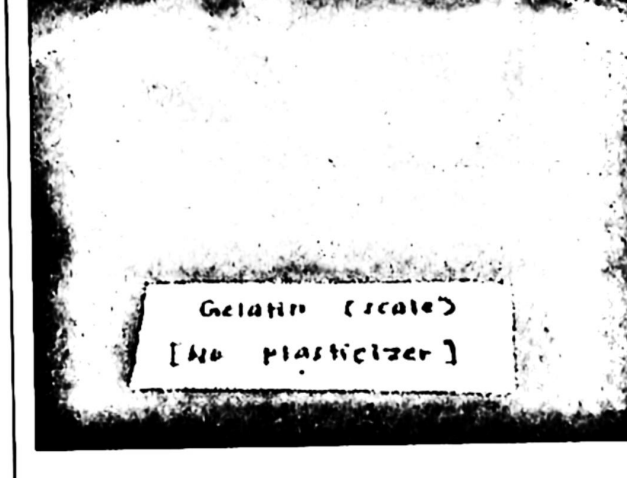
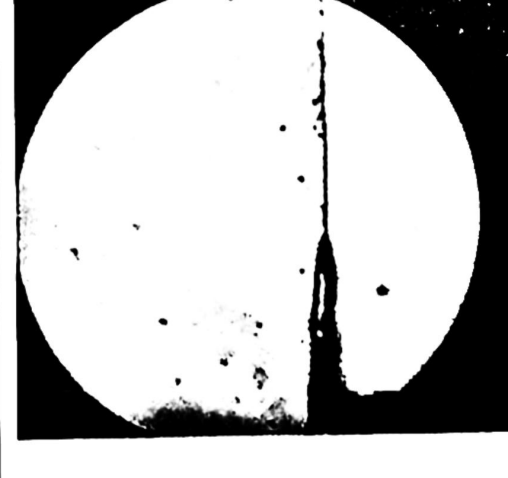
First picture in Figure 4.3 to Figure 4.7 shows different types of plastic that made up of gelatin which were gelatin from powder, gelatin extracted from scale, gelatin from powder and added with glycerol, gelatin extracted from scale and added with glycerol, and the commercial plastic which is low density polyethylene (LLDPE) that were cut with the razor blade until its underlying substrate in order to observe the self-healing properties by water assistance. Figure 4.3 to 4.7 shows the self-healing processed of that four different types of plastic that made up of gelatin and also the commercial plastic which was LLDPE that use as a controlled. As shown in the Figure 4.3, the fresh cracked of the film could be seen clearly under the microscope and faded or could not be seen clearly after several minutes of dropping the water on the films except for LLDPE film. The results of the healing processed in every two minutes of 10 minutes, one hour and after 24 hours were showed in second picture and afterwards of Figure 4.3 to 4.7 respectively. Second picture in Figure 4.3 to Figure 4.7 shows the condition of the films after 2 minutes of experiments in which showing the starting of the healing process of the gelatin based films meanwhile there were no change observed for LLDPE film. This is because of the new film segment that emerged between the gap due to the swelling of the damaged films and versatility of the polymer chains under the stimulus of water (Hu et al., 2018). The excellent self-

healing ability of the films is due to the high mobility of the polymer network that facilitates the reconstruction of Schiff base linkage under water assistance (Hu et al., 2018). Highly hydroscopic nature of gelatin will promote the tendency to swell or to be dissolved when it is put in contact with the surface or foodstuffs that have high moisture content (Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, 2011). The film were than observed after 1 hour and 24 hours of conducting the experiment to see the further results of the self-healing test. The results in Figure 4.3 to 4.7 show that the films were self-repairing but the healed surface appeared not as smooth as the original films. There were no significant effect of the film weather the gelatin based film were made up from commercial tilapia fish gelatin powder which were used in preliminary study or made from extract tilapia fish scale that were originally extracted by chemical pre-treatment and thermal denaturation at the laboratory. However, there were significant effect on the physical appearance and tensile properties of both films that made up from commercial tilapia fish gelatin powder and extract tilapia fish scale that were originally extracted by chemical pre-treatment and thermal denaturation when the films were added with glycerol. Glycerol that added to the films gave the plasticization effect in which resulting in good films that is not brittle and not easily tearing apart. The gelatin based films that are not added with glycerol appeared to be physically more brittle than the gelatin based films that added with glycerol. Recent

studies shows that the functional properties of the gelatin and the shelf life of the food products can be improve with the addition of different substances such as strengthening agent, cross-linkers, plasticizers or additives with antimicrobial or antioxidant properties (Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, 2011)



Time	Sample that have been cut until underlying substrate	Condition of the plastic after a drop of water is added under microscope
0 minutes	<p style="text-align: center;">A</p> 	<p style="text-align: center;">B</p> 
2 minutes	<p style="text-align: center;">C</p> 	<p style="text-align: center;">D</p> 
4 minutes	<p style="text-align: center;">E</p> 	<p style="text-align: center;">F</p> 

6 minutes	<p style="text-align: center;">G</p> 	<p style="text-align: center;">H</p> 
8 minutes	<p style="text-align: center;">I</p> 	<p style="text-align: center;">J</p> 
10 minutes	<p style="text-align: center;">K</p> 	<p style="text-align: center;">L</p> 
1 hour	<p style="text-align: center;">M</p> 	<p style="text-align: center;">N</p> 

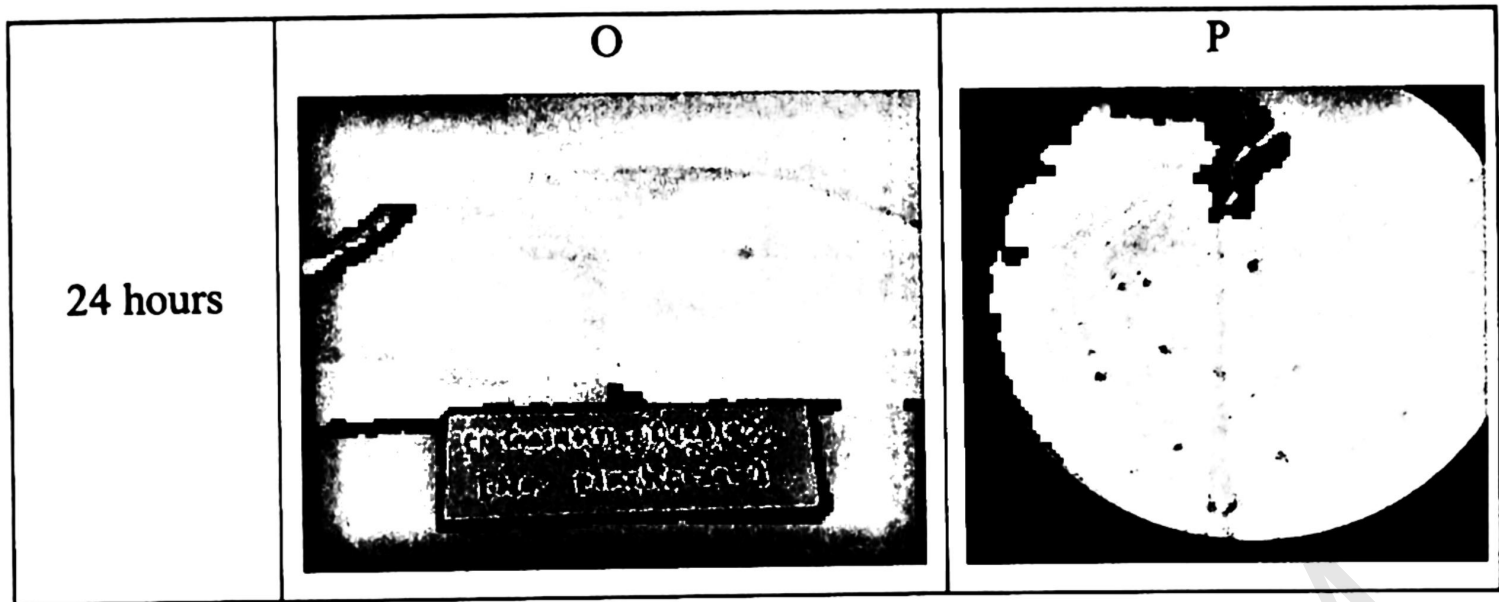
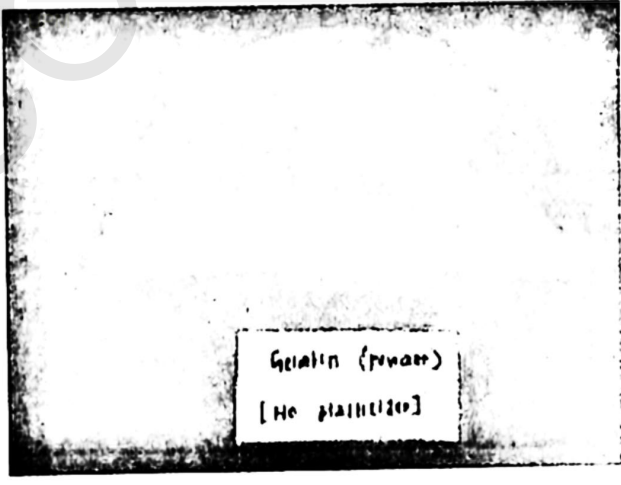

