THE EFFECTS OF TANNIC ACID ON PHYSICOCHEMICAL, BARRIER, AND MECHANICAL PROPERTIES OF BOVINE GELATIN FILMS

by

FARAH NABILA BINTI MOHD SHAFULLAH

A dissertation submitted in partial fulfilment of the requirement for the Degree of
Bachelor of Technology (B. Tech) in the field of Food Technology
School of Industrial Technology
Universiti Sains Malaysia

August 2021

PUSAT PENGAJIAN TEKNOLOGI INDUSTRI UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN DISERTASI MUTAKHIR SATU (1) NASKAH

Nama penyelia: Dr. Abdorreza Mohammadi Nafchi

Bahagian: Teknologi Makanan

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh

Encik/Puan/Cik Farah Nabila Binti Mohd Shafullah

mengenai disertasinya sebagaimana yang dipersetujui oleh Panel Pemeriksa di *Viva Voce*-nya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembetulan/pindaan yang dilaksanakan oleh calon.

Sekian, terima kasih.

A. Mohammadi

(Tandatangan dan cop)

21/08/2021

(Tarikh)

DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person expect where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my search project and does not include a substantial part of my work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

Farah Nabila

……

FARAH NABILA BINTI MOHD SHAFULLAH

AUGUST 2021

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my sincere gratitude to my final year project supervisor, Dr. Abdorreza Mohammadi Nafchi for his dedication, valuable advice, constant supervision and guidance throughout this research and the writing of this dissertation. Without his dedication, this thesis would not be possible.

A special word of thank to laboratory assistants of School of Industrial Technology especially Mr. Maarof, and Mr Rahim for all the helps given throughout this research was carried out. Moreover, I also would like to express my appreciation to my final year project partner, Sazereen binti Amir and post-graduate student, Ms. Nazila for accompanying and help me during the project. This project would not be successfully accomplished without all the helps and guidance from everyone.

Last but not least, I would like to thank my beloved family members all my friends who has support me continuously throughout this project.

Farah Nabila Binti Mohd Shafullah

August 2021

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Caption
ASTM	America Society for Testing and Materials
EAB	Elongation at break
FTIR	Fourier Transform Infrared Spectroscopy
RH	Relative humidity
SEM	Scanning Electron Microscope
TA	Tannic acid
TS	Tensile strength
WVP	Water vapor permeability
WVTR	Water vapor transmission rate
YM	Young's Modulus

KESAN ASID TANIK KE ATAS CIRI-CIRI FISIKOKIMIA, PENGHALANG, DAN MEKANIKAL FILEM GELATIN LEMBU

ABSTRAK

Filem gelatin lembu ialah salah satu alternatif filem biodegradasi yang baik untuk pembungkusan makanan kerana sifat fungsinya yang hebat. Walau bagaimanapun, ia mempunyai sifat mekanikal dan penghalang wap air yang buruk yang mungkin membataskan penggunaannya. Dalam kajian ini, filem gelatin lembu dihubung silang dengan asid tanik (TA) pada kepekatan yang berbeza iaitu 0%, 2%, 4%, dan 6%. Objektif utama kajian ini adalah untuk menilai kesan asid tanik terhadap sifat fizikokimia, penghalang, dan mekanikal filem gelatin lembu. Morfologi, warna, konformasi, kelarutan air, sifat mekanikal, dan kebolehtelapan wap air (WVP) filem disiasat. Gliserol ditambah yang bertindak sebagai pemplastik. Dapatan kajian ini menunjukkan bahawa terdapat peningkatan ($p > 0.05$) sifat mekanikal filem, dimana kekuatan tegangan (TS) meningkat dari 2.85 MPa menjadi 10.61 MPa dan pemanjangan pada pemecahan (EAB) meningkat dari 226.09% menjadi 400.99%. Sifat fizikokimia filem seperti kelarutan dalam air menurun (dari 35.42% menjadi 24.85%) dengan ketara apabila kepekatan TA meningkat. Ini menunjukkan bahawa peningkatan kepekatan TA akan meningkatkan ketahanan air pada filem. Walau bagaimanapun, peningkatan kepekatan TA tidak mempunyai kesan yang signifikan terhadap kebolehtelapan wap air filem. Kesimpulannya, berdasarkan hasil yang diperoleh, peningkatan kepekatan TA dapat membantu meningkatkan kelarutan air, kekuatan tegangan, dan pemanjangan pada pemecahan filem gelatin lembu sebagai filem terbiodegradasi dalam pembungkusan makanan.

