UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION AND APPLICATION OF SOLUBLE PINEAPPLE PUREE PECTIN-BASED FILMS ON STORED AGAR-AGAR POWDER PROPERTIES

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PROJECT REPORT SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF PROCESS AND FOOD ENGINEERING WITH HONOURS IN THE UNIVERSITI PUTRA MALAYSIA

DEPARTMENT OF PROCESS AND FOOD ENGINEERING

FACULTY OF ENGINEERING

UNIVERSITI PUTRA MALAYSIA

2020/2021

ACKNOWLEDGEMENT

First and foremost, praises and thanks to God, the Almighty, for His blessings for giving me good health and strength to complete my final year project successfully. I am overwhelmed and grateful to acknowledge the effort of all those who have helped me throughout my journey to complete my final year project.

I wish to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Rosnita A. Talib for providing me her guidance, encouragement and useful critiques along the way of completing the project. Her assistance, times and encouragement truly help my progression in learning and gaining new knowledge, skills and experience. This works would be impossible without her guidance.

I also express my deepest appreciation to both of my examiners, Assoc. Prof. Dr. Siti Hajar Othman and Dr. Affandi P. Mohammed for the comments, additional information, and ideas that helped me from the start of my research. Truly, their comments and ideas contribute greatly for the success of my journey.

I also wish to express my gratitude to all the staff of Department of Process and Food Engineering, Universiti Putra Malaysia for their generous attitude and help during my labwork activity. A special thanks to Pn. Siti Hajar Zakaria as our laboratory assistant, that takes responsibility in guidance and times spent for us to use the lab facilities. I also would like to thank Nurzia Mohamad, a Master student also under the supervision of Assoc. Prof. Dr. Rosnita, for guiding me throughout my project

Last but not least, I am thankful and grateful to all my lecturers, family and friends who have been always helping, giving encouragement and prayers for me throughout all the struggles and hardships. All the supports truly gave me a healthy mental, emotion and physical to finally finish the project.

ABSTRACT

The growing production of plastics food packaging possess negative impact to the environment and human. Therefore, this study aims to explore the application of soluble pineapple puree pectin-based (PPA) packaging films on the quality changes of agar-agar powder properties under different storage temperatures. Two objectives of the study were to characterize the soluble PPA films properties, including water solubility, tensile, seal strength, water vapour permeability, surface roughness and optical, and to evaluate the effect of the films on the quality changes in stored agar-agar powder. The soluble PPA films were made using a formulation ratio of 1:0.5:1 (pectin:glycerol:pineapple puree). For soluble pectin-based (PG) film, which acts as a control, the formulation is 1:0.5 (pectin:glycerol). Water solubility test, texture analyzer, water vapour permeation test, atomic force microscopy (AFM) and colorimeter were carried out to characterize the films. The agar-agar powders were stored for 39 days at ambient (28 °C) and chill (4 °C) temperatures. At selected storage time, moisture content, true density and colour of agar-agar powder were evaluated using moisture analyzer, gas pycnometer and colorimeter, respectively. The results revealed that soluble PPA film has a higher solubility percentage than soluble PG film, where the solubility percentage of soluble PPA and PG films were 97.3 ± 1.7% and 93.7 ± 5.8%, respectively. The tensile properties of the soluble PPA film were 3.5 ± 0.1 MPa for tensile strength (TS), 34.2 ± 3.9% for elongation at break (EAB), 0.2 ± 0.0 MPa for Young Modulus (YM) and 0.7 ± 0.0 MJ/m^3 for toughness. Meanwhile, the soluble PG film showed values of TS, EAB, YM and toughness of 4.3 ± 0.1 MPa, 42.1 ± 1.2%, 0.2 ± 0.0 MPa and 1.1 ± 0.0 MJ/m^3, respectively, which were slightly higher than the soluble PPA film. The seal strength result showed insignificant difference between both types of film. Additionally, the optical properties showed that

PPA film was insignificantly less transparent compared to soluble PG film. Moreover, the water vapour permeability (WVP) of soluble PPA film was slightly better than soluble PG film, although there was no significant improvement. The AFM test revealed that soluble PPA film has a significantly smoother surface than soluble PG film. For the studies of quality changes on the agar-agar powder at selected storage temperature, the result for all the tests (moisture content, true density and the colour changes of agar-agar powder) showed the quality changes were not significantly different by the type of films and storage temperatures. However, the results were significantly difference by the storage period. Packaging tests revealed that both soluble PPA and PG films showed potential soluble material in food powder packaging application.

ABSTRAK

Peningkatan produksi plastik pembungkus makanan memberi impak yang negative terhadap alam sekitar dan manusia. Oleh itu, kajian ini bertujuan untuk meneroka aplikasi pembungkusan untuk filem larut berasaskan puri nanas-pektin (PPA) mengenai perubahan kualiti sifat serbuk agar-agar di bawah suhu penyimpanan yang berbeza. Dua objektif kajian adalah untuk mencirikan sifat filem PPA larut termasuk kelarutan air, tegangan, kekuatan meterai, kebolehtelapan wap air, kekasaran permukaan dan optik, dan untuk menilai kesan filem terhadap perubahan kualiti serbuk agar-agar yang tersimpan. Filem PPA larut dibuat menggunakan nisbah formulasi 1: 0.5:1 (pektin: gliserol: puri nanas). Untuk filem berasaskan pektin larut (PG), yang berfungsi sebagai kawalan, formulasi adalah 1: 0.5 (pectin: gliserol). Ujian kelarutan air, penganalisis tekstur, ujian penyerapan wap air, mikroskopi daya atom (AFM) dan jangka warna dilakukan untuk mencirikan filem. Serbuk agar-agar disimpan selama 39 hari pada suhu sekitar (28˚C) dan sejuk (4˚C). Pada masa penyimpanan yang dipilih, kandungan kelembapan, ketumpatan sebenar dan warna serbuk agar-agar dinilai masing-masing menggunakan penganalisis kelembapan, pycnometer gas dan jangka warna. Hasilnya menunjukkan bahawa filem PPA larut mempunyai peratusan kelarutan yang lebih tinggi daripada filem PG larut di mana peratusan kelarutan filem PPA dan PG larut masing-masing adalah 97.3 ± 1.7% dan 93.7 ± 5.8%. Sifat tegangan filem PPA larut adalah 3.5 ± 0.1 MPa untuk kekuatan tegangan (TS), 34.2 ± 3.9% untuk pemanjangan pada waktu rehat (EAB), 0.2 ± 0.0 MPa untuk Modulus Muda (YM) dan 0.7 ± 0.0 MJ/m^3 untuk ketangguhan. Sementara itu, filem PG larut menunjukkan nilai TS, EAB, YM dan ketangguhan masing-masing 4.3 ± 0.1 MPa, 42.1 ± 1.2%, 0.2 ± 0.0 MPa dan 1.1 ± 0.0 MJ/m^3, yang sedikit lebih tinggi daripada filem PPA larut. Filem PG larut juga mempunyai kekuatan meterai yang lebih baik

berbanding dengan filem PPA larut, yang mana filem PG mempunyai kekuatan meterai 0.4 ± 0.1 N/mm sementara filem PPA adalah 0.3 ± 0.0 N/mm. Selain itu, sifat optik filem PPA larut menunjukkan bahawa filem PPA kurang telus berbanding dengan filem PG larut. Selain itu, kebolehtelapan wap air (WVP) filem PPA larut lebih baik daripada filem PG larut, di mana nilai WVP filem PPA dan PG adalah 8.3E-09 ± 3.5E-09 g/m.day.Pa dan 1.2E-08 ± 9.9E-10 g/ m.day.Pa, masing-masing. Ujian AFM menunjukkan bahawa filem PPA larut mempunyai permukaan yang lebih halus daripada filem PG larut. Untuk kajian perubahan kualiti pada serbuk agar-agar pada suhu penyimpanan terpilih, kandungan kelembapan serbuk agar-agar yang dibungkus dengan filem PPA larut lebih rendah daripada yang dibungkus dengan filem PG larut pada kedua suhu penyimpanan. Sementara itu, ketumpatan sebenar serbuk agar-agar menunjukkan trend penurunan pada suhu penyimpanan dan jenis filem larut. Warna serbuk agar-agar juga menunjukkan penurunan pada kedua suhu penyimpanan. Penurunan perbezaan warna total (ΔE) pada suhu penyimpanan dan jenis filem menunjukkan bahawa serbuk agar-agar kehilangan intensiti warna kekuningan selama 39 hari masa penyimpanan. Ujian pembungkusan terhadap serbuk agar-agar menunjukkan bahawa kedua-dua filem PPA dan PG mempunyai potensi untuk diaplikasikan sebagai bahan pembungkusan makanan serbuk.

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LIST OF ABREVIATION

ΔE	Total colour difference
AFM	Atomic Force Microscope
EAB	Elongation at break
PG	Pectin-based
PPA	Pineapple puree pectin-based
TS	Tensile strength
WVP	Water vapour permeability
WVTR	Water vapour transmission rate
YM	Young modulus

CHAPTER 1

INTRODUCTION

1.1 Background

The increment of waste generated each year has become a serious concern to society. Based on the article, Global Waste Generation – Statistics & Facts, (Tiseo, 2020) municipal solid waste generation in worldwide is expected to increase by 70% to 3.4 billion metric tons by 2050. A huge quantity of waste is still sent to landfill sites, while only 20% of the waste is recycled each year. Plastic waste especially has garnered huge attention in the countries worldwide due to its negative impact on marine life. Thushari & Senevirathna (2020) stated that the lack of proper waste disposal has resulted in plastic debris with counts of five trillion, weighing 260,000 tonnes floating over the world ocean surface. This fact shows how bad plastic pollution is across the world as it can harm the ecosystem of the marine life and land life. For example, the most critical issue associated with microplastic fragments which individuals often encountered is the entanglement and ingestion of plastic materials by marine animals (Thushari & Senevirathna, 2020).

The municipal solid waste generated from households, industrial, shops and other institutions is one of the biggest contributors of plastic waste. These plastic wastes usually come from polymer-based packaging. The principal role of packaging is to protect the products from outside influence and damage, as in food industry; it is a protection against the influence that is capable of containing the food, and ingredient as well as nutritional information, can be provided to consumers (Marsh et al., 2007). Polymer-based packaging are one of the most used packaging materials among

manufacturers as plastics have a wider variety of being introduced ranging from rigid to flexible forms.

Food packaging can be defined as the system that has been traditionally considered as simple containers for the transportation of food from production place to the retail outlet and then to the consumer with no alteration of its nutritional and organoleptic characteristics (Valdes et al., 2015). Since the ultimate function of packaging is to preserved food from external contamination, the packaging materials should be considered. Furthermore, it ensures that the food is free from environmental influences such as heat, moisture, oxygen, enzymes, loss of aromas and unpleasant odour components. Marsh et al. (2007) states that the U.S. Food and Drug Administration (FDA) has carefully reviewed and regulates the substances used to make plastics for packaging purposes to ensure public safety. Due to the health concerns regarding residual monomers and components in plastics, including stabilizers in polymer-based food packaging. Although polymer-based packaging is often the ideal packaging for most food, soluble and edible films for packing food especially ready-to-eat food should be developed for greener approach regarding the health concerns due to plastics.

Soluble and edible films made of pectin with pineapple puree can replace the existing polymer-based packaging for food. Pectin is one of the most abundant polysaccharides used in the making of soluble films due to its properties, such as being biodegradable and derived from renewable raw materials (Valdes et al., 2015). Pectin is widely distributed in primary cell wall and the middle lamella of the higher plant (Dranca & Oroian, 2018). Traditionally, pectin was used as a gelling agent, thickening agent and stabilizer in the food sector. Depending on the source of pectin, their structural and macromolecular properties such as galacturonan methoxylation,

galacturonic acid content, neutral sugar composition and molecular weight are diverse (Drance & Oroian, 2018). Besides apple pomace and citrus peel, other sources were considered for the productions of commercial pectin due to the rising demand and growing interest in valorizing side streams to obtain pectin with diverse functional properties.

The uprising trend of using pectin as biopolymer for food packaging application is highly driven by its biodegradability and bio-compostable, sustainability and non-toxic characteristics (Mellinas et al., 2020). As pectin is a type of polysaccharide, it has better thermal stability than other biopolymers such as lipids and proteins since pectin are not irreversibly denatured via heating. However, its main drawbacks for food packaging applications includes the high sensitivity to moisture and low mechanical resistance (Damm et al., 2016). To overcome these disadvantages, Mellinas et al. (2020) proposed that the pectin-based biofilms can be improved by incorporating different reinforcing additives to the polysaccharide matrices, the combination with different polymers to obtain blends or making multilayer films.

In this research, the bioplastic was developed using pectin as a matrix component and glycerol as a plasticizer. Lutfi et al. (2017), claims that the glycerol reduces the stiffness and improves the flexibility of the films through both functions of increasing the mobility of the polymer bonding as well as improving the water holding capacity. In addition, pineapple puree was incorporated into the pectin mixture where it is believed to create bioplastic that has better mechanical properties. Pineapple is a tropical fruit that can be found easily in Malaysia. Previous preliminary studies done by Raduan (2020) and Kamal (2020), on the effect of chosen tropical fruit puree (dragon fruit, mango, papaya and pineapple) have shown pectin-based films incorporated with a puree of papaya and pineapple shows the highest tensile strength.

This may be due to the high content of polysaccharides in the tropical fruits. Kamal (2020) also reported that pectin-based film incorporated with pineapple-puree had high solubility percentage of 97.9%.

In correlation with the FDA concern regarding the safety issue of the substances used to make plastics, this soluble film is fabricated fully from edible natural sources which is suitable for applying primary packaging. The ingredients used in developing the pectin-based film include pectin, glycerol and fruit puree (pineapple) only. It is important to ensure the quality parameters of the food product packed with the soluble films are being maintained throughout the storage period. Therefore, studies such as the moisture content and colour intensity changes of the food samples should be performed prior to pack the food. Liu et al. (2020) reported that the colour intensity of coconut and coffee powder packed using soluble polysaccharide-based films were able to be maintained for storage period of 7 days.

Due to its hydrophilicity, it is highly soluble in water and is compatible with packing a product with high pectin content. Through this research, several tests have been carried out to study the characteristic of the film. In addition, the effect of storage temperature on the agar-agar properties was also studied. The method used in developing the film is through solvent casting.

1.2 Problem statement

Petroleum-based packaging such as plastics is versatile packaging material as it poses high durability, giving products long shelf life, and is convenient in forming them into desired shapes. Plastics which are made up of synthetic organic polymers

currently being widely used in different applications ranging from water bottles, clothing, food packaging, medical supplies, electronic goods, construction material and others (Alabi et al., 2019). It is also inexpensive compared to other materials for packaging purposes, thus making it and the product affordable to consumers. This is also why most manufacturers prefer petroleum-based films for various applications, especially food packaging.

However, this polymer-based packaging negatively impacts both environment and human health especially packaging for food material. As known, the material used to make plastics comes from petroleum which is a non-renewable energy. The growing amount of manufacturing plastic materials might limit the source of non-renewable energy, which can bring imbalance to the ecosystem.

Moreover, petroleum-based food packaging takes a long time to degrade due to its non-biodegradability nature. This problem leads to the increment of municipal solid waste, which further limits landfill space. Alabi et al. (2019) reported that approximately 10% of waste generated from households is plastics and most likely end up on the landfill. Historically, landfilling has always been the preferred method for waste management as it is relatively cheap and simple without necessary treatment requirements such as cleaning or separation. Even so, the government of many countries is beginning to use a greener approach for waste management since there is a growing environmental and public health concern about the potential effects of landfills. This includes the various types and quantities of toxic chemicals and their potential for leaching at landfill sites.

That leakage could generate the possibilities of chemical contamination to the soil and groundwater by disintegrated plastic by-products and additives that can persist

in the environment on long term basis (Alabi et al., 2019). Although environmental pollution and risks to public health can be reduced if the landfills are well-managed, today's technology enables researchers to develop bioplastics as one of the greener approaches in managing municipal solid waste. Thus, biopolymer-based plastics such as pectin-based soluble film can reduce the environmental issue as it is designed to directly be consumed together with the product in the package hence minimizing the amount of solid waste generated.

Although bio-based films such as those made from pectin are a great alternative for primary packaging application, these films have some drawbacks that cannot be overseen. Such disadvantages are poor mechanical properties, short shelf life, and poor water vapour barrier compared to the existing polymer-based plastics. Therefore, glycerol as a plasticizer and fruit puree are added into the basic film mixture to overcome those weaknesses. The glycerol is used to improve the film's flexibility and reduce the brittleness of neat pectin film. While, the fruit puree (pineapple) might help enhance the film's water solubility, tensile properties and WVP due to its polysaccharides content. The application of the films on the stored agar-agar quality properties should also be taken into consideration. The WVP of the films might not be sufficient enough to prevent agglomeration due to moisture gain of the agar-agar powder for a long storage time.

1.3 Objectives

This research was developed in accordance with the objectives as follows:

1. To characterize pectin-based films incorporated with pineapple puree of formulation ratio of 1:0.5:1 (pectin:glycerol:pineapple puree).
2. To evaluate the application of the films on stored agar-agar powder quality properties.

1.4 Scope of study

The research presents an opportunity to develop the pineapple puree pectin-based films (PPA) and then characterize the pectin-based film followed by packing them with agar-agar powder for storage studies at different temperatures. To achieve the first objective of this research which is the characterization of the soluble film, the scope of the study includes the preparation of pineapple puree pectin-based films. The film preparation will require defrosted pineapple puree, pectin powder, and glycerol using the formulation 1:0.5:1 (pectin:glycerol:pineapple puree). The characterization of the pineapple puree pectin-based films will focus on water solubility test, tensile properties, seal strength, water vapour permeability, surface roughness, and optical properties.

Next, to achieve the second objective, which is to study the shelf life of a product packed using the PPA film, it requires preparing of package made from pectin-based films incorporated with pineapple puree. The film will be made into a pouch or sachet of dimension 5cm × 6cm, where the agar-agar powder will be placed inside the sachet and sealed it. From this package, a series of testing will be done, such as

moisture content, true density and colour changes of the agar-agar powder during storage.

1.5 Flow chart of study

Figure 1.0 shows the flow chart of experiments carried out in the study. The study started with the preparation of pineapple puree and the preparation of soluble PPA films. The experiments involved in the first objective of the soluble PPA films include water solubility test, tensile properties test, seal strength test, water vapour permeability test, AFM test and optical properties. Meanwhile, evaluating films' application on stored agar-agar properties involved moisture content, true density, and colour changes.