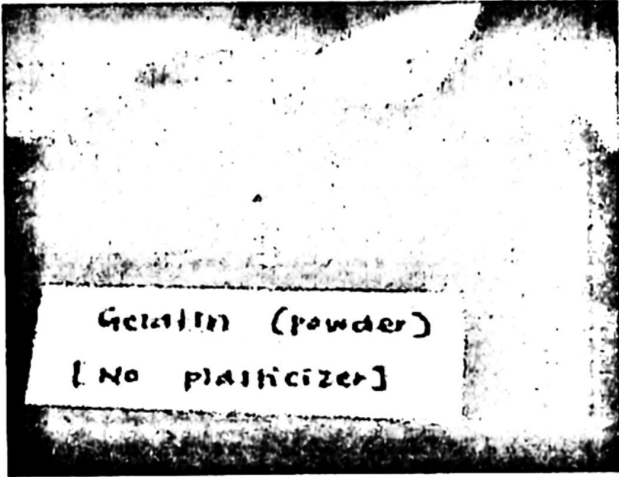

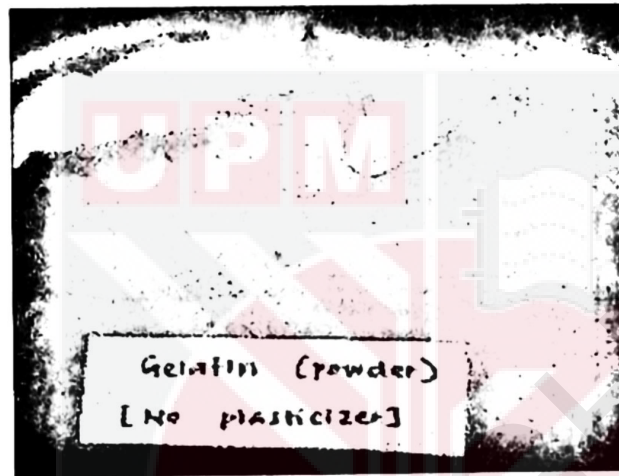

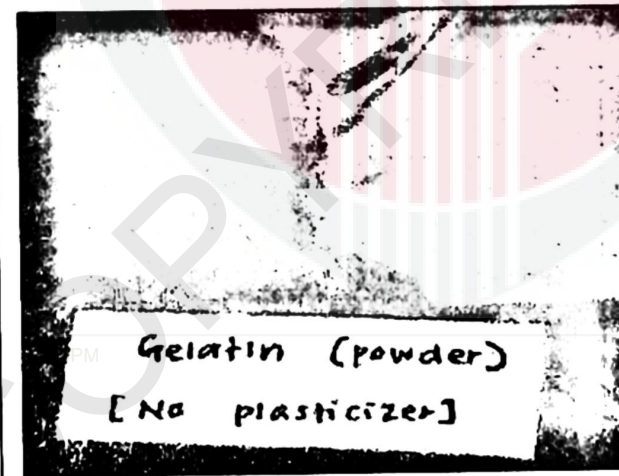

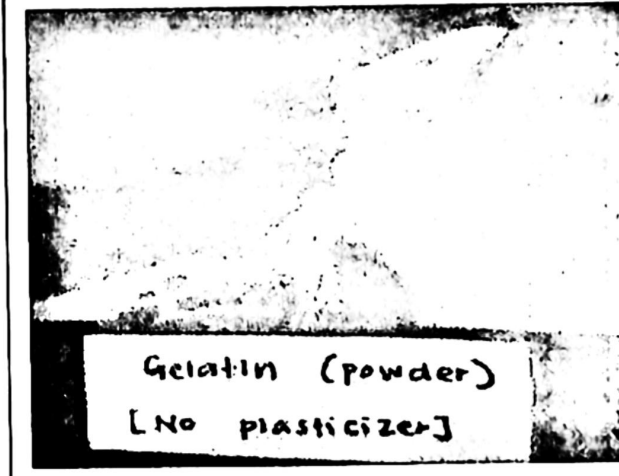



Figure 4.4: Gelatin film without glycerol (extract), (A,B = 0 minutes; C,D = 2 minutes; E,F = 4 minutes; G,H = 6 minutes; I,J = 8 minutes; K,L = 10 minutes ; M,N = 1 hour; O,P = 24 hours)

Time	Sample that have been cut until underlying substrate	Condition of the plastic after a drop of water is added under microscope
0 minutes	A 	B 

2 minutes	<p style="text-align: center;">C</p>  <p style="text-align: center;">Gelatin (powder) [No plasticizer]</p>	<p style="text-align: center;">D</p> 
4 minutes	<p style="text-align: center;">E</p>  <p style="text-align: center;">Gelatin (powder) [No plasticizer]</p>	<p style="text-align: center;">F</p> 
6 minutes	<p style="text-align: center;">G</p>  <p style="text-align: center;">Gelatin (powder) [No plasticizer]</p>	<p style="text-align: center;">H</p> 
8 minutes	<p style="text-align: center;">I</p>  <p style="text-align: center;">Gelatin (powder) [No plasticizer]</p>	<p style="text-align: center;">J</p> 

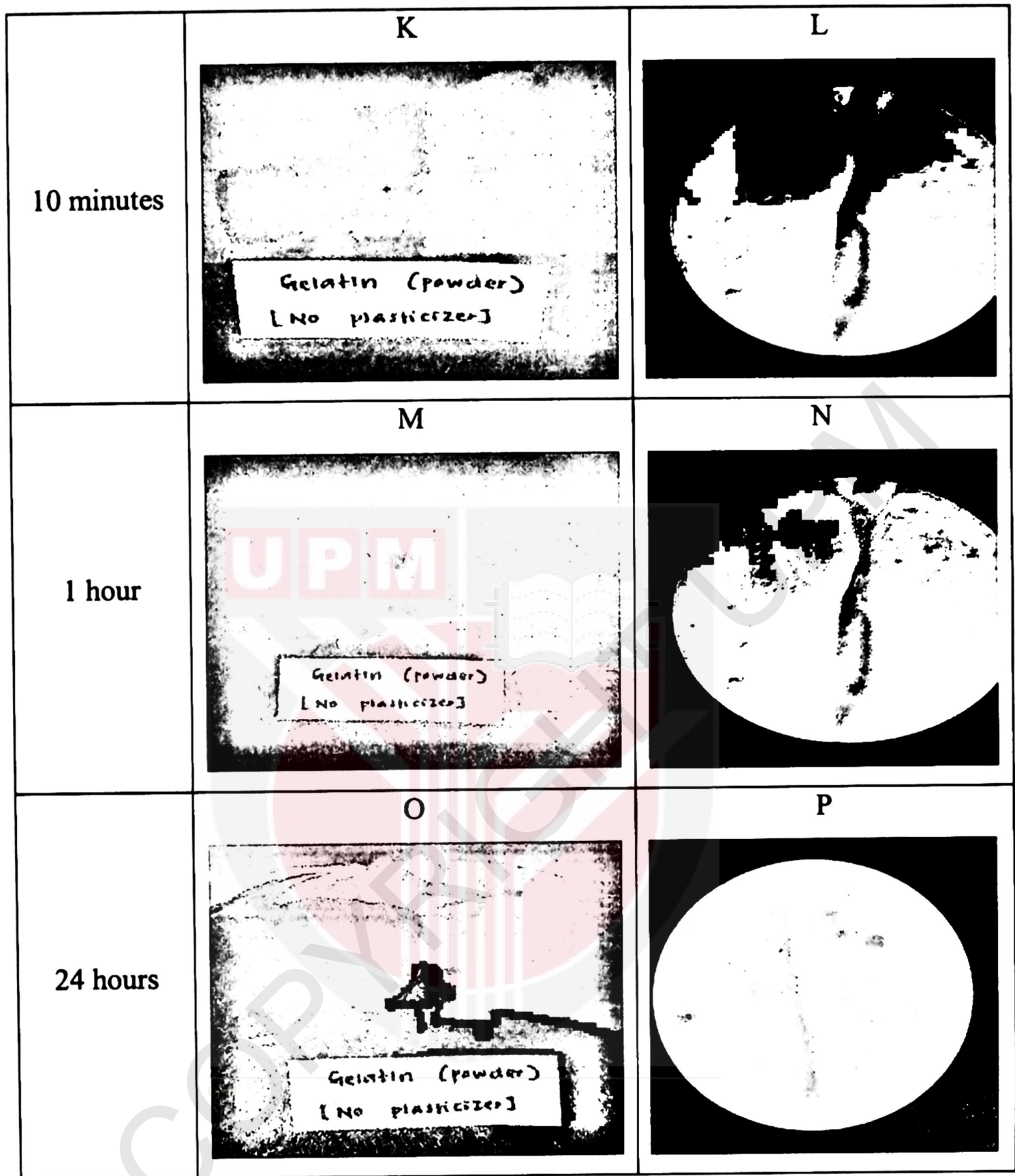
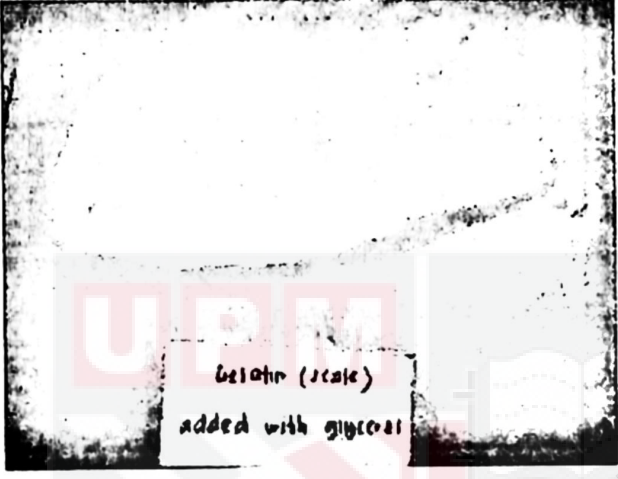
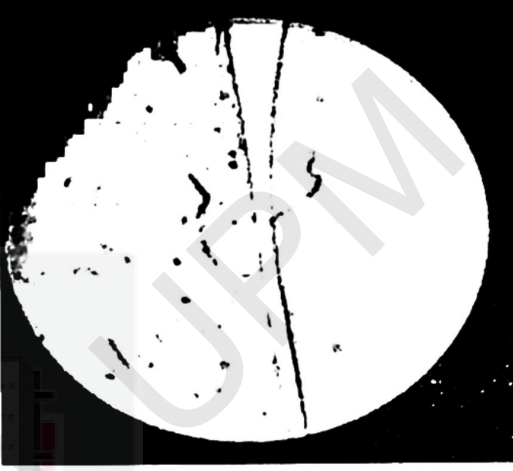
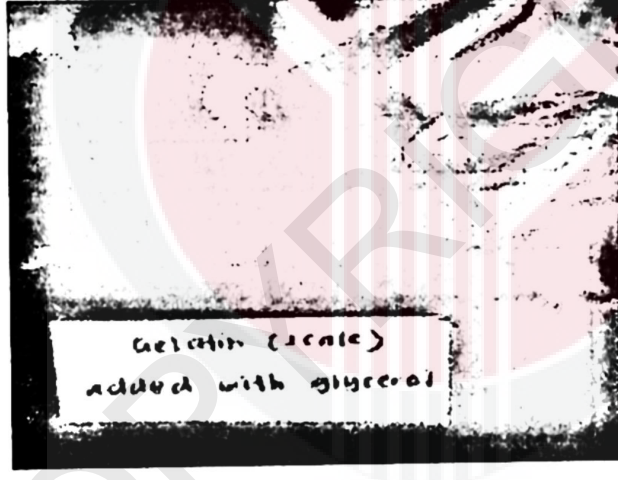