THE EFFECTS OF TANNIC ACID ON PHYSICOCHEMICAL, BARRIER, AND MECHANICAL PROPERTIES OF BOVINE GELATIN FILMS

ABSTRACT

Bovine gelatin film is one of the good alternatives of biodegradable film for food packaging because of their great functional properties. However, it has poor mechanical and water vapor barrier properties which may limit its application. In this study, bovine gelatin films were crosslinked with tannic acid (TA) at different concentration which is 0%, 2%, 4%, and 6%. The main objective of this study is to evaluate the effect of tannic acid on the physicochemical, barrier, and mechanical properties of the bovine gelatin films. The morphology, color, water solubility, mechanical properties, and water vapor permeability (WVP) of the films were investigated. Glycerol was added which act as a plasticizer. The findings of this study showed that there were increase ($p > 0.05$) in the mechanical properties of the films, where tensile strength (TS) was increased from 2.85 MPa to 10.61 MPa and elongation at break (EAB) was increased from 226.09% to 400.99%. The physicochemical properties of the films such as water solubility decreased (from 35.42% to 24.85 %) significantly when TA concentration increases. This indicates that increasing concentration of TA will increases the water resistance of the films. However, the increasing concentration of TA has no significant effect on the water vapor permeability (WVP) of the films. In conclusion, based on the obtained results, increasing the concentration of TA may help to improve the water solubility, tensile strength, and elongation at break of the bovine gelatin films as a biodegradable film in food packaging..

CHAPTER 1

INTRODUCTION

1.1. Research background

Over the past years, due to environmental concerns regarding the use of synthetic petrochemical-based polymers, the demand for ecologically friendly packaging materials was increasing considerably. For many years, plastics have been widely used in food and packaging industries, including polystyrene, polyethylene, polypropylene and polyethylene terephthalate (Meritaine da Rocha et al., 2018; Madera-Santana et al., 2014). They are commonly used as they are cheap, stable, lightweight, easy to be processed and available in various form such as film, foam, elastic or hard material. However, the issue of this synthetic polymers is that it is not naturally biodegradable and required many years to degrade which will accumulate over years and led to high environmental problem (Menezes et al., 2019). Geyer et al. (2017) reported that about 79% of 6300 metric tonnes of waste generated in 2015 was accumulated in the natural environment (Picchio et al., 2018). Thus, there is a great interest in the development of renewable and environmentally friendly bio-based polymeric materials in recent years due to ecological awareness that has been growing among our population (Meritaine da Rocha et al., 2018; Moreno et al., 2015; Shankar and Rhim, 2015; Benbettaïeb et al, 2014). Various source of biodegradable films which including from lipids, polysaccharides, and proteins to be employed as the alternative of the synthetic polymers.

Gelatin as a kind of protein have been widely used among the other biopolymers to form a clear, flexible, and strong film due to its unique functional and technological properties (Karim and Bhat, 2009). Gelatin is a water-soluble, biodegradable protein that is produced through partial collagen hydrolysis (Choi et al., 2018). It also has excellent versatility because of its composition of α amino acid as different amino acid composition gives different properties of the gelatin such as melting and gelling temperature, and gel strength (Peña et al., 2013; Venkateshwarlu Gudipati, 2013).

1.2. Rationale of the study

However, as with the majority of protein films, gelatin film has poor mechanical properties and water vapor barrier which limit its application despite of having unique properties (Cao et al., 2007). In order to be a good biodegradable packaging film, the bovine-gelatin film should have a good mechanical and barrier properties. This can be an issue for high moisture food products because films are able to swell, dissolve or disintegrate after in contact with water (Gómez-Guillén et al., 2009). Several research studies have been conducted to evaluate the effect on gelatin-based products to enhance the functional properties of gelatin and food-life products with the addition of different materials, such as crosslinkers, strengthening agents, plasticizers or antioxidant-based additives (Ramos., 2016)

To resolve such limitations, chemical cross-linkages are a commonly used and highly effective way of improving the mechanical, barrier and water resistance properties of the gelatin by introducing covalent stable bonds between gelatin chains (Choi et al.,

2018; Peña et al., 2013). Cross-linking agents such as natural phenolic compounds, seems to be preferable in order to reduce the toxicity risks. Tannic acid is a plant polyphenol that exists naturally and can be found widely in nearly all plants (Robles, 2014), could be employed as a safe alternative cross-linker since they are able to complex with conformationally open proteins like gelatin, mainly through hydrogen bonds and hydrophobic interactions (Zhang et al., 2011; Xing et al., 2004; Hagerman and Butler, 1981)

1.3. Objectives

The objectives of the project were:

i. To study the effects of different concentration of tannic acid on physicochemical of bovine gelatin films.
ii. To evaluate the effect of concentration of tannic acid on mechanical properties of bovine gelatin films.
iii. To determine the effect of tannic acid on barrier properties of bovine gelatin films.