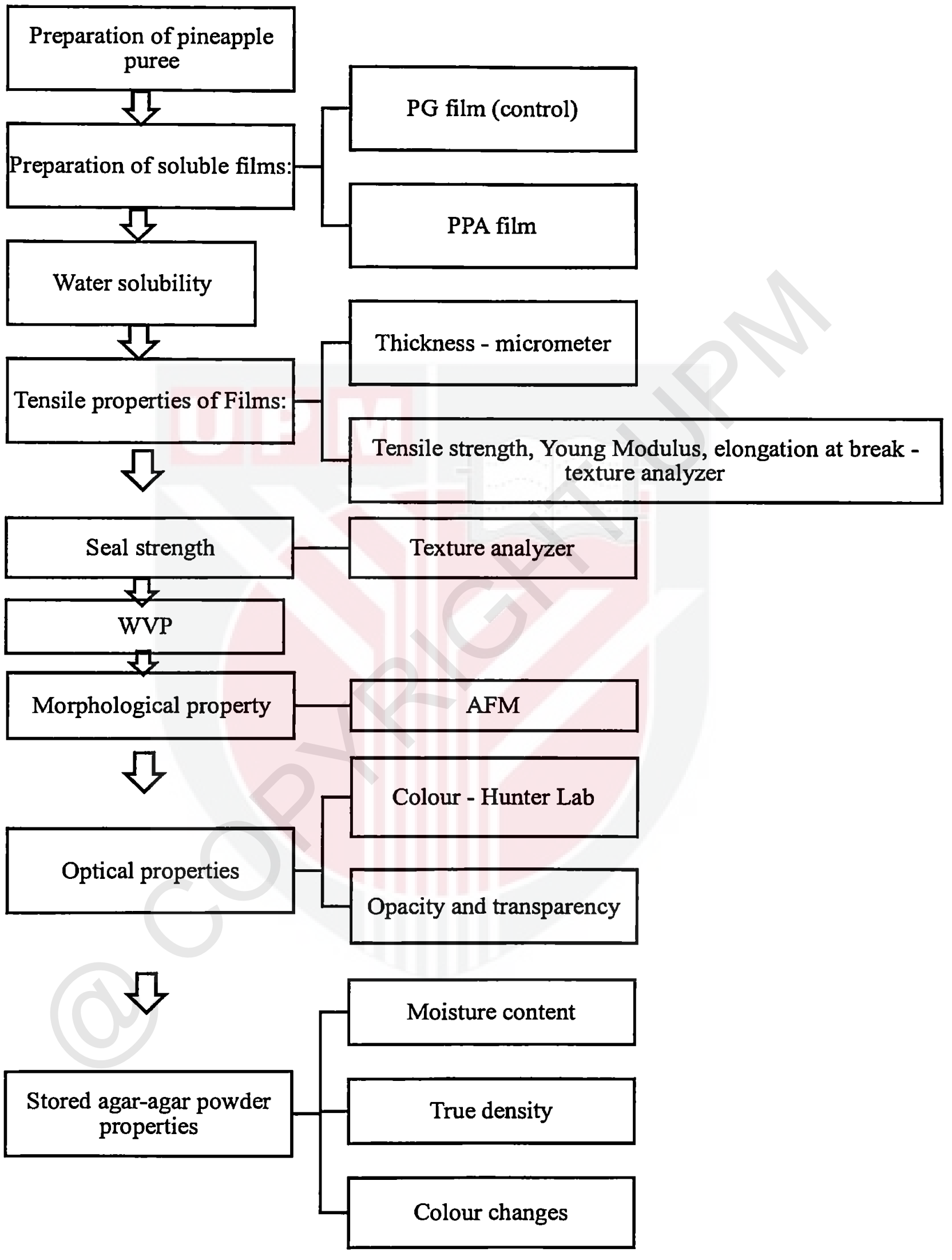


Figure 1.0: Process flow chart of characterization of PPA films and film application on stored agar-agar powder properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthetic polymer-based food packaging

2.1.1 Common food packaging plastics

By definition, synthetic polymers are materials derived from petroleum oil. Plastics are polymers that are made from the process of condensation polymerization (polycondensation) or addition polymerization (polyaddition) of monomer units (Marsh & Bugusu, 2007). During polycondensation, the condensation reaction between the molecules caused the polymer chain to grow and is accompanied by low molecular weight by-products such as water and methanol. It involves monomers with at least two functional groups such as alcohol, amine or carboxylic groups. Meanwhile, polyaddition involves the addition reaction where the polymer chains grow, in which two or more molecules combine to form a larger molecule without the liberation of by-products. This process involves unsaturated monomers by which double or triple bonds are broken to link monomer chains.

One of the major polymers used in daily life or for packaging application is probably polyethylene. Polyethylene is a versatile material with the simplest structure among the polymers, which is often applicable to grocery bags, shampoo bottles, children's toys and even bulletproof vest (Plastics and Their Roles in Food Packaging, n.d.). There are several types of polyethylene depending on the structure of the carbons chain. Each structure gives different properties such as tensile strength, durability and others. Polyethylene types widely used in food packaging include low-density polyethylene (LDPE) and high density polyethylene (HDPE).

LDPE is a slightly translucent and tough material made by blow extrusion into tubular film or extruded through a slit die and chill-roll cast, giving a clearer film. This type of film is known for its good tensile strength, burst strength, good impact resistance and tear strength, hence enabling it to retain its strength down to -60 °C (Plastics and Their Roles in Food Packaging, n.d.). Although it lacks in its capability of barrier to gases, it is an excellent barrier to water and water vapour. Due to its inert property, it causes no hazard in normal handling. Its excellent chemical resistance, particularly to acids, alkalis and inorganic solutions, has made LDPE to be one of the most popular packaging materials, such as rigid packaging. It can be easily moulded into various containers or bottle shapes, where its flexibility enables the contents to be squeezed out.

In addition, a much more linear structure than LDPE and has up to 90% crystallinity compared to LDPE, which possesses 50%, is HDPE (Plastics and Their Roles in Food Packaging, n.d.). Compared to LDPE, HDPE is stiffer and harder; hence, its tensile and bursting strengths are higher. This type of plastic can withstand high temperatures up to 121 °C. However, due to the linear nature of molecules, the impact and tear strengths are weaker than LDPE. In addition, it tends to align itself in the flow direction causing the tear strength much lower in the machine direction compared than the transverse direction. Selke & Hernandez (2001) claims that another advantage of HDPE, it is relatively good as a water vapor barrier. However, it is generally a poor barrier (gasses etc.) as it is a non-polar material. Thus, it is not a suitable package for oxygen and carbon dioxide-sensitive food products because it does not provide a sufficient barrier to transferring both gases.

Polypropylene (PP) is another type of plastic material mainly used for food packaging applications. PP is a vinyl polymer with a similar structure to polyethylene but has a methyl group attached to every other carbon atom on the backbone chain (Klein, 2011). It is one of the most versatile polymers where it serves dual duty, both as plastic and as a fiber. It is often used to make safe food containers because it has a high melting point of 160 °C. Maddah (2016), writes that the glass transition temperature of PP is within the range of 10 °C and -20 °C. It results in the polymer becomes brittle as subzero temperatures are approached.

Next, polyethylene terephthalate (PET) is another common polymer used in food packaging. It is one of the most diffused thermoplastic polymers available on the market (Nistico, 2020). This type of polymer is characterized by having esters functionalities attached to the main macromolecular chains, (Krishnan et al., 2008). Based on the journal "Plastics and Their Roles in Food Packaging" (n.d.), PET has the higher melting point among the three polymers mention above, where its melting temperature is 267 °C and its glass transition point is between the range of 67 °C and 80 °C Nistico (2020), writes that PET has been regarded as the third most commonly used plastic in the packaging industry (covering 16% of the European consumption) and is continuously growing in demand. The film has outstanding properties such as great tensile strength, excellent chemical resistance, lightweight, elasticity and good thermal stability, making it a good material for food packaging application.

Another type of plastic material used in food packaging is polyvinyl chloride (PVC). It is a polymer in which more than 50% of the content by weight consists of chlorine (Basmage et al., 2020). This type of plastic is more commonly used in manufacturing pipes or hardware materials rather than food packaging material, yet

Pearson (1981) claims that western Europe country such as the United Kingdom uses a total tonnage of 400kt of PVC for foodstuff packaging. Several basic types of PVC material are used for the application, namely bottles, rigid thermoformed foil, cling film, and cap sealing. The article "Plastics and Their Roles in Food Packaging" (n.d.), reported that this polymer was previously used in food packaging applications as the FDA approves certain octyltin compounds for stabilizing PVC during blow moulding of containers.

Polystyrene (PS) is another polymer used in food packaging where there is an addition of styrene in the polymer structure. This material is translucent, hard and brittle that has a relatively low melting point. PS is a vinyl polymer that can be mono-extruded, co-extruded with other plastics, injection molded or foamed to produce a range of products (Marsh et al., 2007). The foaming process will produce opaque, rigid, lightweight material capable for impact protection and thermal insulation properties. PS typically utilizes in protective packaging such as egg cartons, disposable plastic silverware, containers, food trays and others.

Thus, these types of plastics are the most common polymer-based food packaging. The polymers mention is relatively inexpensive which is why it is widely applied in packaging either food or non-food. The characteristic of these plastics can be summarised in the following table.

2.1.2 Issues with the current food packaging materials

Polymer-based food packaging is often associated with environmental issues as it negatively affects the environment and society. For example, the US EPA's most recently compiled waste generation statistics indicate that a total of 292.4 million

tonnes of municipal solid waste (MSW) generated or 2.22 kg per person per day, (National Overview: Facts and Figures on Materials, Wastes and Recycling, 2021). Meanwhile, in Malaysia, a total of 4.0 million tonnes of scheduled waste and 33.8 thousand of clinical waste were generated in 2019, 7.5% more than the previous years (Compendium of Environment Statistics Malaysia, 2020). Globally, it is expected that the MSW generation will be up to 3.4 billion metric tonnes by 2050 (Tiseo, 2020).

Generally, plastics are known to be one of the slowest materials to degrade. When released into the environment, plastics do not naturally degrade to a large degree. Webb et al. (2012) write that the natural degradation of plastics starts with photodegradation, leading to thermal oxidative degradation where the sunlight provides activation energy through ultraviolet light that will initiate the incorporation of oxygen atoms into small amounts of polymer. This process eventually causes the plastics to become brittle and break down into smaller pieces continuously until the polymer chains' molecular weight is sufficiently low for microorganisms to metabolize them. This entire process of plastic degradation is very slow, and it can take 50 years or more to break down fully. Some studies claim that plastics items from landfills can take up to 1000 years to decompose (Webb et al., 2012). The degradation of plastics in seawater is much slower than in lands due to the lower temperature and oxygen availability.

The slow process of degradation becomes the main reason for the environment to be negatively affected. One of the disadvantages of plastic food packaging to nature is landfilling. As polymer-based food packaging is growing in numbers, the landfill sites become more compact hence reducing the availability of spaces for landfilling are limited. MSW disposal by landfilling brings out several pollutions to the community, including air and odour pollution. The high amount of waste in a particular

landfill does not only cause terrible smells to release into the air and gas emissions that contain methane, carbon dioxide, and more than 100 different nonmethane organic compounds (Marsh et al., 2007). The emission of these gases will affect the quality of air and cause greenhouse effect and thus global warming. The air quality also deteriorated because of the MSW combustion through incineration due to the limited capacity of the landfills.

Contamination of groundwater can also potentially cause by improperly designed landfills. Marsh et al. (2007) reported the emergence of evidence that landfills sit in wetlands areas is more prone to creating contaminated groundwater which was caused by the promulgation of MSW Landfills Criteria (40 CFR part 258). This problem happened when rainwater for liquid from the waste itself permeates the landfill and dissolves the substances in the waste. Acidic or alkaline conditions enhance the extraction of certain substances into the soil hence to the groundwater. Even though composite liners (under the standards) are used to avert the leachate from reaching the groundwater collection to reduce contamination, it hinders the degradation of organic material within the landfills due to limited air and water. Contaminated groundwater will potentially harm human health as water is important for domestic use.

Other than harm to human health, plastics materials also pose a danger to animal health. The most frequent news that can be seen regarding the impact of plastics to the animal is they get tangled or drown in plastics materials such as plastic bags. It happens because they often confuse the bags for food and consume them, resulting in blockage of their digestive processes (The Negative Environmental Effects of Plastic Shopping Bags, n.d.). As a result, the animal that caught in entanglement in marine debris may experience starvation, choking, laceration, infection, reduced reproductive

success and mortality (Katsanevakis, 2008). Thiel et al. (2003) also reported that many endangered tortoises were found suffocated due to the mistaking of swallowing of plastic bags combined with seaweed.

In addition to environmental problems, polymer-based food packaging can give risk to human health due to the chemical used in plastic materials. Chemicals can leach from the packaging into the food materials such as bisphenol A (BPA) and phthalates. These chemicals are endocrine disruptors, where they can mimic the body's natural hormones, therefore, can cause a raft of health problem ("Is Plastic Food Packaging Dangerous" (2014), retrieved from https://www.choice.com.au/food-and-drink/food-warnings-and-safety/plastic/articles/plastics-and-food). Some of the health implication caused by these chemicals includes infertility, obesity, breast cancer, prostate cancer, heart disease and diabetes.

In conclusion, plastic food packaging affect negatively to the environment, humans and animals. The growing concern of this epidemic problem, especially towards the environment, has urged the man to find various solutions to overcome it. Researchers are now looking into a greener solution of turning the non-biodegradable plastics packaging into biodegradable one. One such solution is the development of bioplastics which poses many positive traits.

2.2 Soluble polymers

2.2.1 Opportunities and application of soluble polymers films

Food wrapping contributes to the increase in waste generation, especially from home or housing areas. This is because most food packaging used in the market

currently is not biodegradable and non-edible. Soluble polymers or bioplastics are polymeric materials that can be consumed by human beings or lower animals in whole or part via oral cavity and poses no threat to the health (Shit & Shah, 2014). It may be applied directly on the product's surface as additional protection to preserve quality and stability. The specific properties of the product and changes in these properties during production and storage were the requirement imposed on the soluble film. Numerous studies were carried out to develop a sustainable soluble polymer packaging that has great stability that can protect and preserve the quality of the food products.

Soluble polymers as food packaging provide beneficial effects on the environment and humans. Due to their distinct taste and biodegradable trait, natural polymers can be an alternative source for packaging development. It has received significant attention in recent years due to its benefits as a synthetic polymer substitute for food packaging. One most obvious advantage is a reduction in waste disposal. It can be consumed with the product and is often used as the natural food grade additives and flavouring or colouring enhancer (Shit & Shah, 2014). Thus, there will be no package to be disposed of, and even if the films are not consumed, they could still contribute to a better environment as they degrade more readily than normal plastics.

Moreover, recent trends also show the application of soluble and edible coatings from biopolymers, particularly pectin-based. Soluble and edible coatings were able to be achieved by researchers through the application of nanocomposites which expand the use of edible and biodegradable polymers (Shit & Shah, 2014). Highly perishable foodstuffs are currently coated with soluble and edible polymers in order to protect their nutritional and organoleptic properties by their potential for shelf life extension and reduction of negative effects caused by processing such as enzymatic browning, texture breakdown, and off-flavours development (Valdes et al.,

2015). The soluble coating can also reduce the respiration and oxidation reaction rates and decrease the risk of pathogen growth on food surfaces during storage because the coating lowered the gas exchange, moisture and solute migration (Valdes et al., 2015). Example of highly perishable food are fresh-cut fruits and vegetables.

In addition, soluble polymers offer the benefits of improving the therapeutic efficacy of drugs. The main objective was to introduce cost-effective, biocompatible, multifunctional, and less toxic polymers so that the delivery system is approved at various phases of clinical trials and benefits society. Such application used is PCL and PLA chemically bonded onto starch and can be directly applied as thermoplastics or compatibilizer (Shit & Shah, 2014). Thus, it behaves with good properties of both components such as processability, hydrophilicity, biodegradability and gelation ability. A natural polymer such as starch has widespread application ranging from a simple filler or binder to a more functional ingredient in the formulation of coatings, capsules, subcutaneous implants, and tablets. This invention makes it convenient for delivery by injection and thus was considered the possible nanoparticle preparation because it was easy to purify and soluble in water. Shit and Shah (2014) also claim that protein-based nanoparticles are more stable during storage, and better pharmacokinetic profile of peptide or protein-based medicine can be achieved.

Finally, it can be suggested that soluble and edible polymers are indeed better than synthetic polymers in many possible ways. It contributes greatly to environmental health by reducing a great amount of non-degradable polymer. It also benefits the pharmaceutical industry, where the invention of a new method of medical is achievable by incorporating natural polymers into the medicine. The advance of today's nanotechnologies aid in the establishment of this achievement. However, care must be taken to use the correct or suitable natural polymers in each application so that the

desired properties such as shelf life and mechanical strength can be tailored. Several types of soluble and edible polymers can be derived from natural sources which will be discussed in the next section.

2.2.2 Categories of soluble polymers

Soluble polymers or biopolymers can be obtained from various natural sources. The edible polymers can be divided into three main categories: polypeptides, lipids, and hydrocolloids (polysaccharides). Figure 2.1 shows the main types of the biopolymers that can be obtained from natural sources or biomass waste and some examples of their source.

The polypeptide or protein-based soluble polymer can be derived from both animal andvegetable wastes. For instance, gelatin can be obtained from the animal origin where inedible tissue or parts of the animal are its main source, while plant origin uses soy protein isolate (Mellinas et al., 2020). It is commonly used for the individual packaging of small portions of food, specifically for products packed in bunches such as beans and nuts (Shit & Shah, 2014). It functions as both carriers for antimicrobial and antioxidant agents and is used in multilayer food packaging materials together with non-edible polymer.

Protein-based polymers were expected to be good oxygen barriers at low relative humidity. However, their moisture-barrier properties were limited because proteins were not totally hydrophobic and contained predominantly hydrophilic amino acid residues. Fortunately, their function relating to mechanical and barrier properties allows the protein-based polymers to substitute synthetic polymer films. For example, collagen polymer has the advantages of biocompatibility and is non-

toxic to most tissues with well-documented structural, physical, chemical, and immunological properties. This allows the polymer to be processed into various forms and readily isolated and purified in large quantities (Shit & Shah, 2014). Additionally, a gelatin-based polymer is useful in pharmaceutical applications as it has a melting point close to body temperature; hence it is readily soluble when to consume.

Next, lipid-based polymers are commonly used in food packaging or 3D printing materials in the last few years. Mellinas et al. (2020) write that extracting lipids from natural sources is necessary to render isolated fatty acids for further used in esterification reactions. Generally, lipid compounds consist of acetylated monoglycerides, natural wax and surfactants. Paraffin wax and beeswax are the most operative lipid substances. Lipid-based polymers are excellent moisture barriers due to their relatively low polarity. However, because of its hydrophobicity, these polymer types form thicker and more brittle films (Shit & Shah, 2014).

Waxes are often directly applied onto fresh fruits creating barrier films to gas and moisture to extend the shelf life and improve the surface appearance of various food. Even so,if waxes are applied as thick layer, they must be removed before consumption, while when used in thin layer, they are considered edible. Examples of wax known as the most efficient edible compounds include paraffin, carnauba, candelilla, and beeswax. They provide a great humidity barrier.

Hydrocolloids are hydrophilic polymers that are obtained from vegetables, animals, microbial, or synthetic origin. These polymers are often known as polysaccharides as they generally contain many hydroxyl groups (Shit & Shah, 2014). Historically, polysaccharides were commercially available for application in

food and non-food industries as stabilizers, thickening and gelling agents. However, recent studies showed polysaccharides offers useful application in the fabrication of polymer-based packaging.

Mellinas et al. (2020) write that the good characteristics of these biopolymers, which include biodegradable, bio-compostable, sustainable and non-toxic characteristics, make them such great candidates in the creation of biopolymers as an alternative to plastics. Besides, they are more thermally stable than other biopolymers such as lipids and protein because of their irreversibility denaturation via heating. Proof that soluble polymers from polysaccharides (starch, carrageenan and chitosan) can extend the shelf life of strawberry fruits was performed mainly for industrial application (Shit & Shah, 2014).

Even so, polysaccharides-based polymers have some drawbacks, such as their sensitivity to moisture and low mechanical resistance. Therefore, to minimize these problems, two different approaches have been proposed, which involve incorporating different reinforcing additives to the polysaccharide matrices and combining with different polymers toobtain blends or multilayer films (Mellinas et al., 2020). The polysaccharide focused on this research will be pectin which will be discussed in the next section.