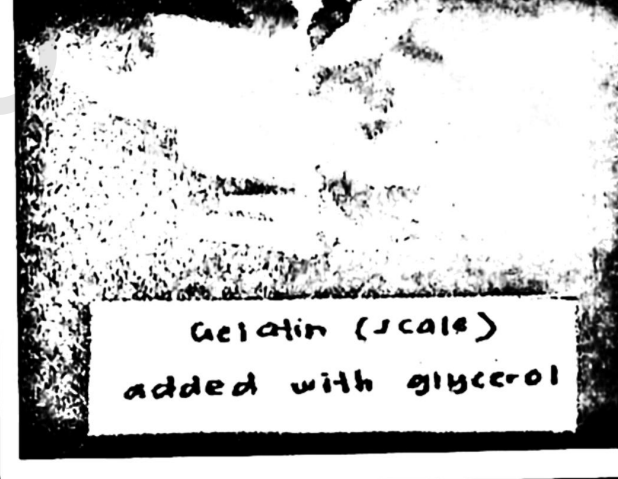

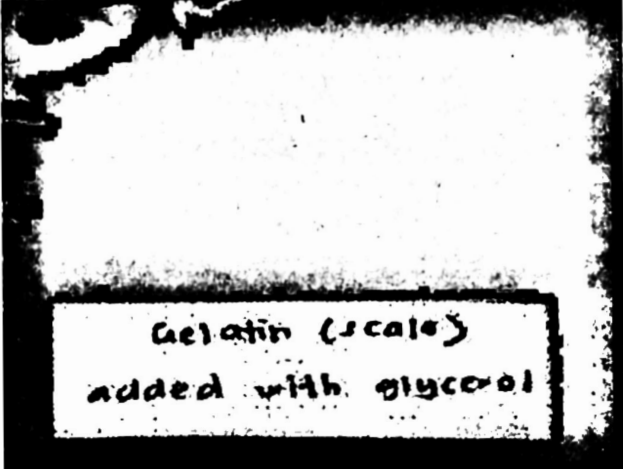
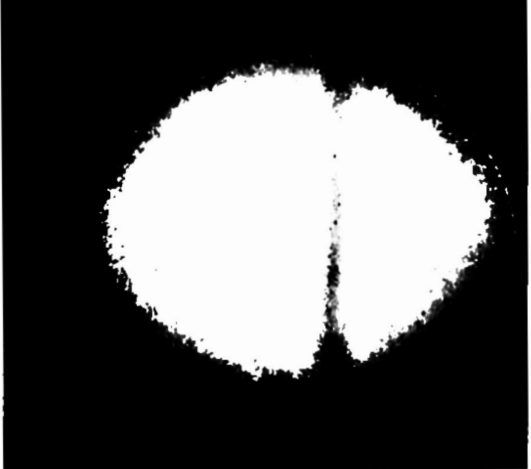
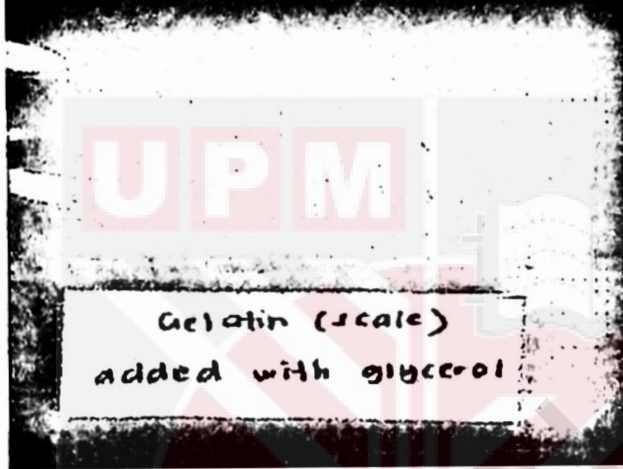

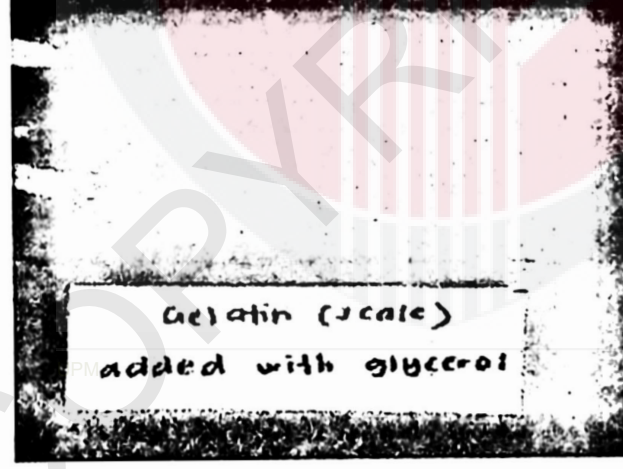
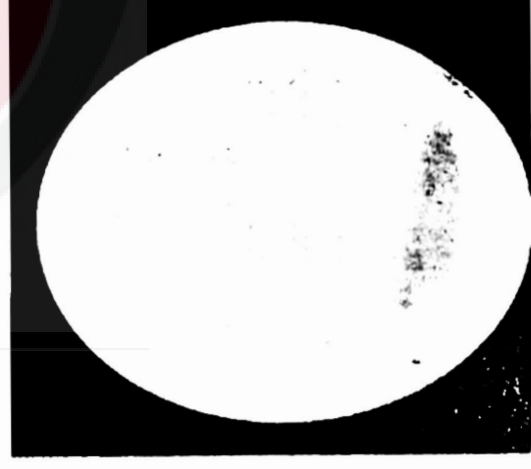
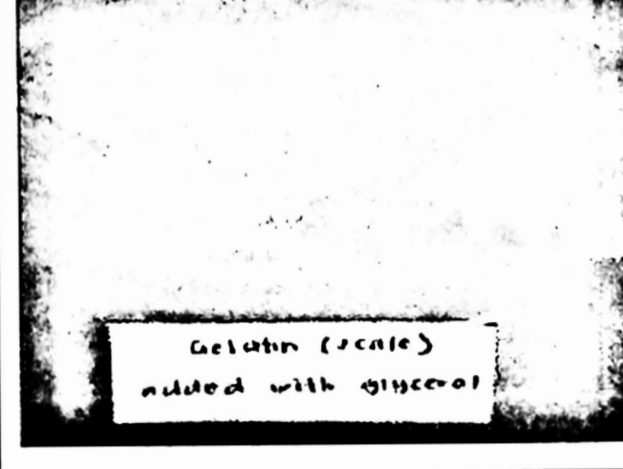
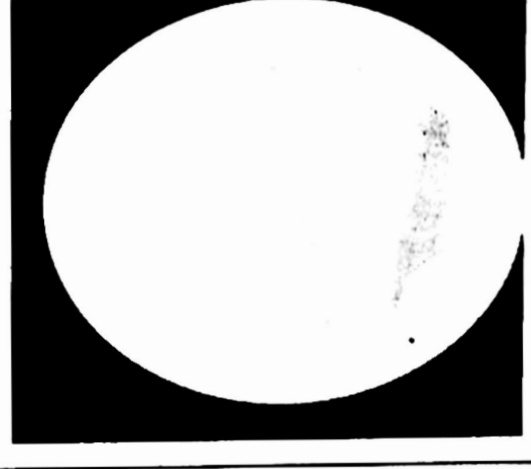


Figure 4.5: Gelatin film without glycerol (commercial) , (A,B= 0 minutes; C,D= 2 minutes; E,F = 4 minutes; G,H = 6 minutes; I,J = 8 minutes; K,L = 10 minutes ; M,N = 1 hour; O,P = 24 hours)

Time	Sample that have been cut until underlying substrate	Condition of the plastic after a drop of water is added under microscope
0 minutes	<p style="text-align: center;">A</p>  <p style="text-align: center;">Gelatin (scale) added with glycerol</p>	<p style="text-align: center;">B</p> 
2 minutes	<p style="text-align: center;">C</p>  <p style="text-align: center;">Gelatin (scale) added with glycerol</p>	<p style="text-align: center;">D</p> 
4 minutes	<p style="text-align: center;">E</p>  <p style="text-align: center;">Gelatin (scale) added with glycerol</p>	<p style="text-align: center;">F</p> 

6 minutes	<p style="text-align: center;">G</p> 	<p style="text-align: center;">H</p> 
8 minutes	<p style="text-align: center;">I</p> 	<p style="text-align: center;">J</p> 
10 minutes	<p style="text-align: center;">K</p> 	<p style="text-align: center;">L</p> 
1 hour	<p style="text-align: center;">M</p> 	<p style="text-align: center;">N</p> 