CHAPTER 2

LITERATURE REVIEW

2.1. Gelatin and its application

2.1.1. Gelatin

Gelatin is a tasteless, good food substance with outstanding biocompatibility, biodegradability and non-toxicity (Ali et al., 2012) which makes it a great potential as a biodegradable and edible films (Bergo and Sorbal, 2007; Etxabide et al., 2017). The abundance, availability and low cost of gelatin make its use convenient for a wide range of applications.

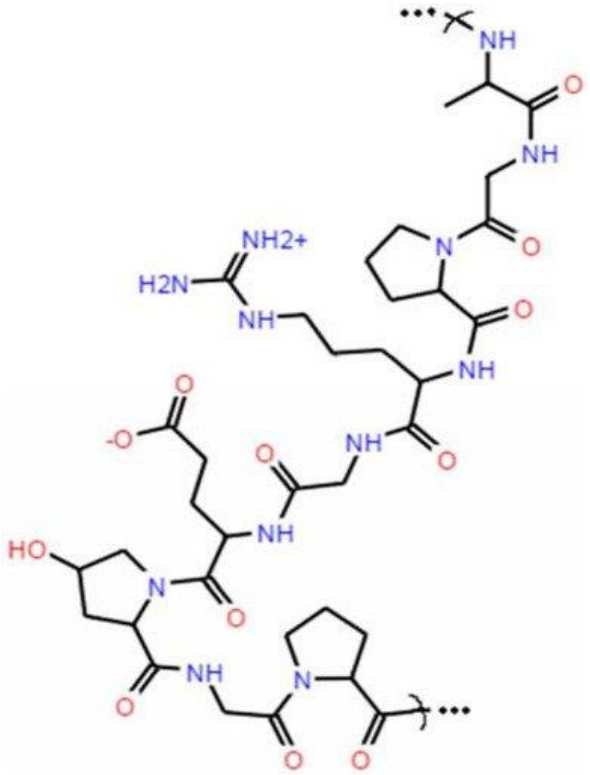


Figure 2.1: Representative gelatin structure according to its typical amino acid composition (Ramos et al., 2016)

According to Haug and Draget (2011) and Liu *et al* (2015), gelatin is heterogeneous polypeptide mixture of α-chain, β-chain and γ-chain which derived from parent protein collagen through process of the destruction of cross-linkages and breakage of the polypeptide chains and peptide bonds with different degree of hydrolysis. Figure 2.1 show the typical amino acid composition of gelatin. The molecular weights, structures, and compositions of the subunits of gelatin influence its functionality (Liu et al., 2015).

The main commercial sources of gelatin are bovine bones, hides, pig skin, fish and recently insects. Porcine and bovine which is categorized under mammalian gelatin are the most popular and widely used subject to major consumer limitations and skepticism due to social, cultural and health concerns (Karim and Bhat, 2009). However, the properties of gelatin may be affected by intrinsic factors including source, age of animal and type of collagen (Nur Hanani et al., 2014).

The method of manufacturing process of gelatin including cleaning of the source tissues accompanied by pre-treatment, gelatin extraction, filtration/purification/sterilization, concentration, drying and finally milling. According to Cheaw et al (2007), gelatin that extracted from different animal species and tissues as well as the severity of the manufacturing method will give different properties of the resulting gelatin that indicate its quality.

Gelatin can be divided into 2 classes which is Type A and Type B depending on the processing method. Type A is where it is obtained from acid treated collagen with isoelectric point at pH 8-9 while Type B has an isoelectric point pH 4–5, from a precursor

treated with alkaline that converts residues of asparagine and glutamines to the respective acids and therefore gives them greater viscosity. The type of acid used may affected the viscoelastic and gelling properties of gelatin (Abdalbasit Mariod and Hadia Fadul, 2013). Porcine (pig skin) gelatin usually is Type A while bovine (beef skin) or pig cattle hides, and bone is Type B (Ramos et al., 2016)

The application of gelatin in food, photographic, cosmetical and pharmaceutical is mainly depended on its gel forming and viscoelastic abilities. Nowadays, mainly in food industry where more applications for gelatin have been identified in products such as emulsifiers, foaming agents, colloid stabilizers, fining agents, biodegradable packaging materials and micro encapsulation agents in accordance with the increasing trend towards the substitution by more natural plastics of synthetic agents (Gómez-Guillén et al., 2011).