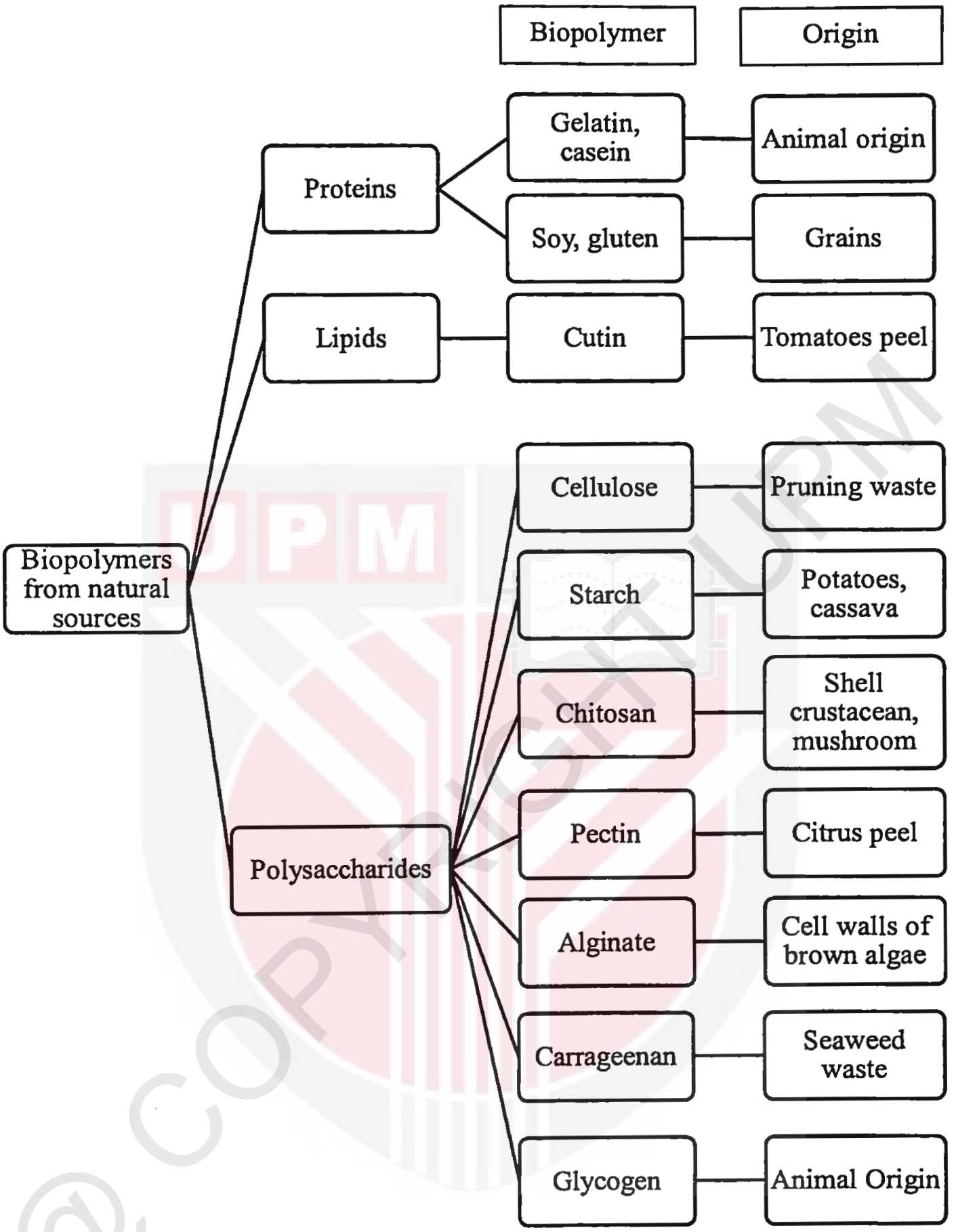


Figure 2.1:Main types of biopolymers and their origin

2.3 Pectin

Pectin is a complex heteropolysaccharide and a major multifunctional component of the cell wall in various terrestrial plants. It generally allows primary cell wall extension and plant growth. Therefore, it could be extracted for it has many useful applications in various fields. In manufacturing industries such as food and pharmaceutical, pectin is utilized as thickeners, stabilizers, and gelling agents. However, the advantages of using pectin as a biopolymer over conventional polymers have been highlighted in recent years, where pectin is being used as the film matrix. Therefore, biopolymers are potentially marketable and becoming preferable over plastics mainly due to their biodegradability.

Pectin, which is usually found in the cell wall of plants with the association with other compounds like cellulose, lignin or polyphenols, is composed of galacturonic acid units, as shown in Figure 2.2 (Mellinas et al., 2020). Valdes et al. (2015) claim that pectic substances are essential in the cell for function, such as adhesion between cells, the mechanical strength of the cell wall, ability to form the stabilizing gels, and they play a significant role in the growth of plant cells. Approximately 70% of the pectin composition comprises galacturonic acid, depending on the plant species. It is linked at the O-1 and the O-4 position in all pectic polysaccharide (Mellinas et al., 2020). Pectin consists of both "smooth regions" and "hairy regions", where it has a region of a linear anionic backbone with no side chains and regions with non-ionic side chains, respectively.

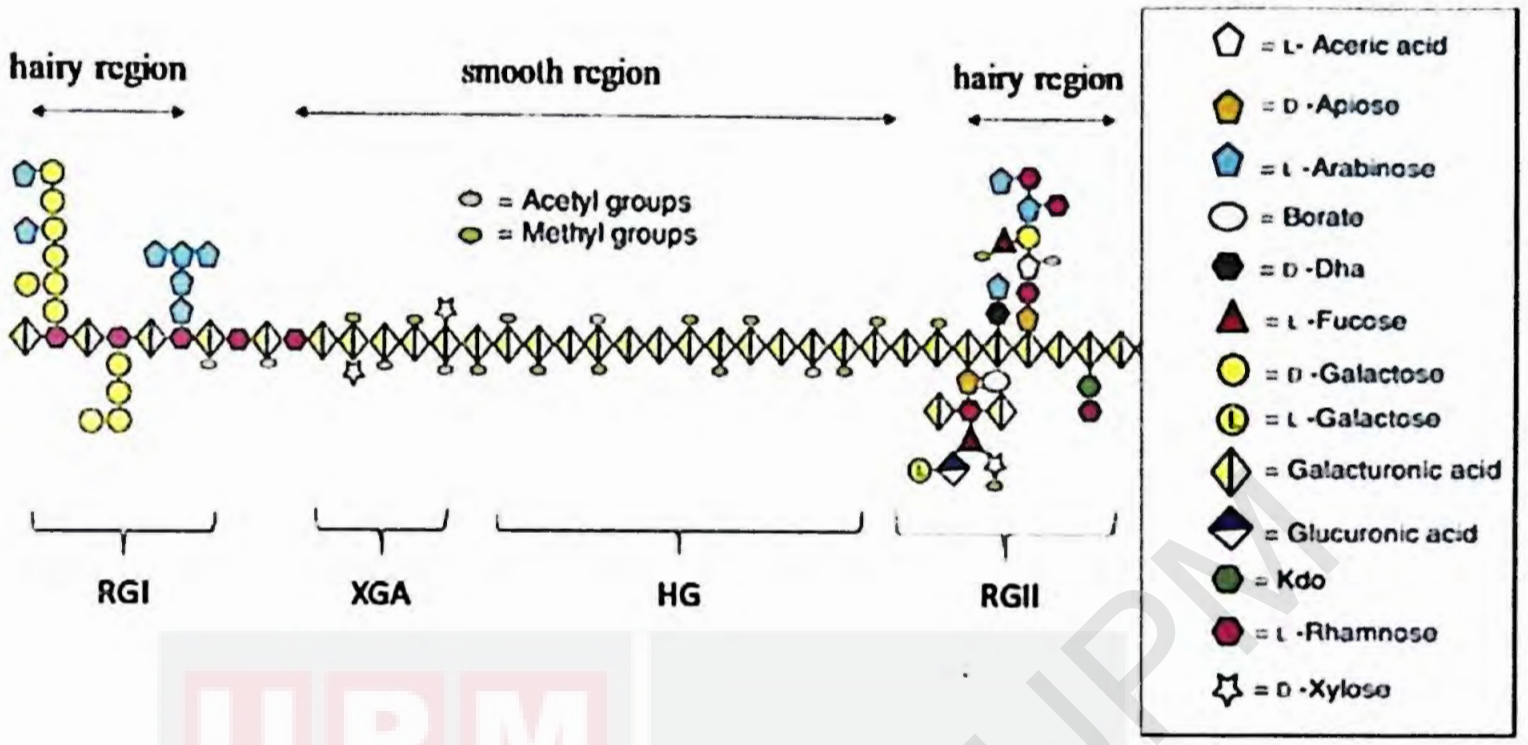


Figure 2.2: Schematic pectin structure (Mellinas et al. (2020)

Valdes et al. (2015) reported that pectin polysaccharides could be classified into three major types. Homogalacturonan (HG) is one of the pectin types, which is a linear polymer formed by D-galacturonic acid, representing approximately 65% of the total pectin content. These domains are the major constituents of the "smooth regions". It can be divided into three families depending on the acetylation or methylation reactions generated during polymerization. These families include (a) pectin with more that 75% of methylated carboxyl groups; (b) pectinic acid with less than 75% of methylation; and (c) pectic acid without the presence of methyl-esterified carboxyl groups.

The next group of pectin polysaccharides is rhamnogalacturonan I (RGI). It generated by the repeating disaccharide rhamnose-galacturonic acid groups acetylated and connected to side chains of neutral sugars, like galactose, xylose and arabinose (Valdes et al., 2015). Mellinas et al. (2020) claim that it contained about 20%-35% of the total pectin and appeared to be a more complex structure than HG.

The final type of pectin polysaccharide is rhamnogalacturonan II (RGII), which is also formed by homogalacturonan chains. Compared to RGI, this group has more complex side groups attached to 12 different glycosyl residues. Those residues include rare sugar species such as 2-O-methylxylose, 2-O-methyl fucose, aceric acid, 2-keto-3-deoxy-D-lyxo heptulosaric acid, and 2-keto-3-deoxy-D-manno octulosonic acid (Valdes et al., 2015). Both RGI and RGII are the hairy regions in the pectin structure.

In general terms, pectin is commonly extracted from various natural sources as well as from biomass waste. Due to their good properties and high yield extraction, Mellinas et al. (2020) reported that the peels of citrus fruits are the main source to obtain pectin at an industrial scale. However, many studies proved that other sources are also capable of pectin extraction, such as vegetable waste or peels, tropical fruits peels, tuber peels and many others. This results in different extraction methods depending on the raw materials.

Valdes et al. (2015) reported several types of extraction methods that have been applied previously and presently: conventional solvent extraction based on stirring and heating, microwave assisted extraction (MAE), electromagnetic induction (EMI), ultrasound-assisted extraction (UAE) and enzymatic extraction. Depending on the extraction methods and raw materials, the parameters such as temperature, pH, time, and solvent:liquid (S:L) ratio are different and the pectin yield extraction. Table 2.1 and Table 2.2 summarise the extraction of pectin using different methods.

Table 2.1: Natural pectin extracted by solvent extraction methods (Valdes et al.,2015)

Natural Source	Extraction Parameters	Pectin Yield Extraction
Okra pods	Citric and phosphate buffer, pH 2 and 6, 60 min, 80°C, 1:15 $g.mL^{-1}$	13.3 (pH2.0); 15.7 (pH6.0)
Durian rinds	HCl, pH 2.8, 43 min, 86 °C, S:L 1:10 $g.mL^{-1}$	9.1
Mango peel	Sulfuric acid in water, pH 1.5, 2.5 h, 90°C	>70
Banana peel	Citric acid solution, pH 2.0, 160 min, 87°C, 1:20 $g.mL^{-1}$	13.89
	Citric acid and HCl, pH 1.5, 4 h, 90 °C	16.54
Tropical fruits peels	Citric acid, pH of 2, 3.3 and 4.5, 120 min, 70 °C	12.56–14.24
Sugar beet pulp	Citric acid, pH 1, 166 min, 99 °C, 1:20 g·mL−1	23.95
Bagasse and pomace lime fruit	Citric acid, 60 min, 90 °C, 1:20 g·mL−1	13.31 (Bagasse); 15.1 (Pomace)
Valencia orange peel	Citric acid extraction, pH 1.5, 75 min, 90 °C	16.7
Watermelon seed	HCl, pH 2, 60 min, 85 °C, 1:15 g·mL−1	19.75
Faba bean hulls	HCl, pH 1.5, 80 min, 85 °C, 1:25 g·mL−1	15.75
Honeydew melon seeds and damaged skin	HCl at pH 1, 80 °C for 4 h	7.9
Tomato peel	Ammonium oxalate and oxalic acid, 90 °C in two	32.0

	extraction steps (24 and 12 h)	
Sunflower head	Sodium citrate, 85 °C, 3.5 h, 1:40 g/mL	16.90

Table 2.2: Innovative extraction methods of pectin

Extraction Method	Natural Source	Extraction Parameters	Pectin Yield Extraction (%)
MAE	Dragon fruit peel	400 W, 45 °C, 20 min, 24 g·mL−1	7.5
		HCl, pH 2.07, 800 W, 65 s, 66.6 g·mL−1	18.57
	Bagasse and pomace of Mexican lime fruit	Citric acid, 800 W, 120 °C, 5 min, 1:30 g·mL−1	16.9 (Bagasse); 8.4 (Pomace)
	Pomelo peel	Tartaric acid solution, pH 1.5, 660 W, 9 min, 1:40 g·mL−1	23.83
	Mango peel	Aqueous solution, pH 2.7, 413 W, 134 s, 1:18 g·mL−1	28.86
	Papaya peel	Aqueous solution, 512 W, pH 1.8, 140 s, 1:15 g·mL−1	25.41
EMI	Citrange albedos	Aqueous solution, pH 1.2 with	29

		H2SO4, 80 °C, 90 min	
UAE	Pomegranate peel	Aqueous solution, pH 1.27, 61.9 °C, 28.31 min, 1:17.52 g·mL−1	24.18
	Sisal fiber	Aqueous solution, 61 W, 50 °C, 26 min, 1:28 g·mL−1	29.32
	Sugar beet pulp	Aqueous solution, 10.70 MPa, 120.72 °C, 30.49 min, 44.0 g·mL−1	24.63
	Grapefruit peel	Deionized water, HCl, pH 1.5, 12.56 W/cm2, 66.7 °C, 27.9 min, 1:50 g·mL−1	27.34
Enzymes	Gold kiwifruit pulp, skin and seed	Purified water, pH 3.7, 25 °C, 30 min,	2.14
	Gold kiwifruit pomace	Celluclast 1.5 L enzyme (1.05 mL·kg−1), 1:3 g·mL−1	4.5

2.4 Glycerol

In manufacturing of plastics, additives are typically used to increase the mechanical strength or the barrier properties of plastics (Chin, 2010). There are various types of additives used in plastics production, ranging from silicon to wood flour,

depending on the purpose of the materials. Generally, plasticizers are colourless and odorless esters produced by a reaction of an alcohol with an acid such as adipic acid, phthalic anhydride and others (Koester, 2015). The combination types of alcohol and acid will determine the set of esters produced, thus the type of plasticizer.

One of the most common plasticizers used in plastics is glycerol. Glycerol serves to improve flexibility by reducing the stiffness of the plastics, where both functions help increase the mobility of the polymer bonding (Lutfi et al., 2017). This is due to the intermolecular forces and the material's glass transition temperature (Blick et al., 2015). Additionally, Lutfi et al. (2017) write that glycerol also helps to improve the water holding capacity of the plastics. In the study of the effect of glycerol on the mechanical behaviour of *Walur (Amorphophallus paenifolius* Var. *sylvestris)* plastic film by Lutfi et al. (2017), the elongation of the film was highly affected by the high portion of glycerol addition. Furthermore, the water content in the Walur plastic was also associated with the glycerol addition resulting in a much more flexible film because water is also considered as a plasticizer.

However, the increase in the glycerol portion in plastic materials will decrease tensile strength. As glycerol improves the elongation and flexibility of the plastics, the tensile strength and the mechanical resistance are lowered because of the weakened intermolecular forces in their chain (Lutfi et al., 2017). In a study on the effect of glycerol on starch-based film, Rodrigues et al. (2006) explain that glycerol disrupts the molecular cohesiveness of starch and lowers the intermolecular interaction in the film. Thus, films with high tensile strength have low glycerol, strengthening the hydrogen bonds between basis polymer chains interaction over the polymer-glycerol attraction.

Zhu et al. (2013) write that glycerol can be generated from biodiesel and is recognized as one of the top 12 building block chemicals from biomass by the US Department of Energy in 2004. Traditionally, biodiesel can be derived from vegetable oils or animal fats through transesterification with methanol or ethanol. The process involves converting triacylglycerol and methanol into glycerol and fatty acids methyl esters known as biodiesel. Alkali or acid catalyst is required to fulfill the process, as shown in Figure 2.3. Due to the variety in biodiesel sources (types of vegetables and species of animals), it is reasonable to deduce that the chemical compositions in crude glycerol vary depending on the sources.

As glycerol is produced from biological origins and chemical catalysis, it is suitable to be used in the production of bioplastics. Furthermore, many studies have been proved the efficiency of using glycerol in the production of a biopolymer-based film (Zhu et al., 2013; Lutfi et al. 2017; Lusiana et al., 2019). Thus, glycerol is applied as the plasticizer for the PG and PPA film.

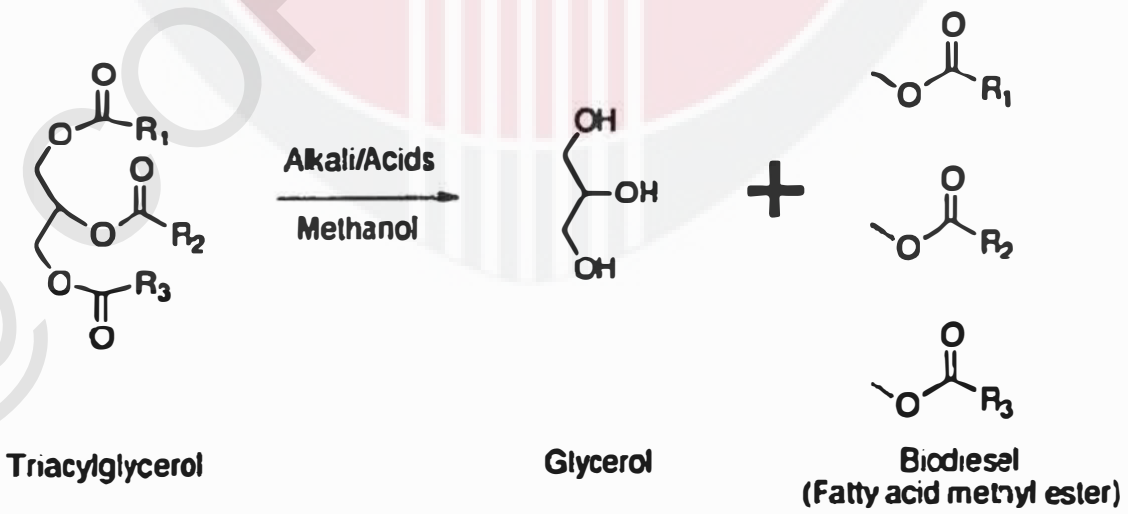


Figure 2.3: Process of transesterification of glycerol production (Zhu et al., 2013)

2.5 Pineapple puree

Pineapple *(Ananas comosus)* is a tropical fruit that is rich in both vitamins and minerals. Pineapple can be easily found in many regions of Malaysia due to its tropical climate. Nowadays, instead of just being sold as whole fruit, pineapples are processed to produce various types of food including canned pineapple slices, pineapple juice, dried pineapple and others. Current studies show that pineapple puree has potential use as a medium to produce bio-based film, (Rodsamran & Sothornvit, 2019).

Additionally, in keeping with the trend of deriving materials featuring distinguish sensory and nutritional properties, fruits as well as vegetables have been utilized as main ingredients in producing soluble and edible films. Otoni et al. (2017), reported that the production of soluble and edible films required the fruits or vegetables to be pureed, extracted or juices, or even as pomace which is a food processing wastes or residues. Pineapple is rich with cellulose, hemicelluloses, lignin and pectin. This might be the reason why pineapple might be effectively used as raw material for pectin production and for production of soluble and edible films.

However, Rodsamran & Sothornvit (2019) reported that pineapple naturally contains high levels of phenolic compounds influencing the cross-linkage between the purity of pectin molecules and phenolic compounds. This reason caused the extraction of pectic substance from pineapple peel has lower yield. Otoni et al. (2017), also explain that films comprised entirely of puree frequently shows poor consistency, mechanical strength and barrier properties. Therefore, it is not practical to use pineapple puree only in making soluble film since it is only comprised of a single biopolymer.