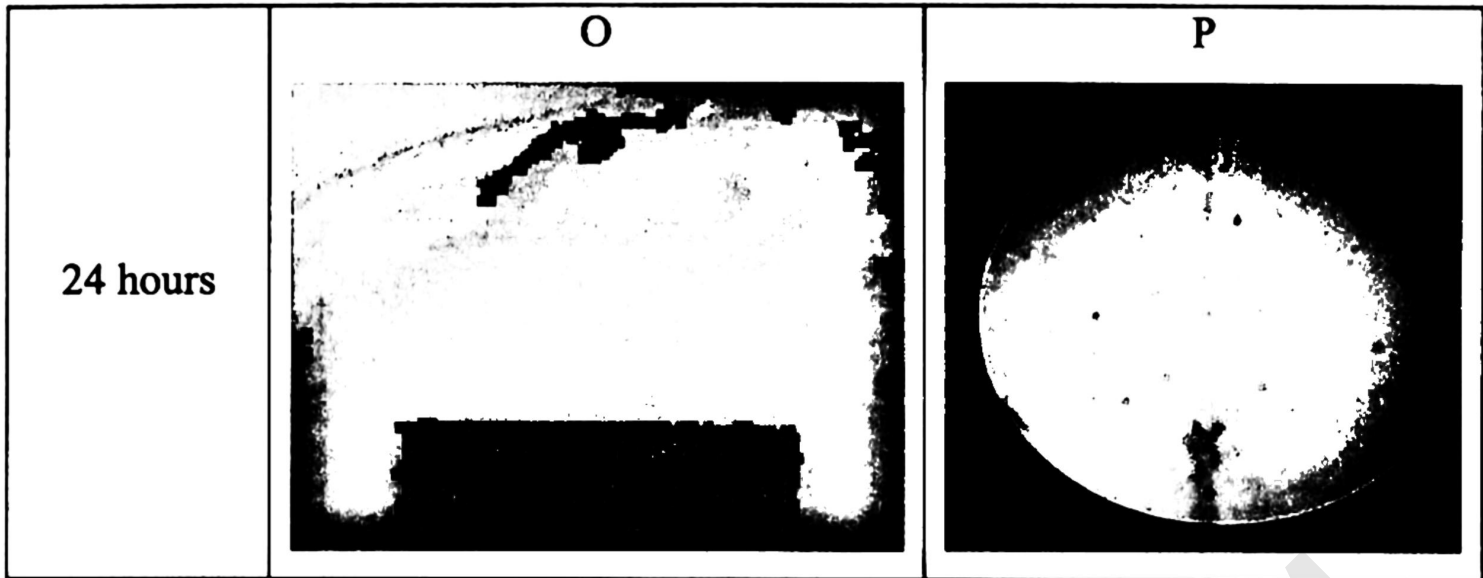
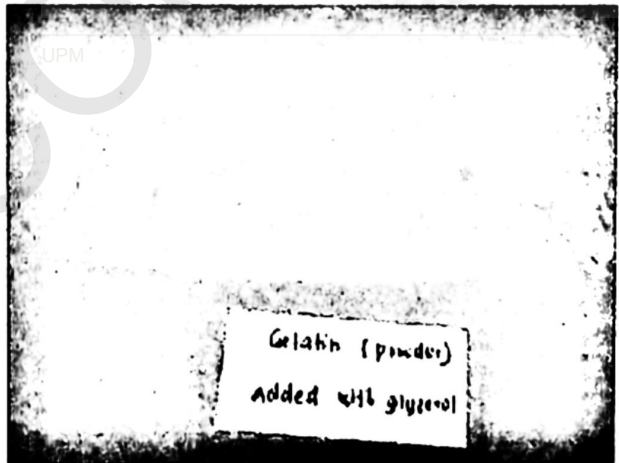
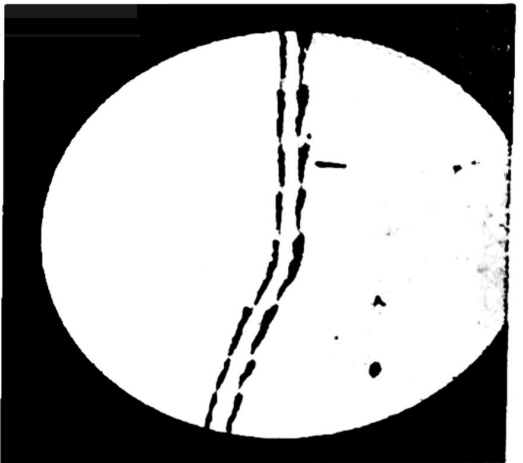
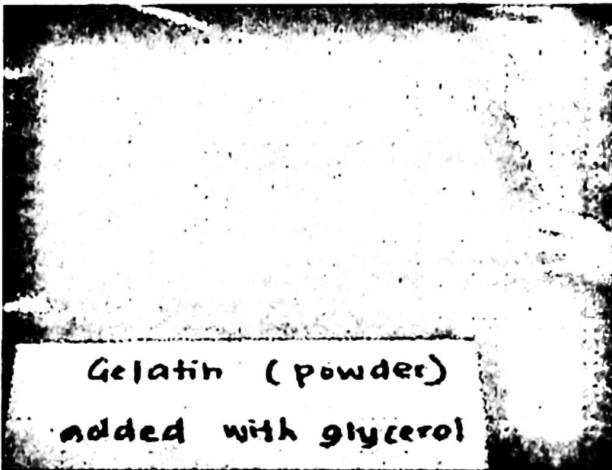
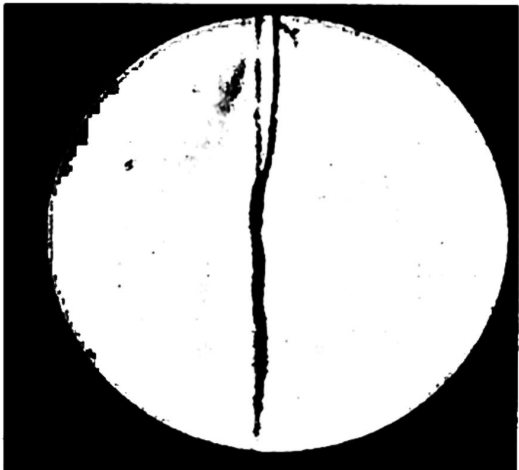
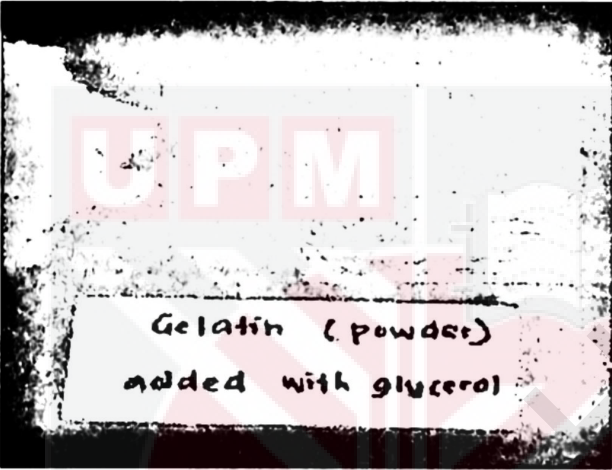

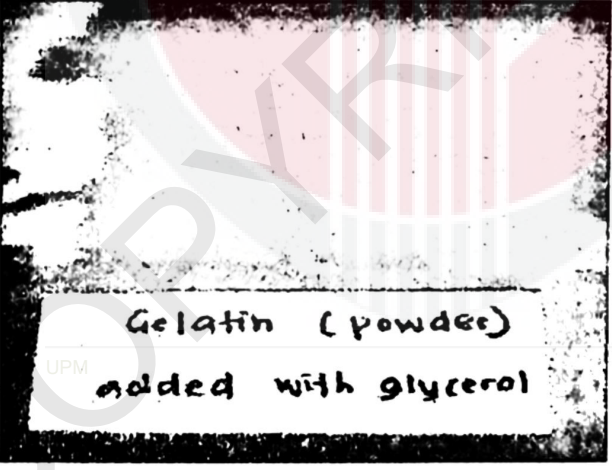

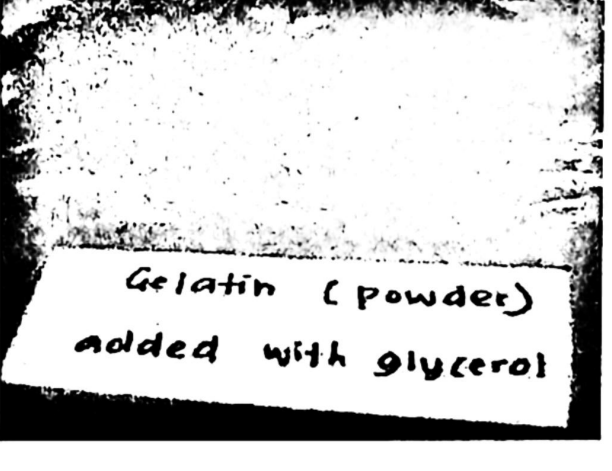



Figure 4.6: Gelatin film added with glycerol (extract), (A,B = 0 minutes; C,D= 2 minutes; E,F = 4 minutes; G,H = 6 minutes; I,J = 8 minutes; K,L = 10 minutes ; M,N = 1 hour; O,P = 24 hours)

Time	Sample that have been cut until underlying substrate	Condition of the plastic after a drop of water is added under microscope
0 minutes	A 	B 

<p>2 minutes</p>	<p>C</p> 	<p>D</p> 
<p>4 minutes</p>	<p>E</p> 	<p>F</p> 
<p>6 minutes</p>	<p>G</p> 	<p>H</p> 
<p>8 minutes</p>	<p>I</p> 	<p>J</p> 