2.1.2. Properties of gelatin

Gelatin consists of 25,2% oxygen, 6,8% hydrogen, 50,5% carbon, and 17% nitrogen, whereas gelatin comprises a combination of single and double unfolded hydrophilic chains. The chemical structure of gelatin comprises of many polypeptide chains such as a-chains, b-chains and c-chains. Furthermore, the gel strength and viscosity of gelatin change with the relative molecular mass distribution, while the electrolyte state, pH and the temperature may be impacted. It is really important to test the quality of gelatin gel strength. Collagen consists of three polypeptide chains. A triple helix stabilizing the interconnected hydrogen bond in structure and interconnected. Proper chemical pre-treatment disrupts non-covalent ties to ensure that the gelatin extraction is sufficiently swelled, and collagen solubilized. The destruction of hydrogen and

hydrophobic ties breaks the structure of a triple helix and dismantles the chains which are followed by molecular separation into a minor part (Janifal Alipal et al., 2021).

2.1.3. Gelatin composites for packaging purposes

Recently, the production and possible use of biodegradable films as alternatives to conventional food packaging polymers has become more interesting. Gelatin that comes from various sources can produced packaging films, and by adding other food ingredients, the behavior and characteristics of gelatin- based films can be modified where the composite film has enhanced physical and mechanical properties. Numerous researches have investigated the mechanical, barrier and humidity ageing properties of films produced by gelatin alone or with incorporation of different types of plasticizers (Liu et al., 2015).

The recent trends in the design of gelatin-based biodegradable materials for food packaging are focusing on the development of the films with enhanced mechanical and water resistant capabilities, through combination of gelatin and biopolymer with varied characteristics including lipids, soy protein isolates and chitosan (Gómez-Guillén et al., 2011). The most important key features of gelatin films including water absorption, water ageing, mechanical properties particularly the resistance and thermomechanical properties, and lastly barrier properties such as water vapor, oxygen or other gases migrations (Liu et al., 2015).

Gelatin has the ability to form thin membranes that are suitable to be used for food packaging. Some products have been made to use gelatin as an external film, from dryness, exhibition to light and/or exposure to oxygen, for the long shelf-life of food and agricultural products (Nur Hanani et al., 2014; Ramos et al., 2016). According to Theeranun Janjarasskul and Krochta (2010) gelatin coatings may lower the oxygen, moisture, and oil migration or carry bioactive ingredients. The drawback of gelatin when is used as packaging film or coatings is its permeability and mechanical resistance even though it has comparatively excellent oxygen barrier properties. It also has an acceptable mechanical resistance only at low or medium relative humidity but not at high relative humidity due to the hydrophilic nature of gelatin (Liu et al., 2015). In order to enhance the properties of the gelatin films, the main goal is to alter the hydrophilic nature of gelatin which can be achieved with either covalent cross linkage agents or incorporation of plasticizers.

2.1.3. Manufacturing of biodegradable films

There are two main technique to produce biodegradable films which is solution casting where it uses wet solvent processing, and extrusion. Solution casting was introduced over 100 years ago and was led by the demands of the new photographic industry. According to Nur Hanani et al. (2014), this method involved the spread of the solution onto a level plates such as silicon plates and acrylic plates, and it is then dried at ambient conditions or under a controlled RH using either hot air, infrared energy, microwave energy or under ambient conditions. However, this technique may have

substantial impact on some of the properties of the films including the appearance, morphology, mechanical, and also barrier properties. Solution casting are extensively used to produce gelatin films that will be used in biodegradable food packaging. The limitation of this method is that it is impractical for commercial scale production as it is energy intensive and the process require a very long time (Nur Hanani et al., 2012).

After 1950s, extrusion method was used in the production of thermoplastic polymers and it became the primary manufacturing process method to produce plastics. Extrusion uses high temperature and pressure to soften and melt the polymer, thus allowed the formation of a cohesive film matrix (Nur Hanani et al., 2014).

2.2. Glycerol as plasticizers

Glycerol is a clear, colorless, hydrophilic, and viscous like liquid with sweet taste belonging to alcohol family of organic compounds. Moreover, glycerol (Figure 2.2) has been recognized and used as favourable plasticizer (Bertuzzi et al., 2007; Rivero et al., 2010; Vanin et al., 2005) and also has been approved by the Food and Drug Administration (FDA) as a food additive (Li and Huneault, 2010).