Although pectin from pineapple puree is not sufficient to be used as biopolymer film material itself, the compound in the pineapple pectin could act as a natural plasticizer and film matrix to produce a soluble film. Otoni et al. (2017), explained that for a stable soluble film successfully created, two or more macromolecules may be combined into blends as proven in carrot-pectin soluble films (Wang et al., 2011), so hydrocolloids are often added as binding agents to enhance films' physical properties.

Rodsamran & Sothornvit (2019) proposed that pureed pineapple mixed with water to dissolves commercial pectin for film forming. The study has been proven that using of pineapple puree as an additive in pectin-based films improved the films' properties especially the water vapor barrier and antioxidant properties. Sonawane (2019) also claims that pineapple fibre which is bio-composite material acts as the reinforced fibers in soluble films and non-soluble films. In addition, the high cellulose content of 70-80% greatly enhances the strength and stiffness of the soluble films.

2.6 Water solubility, water vapour permeability and moisture content

For edible and soluble films, water solubility is a very important property that affects their future applications. By definition, solubility refers to the ability of films to dissolves in water for proper digestion when consumed, or it can decompose naturally when discharged into the environment. Since the soluble films produced in this study are to be used in the packaging of agar-agar powder, it is important and convenient for the films to be readily soluble when immersed in water to reduce packaging waste.

Water solubility study performed by Liu et al. (2020) on soybean polysaccharide (SSPS) blended with gelatin, where a piece of film was immersed in 50 ml of distilled water. It was reported that the higher percentage of SSPS on gelatin-based matrix result in higher solubility of film. In contrast, the water solubility of blended films decreased with the increasing weight fraction of gelatin. It can be explained that the incorporation of gelatin help to establish more compact polymeric matrices, thus permitting the free volume decrease in blend film with higher gelatin content. Since both gelatin and pectin are thickening agents, it can be expected that pectin-based film incorporated with pineapple puree has higher water solubility than PG film. For packaging application test, previous study on coffee and coconut powder packed using polysaccharide-based film showed good solubility when dissolves in water where it dissolves completely in less than 30 seconds (Liu et al, 2020).

Additionally, water vapour permeability (WVP) and water vapour transmission rate (WVTR) are important properties that affect the soluble film's application performance in food packaging. It is expected that the PPA film has a lower WVP value than the PG film. Alves et al. (2011) also claimed that the vapour barrier properties could be enhanced by incorporating fruit macromolecules into the polymer matrix. The dry cup method the most common method for testing the WVP of a film, is as it is inexpensive and simple. A similar method was performed by Liu et al. (2020) and Chambi & Grosso (2010).

The moisture content of soil (sample) referred to the amount of water present in the soil. It is the ratio of the mass of water in a sample to the mass of solids in the sample, which is expressed as a percentage. The moisture content of the powder can be measured using a moisture analyzer because it is fast and inexpensive. Moisture content is related and highly influenced by the WVP and WVTR of the type of film

used for the packaging of the food material. Alves et al. (2011) and Liu et al. (2020) reported that incorporating fruit and vegetable macromolecules improve the stability of barrier ability against moisture. It is found that increasing concentration of gelling agent in pectin-based films reduce the water vapor permeability, thus lowering the rate of moisture content gain (Alves et al., 2011; Liu et al. 2020).

2.7 Tensile strength properties and heat seal strength

The tensile properties are important characteristics of films to maintain the mechanical integrity of food packages. In order to study the strength comparison of both types of film, measurement of the TS, EAB and YM need to be performed in developing a sustainable soluble and edible film. One of the most common methods to measure tensile strength and breaking strength is a texture analyzer based on the standard D882-02 (ASTM, 2002). It is a texture measurement system that moves in either an up or down direction to compress or stretch a sample suitable for measuring the TS and EAB of film. A similar method also has been used by several studies on measuring the TS and EAB of biopolymer film, such as Rodsamran & Sothornvit (2019); Liu et al. (2020); Chambi & Grosso (2010).

In order to prevent leakage of the product inside the package during storage, processing or handling, it is required to have a sufficient seal strength of the packaging material. Liu et al. (2020) stated that it had been recognized that a major drawback for soluble and edible films has a poor heat seal property. A preliminary study showed that using heat seal level 8 of the impulse heat-sealer Model SP-300H and heat seal the film strip 3 times could provide sufficient melting and fusion of pectin-based and

PPA films. The seal strength test method usually was done according to the ASTM standards (D882, E1771 and E691), where the equipment involved also use a texture analyzer. This is because texture analyzer can generate a digitized plot of force versus grip travel. Liu et al. (2020) and Raduan (2020) also reported that the same technique was used to perform heat seal strength on soybean polysaccharide/gelatin and pectin-fruit puree film, respectively.

2.8 Atomic force microscopy of bio-based film

AFM is a common method to perform topography images of a sample surface using scanning the cantilever over a region of interest, in this case, the soluble films. The peak and trough feature on the sample surface influence the deflection of the cantilever that is monitored by the PSPD. Thus, AFM enable researchers to study the surface roughness of the samples, which is suitable for analyzing the surface roughness of a film. Dai et al. (2018) ad Liu et al. (2020) also performed the same method for analysing the surface roughness.

Previous studies reported that bio-based films that contain high concentration or weight fraction of plasticizer have smoother surface area and better appearance, (Sanyang et al., 2016; Liu et al., 2020; Dai et al., 2018). Sanyang et al. (2016) also reported that plasticizers have low molecular size which enable them to creep within the intermolecular spaces of polymer chains. It leads to the reduction of intermolecular hydrogen bond strength, increasing the film molecular mobility and eventually creating a smooth surface. In contrast, thickening agent such as pectin and gelatin induced the formation of small clusters on the surface of the film creating a rougher

surface when the concentration of these material is high in the matrix film, (Liu et al., 2020). Thus, it is expected for the PPA films to have a smoother surface than PG films as PPA films contain pineapple puree which acts as a plasticizer as well as matrix.

2.9 Optical properties

In practical food packaging applications, it is most preferred to have films packaging that is both colourless and transparent. The most suitable and common method of performing analysis of optical properties is by using colorimetry. Through colorimetry values of L*, a* and b* can be generated, which are used to determine a sample's colour pigment and the total colour difference. A similar method was used by Pathare et al. (2012); Sircusa et al. (2018); Liu et al. (2020) on the optical properties of biopolymer-based films. The transmittance of the film, which will be used to measure the transparency and opacity, is often measured by using a spectrophotometer.

The values of transparency and opacity show similar values; however, both are opposite in terms of meaning. The higher the value of transparency, the less transparent film, which indicates that the film was more opaque. Pathare et al. (2012) stated that the total colour difference indicates the magnitude of colour difference between stored cand control samples (standard plate). It can be analytically classified into three which are very distinct ($\Delta E>3$), distinct ($1.5<\Delta E<3$), and small differences ($1.5<\Delta E$). A bio-based film made with pectin and citrus essential oil showed a slightly yellowish hue (Siracusa et al., 2018). It is important to preserve the transparency of the film or at least display a colour similar to the natural pigment of the food on which the film is

going to be used, since the colour of the film may affect the consumer acceptance of the product.

2.10 True density

The true density of the powder is often measured using the gas pycnometer instrument. This instrument measures the true density of a porous sample using of gas displacement. An inert gas such as helium is often used as the displacing medium. First, the sample is sealed in the instrument chamber of known volume; then, the helium gas is admitted to expanding into another precision internal volume. The pressures observed upon filling the sample chamber and then discharging it into a second empty chamber allow of the sample solid phase volume computation. True density is the density of the solid material, excluding the volume of any open and closed pores. A similar method was used by Yu et al. (1995) on the study of coal agglomerates during storage.

It has been reported that the true density of the powder increases to a maximum during storage and the decreases due to increasing moisture content (Yu et al., 1995). The moisture gained by the powder particles will cause the powder to agglomerates and generate intraporosity between the powder particles which eventually reduce the true density.

CHAPTER 3

METHODOLOGY

3.1 Materials

The raw materials used include pectin, glycerol and pineapple puree. Both pectin and glycerol were purchased from R & M Chemicals, R & M Marketing, Essex, UK. Meanwhile, the puree was extracted from pineapple (*Ananas comosus*) by which the fruit was purchased from TSM Selangor Trading Sdn. Bhd. The pineapple fruit was selected in the commercial ripening stage and was used as soon after the purchase.

3.2 Preparation of pineapple puree

Before making the pineapple puree, all apparatus and equipment were cleaned and sanitized using 70% alcohol solution. The pineapple was washed and air dried before peeling using a knife. In this research, the flesh of the fruit was used to make the puree instead of the peels. The peeled pineapple was then cut into cubic for an easier blending process. 100g of the pineapple was blended using a food blender (MX-GM1011, Panasonic Co., Japan). The blended pineapple was then filtered using cloth for fibers removal. The pineapple puree was kept in a cleaned and sanitized glass bottle wrapped with aluminium foil to prevent vitamin C degradation by deoxidation. The puree was stored in the freezer for further use. The process flow chart of this process is shown in Figure 3.1 to summarise the process.

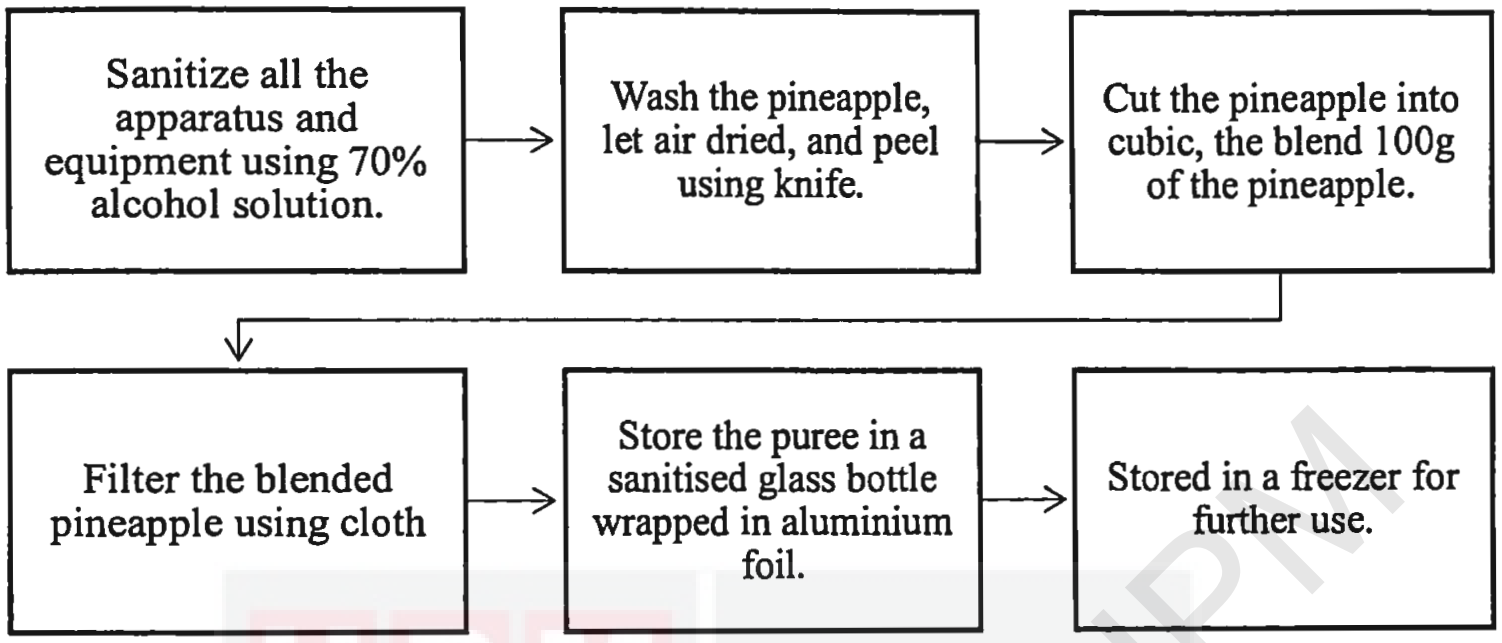


Figure 3.1: Pineapple puree preparation procedure

3.4 Preparation of soluble pineapple puree pectin-based film

The method of preparing both PG films (control) and PPA films was based on C. Liu et al. (2020), with some modifications done on preliminary studies. The formulations of the films used were according to the ratio of 1:0.5 and 1:1:0.5 for PG films and PPA films, respectively. The formulations in unit weight include 4 g of pectin powder, 4 g of pineapple puree and 2 g of glycerol.

The procedure of preparing PPA films started with dissolving the weighted pectin powder, glycerol and the pineapple puree into 100 ml of distilled water at a temperature of 70 °C Then, the solution was stirred at 100 rpm using a magnetic stirrer. The solution was kept at 95 °C for 30 minutes while stirred for cross-linked structure and pectin molecule formation. The solution was then cooled down to 50 °C before incorporating the pineapple puree into the pectin solution. The mixture was then stirred

for another 5 minutes at 100 rpm to obtain a homogenous mixture. Next, the mixture was allowed to be refrigerated at temperature of 4 °C for 1 hour in a chiller. Next, the mixture was allowed for sonification using a digital sonifier (Branson, Branson Ultrasonic Corporation, Danbury, U.S.A) at 50% amplitude for 10 minutes for bubble removal. Then, 20 ml of the mixture was cast onto a glass petri dish and let dry for 4 hours in the oven (Memmert, Memmert GmbH + Co., Schwabach, Germany) with a 0% fan setting. Another 30 minutes of oven drying (with 10% fan setting was performed to ensure the films were dried completely. Lastly, the dried films were peeled off from the petri dish and conditioned under controlled relative humidity (RH) of 50% and temperature of 28 °C in an environmental chamber (BH-TH_50, SH Guangpin (Bogong) Test Equipment Manufacturing Co.Ltd., Shanghai, China) for 48 hours before analysis.

Table 3.1 and Table 3.2 show the film mixture formulations in ratio and in unit weight, respectively. Meanwhile, Figure 3.2 shown below is the flow diagram of the film preparation procedure.

Table 3.1 The formulation of soluble films in ratio.

Film	Components	Ratio
PG	Pectin:Glycerol	1:0.5
PPA	Pectin:Glycerol:Pineapple Puree	1:0.5:1

Table 3.2:The formulation of soluble films by weight (g)

Film	Components	Weight ratio (g)
PG	Pectin:Glycerol	4:2
PPA	Pectin:Glycerol:Pineapple Puree	4:2:4

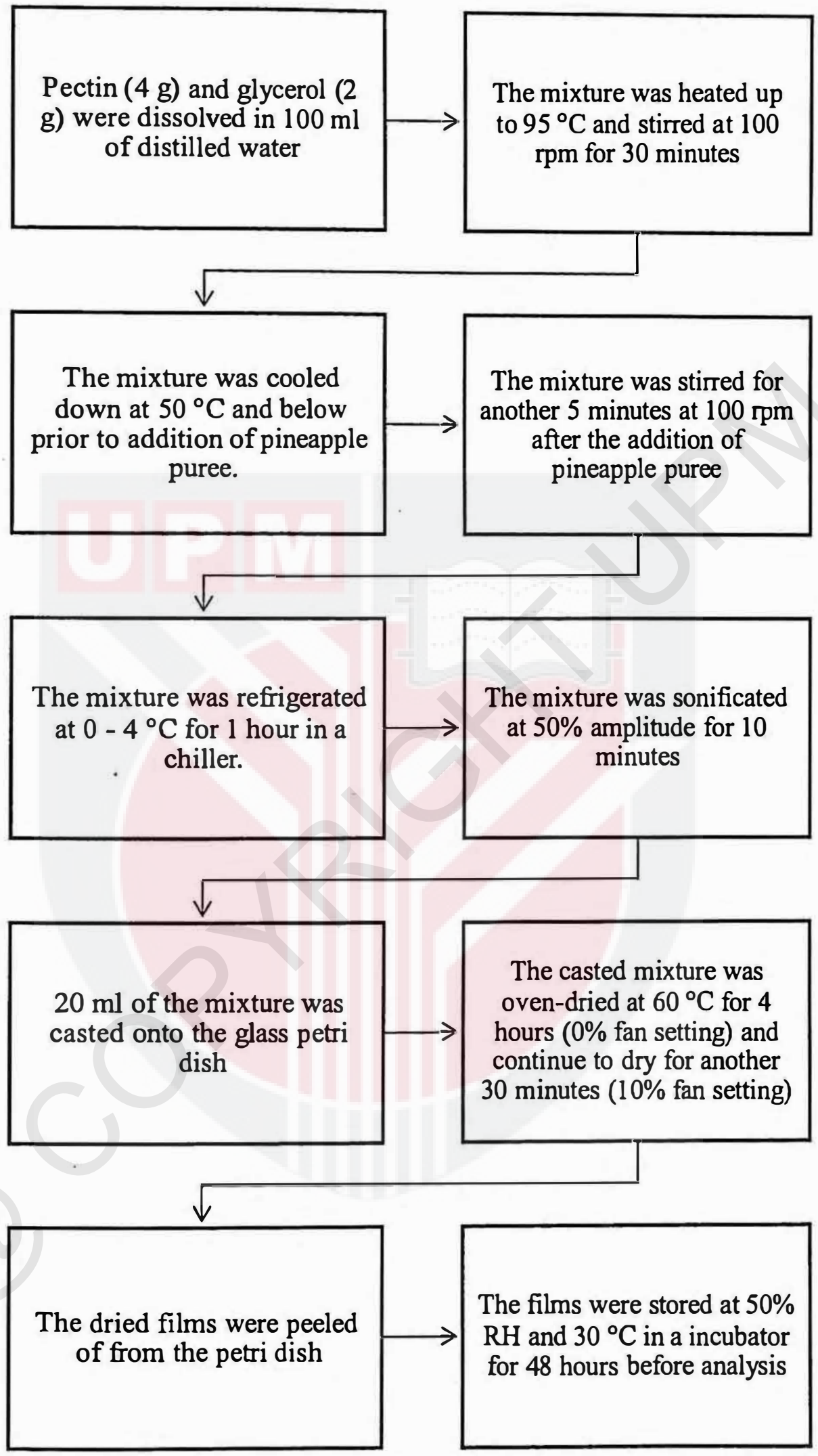


Figure 3.2: Flow diagram of preparation of soluble films procedure

3.5 Characterization of soluble pineapple puree pectin-based films

3.5.1 Water solubility test

The water solubility of the film at 27 was measured according to Liu et al. (2020) with slight modifications. First, the films were cut into square pieces of 3 for each film (replicates) with the dimension of 40 mm x 40 mm using a sharp razor blade. The samples were then weighed and dried in an oven at 105 °C to constant weight for 24 hours. The films were then placed in a desiccator containing silica gel with RH 0% for 30 minutes prior to weighing to obtain initial weight of the films (W_0).

Then, each sample was immersed in 50 ml of distilled water and placed in a Shaking Water Bath (Model BS-21, Jeio Tech Inc., Korea) at room temperature for 24 hours. Then, each solution containing the films was filtered through filter paper (Whatman No.541), which was previously dried and weighed at the same condition as the films. After the filtration process, the filter papers used were further dried with the same condition at previous for 24 hours and the final dry weight (W_i) was obtained. The water solubility test was done triplicate for each type of film. The film water solubility was calculated as follows:

$$water\ solubility\ (\%) = \frac{W_i - W_0}{W_i} \times 100 \quad (3.1)$$

Where, W_0 is the initial dry weight of film (g) and W_i is the final dry weight of the film.

3.5.2 Tensile test

There are several main characteristics of the films to be tested for tensile properties as recommended by the American of Testing and Materials (ASTM),

including thickness, TS, EAB, YM and toughness. For the measurement of films thickness, it was performed using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Average thickness value was taken by measuring five random spots on the film.