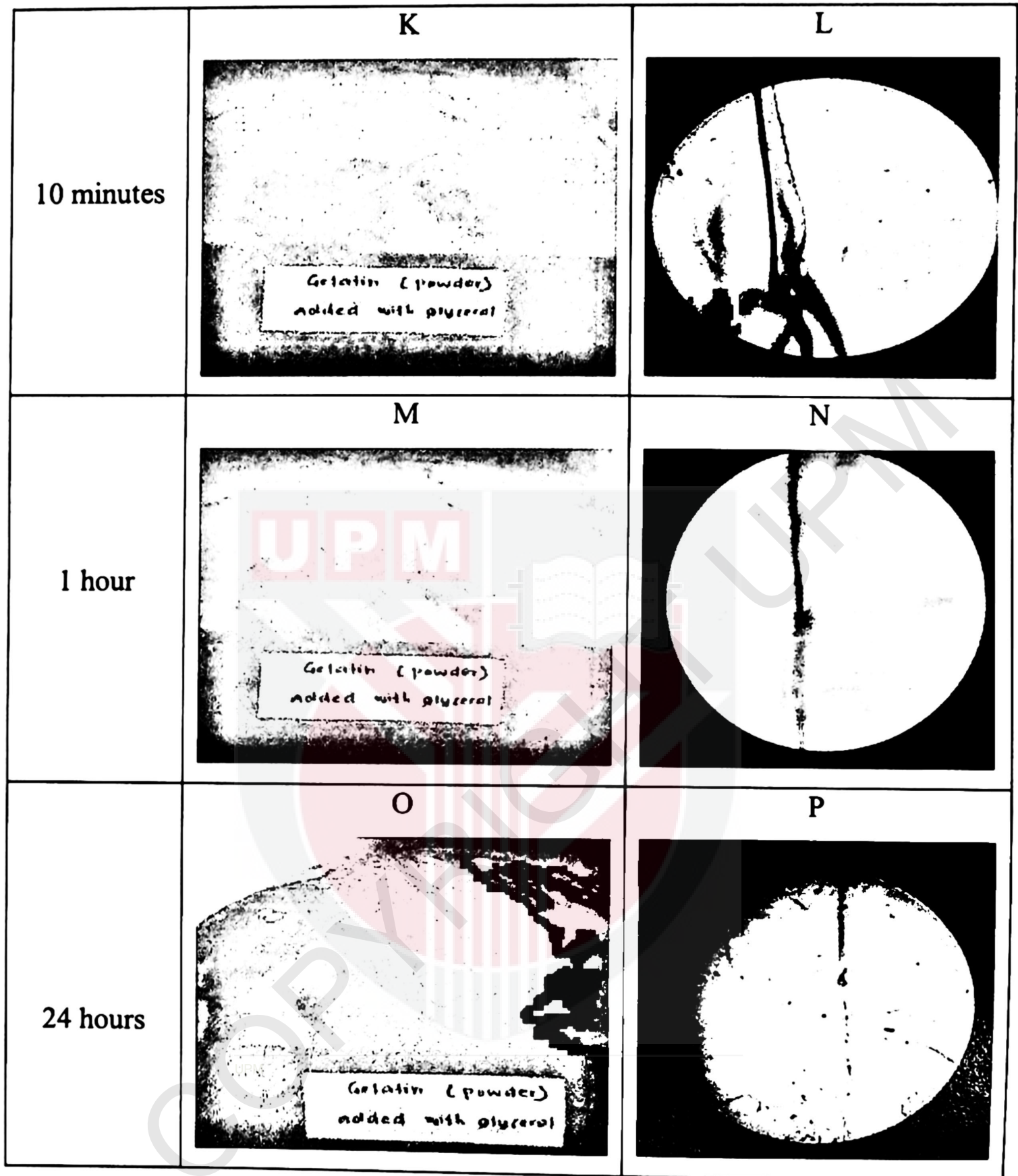



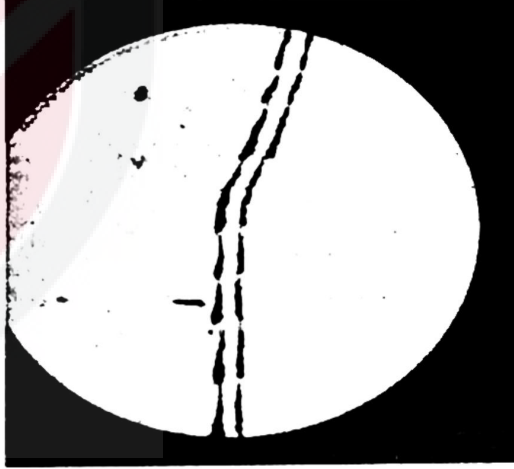
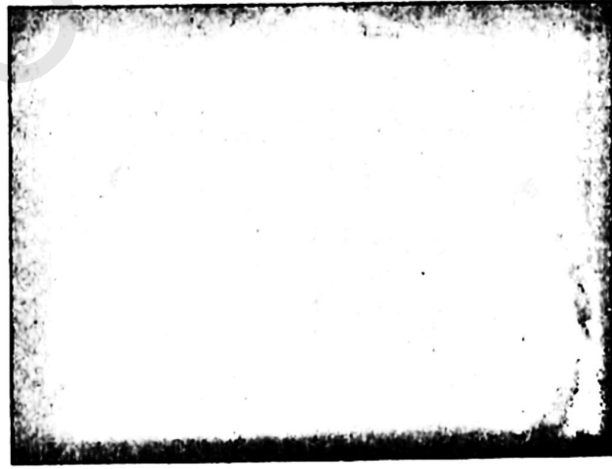
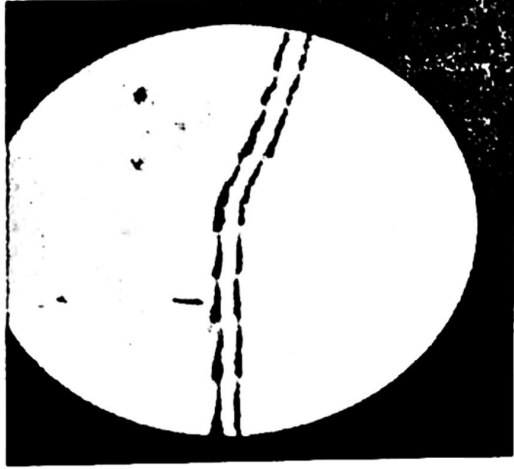

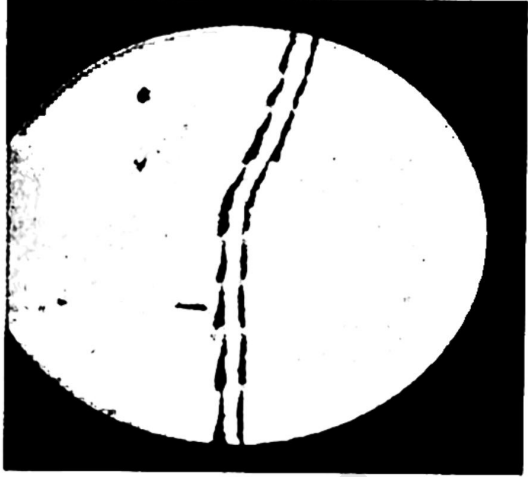
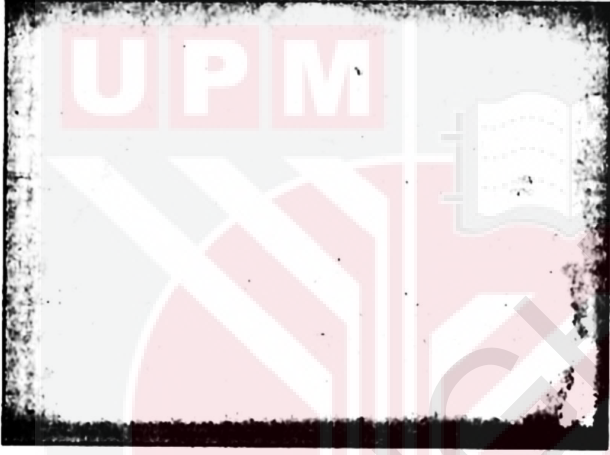


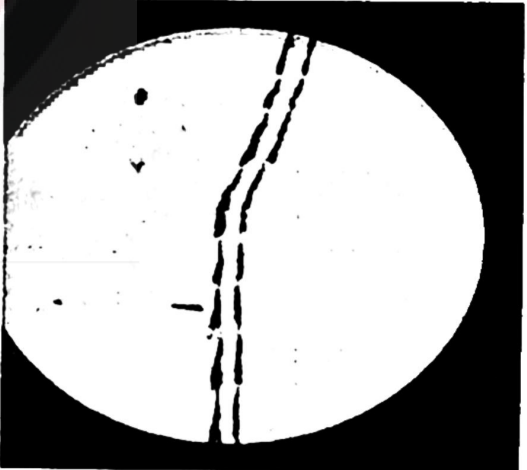
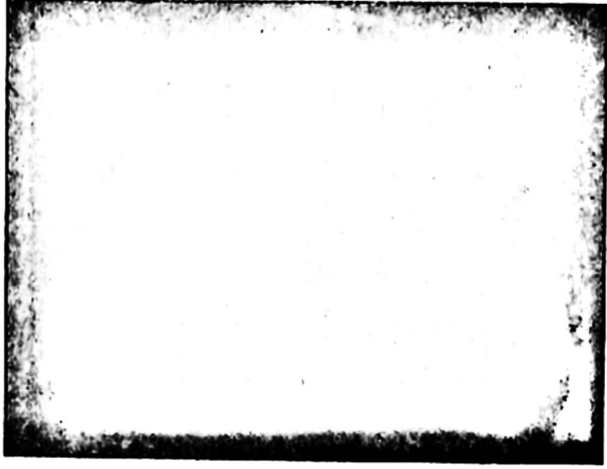
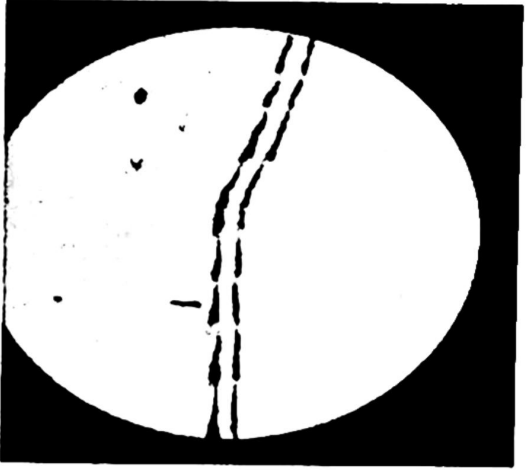