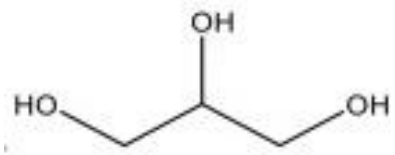


Figure 1 Structural formulae of glycerol
(Koupantsis et al., 2016)

Polyols such as glycerol, sorbitol, and propylene glycol are the common plasticizer used in biodegradable and edible film (Dangaran et al., 2009) in order to make the films more flexible, softer and prevent the polymeric matrix from any pores and cracks (Nur Hanani et al., 2014). However, some other properties of the film may change including tensile strength reduction, increases of water permeability and film capacity to adsorb water (Olivas and Barbosa-Cánovas, 2008).

According to Tajik et al. (2013) when incorporating glycerol at the correct level with consideration to the biopolymer content, it is able to lower intermolecular forces and increase the mobility of the polymer chains which basically used in order to improve the mechanical properties of edible films.

The type and quantity of plasticizers used may strongly affect film formation from aqueous dispersions of polymers and the performance of the films. Unless a plasticizer is employed, biodegradable films are usually fragile because of large intermolecular forces that involve interacting with polymers chain to chain (Nur Hanani et al., 2013).

2.3. Tannic acid

Tannic acid is a type of polyphenol and a commercial form of tannin that are rich in OH groups which can interact strongly with macromolecules such as proteins and polysaccharides (Patel et al., 2013; Xie et al., 2017). Tannic acid is an amorphous powder

with light-yellowish color, with a strong astringent taste. When exposed to light, it becomes yellowish (Har Bhajan Singh and Bharati, 2014)

Tannic acid is water soluble and has a high molecular weight. It has several useful properties such as antioxidant, antibacterial, and antiviral. $C_{76}H_{52}O_{46}$ is the chemical formula of commercial tannic acid as shown in Figure 2.3. With the structure of tannic acid that consist of gallic units and extensive terminal phenolic hydroxyl groups, it has a distinctive properties and often used in many application including coatings, adsorption and antibacterial materials, mucoadhesive compounds, separator for lithium-ion batteries, and nanomaterials (Liu., 2014; Luo et al., 2016; Shin et al., 2016).

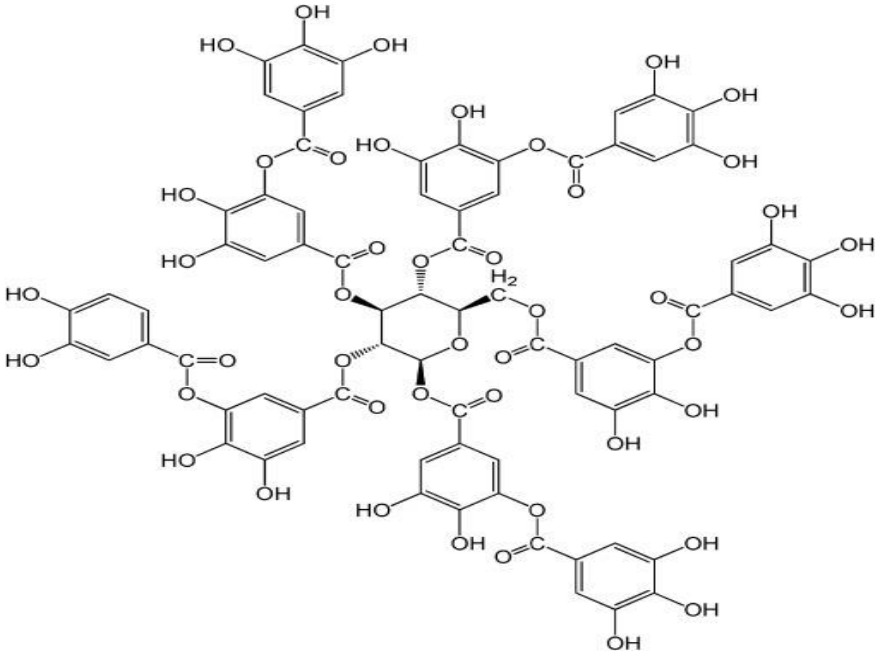


Figure 2.3: Chemical structure of tannic acid

With the presence of multiple phenol groups in the structure of TA, it has the ability to bind with ionic pairing, hydrogen bonding and metal coordination (Koupantsis., 2016). A study by Picchio et al. (2018) reported that plant-derived TA has demonstrated an

effective casein crosslinker agent which produce protein films with improved physicochemical characteristics. Menezes et al. (2019) also reported that the tannic acid incorporated in gelatin-AgNPs nanocomposite film has improved the water vapor barrier properties and it is a significant factor in maintaining or extending the shelf life of the package products.

2.3.1. Tannic acid as crosslinking agent

The cross-linking of bovine gelatin by chemical agents may also help to improve its functionality. Many studies involving the crosslinking of gelatin with phenolic compound such as ferulic acid, caffeic acid, tannic acid and gallic acid has been successfully done in order to improve the functional properties of gelatin via interactions of covalent and non-covalent (Huang et al., 2019).