With reference to C. Liu et al. (2020), the TS and EAB of the films were measured using a texture analyzer (Stable Micro System, model TA. TXplus, Surrey, England) according to the standard method D882-02 (ASTM, 2002). The films were cut into long strip of dimension 15 mm x 100 mm (length x width) as shown in Figure 3.3 and then conditioned at 25 ± 1 °C and 50 ± 5% RH for 48 hours in an environmental chamber (BH-TH_50, SH Guangpin (Bogong) Test Equipment Manufacturing Co.Ltd., Shanghai, China) before testing. The testing was performed where the rubber-coated pneumatic grips enable accurate control of the gripping pressure. A 5 kN load cell with 0.5 mm/sec crosshead speed was applied to the testing. The film will be clamped between two grips and the fixtures start to move until the film breaks. At its point of breaking, the force (N) and elongation (mm) were recorded. Five replicates of each film were tested to calculate the TS, EB and Young Modulus (YM).

The texture analyzer generate a load extension graph to report tensile strength, percentage elongation at break, and toughness. TS is the strength of the sample under a loading which can be calculated as the following equation:

$$TS = \frac{F_B}{A_i} \tag{3.2}$$

Meanwhile, the EAB can be calculated using the equation below:

$$EAB = \frac{L_B}{L_i} \times 100 \tag{3.3}$$

YM is the slope of the initial linear portion of stress versus strain curve that can be estimated as the following equation:

$$YM = \frac{\Delta F / A_i}{\Delta L / L_i} \quad (3.4)$$

Where F_B is the force at breaking point, L_B is the elongation at breaking point, Ai is the initial minimum cross-sectional area of the film sample, L_i is the primary length of the sample, while ΔF and ΔL are the alteration in force and length.

Figure 3.3: Dimension of the films strip for tensile strength test

3.5.3 Seal strength test

The seal strength test for the films was performed according to Liu et al., (2020) with some modifications. First, the films were cut into strips of 15 mm × 100 mm. Two strips of the same type of film were faced together, and an area of 15 mm × 10 mm^2 near the edge of the strip was sealed using an impulse heat sealer Model SP-300H

(Triumph Mercantile Corp, Taiper, Taiwan). The microprocessor maintained the dwell time and temperatures of each pair of films strip throughout the experiment. After heat sealing, the specimens were conditioned at 28 ± 2 °C and 50 ± 5% RH in an environmental chamber (BH-TH_50, SH Guangpin (Bogong) Test Equipment Manufacturing Co.Ltd., Shanghai, China) for 48 hours before the testing. Each type of films was done 5 replicates.

After 48 hours, each leg of the specimens was clamped to the machine and peeled open at 180° angle (Figure 3.5). The measurement was done using a texture analyzer (Stable Micro Systems, model TA. TXplux, Surrey, England) according to the ASTM standard method D882-02 (ASTM, 2002). The constant rate of loading 200 mm/min and initial distance between the clamps (grip length) 10 cm were chosen.

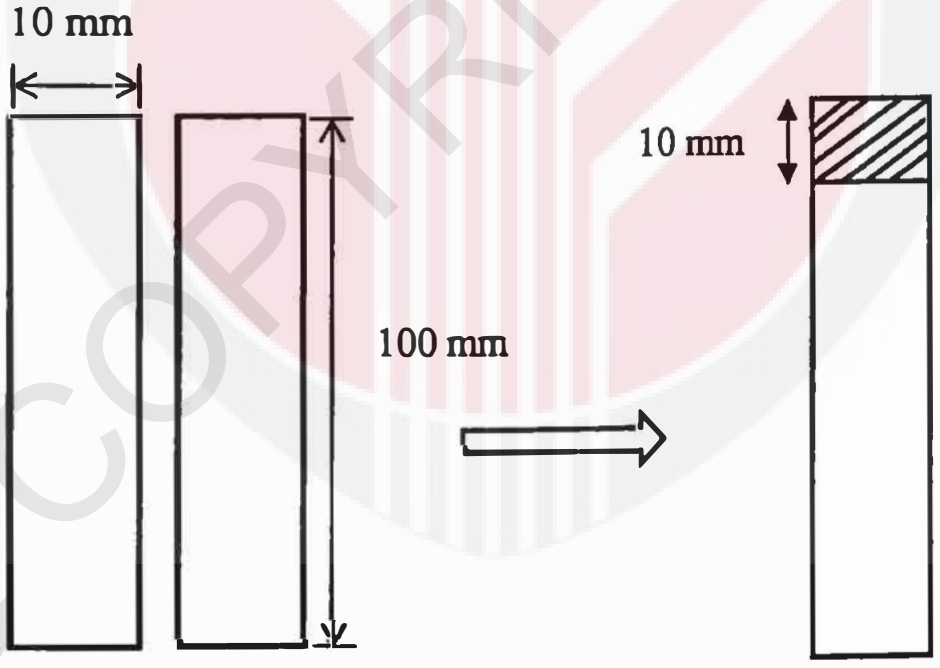


Figure 3.4: Fabrication progress of heat-sealed specimens and samples after cut and heat-sealed

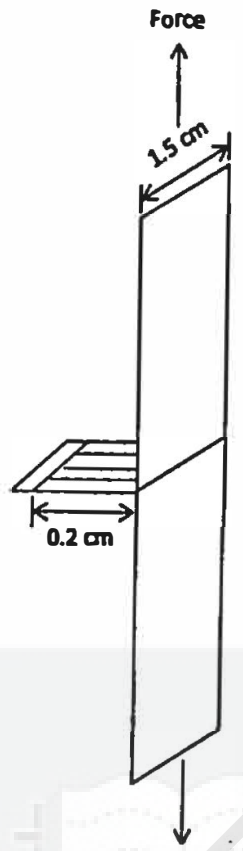


Figure 3.5: Overview of seal strength test of heat-sealed specimens

3.5.4 Water vapour permeability (WVP) test

The water vapour permeability (WVP) of films was evaluated according to the modified method of ASTM standard E96-05 used by C. Liu et al. (2020) with some modifications. The films were cut into circular samples and sealed onto circular test cups (GARDCO Perm Cup, BYK Int. Co., U.S.A) with a diameter of 60 mm. A small glass dish was used to place calcium chloride ($CaCl_2$) with approximately 1.5 cm between the film and the surface of $CaCl_2$. The test cups were placed inside a desiccator over a magnesium hydroxide ($Mg(OH)_2$) solution (50% RH) at ambient (28 °C) The weight of each cup was recorded at intervals of 24 hours over a 7 days period. The WVP value of the of the film was calculated using the following equation:

$$WVP = \frac{WVTR \times L}{\Delta P \times A} \tag{3.5}$$

$$WVTR = \frac{dm/dt}{A} \tag{3.6}$$

Where WVTR represent the water vapour transmission rate obtained for the slope of plot loss vs. time. ΔP is the water vapor pressure difference across the film (kPa), and A is the area of the film in unit m^2. The test was done in two replicates of each type of film.

3.5.6 Atomic force microscopy (AFM)

The surface properties such as roughness of the films were investigated based on Liu et al. (2020). For AFM analysis, the 3D surface topography of each film was recorded using a Keysight's 5500 (N9410S, Keysight Technologies Canada Inc., Canada) atomic force microscope. All the image of 256 × 256 pixels were acquired by scanning square areas of 10 mm × 10 mm.

3.5.6 Optical test

The optical properties of both films (PG and PPA) were studied based on their colour, opacity and transparency properties. The colour of the films samples was measured using an instrument called HunterLab ColorFlex EZ45/0° colour spectro-photometer (HunterLab, Reston VA< USA), with D65 illuminant, 10° observer, based on ASTM E308 and with reference to Siracusa et al., (2018). The measurements were carried out by applying CIE Lab scale. Before performing the test, the instrument was calibrated using a black and white tile. The films were analysed where five random spots were chosen. The results obtained are expressed as L* (luminosity), a* (red/green) and b* (yellow/blue) parameters. The total colour difference (ΔE) can be calculated using the equation as follows:

$$\Delta E = [(L^* - L')^2 + (a^* - a')^2 + (b^* - b')^2]^{1/2} \quad (3.7)$$

Where L*, a* and b* are the colour parameter values of the sample and L', a' and b' are the colour parameter values of the standard white plate used as the film background. The values of L', a' and b' are 100.9, 0.04, and 0.09 respectively. From the five repetitions for each film made, a mean value is recorded.

Meanwhile, for opacity and transparency properties, it was calculated according to the method by Liu et al. (2020). Upon performing the colour test, the wavelength value at 600nm of the specimens were obtained and used to calculate the transparency of the films. The transparency and the opacity of the films can be calculated by using the equation as follow:

$$Transparency = -logT_{600nm}/L \quad (3.8)$$

$$Opacity = \frac{A_{600}}{L} \quad (3.9)$$

Where T_{600nm} is the transmittance of film specimens at 600nm, L is the average thickness of the specimen of the specimens (mm). The lower the value of transparency, the higher the transparency of the films. Thus, the opacity is the opposite of the transparency value. The higher the value of transparency, the higher the opacity of the films.

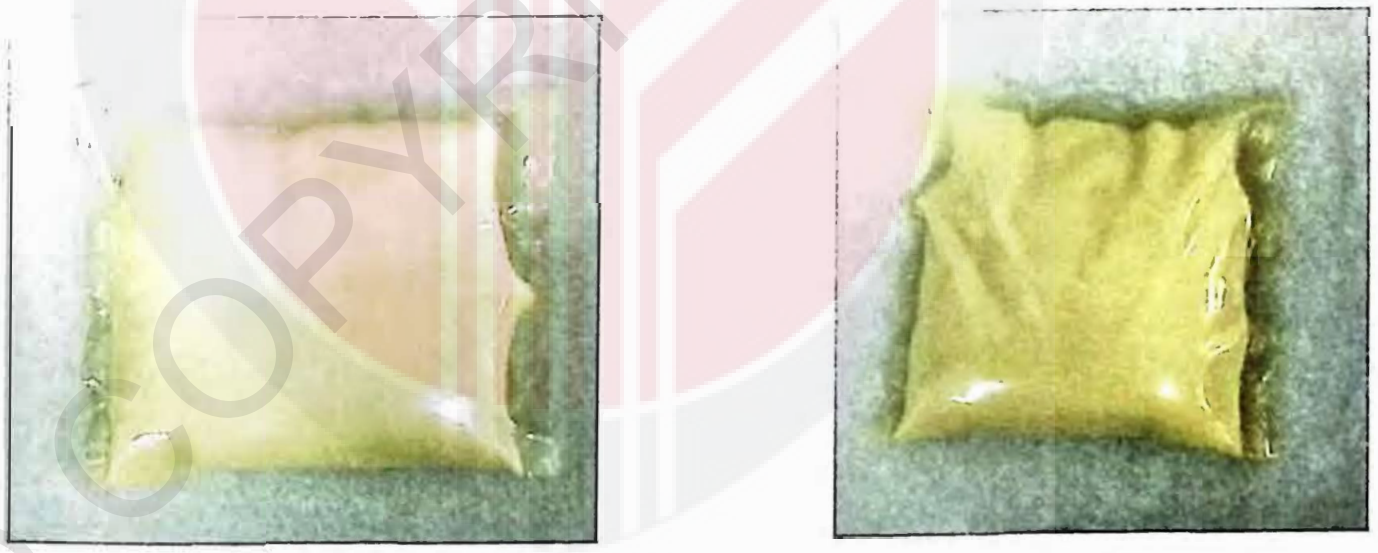
3.6 Agar-agar powder packaging test during storage

3.6.1 Packaging and storage method of agar-agar powder

The packaging process of the agar-agar powder was performed by transferring 8 g of agar-agar powder (NONA. Sri Nona Food Industries Sdn. Bhd., Malaysia) from

the original packet into the soluble PG and PPA film pouches. Each pouch with the dimension of 5 cm × 6 cm by using 1 piece of soluble film which has been folded into half and two sides of the folded film were sealed. The agar-agar powder was then transferred into the sealed pouch from the opening on the top. Then, the final seal was applied on the top edge of the pouch. The pouches were trimmed off the excess edges. The pouches containing the agar-agar powder were shown on Figure 3.6.

1 batch of the samples were place on trays and kept at ambient with temperature of 28 °C and 50% RH. The temperature and the RH of the ambient storage were maintained by keeping the fans switched on and providing good ventilation by opening some of the windows in the laboratory open for the entire storage period. The trays were covered using foldable mosquito net for pest control. Another batch of samples were also place on trays and kept at chiller with 4 °C.



PG film PPA film

Figure 3.6: Soluble PG and PPA film pouches containing agar-agar powder for storage.

3.6.2 Moisture content of agar-agar powder

The moisture content of the agar powder (product for films packaging application) was analyzed using a moisture analyzer (AND MX-50, A&D Co., Ltd, Japan). Prior to performing the analysis, the instrument was calibrated first using a

dummy agar-agar powder trice, or until a constant reading has achieved. 2 g of the specimen was placed on the metal petri dish and its moisture was analyzed at 105 °C until there is a beep sound indicating the analysis is complete.

3.6.3 True density of agar-agar powder

Generally, density is defined as the quotient from the mass by volume. The agar-agar powder density test was performed using an instrument called gas pycnometer (AccuPyc 10-cc, One Micromeritics Dr., Norcross, Ga 30093-1877, U.S.A.). The analysis involves a helium gas where it was used to displace the amount of the powder particle in term of volume. From this method, the volume and density of the powder can be obtained.

To perform the analysis, the software was first setup. The weight of the specimen was determined by weighing using electronic balance (SHIMADZU ATY224, Shimadzu Co., Philippines) the empty cell and its weight containing the specimen. The final weight subtracted the initial weight to obtain the specimen weight. The cell was then placed inside the gas pycnometer and the sample weight were input in the software. The analysis was then performed where 10 readings of the volume and the true density were generated at the end of the analysis.

3.6.4 Colour changes

The colour of agar-agar powder during storage at two different storage temperatures was analyzed using the Precise Color Reader (Model WR-18, Shenzhen Wave Optoelectronics Technology Co., Ltd., China). The instrument was first calibrated using the standard white tiles, to perform the test, where the reading was

taken at least trice. Then, after constant reading of the white tiles was done, about 2 g of the agar-agar powder was placed on the white tiles for the colour measurement. The measurement was also done trice. The values of L*, a* and b* were recorded for the calculation of ΔE. The ΔE can be calculated as follows:

$$\Delta E = [(L^* - L')^2 + (a^* - a')^2 + (b^* - b')^2]^{1/2} \quad (3.10)$$

Where, L', a' and b' were the values of the standard white tiles of 88.14, 0.49, and 1.21 respectively.

3.7 Statistical analysis technique

Statistical analysis was performed using Minitab Statistical Software Version 19 (Minitab for Windows and Mac, Minitab inc., State College, Pennsylvania, USA). The data was expressed as mean ± standard deviation (SD), where the minimal level of significance taken was $p < 0.05$. The means were compared using Tukey Test and One-way analysis of variance (ANOVA) approach was applied for analysing the data for the characterisation of the soluble PG and PPA films. The data of packaging test for agar-agar powder application was analysed using Two-way analysis of variance (ANOVA) approach and the means were compared using Tukey Test. The minimal level of significance taken was also $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterisation of soluble pineapple puree pectin-based film

4.1.1 Water solubility

Figure 4.1 shows the solubility percentage of both PG film and PPA film represented by a bar graph. The result showed that the PPA film was more soluble than PG film, where the PPA film is 97.3 ± 1.7% soluble and 93.7 ± 5.8% for PG film. This result suggests that PPA film is better used in ready-to-eat food packaging applications, as mentioned by Liu et al. (2020).

Arham et al. (2015) reported that the increasing concentration of glycerol as plasticizer influence the improvement of the film solubility, which is caused by the hydrophilic and hygroscopic plasticizer. Since the formulation used in producing of the soluble films of both types required a fixed amount of glycerol (2 g), it can be concluded that the addition of pineapple puree, which acts as a natural plasticizer and matrix, increase the concentration of plasticizer. The hygroscopic properties of plasticizer attract and bind water molecules, supporting the film surface wetting and moisture absorption. In addition, the hydrophilic properties of plasticizer enable the increased formation of solubilized dry matter, thus increasing the solubility. However, statistical analysis showed that the result was not significantly difference. Thus, it can be concluded that the incorporation of pineapple puree has no significant effect on the improvement of film solubility.

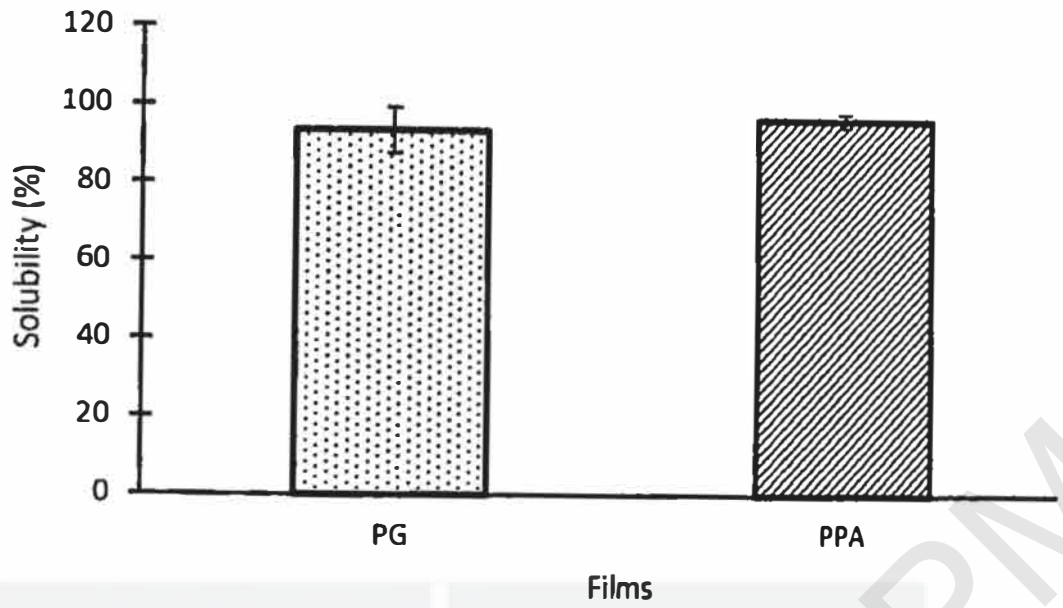


Figure 4.1: Percentage of water solubility of soluble films

4.1.2 Tensile properties

Table 4.1 shows the tensile properties for both film types, including TS, EAB, YM and toughness. The TS, EAB, and YM values of the soluble PG film were 4.3 ± 0.1 MPa, 42.1 ± 1.2%, and 0.2 ± 0.0 Pa, respectively which are higher than soluble PPA film. The soluble PPA film had a TS of 3.5 ± 0.1 MPa, EAB of 34.2 ± 3.9 %, and YM of 0.2 ± 0.0 Pa. Statistical analysis shows that both TS and EAB values of soluble PPA film are significantly different from TS and EAB values of soluble PG film ($p < 0.05$). The toughness value of the soluble PPA film is also significantly lower value than the PG film, indicating that PPA film was more flexible yet less tough than PG film due to pineapple puree acting as a natural plasticizer (Otoni et al., 2017). Table 4.2 shows the thickness of both soluble PG and PPA films, where both films have 0.1 ± 0.0 mm of thickness which is not significantly different.

Although previous studies suggested that soluble films incorporated with pineapple puree able to improve the mechanical properties of the soluble film (Wang

et al., 2011; Rodsamran & Sothornvit, 2019; Sonawane, 2019), specific interactions between the film constituents and the pineapple puree such as crosslink, different structural arrangements of the components or the formation of heterogeneous biphasic structures, must be taken into consideration. Dai et al. (2018) also reported that the fruit puree's cellulose component might aggregate the pectin-based matrix due to Van der Waal's forces, leading to lower TS and EAB values.

Another influence that might affect the strength of the PPA film might be due to the storage period of PPA film after production was longer compared to PG film. This reason can be supported by the preliminary studies done by Raduan (2020). The tensile properties of bio-based film degraded when stored longer by 35% from day 0 to day 7 of the storage period at ambient with RH 50%.