Figure 4.7: Gelatin film added with glycerol (extract), (A,B = 0 minutes; C,D = 2 minutes; D,E = 4 minutes; E,F = 6 minutes; G,H= 8 minutes; I,J = 10 minutes ; K,L = 1 hour; M,N = 24 hours)

Time	Sample that have been cut until underlying substrate	Condition of the plastic after a drop of water is added under microscope
0 minutes	<p style="text-align: center;">A</p> 	<p style="text-align: center;">B</p> 
2 minutes	<p style="text-align: center;">C</p> 	<p style="text-align: center;">D</p> 
4 minutes	<p style="text-align: center;">E</p> 	<p style="text-align: center;">F</p> 

6 minutes	<p style="text-align: center;">G</p> 	<p style="text-align: center;">H</p> 
8 minutes	<p style="text-align: center;">I</p> 	<p style="text-align: center;">J</p> 
10 minutes	<p style="text-align: center;">K</p> 	<p style="text-align: center;">L</p> 
1 hour	<p style="text-align: center;">M</p> 	<p style="text-align: center;">N</p> 

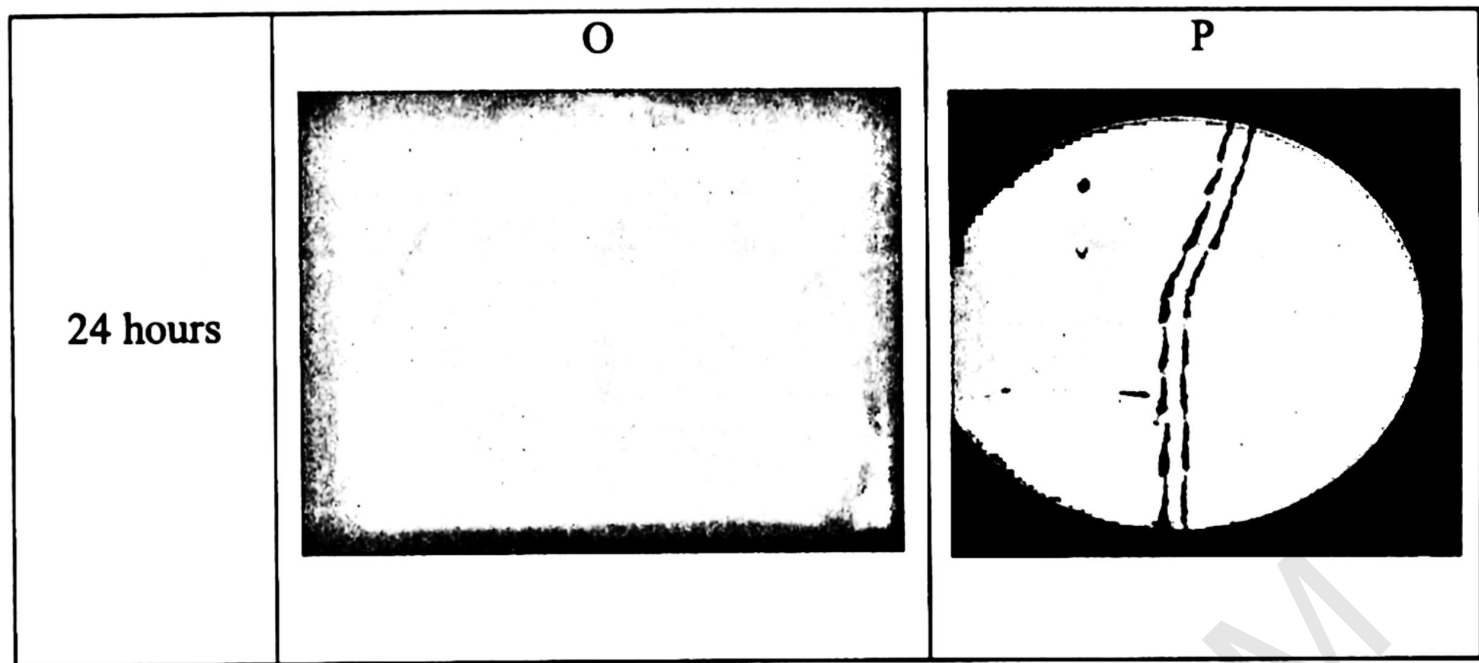


Figure 4.8: Linear low density polyethylene (LLDPE) (A,B = 0 minutes; C,D = 2 minutes; D,E = 4 minutes; E,F = 6 minutes; G,H= 8 minutes; I,J = 10 minutes; K,L = 1 hour; M,N = 24 hours)

4.6 Fogging Test

The fogging test had been done by using a piece of mushroom in order to observe the fogging behaviour of four types of gelatin based film which are commercial gelatin, gelatin extracted from scale, commercial gelatin and added with glycerol, gelatin extracted from scale and added with glycerol and linear low density film (LLDPE). Figure 4.8 shows the picture taken before the fogging test were done.

It can be seen clearly that there were no fog for all types of plastic film that used. After the 24 hours of fogging test that had been done referring to Figure 4.9, the four types of gelatin based films show no fog and the mushroom in the container can be seen clearly while the LLDPE film shows the fog behaviour which cover the appearance of

the mushroom in the container. This was probably because of the hydrophilic nature and water absorbing ability of the films. The antifogging property of the films were derived from its hydrophilic behaviour and its ability to absorb water. The water molecules in the condensed droplets is said to be quickly absorbed into the hydrophilic domains of the film by hydrogen bonds and dipole-dipole interaction. Meanwhile the water droplets that not being absorb is said to form a thin sheets-like layer on the surface of the smooth film which reduced light scattering (Hu et al., 2018).

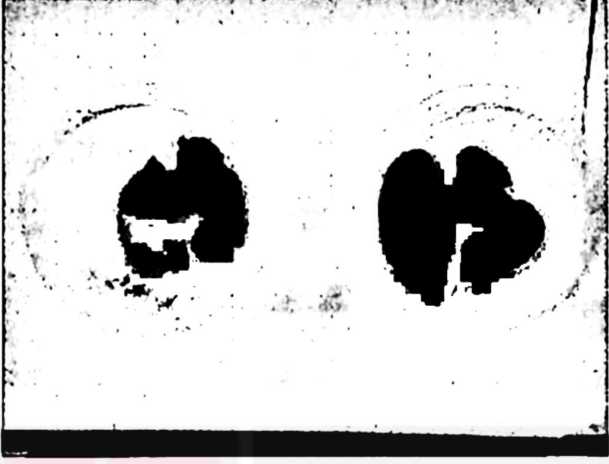
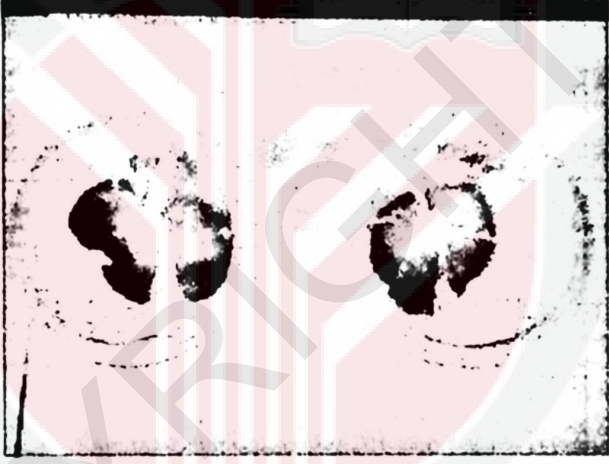

Then, the mushroom (1 piece) were replaced with new mushrooms (3 pieces). The study on different pieces of mushroom were only to observe and to capture a clear fogging behaviour of the films. The results in Figure 4.11 shows that the gelatin based plastic films still maintaining its antifogging behaviour while the linear low density (LLDPE) film shows heavy fogging than before (with on piece of mushroom). It is clearly showed that the gelatin based plastic films have good permeability to oxygen and carbon dioxide, water vapour and flavours for fruits and vegetable (Alfaro et al., 2014). Based on previous study, it is stated that gelatin based films can be used to coat

fruits and vegetables in order to retard the degradation process that occurred due to the transportation oxygen (O₂), carbon dioxide (CO₂) and water vapour. It is also clearly stated that glycerol and sorbitol are hydrophilic plasticizers that increase the water vapour permeability of hydrocolloids based films (Al-Hassan & Norziah, 2012a). Al-Hassan & Norziah (2012) study also reported that both types of films (starch/gelatin) with either sorbitol or glycerol added showed lower water vapour permeability (WVP) value in which around (1.8-2.2) g.mm/m².h.Pa x 10⁴. Based on the results showed, the gelatin films with and without glycerol have lower water vapour permeability which incorporating to no fog occurred when the mushrooms were coated with the gelatin based films. Previous study had stated that 90% of the weight of freshly harvested mushrooms is make up of water and they give off large amounts of water vapour due to their porous skins. Current packaging that being used only allow gases to pass through a plastic film but do not allow enough water vapour from the respiring mushrooms to flow out in which too much moisture builds up in the package would spoil the mushrooms. Moisture build up result in high-in package humidity which due to condensation that happened on the film that is driven by the temperature fluctuations. In order to retard degradation processes due to the excellent transport of gases (O₂ and CO₂) and water vapour, fruit and vegetables also can be coated with gelatin based film (Soulestin et al., 2011). Potential applications of gelatin edible films in the food industry may include the transport of gases (O₂ and CO₂), water vapour, and flavour for fruits and vegetables. Most commonly, gelatin is use in packaging of highly perishable food products such as meat and fish. It is based on some particular properties that gelatin have such as minimal cost needs, its availability, functional attributes, mechanical (flexibility, tension) and optical (brightness and opacity) properties, barrier effect against gas flow, structural resistance to water and

microorganisms and sensory acceptability (Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, 2011)



