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# Extraction of Edible Superhydrophobic Membrane Using Tomato-peel and Beeswax

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## Abstract:

This thesis is about making an edible superhydrophobic film using tomato peels and beeswax. The extraction process was done twice, first by following the article and second by changing the process according to the requirement. The films were made by cooking tomato peels with 3 wt% NaOH solution at 93°C. Then 6 mol/L HCl was used to acidify the mixture until it reaches pH level around 5-6 and cutin was extracted by gravity separation method. Then the obtained cutin was mixed with pectin to create the film and then it was treated with beeswax, to make the film superhydrophobic. Then the contact angle test and biodegradability test were done on the film (by colleagues). Also, the FTIR test was done to ensure the hydrogen binding bond in the film and to compare the result with the previous results.

**Keywords:** Superhydrophobic, edible films, biofilms, tomato peel, beeswax, cutin, pectin

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# 1 INTRODUCTION

## 1.1 Background

In recent years, the world has been moving towards environmental sustainability which has led to growing concern for eco-friendly materials that will reduce waste and promote a circular economy. Materials used for recent food packaging are usually plastic and Styrofoam, which are not biodegradable and can take hundreds of years to decay. In addition, these materials can release harmful chemicals into the surroundings which might be toxic to all living organisms including humans.

In this context, extraction of the superhydrophobic membrane using bio-waste materials is a unique idea. Superhydrophobic materials have turned up as a promising technology in recent years due to their rare properties of water or liquid repellency, corrosion resistance, and self-cleaning ability. (Razavi et al., 2017) The property, self-water repelling, helps to make the surface dry even when it is exposed to wet environments. Due to its rare properties, it is useful in various industrial applications such as waterproof coatings, anti-fouling materials, and biomedical devices. The superhydrophobicity is achieved by making the surface with high contact angle to low contact angle, which results water droplets sliding easily without sticking to the surface rather than making the surface wet. (Bake et al., 2018)

Various kinds of synthetic materials are widely used to make the materials superhydrophobic, while researchers are exploring the different natural sources to produce such materials. Natural sources offer several advantages, including biodegradability, less environmental impact, and sustainability. Natural sources include plant leaves, different fruits, animal skins, and insect wings. In this context, tomato peels and beeswax will be used to complete the project.

Tomato peels and beeswax are two natural sources with easy availability and cost-effectiveness. Tomato peels are a by-product from the food processing industries and are often discarded as waste, which can be available at low cost. While beeswax is a natural wax prepared by bees and is being used for various applications, such as cosmetics and candle making. Both tomato peels and beeswax have superhydrophobic properties, their combination can be used to produce superhydrophobic materials at a low cost and sustainable manner. (Wang et al., 2020)

Lotus leaf is a natural superhydrophobic material and this research was inspired by it. To prepare an edible superhydrophobic membrane, cutin-pectin mixture was added to make the membrane, and a beeswax coating was used to make it superhydrophobic. Cutin was first extracted from the tomato peel, which itself has a hydrophobic property. Then commercial apple pectin was used to make the cutin-pectin membrane and beeswax, which we got from the bee farm, was used to make the coating for the membrane which makes it superhydrophobic. Since the membrane has been used in all agricultural goods, making it edible. (Wang et al., 2020)

## 1.2 OBJECTIVE

In this thesis, we aim to investigate the extraction of edible superhydrophobic materials using tomato peel and beeswax. For the extraction process, research done by Daheng Wang, Jinxia Huang, and Zhiguang Guo on "Tomato-lotus inspired edible superhydrophobic artificial lotus leaf" will be followed. However, this study does not cover the commercial-scale production of the membrane. Also, this study was conducted in a group and divided into three parts. This report will mainly focus on the extraction or development process of the membrane.

The main objective of this thesis is to (i) create the superhydrophobic membrane using bio-waste (i.e., tomato peel and beeswax) in a lab, and (ii) FTIR analysis of the samples (cutin, pectin, membrane, and beeswax), to ensure the hydrogen bond binding between the membrane and coating. Also, to ensure the samples show the same properties as the article followed.

This thesis consists of six chapters. The Introduction chapter covers the basic knowledge of the superhydrophobic surface, which can be used to control food waste. On the other hand, the literature review covers a brief description of superhydrophobic surfaces, their natural sources, and their applications. Also, this chapter includes a discussion of edible membranes, tomatoes and beeswax as raw materials to produce membranes and a small description of cutin and pectin.

Moreover, the production process of the membrane is discussed in the Experimental Part chapter. Whereas the fifth chapter covers the results obtained after the film production and the fifth chapter covers the discussion and conclusion of the thesis. Finally, the last chapter includes all the references used for writing the thesis.

# 2 LITERATURE REVIEW

## 2.1 Superhydrophobic Materials

In recent decades, there has been serious interest in superhydrophobic materials due to their extraordinary water-repellent properties. When the water contact angle (WCA) on a surface is below 90 degrees and exceeds 90 degrees, the surface is classified as hydrophilic and hydrophobic respectively(Liu et al., 2016). The superhydrophobic surface on the other hand, possesses a WCA above 150 degrees (as shown in Figure 1) and a sliding angle of fewer than 5 degrees, which represents the minimum incline required for a water droplet to slide or roll off the surface with its self-cleaning property(Ali et al., 2018).