Table 4.1:Tensile properties of soluble films

Film	Tensile strength (MPa)	Elongation at break (%)	Young modulus (MPa)	Toughness MJ/m^3
PG	4.3 ± 0.1^a	42.1 ± 1.2^a	0.2 ± 0.0^a	1.1 ± 0.0^a
PPA	3.5 ± 0.1^b	34.2 ± 3.9^b	0.2 ± 0.0^a	0.7 ± 0.0^a

Result for each film is presented as means ± SD (n=3). Mean values in the same column with different letters (a and b) are significantly different ($p<0.05$).

Table 4.2 Thickness of soluble PG and PPA films

Film	Thickness (mm)
PG	0.1 ± 0.0^a
PPA	0.1 ± 0.0^a

Result for each film is presented as means ± SD (n=2). Mean values in the same column with different letters (a and b) are significantly different ($p<0.05$).

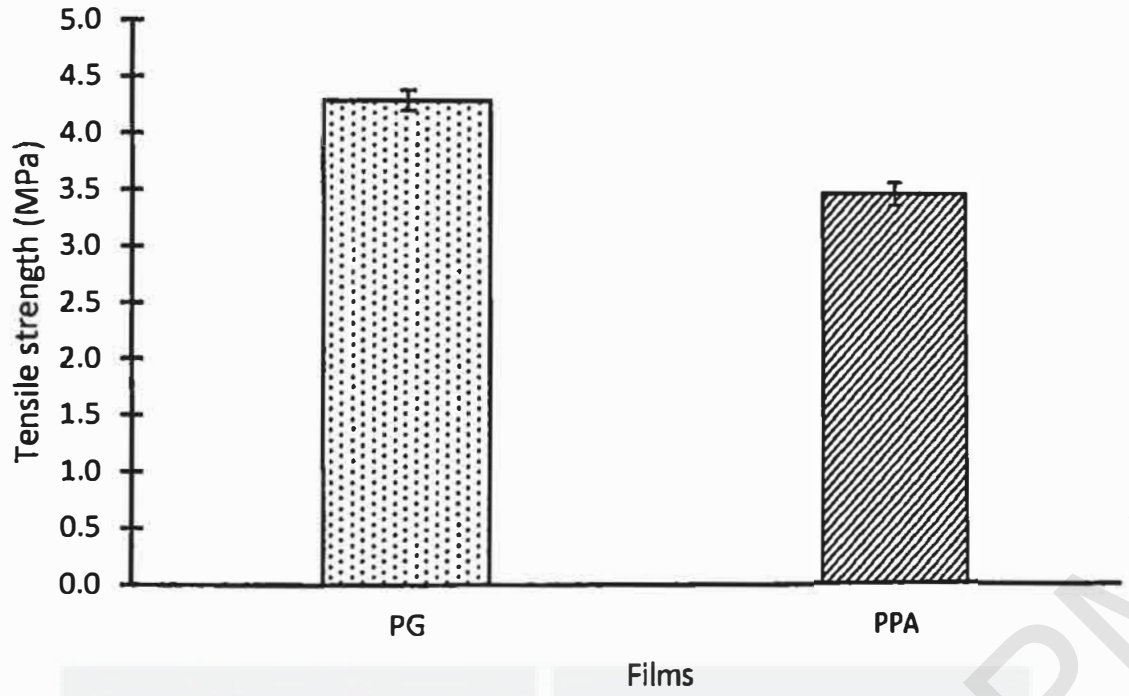


Figure 4.2: Tensile strength (MPa) of soluble films

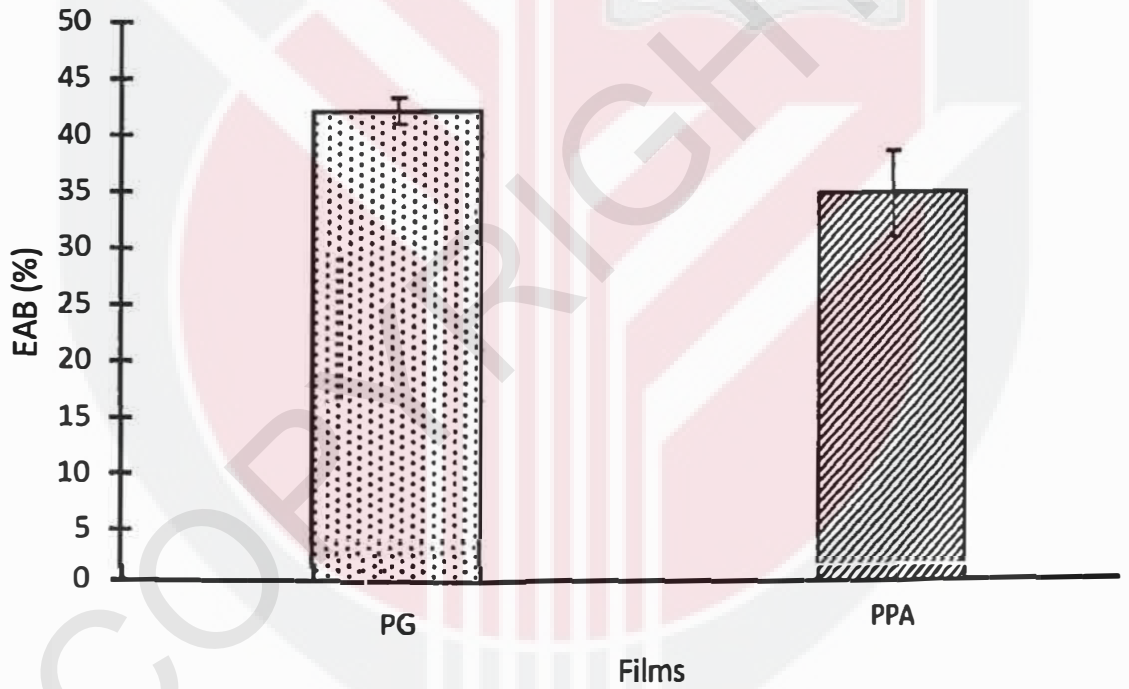


Figure 4.3: Elongation at break (%) of soluble films

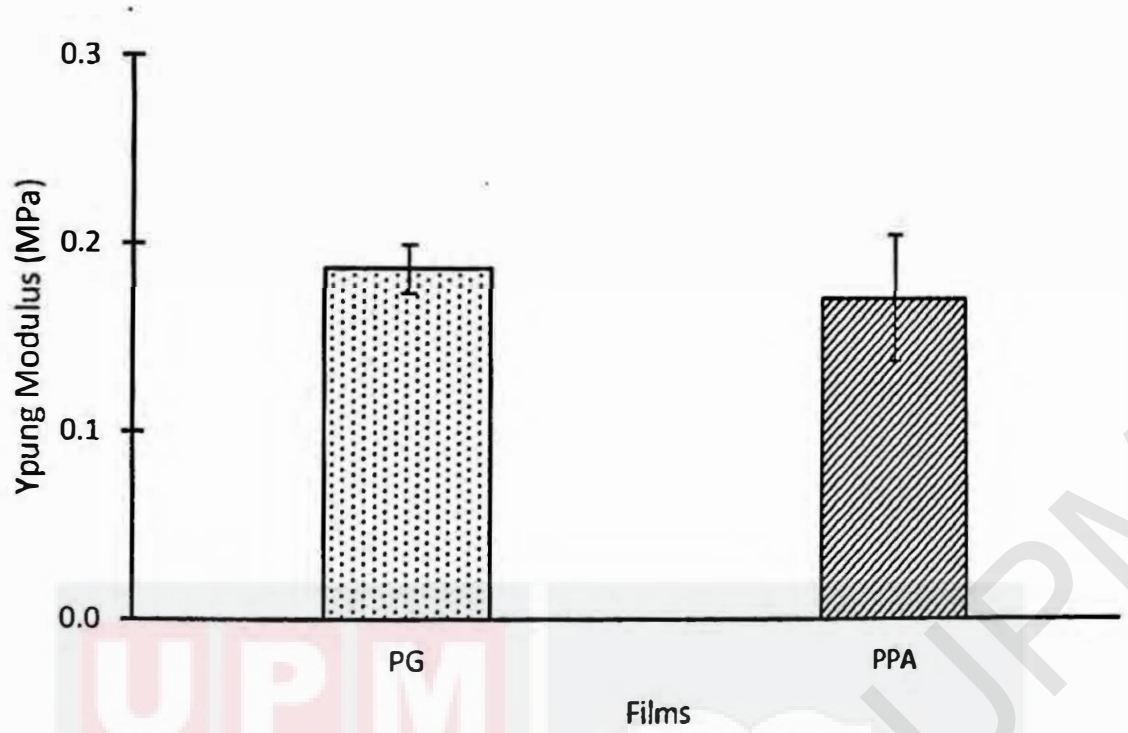


Figure 4.4: Young Modulus (MPa) of soluble films

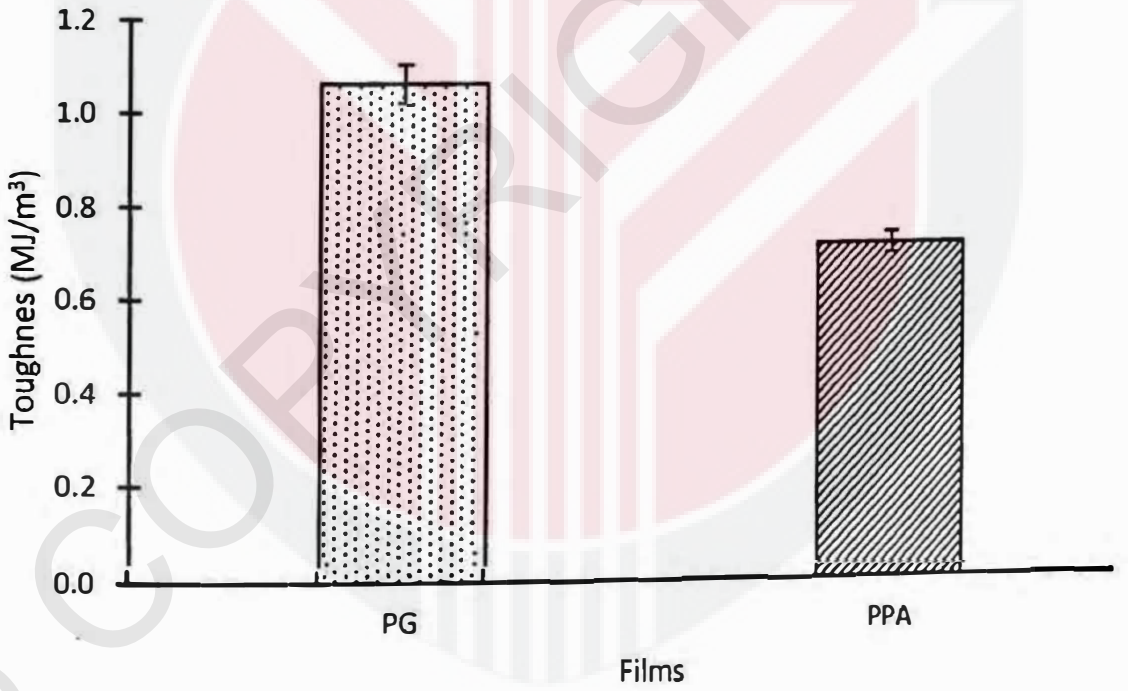


Figure 4.5: Toughness (MJ/m^3) of soluble films

4.1.3 Seal strength

The seal strengths of both soluble PG and PPA films are shown in Figure 4.6. The result shows that PG film has a higher strength of seal than PPA film. The seal strength of PG film was 0.4 ± 0.1 N/mm, while PPA film was 0.3 ± 0.0 N/mm, which is not significantly different between both films ($p > 0.05$).

Supposedly, the addition of pineapple puree in the PG film can significantly increase the seal strength of the film, as suggested by Liu et al. (2020). However, the soluble PPA film had lower seal strength than soluble PG film because the PPA film was produced way earlier and stored longer than PG film. Furthermore, it was proven by Raduan (2020) that the storage period of the edible film affects the seal strength of the film, where the soluble film's seal strength lowered with a longer period of storage.

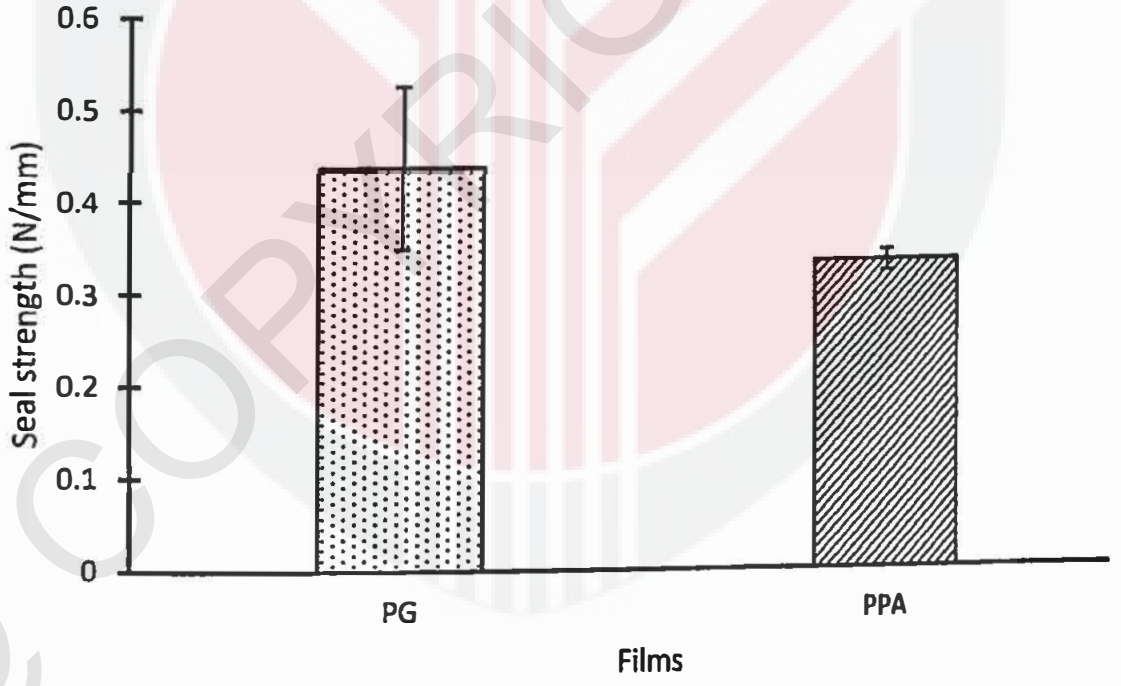


Figure 4.6: Seal strength of soluble films

4.1.4 Water vapour permeability

With the transmission area of the films is 28 cm^2, the WVTR value of the PPA film is 214.5 ± 89.4 $g/day.m^2$, which is 28% lower than the WVTR value of PG film

(297.4 ± 25.5 g/day.m^2), as shown in Table 4.3. The value of WVP calculated for PPA film also was lower than PG film, where the WVP value of the PPA film was 8.8 x 10^{-9} g/m.day.Pa while for PG film was 1.2 x 10^{-8} g/m.day.Pa. The graph of weight changes (g) against time (day) of both types of film, PG and PPA films, were plotted as shown in Appendix 4. Based on the graphs, the soluble PPA film show lower weight changes against time than the PG film, which result in a less steep slope than the PG film. Therefore, the slopes of the graph were used to obtain the values of WVTR of both types of film.

Statistical analysis shows that the values of WVTR and WVP of both types of film were not significantly different. Similar result was observed by Chambi and Grosso (2011) on the WVP study of pectin-based film. The insignificant improvement of WVP of the PPA film might be due to the ratio of the formulation (Chambi & Grosso, 2011).

Table 4.3: Water vapour permeability properties of soluble films

Film	WVTR (g/day.m^2)	WVP (g/m..day.Pa)
PG	297.4 ± 25.5[a]	1.2E-08 ± 9.9E-10[a]
PPA	214.5 ± 89.4[a]	8.3E-09 ± 3.5E-09[a]

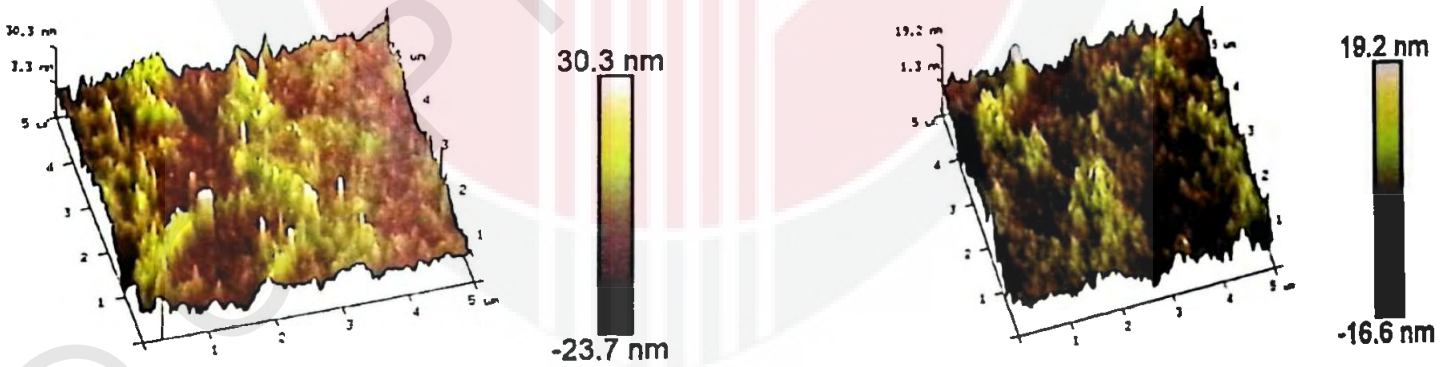
Result for each film is presented as means ± SD (n=2). Mean values in the same column with different letters (a,b, c…) are significantly different ($p<0.05$).

4.1.5 Surface roughness

The representative 3-D images for the scanning area of 5 µm x 5 µm, of the surface of the PG film and the PPA films are shown in Figures 4.7a and 4.7b, respectively. The initial value of average surface roughness (Ra) of PG and PPA films was 8.03 nm and 31.5 nm, respectively. While the root mean square roughness (Rq)

was 10.4 nm (PG film) and 38.2 nm (PPA film). The topographical image of the films gives the final value (after flatten) of the Ra and Rq of the PG film with 5.22 nm and 7.14 nm, respectively. Meanwhile, for PPA, the value of Ra and Rq are 3.70 nm and 4.85 nm, respectively.

Statistical analysis revealed that the film incorporated with the pineapple pure shows a significant smoother surface than the PG film. In other words, the pineapple puree enhanced the surface features of the film by lowering its roughness. This might be because pineapple puree acts as a natural plasticizer. As mentioned before, Rodsamran & Sothornvit (2019) explained that pineapple puree which contains high levels of phenolic compound and pectic, can act as natural plasticizer that reduces the film brittleness, cracks and therefore improves the surface roughness. A Roy et al. (2009), also reported that plasticizers used on film forming have a significant positive effect on the surface roughness.



(a) (b)

Figure 4.7: Topographical images performed by AFM on PG film (a) and PPA film (b)

Table 4.4: Surface roughness of films

Film	Ra (nm)	Rq (nm)
PG	5.22[a]	7.14[a]
PPA	3.70[b]	4.85[b]

Result for each film is presented as means ± SD (n=2). Mean values in the same column with different letters (a and b) are significantly different ($p<0.05$).

4.1.6 Optical properties

The measurements of the total colour difference (ΔE), transparency and the opacity of the soluble PG and PPA films were performed. The results are shown in Table 4.5, where the transparency value of PG film was 0.9 ± 0.0 and 1.0 ± 0.2 for PPA film. The a* value of the soluble PPA film also shows a higher negative value than the PG film, which indicates that the PPA film is more into greenish scale. In comparison, the b* value for PPA film has a higher positive value than the soluble PG film, indicating it is more yellowish. Table 4.5 also showed that the ΔE values indicate that both the films have a very distinct difference from the standard tiles where the ΔE value of PG and PPA film are 13.0 ± 0.3 and 13.7 ± 1.2, respectively.