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Type of plastic	Sample	Description
<p>1. Gelatin film (extract)</p>		<p>Clear</p>
<p>2. Gelatin film (commercial)</p>		<p>Clear</p>
<p>3. Gelatin film added with glycerol (extract)</p>		<p>Clear</p>






<p>4. Gelatin film added with glycerol (commercial)</p>		<p>Clear</p>
<p>5. Linear low density polyethylene</p>		<p>Clear</p>

Figure 4. 9: Before fogging test for 1 piece of mushroom

Type of plastic	Sample	Description
1. Gelatin film (extract)		Clear
2. Gelatin film (commercial)		Clear
3. Gelatin film added with glycerol (extract)		Clear






<p>4. Gelatin film added with glycerol (commercial)</p>		<p>Clear</p>
<p>5. Linear low density polyethylene (LLDPE)</p>		<p>Not clear (fogging)</p>

Figure 4.10: After fogging test for 1 piece of mushroom (put in the chiller for more than 24 hours, at 2 to 5 °C)

Type of plastic	Sample	Description
1. Gelatin film (extract)		Clear
2. Gelatin film (powder)		Clear
3. Gelatin film added with glycerol (extract)		Clear



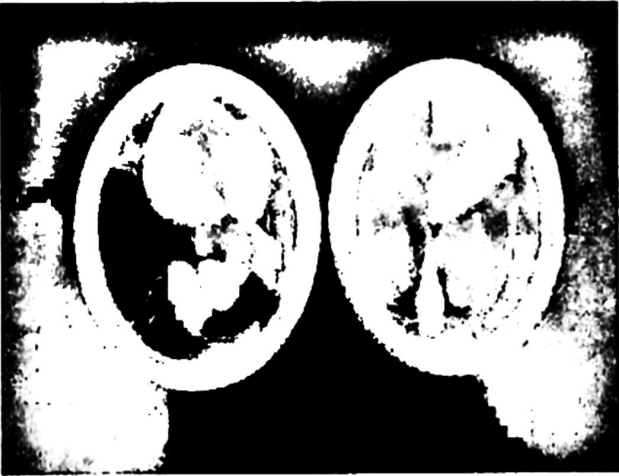
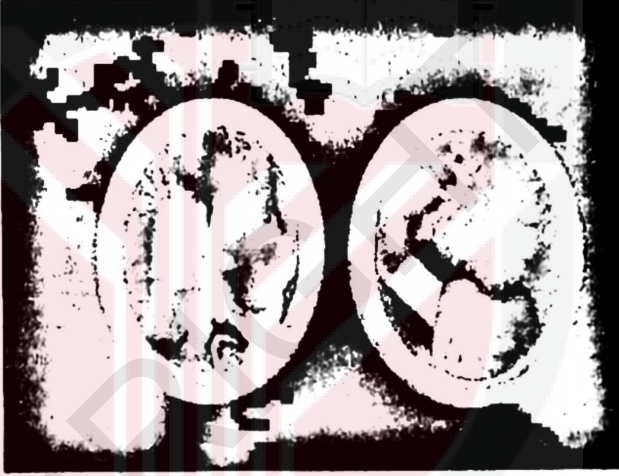

<p>4. Gelatin film added with glycerol (powder)</p>		<p>Clear</p>
<p>5. Linear low density polyethylene (LLDPE)</p>		<p>Clear</p>

Figure 4.11: Before fogging test for 3 pieces of mushroom

Type of plastic	Sample	Description
1. Gelatin film (extract)		Clear
2. Gelatin film (powder)		Clear
3. Gelatin film added with glycerol (extract)		Clear



<p>4. Gelatin film added with glycerol (powder)</p>		<p>Clear</p>
<p>5. Linear low density polyethylene (LLDPE)</p>		<p>Not clear (fogging)</p>

Figure 4.12: After fogging test for 3 pieces of mushroom (put in the chiller for more than 24 hours, at 2 to 5 °C)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Objective of this study has been achieved by conducting mechanical test, thermo-gravimetric analysis (TGA), and differential scanning calorimetry (DSC). The results of the experiments showed that gelatin films added with glycerol had lower tensile strength compared to gelatin film without glycerol in which the tensile strength of the gelatin without glycerol was approximately 69.04 MPa with 3.3% elongation at break (EAB) while gelatin that added with glycerol has a tensile and elongation at break (EAB) value of 14.98 MPa and 95.5% respectively. TGA analysis showed that that gelatin film that added with glycerol had a lower weight losses which was 46.01% as compared to gelatin film without glycerol that had a weight loss of 51.08% at temperature of 455.2°C and 486.6°C respectively. Gelatin film that added with glycerol had good thermal stability compared to gelatin film without glycerol as referred to the decomposition temperature and the incorporation of the weight loss of both type of films. On the other hand based on DSC analysis, gelatin film that added with glycerol had T_g and T_m value of 28.3°C and 41.0°C respectively while gelatin based film without

glycerol had T_g and T_m value of 36.5°C and 92.5°C. Lower T_g and T_m value of gelatin film that added with glycerol was due to the ability of the plasticizer (glycerol) to penetrate between the polymer chains in order to weaken the interaction between proteins.

Furthermore, self-healing also has been achieved in which the gelatin based films was a self-healing biopolymer that were able to heal injury and can restore the film function. The results of the experiment show the progress of the self-healing process of the gelatin based films in several minutes and after 24 hours. Anti-fogging properties of the gelatin based films also have been achieved in which the gelatin based films have low oxygen and water vapour barrier that helps in maintain the freshness of the vegetables or food that being covered or coated with the films. From this study, gelatin based films maintain the freshness of mushrooms well rather than low density polyethylene (LDPE). Moreover the incorporated hydrophilic plasticizer increase the water vapour permeability of the hydrocolloids based films that act as an added value to the films. It also can be conclude that the materials that can self-heal also has the antifogging behaviour due to their hydrophilic nature.

5.2 RECOMMENDATION

As for the recommendation for the future studies of the gelatin based films, it is recommended to acknowledge more types of gelatin sources rather than only one source that can be recycle into something useful such as edible coating food. It is because gelatin is a protein that are produce abundantly and have a good film forming properties that is generally low cost (Shehap et al., 2015). Therefore it can sustain our living by producing plastic films from renewable energy sources and can help in reducing waste.

Other than that, further research on the cross linkers that can help in inducing the self-healing behaviour of the films is also one of the recommended studies as it can improve the time taken for the self-healing process of the films. It is important because films serve as protection for food products and it need to be improve so that it can reduce cross contamination problems regarding food products when there is tear occur to the plastic films.

Moreover, further research on antimicrobial properties that can be added value to the gelatin films. As we know, antimicrobial agent is recently added to the packaging films so that it can avoid any contamination due to microbes. Regarding this matter, antimicrobial agent that suitable to the gelatin films need to be choose so that it can give advantage without affecting the physical appearances of the gelatin films itself.

Lastly, improvement on testing of mechanical resistance and self-healing ability of the films using quantitative analysis such as abrasion tests that monitored by relative optical transmission. Meanwhile, improvement on anti-fogging test using

water vapour permeability (WVP) and oxygen transmission rate (OTR) in order to have a significant value about the gelatin films.