Hydroxyl group in tannic acid may interact with carboxyl group of the gelatin through the hydrogen bond and hydrophobic interactions between aromatic ring of tannic acid and gelatin side chain rings may occur, thus improving the functionality of gelatin (Huang et al., 2019). Oxidized phenolic group can react to amino acids or sulfhydryl side chain in the gelatin thus forming C-N or C-S bonding and generate crosslinked network (Yan et al., 2011). There are several factors affecting the interactions of gelatin and phenolic including temperature, pH, types of gelatin, and types and structure of phenolic compounds (Huang et al., 2019).

According to Muhoza Bertrand et al. (2019) the crosslinking of proteins with different pH polyphenols altered the secondary protein structure, improved thermal stability and rheological properties because of the formed protein - polyphenol complex.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

Bovine gelatin powder was purchased from the local manufacturer, Halagel (M) Sdn Bhd, Malaysia. Chemical that used during preparation of the film including tannic acid, sodium hydroxide, and glycerol. Glycerol was obtained from MERCK Company, USA. Tannic acid was obtained from HmbG Chemicals. Sodium hydroxide was obtained from BDH Prolabo, VWR International, USA.

3.2. Methods

3.2.1. Experimental methods

Film-forming solution was prepared by adding tannic acid into gelatin solution in 50 mL of deionized water with 0.16 g plasticizer (glycerol). The variables planned was the film formulation with different concentration of tannic acid which is 0%, 2%, 4%, and 6%. The effect of different concentration of tannic acid on the properties of the films were investigated. The analysis involved mechanical, barrier, and physicochemical properties including thickness, color, water solubility, SEM and FTIR. The properties of the films were compared with the control. The experimental methods in this study are shown in Figure 3.1.