Statistical analysis shows that the transparency, opacity and ΔE of the PPA film were not significantly different from PG film. This indicates that both films produced are translucent which favour the ideal colour for food packaging. Comparing the values of transparency and ΔE of the PG and PPA films shows that the PG film is slightly more translucent and less distinct different from the standard white tiles than the PPA film. This might be due to the carotene compound found in pineapple, which contributes to the yellow colour of the film. In the UV of 600 nm, both film types showed a similar range of transparency value as Liu et al. (2020).

Table 4.5: Optical values of films

Film	L*	a*	b*	Transmittance at 600 nm	Transparency	Opacity	ΔE
PG	91.9 ± 0.3^{a}	-0.9 ± 0.1^{a}	9.4 ± 0.3^{a}	83.6 ± 0.6^{a}	0.9 ± 0.0^{a}	0.9 ± 0.0^{a}	13.0 ± 0.3^{a}
PPA	90.8 ± 2.1^{a}	-1.2 ± 0.1^{b}	9.2 ± 0.8^{a}	82.5 ± 3.4^{a}	1.0 ± 0.2^{a}	1.0 ± 0.2^{a}	13.7 ± 1.2^{a}

Result for each film is presented as means ± SD (n=3). Mean values in the same column with different letters (a and b) are significantly different ($p<0.05$).

4.2 Packaging test for agar-agar powder application

4.2.1 Moisture content of agar-agar powder

Table 4.6 and Table 4.7 below show the moisture content of the agar-agar powder packed with soluble PG and PPA films stored at two different storage conditions, ambient (28 °C, RH 50%) and chilled (4 °C) respectively. Generally, the moisture content of the agar-agar powder packed with PPA film at both ambient and chiller are lesser than those samples packed in PG film. This indicates that less moisture (water vapour) was absorbed by the samples using PPA film pouches. Since there was no significant improvement of WVP values of PPA films, the moisture content of the agar-agar powder was also not significant different between both types of film.

Based on statistical analysis shown in Appendix 7 and 8, the types of film used to pack the agar-agar powder has no significant effect on the rate of moisture content gained by the agar-agar powder at ambient ($f(1) = 4.07$, $p > 0.05$), and chilled temperature ($f(1) = 1.81$, $p > 0.05$) . However, the amount of storage days significantly affects on the rate of moisture content gained by the agar-agar powder for ambient ($f(4) = 337.57$, $p < 0.05$) and chilled temperatures ($f(4) = 394.07$, $p < 0.05$). A large increment of moisture content can be observed between day 0 and day 7 for ambient and day 0 to day 27 for chilled temperature, where the agar-agar powder gained moisture from 11.8% to around 20% for both types of film.

Thus, it can be suggested that the incorporation of pineapple puree into the pectin-based matrix has no significant improvement on the moisture barrier as mention in Section 4.1.4. In terms of storage conditions, it is better to store the agar-agar powder

at room temperature as suggested by FDA, Anonymous (2012), since the moisture content at chill temperature was slightly higher than at ambient.

Table 4.6: Moisture content (%) of agar-agar powder stored at ambient

Time (Day)	**Moisture content (%) at ambient (25 °C, RH 50%)**	
	PG	PPA
0	11.8 ± 0.0	11.8 ± 0.0
7	20.5 ± 0.0	20.0 ± 0.1
32	19.5 ± 0.9	19.5 ± 0.5
36	20.0 ± 0.7	19.0 ± 0.1
39	20.2 ± 0.4	19.9 ± 0.0

Table 4.7: Moisture content (%) of agar-agar powder stored at chill temperature

Time (Day)	**Moisture content (%) at chilled storage (4 °C),**	
	PG	PPA
0	11.8 ± 0.0	11.8 ± 0.0
27	20.9 ± 0.5	20.2 ± 0.8
31	20.4 ± 0.3	20.5 ± 0.2
34	20.5 ± 0.0	19.8 ± 0.5
39	20.3 ± 0.4	20.3 ± 0.1

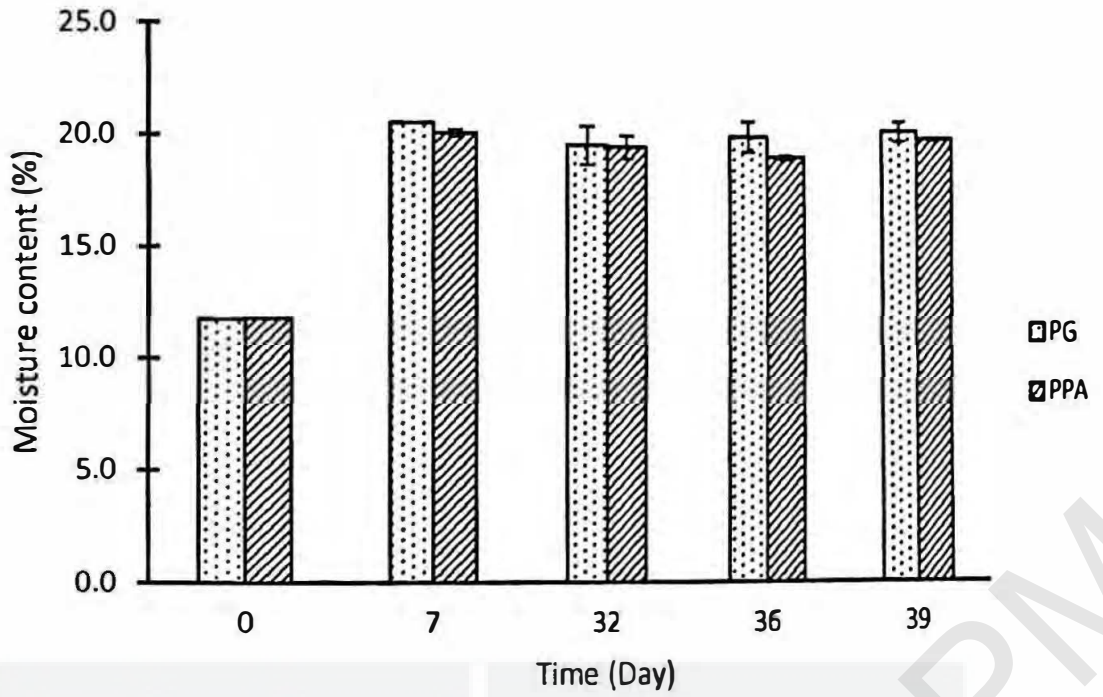


Figure 4.8: Moisture content (%) of agar-agar powder stored at ambient

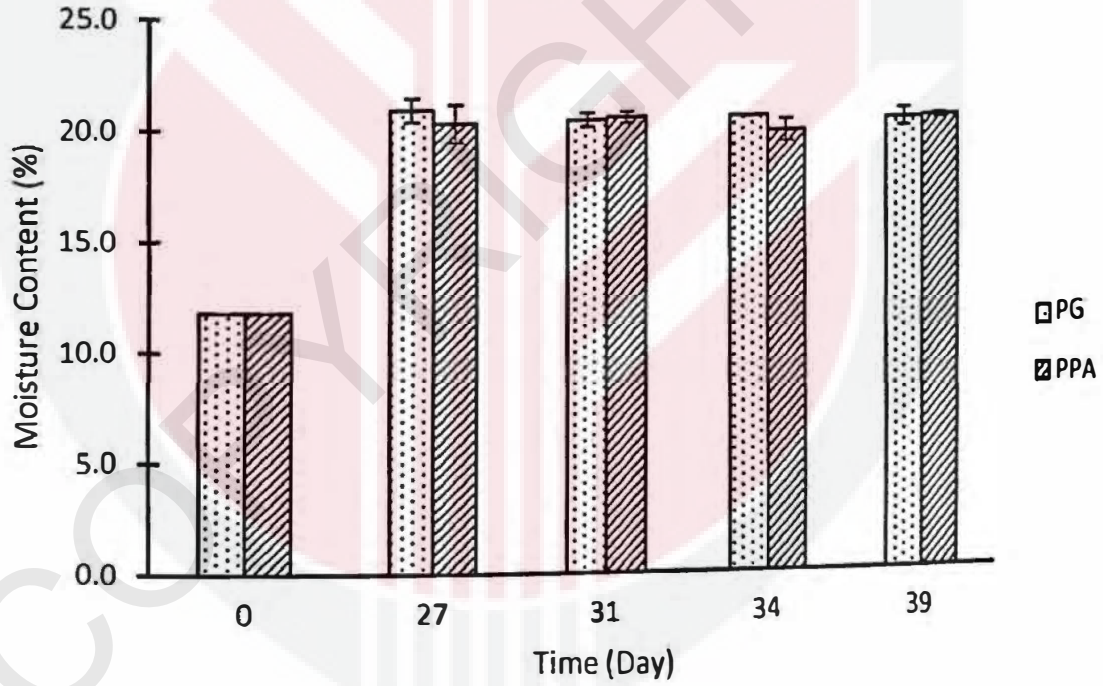


Figure 4.9: Moisture content (%) of agar-agar powder stored at chilled condition

4.2.2 True density of agar-agar powder

Figures 4.10 and 4.11 show the true density of agar-agar powder changes for a total of 39 days of the agar powder stored at ambient and chilled temperatures, respectively. From the graph, the true density of the samples had a slight decrement from day 0 to day 7 for samples stored at ambient and from day 0 to day 27 for samples stored at chilled temperatures. Both graphs showed that the major decrement of true density of the agar-agar powder only happened during day 0 to day 7 for ambient and day 0 to day 27 for chilled temperatures. Beyond that storage period, there seems to be no significant difference in the true density of the samples for both types of film and storage temperatures. Statistical analysis (Appendix 9 and 10) showed that the types of film used to pack the samples did not significantly affects the changes of true density in both ambient ($f(1) = 3.59$, $p > 0.05$) and chilled temperatures ($f(1) = 0.65$, $p > 0.05$). Meanwhile, the storage period significantly affects the changes in the true density of the samples for both ambient ($f(4) = 175.2$, $p < 0.05$) and chilled temperature ($f(4) = 307.45$, $p < 0.05$).

The decrement of true density (both types of film and storage temperature) may be due to the agglomeration of powder particles. Agglomeration of powder particles was mainly affected by the moisture content of the samples. The moisture content of the samples increased from 11.8% to about 20% and fluctuated between 20.0% to 20.5% for both storage temperatures. The moisture content fluctuation can be assumed that the samples' moisture content became constant, resulting in constant true density after day 7 of ambient storage and day 27 of chiller storage. Agglomeration of the powder particle creates intraporosity, which will increase with increasing moisture content. Since true density is a density that excluded all open and closed pores, the increasing intraporosity due to the agglomeration of agar-agar powder particles caused

the decrease in the true density of the agar-agar powder. This reason can be supported by the study on coal agglomeration due to moisture content (Yu et al. 1995).

In terms of storage temperatures, it can be seen Appendix 6 that both the true density (g/cm^3) for both types of film were lower when stored at ambient compared to chilled temperature. Hence, it supports the fact that the agar-agar powder is better stored at room temperature.

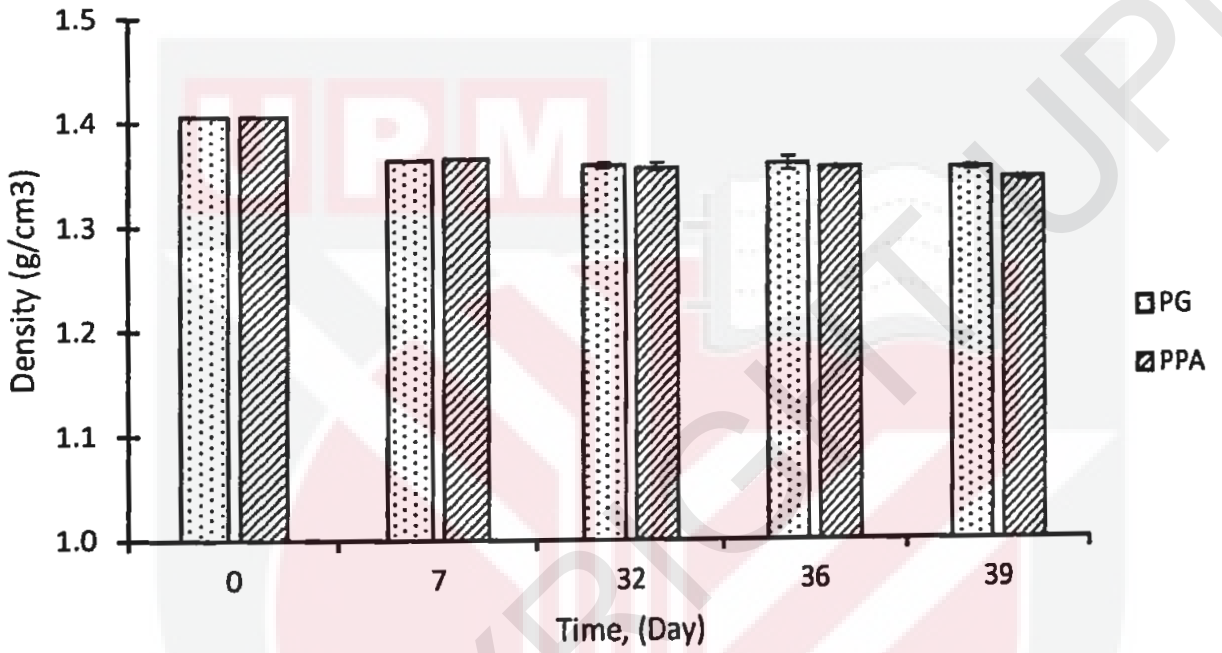


Figure 4.10: True density of agar-agar powder packed with the soluble films stored at ambient

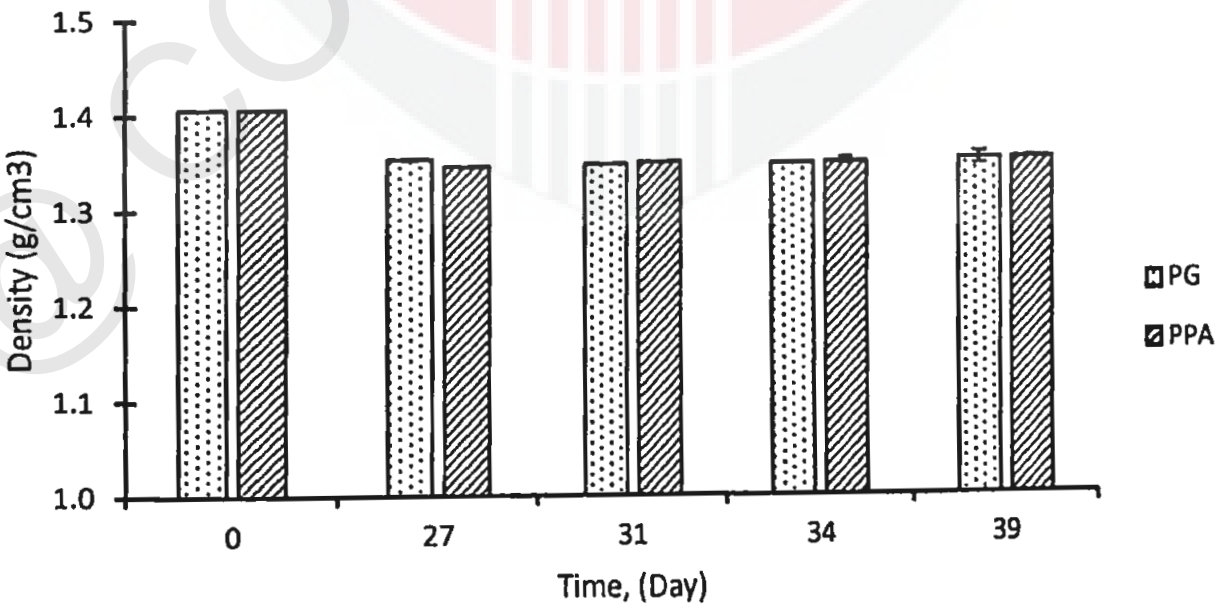


Figure 4.11: True density of agar-agar powder packed with the soluble films stored at chilled temperature

4.2.3 Colour changes of agar-agar powder

The values of ΔE for both types of film and storage temperatures are shown in Tables 4.8 and 4.9. There is no significant difference between the value of ΔE by the type of films packaging for both ambient ($f(1) = 0.21$, $p > 0.05$) and chilled temperatures ($f(1) = 1.50$, $p > 0.05$) in 39 days. This indicates that the storage condition has very little effect on the colour degradation of the agar powder. However, the value ΔE shows significant differences by the of storage period (days) for both ambient ($f(3) = 85.90$, $p < 0.05$) and chilled temperatures ($f(4) = 29.04$, $p < 0.05$). Thus, the degradation of colour was not significantly affected by the types of film but it was significantly affected by the number of days for storage.

Since there is no significant reduction of colour intensity for a total of 39 days in both storage temperatures, it shows that the storage conditions do not cause great colour degradation. However, it is revealed that the storage temperature at the chiller loses its colour intensity more than the one stored for both types of films. This reason suggest that the agar powder should be stored better at room temperature than at chilled condition supported by FDA (Anonymous, 2012).

Table 4.8: Total colour difference of soluble films stored at ambient temperature.

Time (Day)	ΔE	
	PG	PPA
0	15.38 ± 0.0	15.38 ± 0.0
32	14.90 ± 0.38	13.99 ± 0.38
36	13.16 ± 0.33	14.47 ± 0.48
39	12.32 ± 0.02	11.74 ± 0.31

Table 4.9: Total colour difference of soluble films stored at chilled temperature

Time (Day)	ΔE	
	PG	PPA
0	15.38 ± 0.0	15.38 ± 0.0
27	14.20 ± 1.38	13.43 ± 0.05
31	12.82 ± 0.11	11.27 ± 0.29
34	11.60 ± 0.66	11.60 ± 0.25
39	11.12 ± 0.17	11.69 ± 0.08

4.2.4 Correlation between the soluble film characterisation and the application on stored agar-agar

The characterisation of the PPA film are mostly not significantly different compared to PG film. It shows that the pineapple puree gave no significant different compared to the control film. The characterisation of the soluble films affects the quality properties of the agar-agar powder during storage. For example, the moisture content gained by the agar-agar powder was highly influence by the water vapour permeability of the film. From the results for WVP and moisture content, both types of films showed quite similar result. It indicates that both types of film might be suitable for the packaging application for powder-based food. Since PG and PPA films both have high solubility, the WVP and moisture content tests considered to be the most critical aspect for the films to be applied in food industries. Therefore, it is important to enhance the water vapour barrier of the soluble PPA films by varying the formulation to reduce the hydrophilicity of the film.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Conclusion

The characterization of soluble films is indeed an important study to determine the influences of the film characteristics on the shelf life of food products for food packaging applications. Improvement of the PG film physical properties such as water solubility, tensile properties, seal strength, WVP, surface roughness and optical properties are important to develop suitable soluble packaging. As a solution, pineapple puree was incorporated into the PG films to prepare blend films by a solution casting method in the present work. With the incorporation of pineapple puree into the PG matrix, the water solubility was improved.

However, the storage period influenced the tensile properties and the seal strength of the PPA films. PPA films also have lower WVP and WVTR, which preventing higher moisture from being absorbed into the agar-agar powder during storage study. The morphology property of the PPA films was also better than the PG films, where the topographical image of PPA films performed by AFM has a smoother surface. Additionally, PPA films produced have good optical properties. The PPA films produced were transparent and able to protect the colour degradation of the agar-agar powder slightly better than the PG films. The true density of the agar-agar powder decreased slightly during the storage study due to the agglomeration of powder particles.

In conclusion, the study shows that the soluble PPA films have a great potential to be used in the food packaging application, particularly for powder-based food, as

PPA film showed high solubility percentage. In addition, the soluble PPA films also have lower WVP value which indicates that it has better moisture barrier.