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REFERENCES

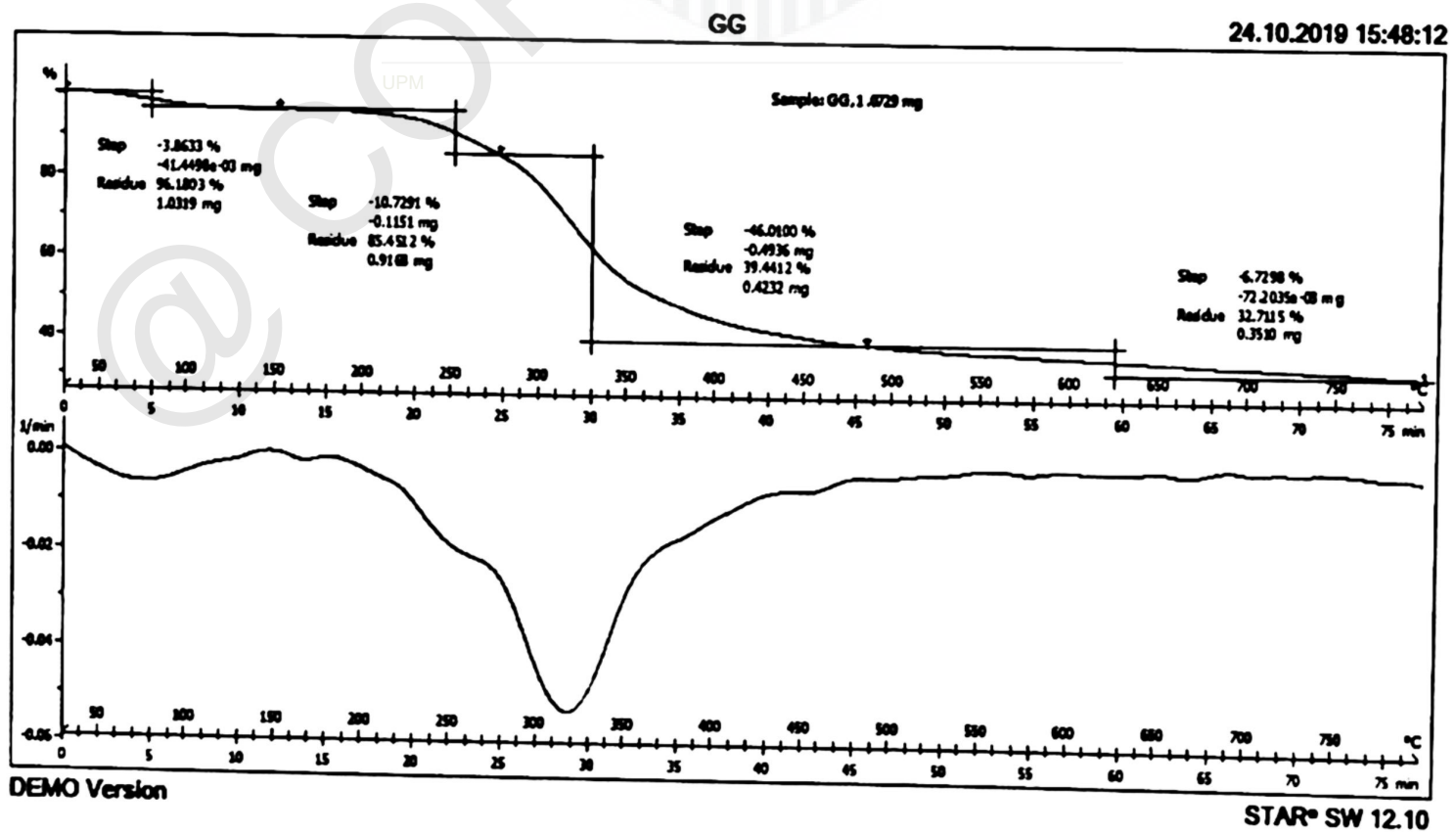
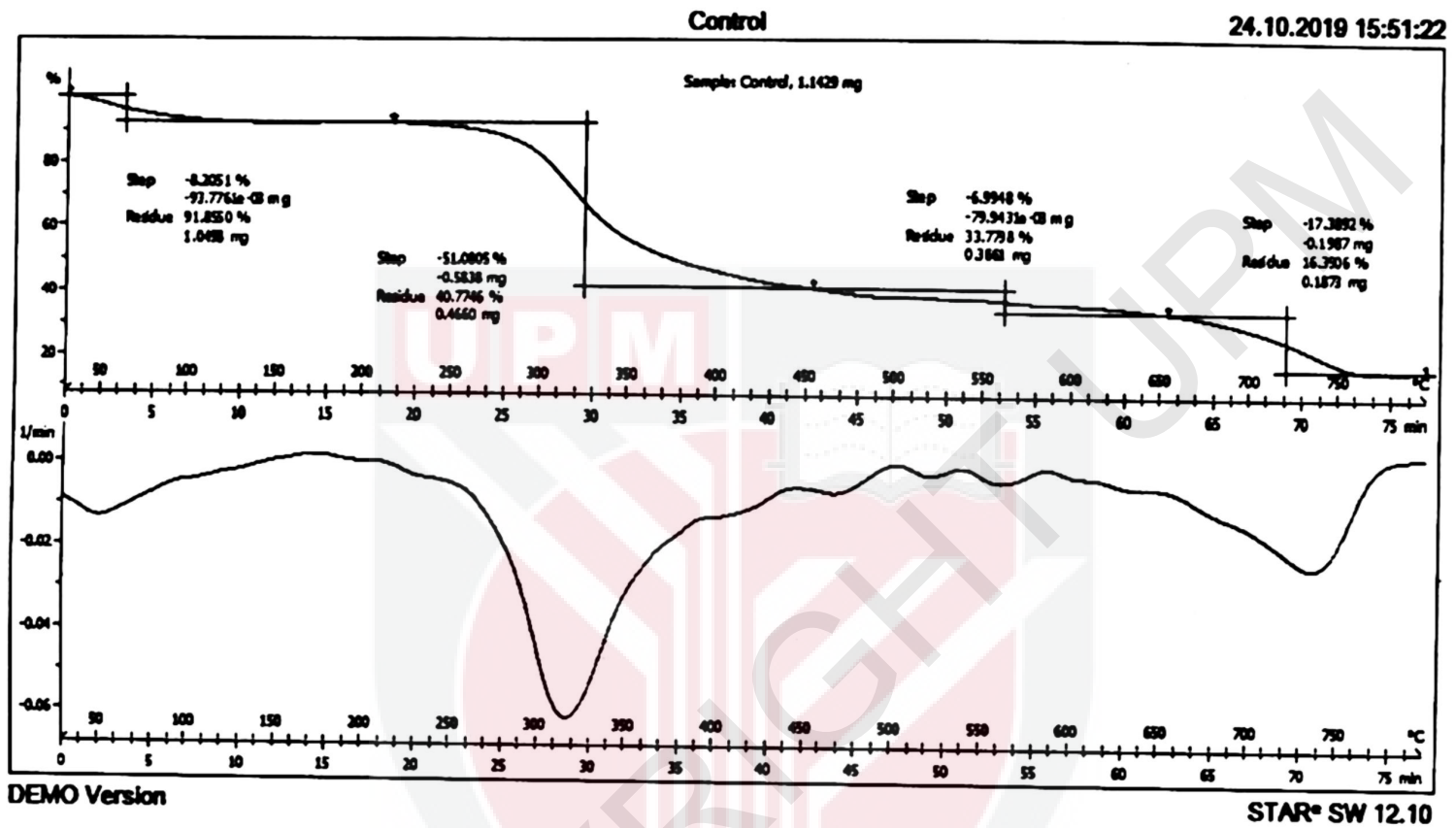
- Al-Hassan, A. A., & Norziah, M. H. (2012a). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, 26(1), 108–117. <https://doi.org/10.1016/j.foodhyd.2011.04.015>
- Al-Hassan, A. A., & Norziah, M. H. (2012b). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*. <https://doi.org/10.1016/j.foodhyd.2011.04.015>
- Alfaro, A. da T., Balbinot, E., Weber, C. I., Tonial, I. B., & Machado-Lunkes, A. (2014). Fish Gelatin: Characteristics, Functional Properties, Applications and Future Potentials. *Food Engineering Reviews*, 7(1), 33–44. <https://doi.org/10.1007/s12393-014-9096-5>
- Applications, B. (2019). *Hydrogels Based on Schiff Base Linkages for*. 1–21.
- Chiou, B. Sen, Avena-Bustillos, R. J., Bechtel, P. J., Jafri, H., Narayan, R., Imam, S. H., ... Orts, W. J. (2008). Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. *European Polymer Journal*, 44(11), 3748–3753. <https://doi.org/10.1016/j.eurpolymj.2008.08.011>
- El-hefian, E. A., Nasef, M. M., & Yahaya, A. H. (2012). *Preparation and Characterization of Chitosan / Agar Blended Films: Part 2 . Thermal , Mechanical , and Surface Properties*. 9(2), 510–516.
- Etxabide, A., Uranga, J., Guerrero, P., & de la Caba, K. (2017). Development of active gelatin films by means of valorisation of food processing waste: A review. *Food Hydrocolloids*, 68, 192–198. <https://doi.org/10.1016/j.foodhyd.2016.08.021>

- Gross, R. A., & Kalra, B. (2002). Biodegradable polymers for the environment. *Science*, 297(5582), 803–807. <https://doi.org/10.1126/science.297.5582.803>
- Hanani, Z. A. N. (2015a). Gelatin. *Encyclopedia of Food and Health*, 191–195. <https://doi.org/10.1016/B978-0-12-384947-2.00347-0>
- Hanani, Z. A. N. (2015b). Gelatin. In *Encyclopedia of Food and Health* (pp. 191–195). <https://doi.org/10.1016/B978-0-12-384947-2.00347-0>
- Hu, B., Chen, L., Lan, S., Ren, P., Wu, S., Liu, X., ... Ding, F. (2018). Layer-by-layer assembly of polysaccharide films with self-healing and antifogging properties for food packaging applications. *ACS Applied Nano Materials*, 1(7), 3733–3740. <https://doi.org/10.1021/acsanm.8b01009>
- Karim, A. A., & Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, 23(3), 563–576. <https://doi.org/10.1016/j.foodhyd.2008.07.002>
- Liang, J., Xia, Q., Wang, S., Li, J., Huang, Q., & Ludescher, R. D. (2015). Influence of glycerol on the molecular mobility, oxygen permeability and microstructure of amorphous zein films. *Food Hydrocolloids*, 44, 94–100. <https://doi.org/10.1016/j.foodhyd.2014.09.002>
- Mendieta-Taboada, O., Sobral, P. J. do A., Carvalho, R. A., & Habitante, A. M. B. Q. (2008). Thermomechanical properties of biodegradable films based on blends of gelatin and poly(vinyl alcohol). *Food Hydrocolloids*, 22(8), 1485–1492. <https://doi.org/10.1016/j.foodhyd.2007.10.001>

- Pettignano, A., Häring, M., Bernardi, L., Tanchoux, N., Quignard, F., & Díaz Díaz, D. (2017). Self-healing alginate-gelatin biohydrogels based on dynamic covalent chemistry: Elucidation of key parameters. *Materials Chemistry Frontiers*, 1(1), 73–79. <https://doi.org/10.1039/c6qm00066e>
- Rivero, S., García, M. A., & Pinotti, A. (2010). Correlations between structural , barrier , thermal and mechanical properties of plasticized gelatin fi lms. *Innovative Food Science and Emerging Technologies*, 11(2), 369–375. <https://doi.org/10.1016/j.ifset.2009.07.005>
- Shehap, A. M., Mahmoud, K. H., Abd El-Kader, M. F. H., & El-Basheer, T. M. (2015). Preparation and Thermal Properties of Gelatin/TGS Composite Films. *Middle East Journal of Applied Sciences*, 5(1), 157–170.
- Soulestin, J., Prashantha, K., Lacrampe, M.F., Krawczak, P. (2011). Packaging Applications. *Handbook of Bioplastics and Biocomposites Engineering Applications*, 77–120. <https://doi.org/10.3390/coatings6040041>
- Therriault, D., Haddad, E., & Jamroz, W. (2012). *Self-Healing Materials Systems : Overview of Major. 2012*. <https://doi.org/10.1155/2012/854203>

APPENDICES

Appendix 1: Thermo-gravimetric Analysis (TGA) for gelatin film without glycerol and with glycerol



Appendix 2: Differential Scanning Calorimetry (DSC) for gelatin without glycerol
and with glycerol