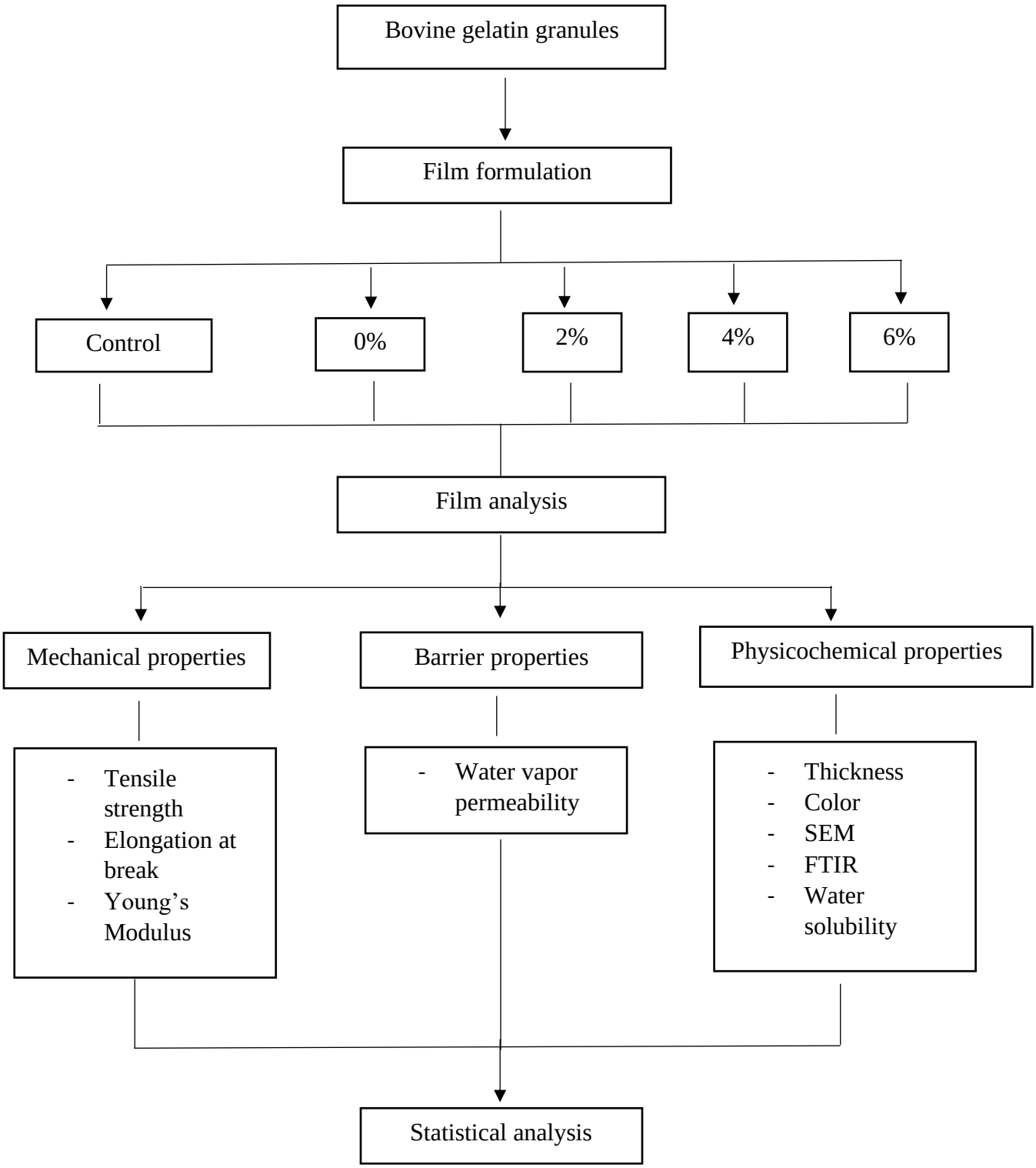


Figure 3.1. Summary of experimental method

3.2.1. Preparation of film

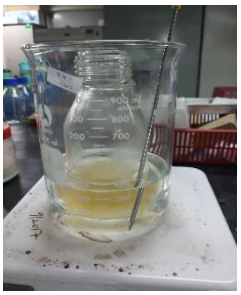
The gelatin film-forming solution was prepared by dispersing tannic acid into deionized water into 50 mL deionized water at different concentration (0%, 2%, 4% and 6%) and their pH was adjusted by sodium hydroxide 1.0 N (pH ≥ 10). Bovine gelatin granules were added into the tannic acid solution to obtain a concentration of 4g/50 mL and then heated (50 °C) for 30 mins under continuous stirring and injection of oxygen to produce a homogenized solution. After completion of gelatinization, glycerol that was used as a plasticizer was added at 1.6g/50 mL. Individual gelatin granules which dissolved in water without addition of tannic acid and injection of oxygen acted as control films. The injection of oxygen facilitates the reaction in order to have a complete reaction. The formulation of the mixtures is shown in Table 3.1. Then the solution was cooled to room temperature before adjusting the pH ≥ 8 with sodium hydroxide 1.0 N.

Table 3.1 Formulation of film-forming solutions of bovine gelatin film with glycerol.

Films	Composition of film-forming solution (g/50 mL deionized water)
Tannic acid 2%	0.08
Tannic acid 4%	0.16
Tannic acid 6%	0.24

A portion (50 mL) of the dispersion was cast on Perspex plates fitted with rims around the edge to yield a 16 × 16 cm^2 film-forming area. The films were let dry at room temperature for 24 h before dried in oven (45 °C) for 20 h. Dried films were peeled and

stored at 23 ± 2 °C and 50 ± 5% relative humidity (RH) until further analysis. The procedures for film preparation are shown in Figure 3.2.



The tannic acid and gelatin powder were dissolved at 50°C using a hot plate

The solution was injected with oxygen with continuous stirring for 30 minutes at 50 °C

After injection of oxygen, the pH of the solution was adjusted to pH 7-8

Then, glycerol was added before the solution was poured into the Perspex plates

Figure 3.2 Film preparation process

3.2. Film characterization

3.2.1. Thickness of Film

The thickness of each film was measured with a hand-held micrometer Dial Thickness Gauge (Mitutoyo, Japan; MI 7305) and accuracy of 0.01 mm at 5 different position of each formulations and the mean thickness value was used in water vapor permeability and mechanical properties analysis.

3.2.2. Film color measurement

The surface color of produced films was measured using a colourimeter Minolta model CM-3500d spectrophotometer (Minolta, Osaka, Japan). The color determination of the bovine gelatin film was done by referring to the test method for color measurement defined by Commission Internationale de I'Eclairage (CIE) L*, a*, b*. L* value represented black (-) to white (+), a* represented green (-) to red (+), and b* represented blue (-) to yellow (+).The white standard color plate that used for the instrument's calibration was used as background for the color measurements. Three replicates of each samples were measured, and the average value was calculated.