5.2 Recommendations for future work

Further, in-depth studies of pectin-based films incorporated with pineapple puree should be continued in order to develop more stable and sustainable soluble and edible packaging. It is recommended to varies the formulation or weight fraction of the raw materials of the films such as pectin, pineapple puree and glycerol for further studies.

Other properties such as thermal stability, antioxidant analysis, ATR-FTIR and others should also be analyzed to obtain a comprehensive characterization of the soluble films to further clarify their potential in food packaging application. This study may contribute greatly to the food industries to achieve sustainable packaging where more bio packaging films can be utilized in the future to overcome the environmental issue of non-environmental-friendly plastics.

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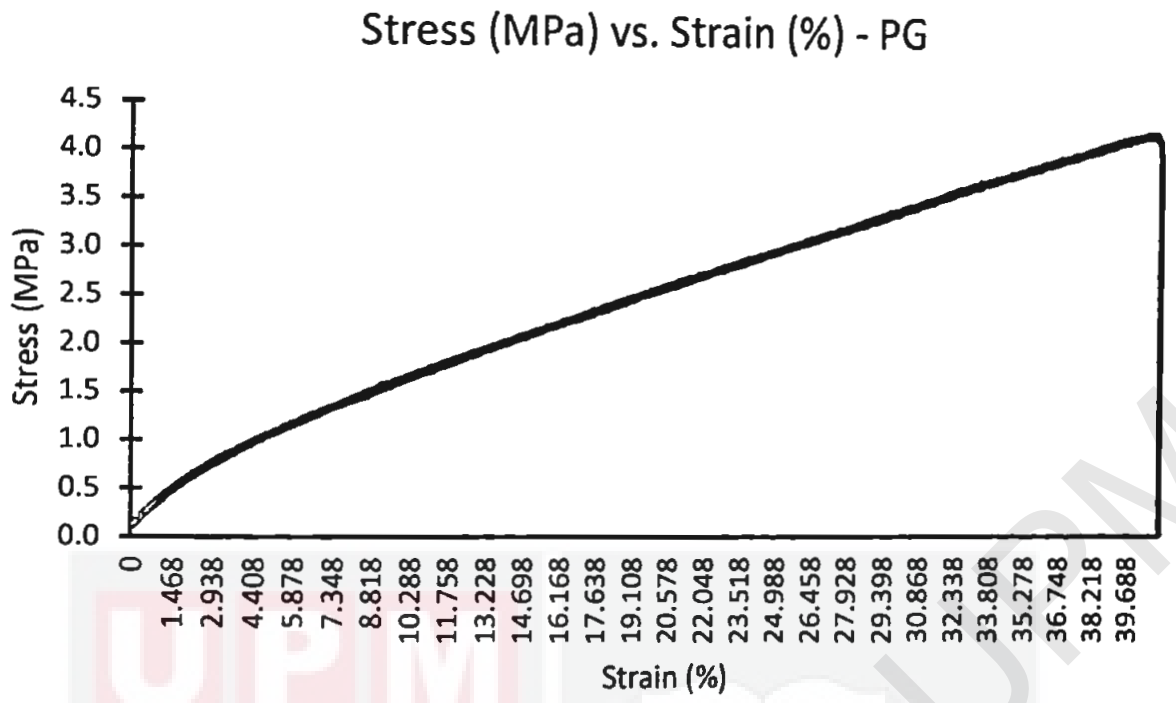
APPENDICES

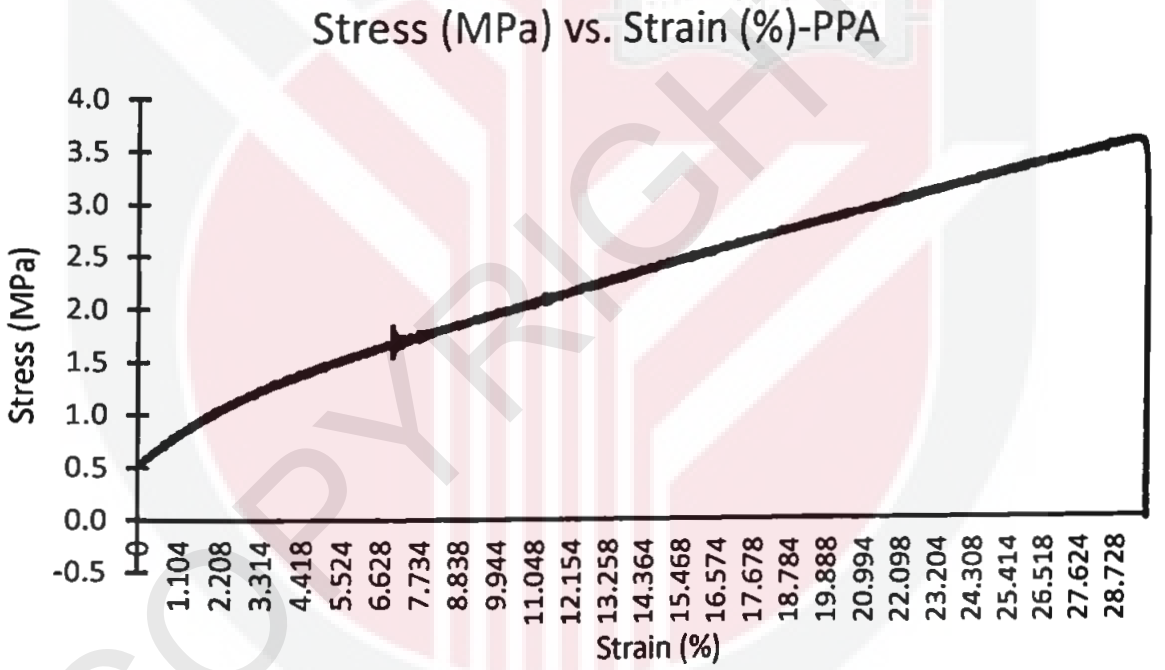
Appendix 1: Water solubility data analysis of PG and PPA films

Sample	Wi (g)	W0 (g)	Solubility
PG1	0.1530	0.0200	86.9281
PG2	0.1690	0.0050	97.0414
PG3	0.2040	0.0060	97.0588
Average	0.1753	0.0103	93.6761
SD	0.0261	0.0084	5.8440
PPA1	0.2190	0.0100	95.4338
PPA2	0.1840	0.0040	97.8261
PPA3	0.2320	0.0030	98.7069
Average	0.2117	0.0057	97.3223
SD	0.0248	0.0038	1.6937

Appendix 2: Tensile properties values and graph of stress vs. strain of PG and PPA films

Sample	Tensile Strength (MPa)	EAB (%)	Young Modulus *obtained from graph	Toughness (MJ/m^3)
PG 1	4.1900	41.0720	0.1929	1.0250
PG 2	4.3570	43.3820	0.1721	1.1070
PG 3	4.3350	41.9120	0.1955	1.0650
Average	4.2940	42.1220	0.1868	1.0657
SD	0.0907	1.1692	0.0128	0.0410
PPA 1	3.6010	29.7140	0.2111	0.6950
PPA 2	3.4260	36.3860	0.1510	0.7270
PPA 3	3.4230	36.6140	0.1547	0.7380
Average	3.4833	34.2380	0.1723	0.7200
SD	0.1019	3.9196	0.0337	0.0223

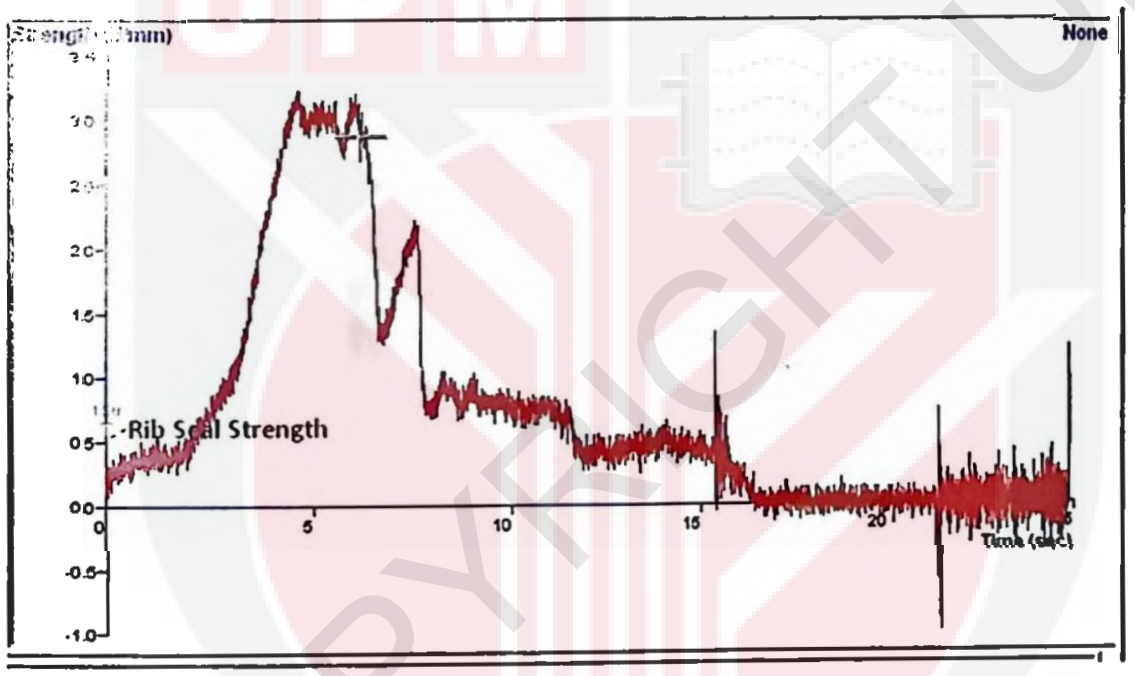




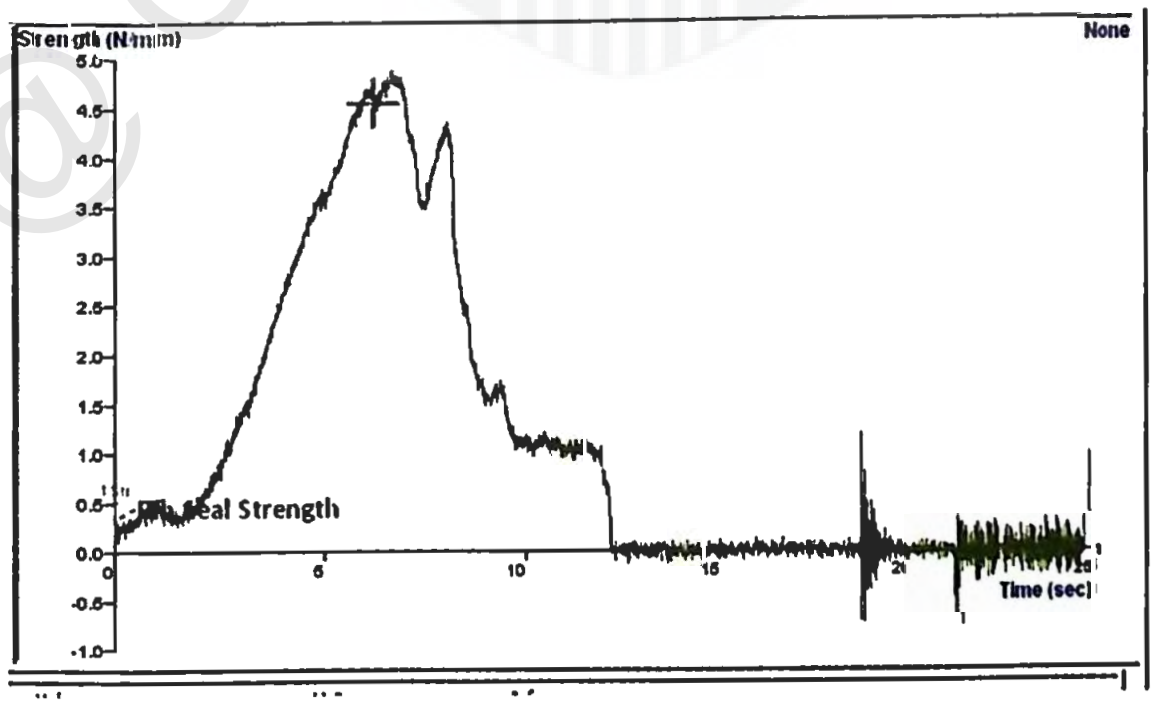
Appendix 3: Seal strength values of PG and PPA films and raw force-deformation curves of PG film (a) and PPA (b) film for seal strength

Sample	Seal strength (N/mm)	
	PG	PPA
1	0.5290	0.3420
2	0.4280	0.3200
3	0.3530	0.3370
Average	0.4367	0.3330
SD	0.0883	0.0115

a) PG film

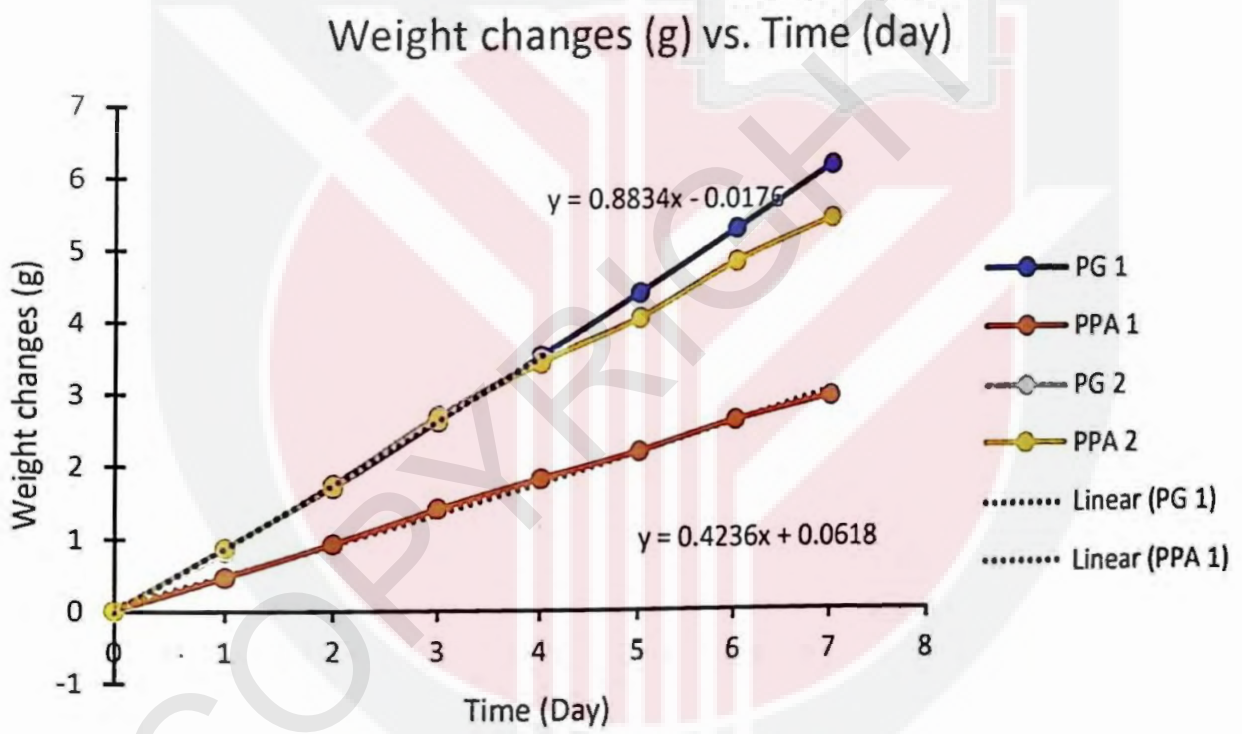


b) PPA film



Appendix 4: Weight changes data of PG and PPA films, and graph of weight changes vs. time for WVP and WVTR.

Time, (Day)	Weight (g)		Weight Changes (g)	
	PG	**PPA**	**PG**	**PPA**
0	89.8641	92.3693	0	0
1	90.7115	93.0308	0.8474	0.6615
2	91.6071	93.6978	1.7430	1.3285
3	92.5438	94.4003	2.6797	2.0310
4	93.3651	94.9917	3.5010	2.6224
5	94.0969	95.4727	4.2328	3.1034
6	94.9298	96.0725	5.0657	3.7032
7	95.6613	96.5494	5.7972	4.1801



Appendix 5: Moisture content values of samples stored at ambient and chilled temperature

Time, (Day)	PG Ambient			PPA Ambient		
	A	B	Average	A	B	Average
0	11.77	11.77	11.8	11.77	11.77	11.8
7	20.5	20.51	20.5	20.15	19.94	20.0
32	20.15	18.93	19.5	19.81	19.11	19.5
36	19.48	20.45	20.0	19.11	18.97	19.0
39	20.5	19.96	20.2	19.88	19.95	19.9

Time, (Day)	PG Chiller			PPA Chiller		
	A	B	Average	A	B	Average
0	11.77	11.77	11.8	11.77	11.77	11.8
27	21.24	20.49	20.9	20.83	19.65	20.2
31	20.58	20.16	20.4	20.63	20.29	20.5
34	20.49	20.46	20.5	19.46	20.15	19.8
39	20.58	20.03	20.3	20.41	20.28	20.3

Appendix 6: True density values of agar-agar powder for PG and PPA film packaging stored at ambient and chilled temperatures

Time (Day)	PG ambient			PPA ambient		
	A	B	Average	A	B	Average
0	1.4060	1.4060	1.4060	1.4060	1.4060	1.4060
7	1.3640	1.3639	1.3640	1.3653	1.3655	1.3654
32	1.3614	1.3578	1.3596	1.3604	1.3540	1.3572
36	1.3663	1.3571	1.3617	1.3579	1.3590	1.3585
39	1.3561	1.3588	1.3575	1.3469	1.3489	1.3479

Time (Day)	PG Chiller			PPA Chiller		
	A	B	Average	A	B	Average
0	1.406	1.406	1.4060	1.4060	1.4060	1.4060
27	1.3542	1.3527	1.3535	1.3456	1.3458	1.3457
31	1.3475	1.3473	1.3474	1.3486	1.3488	1.3487
34	1.3462	1.346	1.3461	1.3505	1.3441	1.3473
39	1.3468	1.3556	1.3512	1.3504	1.3520	1.3512

Appendix 7: Analysis of variance (ANOVA) for moisture content of agar-agar powder stored at ambient temperature.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	1	0.634	0.6337	4.07	0.063
Day	4	210.162	52.5404	337.57	0.000
Error	14	2.179	0.1556		
Lack-of-Fit	4	0.539	0.1348	0.82	0.540
Pure Error	10	1.640	0.1640		
Total	19	212.974			

Appendix 8: Analysis of variance (ANOVA) for moisture content of agar-agar powder stored at chilled temperature

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film chiller	1	0.271	0.2714	1.81	0.200
Day chiller	4	236.378	59.0944	394.07	0.000
Error	14	2.099	0.1500		
Lack-of-Fit	4	0.578	0.1444	0.95	0.475
Pure Error	10	1.522	0.1522		
Total	19	238.748			

Appendix 9: Analysis of variance (ANOVA) for true density of agar-agar powder stored at ambient temperature.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film ambient	1	0.000038	0.000038	3.59	0.079
Day	4	0.007377	0.001844	175.20	0.000
Error	14	0.000147	0.000011		
Lack-of-Fit	4	0.000072	0.000018	2.38	0.122
Pure Error	10	0.000076	0.000008		
Total	19	0.007562			

Appendix 10: Analysis of variance (ANOVA) for true density of agar-agar powder stored at chill temperature

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film chiller	1	0.000006	0.000006	0.65	0.435
Day Chiller	4	0.010485	0.002621	307.45	0.000
Error	14	0.000119	0.000009		
Lack-of-Fit	4	0.000058	0.000014	2.34	0.126
Pure Error	10	0.000062	0.000006		
Total	19	0.010610			