3.2.3. Water Vapor Permeability of Film

Water vapor permeability (WVP) of the gelatin films were measured according to method of Araghi et al., (2015) with modified gravimetric cup method based on ASTM Standard E96/E96M-16 (ASTM, 2016). The glass cell was filled with silica gel (desiccant) until two-third of its height to produce a 0 % relative humidity (RH) below the film. The gelatin film sample was placed between the glass cell and the ring cover of each glass cell coated with parafilm. The air gap was at approximately 1.5 cm between the film surface and desiccant. The water vapor transmission rate (WVTR) of each films were measured at 55 ± 2% RH and 25 ± 2 °C. The initial weigh of the glass cell was measured using an analytical balance (Metter Toledo, B204-S, Switzerland) with accuracy of 0.0001 g and the reading was recorded as the weight of 0^{th} day. The glass cell then was placed into desiccator which has been filled with concentrated magnesium nitrate solution ($Mg(NO_3)_2$. The cell weight gain measurement was taken by weighing the glass cell for 7 days.

A linear regression of glass cell weight as a function of time was plotted to get the slope value. The analysis was done in triplicates for each sample. The WVP of each film was calculated according to the following equation (1):

$$\mathrm{WVP} = \frac{\mathrm{w}}{\mathrm{t}\,\mathrm{A}}\frac{\chi}{\Delta \mathrm{P}}$$

Where, $\frac{\mathrm{w}}{\mathrm{t}}$ (g/h) indicates the slope of the graph of the weight gain against time, A (m^2) is the exposed film area, χ (mm) is the thickness, and ΔP is the partial pressure difference through the film calculated through Equation (2):

$\Delta P = S (R_1 - R_2)$

S, is the saturated vapor pressure at 25 °C (3166 kPa), R_1 and R_2 are the relative humidity in the desiccator (0.55) and in the interior of the cell (0.0), respectively, expressed in fractions.

3.2.4. Mechanical Properties of Film

The tensile strength (TS), Elongation at break (EAB), and Young's Modulus (YM) of the gelatin films were analyzed by using a TA-TX2 texture analyzer (Stable Micro System, Surrey, UK) according to the ASTM standard method D882-18 (ASTM, 2018) with some modification. The films samples were cut into strips with a dimension of 10cm × 2 cm for each formulation prior to analysis.

For each type of film sample, 8 strips were tested, and the best 5 readings were taken for data analysis. A 5 kg load cell was installed on the texture analyzer. First, a proper calibration must be conduct before starting the analysis. The measurement started by clamping the sample strip between the tensile grip which had an initial grip distance of 50 mm. The tensile strength, elongation at break and Young's Modulus were determined according to the formula shown as follow:

$$TS\ (MPa) = \frac{F}{A},$$

Where, F_{max} = maximum force acting

A= Cross sectional area of film (m^2)

$$\text{EAB (\%)} = \frac{\Delta l}{l_{\circ}} \times 100,$$

Where, Δl = film extension at the point of sample rupture

$l_{\circ}$ = initial length of the sample

$$\text{YM (MPa)} = \frac{\text{Tensile stress}}{\text{Tensile strain}}$$

3.2.5. Water Solubility

The water solubility of the film was determined according to the method of Benbettaïeb et al. (2014). The percentage of dry matter solubilized after 24 hr immersion of the film was measured in order to determine the film solubility. Each film was cut into 3 discs with dimension of 2 cm × 2 cm and dried to a constant weight (W_i) in an oven at 105 °C to determine the initial dry matter content. The film disc then was immersed in 30 mL of distilled water for 24 h at 25 °C. After 24 h of immersion, the film disc was removed from the solution and dried to a constant weight (W_f) in an oven at 105°C to determine the weight of dry matter that was not solubilized in the water. The film solubility was calculated by the equation:

$$\text{Film solubility (\%)} = \left(\frac{Wi - Wf}{Wi}\right) \times 100$$

3.2.6. Scanning Electron Microscopy (SEM)

The morphology of the bovine gelatin film was investigated using a scanning electron microscope according to Carvalho and Grosso (2004). The film was cut into smaller pieces then was fixed evenly and erectly on stubs using copper-conducting adhesive tape to observe the cross-section morphology of the film. Then it was coated with gold to make the samples conductive. Finally, the coated sample was observed and photographed under the SEM at 10 kV at 1000x magnification.

3.2.7. Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of the bovine gelatin films were determined using a FT-IR spectrophotometer (Nicolet iS10, USA) according to Benbettaïeb et al, (2014) with some modification. The film was placed on the magnetic sample holder and the transmittance spectra versus the wave number was collected to determine any potential interactions between the bovine gelatin and tannic acid in the film. The measurement of the FTIR spectra in the range of 600- 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3.3. Statistical Analysis

The statistical analysis was performed using SPSS statistical software version 27.0 (SPSS Inc, Chicago, IL) and excel (Microsoft, Inc). Analysis of varience (ANOVA) and followed by Duncan test were carried out to perform comparison mean at ($p < 0.05$) significant level.