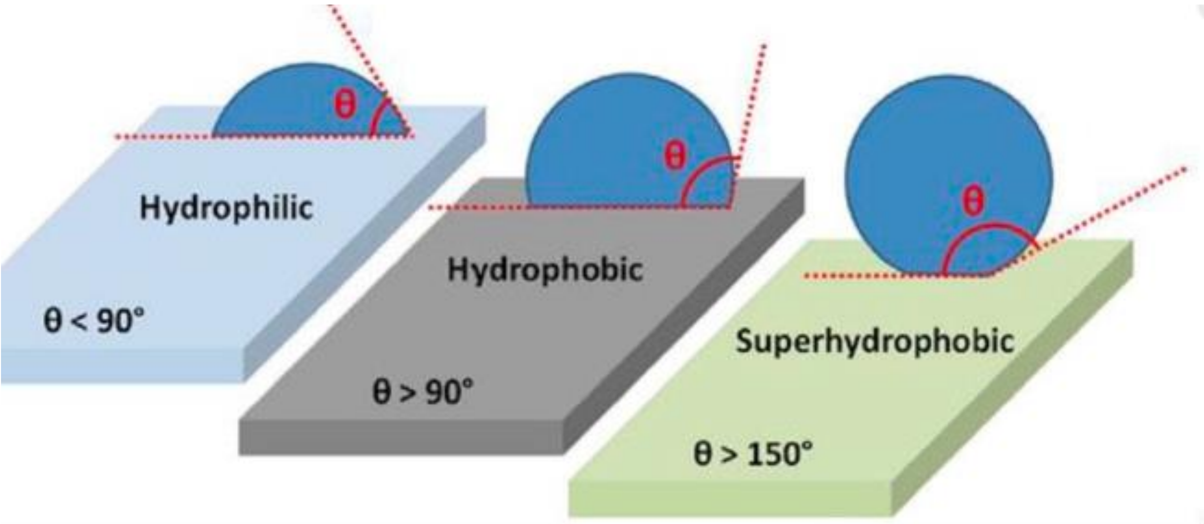


*Figure 1:Hydrophilic, Hydrophobic, and Superhydrophobic surfaces (L iu et al., 2016)*

The wetting behavior of water droplets on solid surfaces can be divided into two main categories: the Cassie-Baxter state and the Wenzel state. In the Cassie-Baxter state, water droplets stay on the surface whereas in the Wenzel state, water drops enter the pores. This phenomenon is well shown in Figure 2.  The superhydrophobicity of a surface is generally explained by the Cassie-Baxter model, where air is trapped in the pores and water droplets rest on the surface of air and pores(Liu et al., 2016).

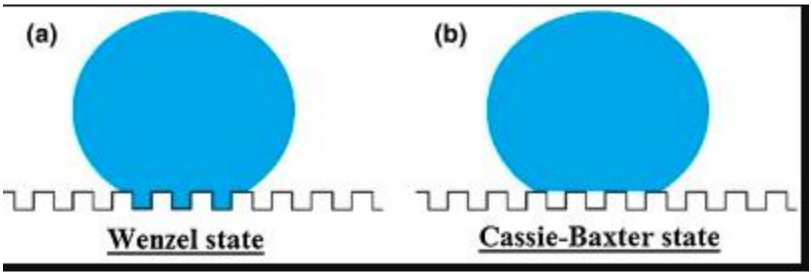


*Figure 2:Wenzel state and Cassie-Baxter state*(Ali et al., 2018)

When any metal encounters water, oxygen present in water oxidizes the metal and leads to corrosion. By reducing the WCA and contact time, a superhydrophobic coating can protect the surface from corrosion. Various methods have been developed in recent decades, some of which include using chemicals and some using bio-products or agricultural by-products. Most of the superhydrophobic coatings are inspired by the microstructure of water-repellent plants or leaves and possess crucial properties for corrosion reduction as well as additional benefits such as anti-fouling, anti-icing, and biocorrosion resistance.(Figueira et al., 2020) Superhydrophobic surfaces can be made using polymers also. To create a successful superhydrophobic surface, it is crucial to control the generation of hierarchical rough morphology. Hydrophobic polymers, such as fluoropolymers and perfluorooctanoic acids, play an important role in achieving superhydrophobicity. Additionally, nanoparticles, lithography, etching, biomimetic approaches, and stamping processes can be used to improve the morphology and roughness of superhydrophobic polymers. These surfaces can also achieve additional features, such as mechanical strength, wear resistance, thermal conductivity, and electrical conductivity. Hydrophobic polymers have been favored with various nanoparticles (carbon nanotubes, graphene, carbon nanofibers, carbon black, zinc oxide, silica, etc.) to create profitable and multifunctional nanocarbon-based superhydrophobic surfaces. These surfaces can be used in electronics, electromagnetic interference shielding devices, materials demanding strength and wear resistance, and biomedical applications. (Kausar, 2019)

### 2.1.1 Superhydrophobic properties found in nature.

Nature is the one who gives life and different survival techniques to all living organisms. Some plants, animals, and insects have got an extraordinary property called hydrophobic or superhydrophobic nature in their leaves, skin, wings, and legs. The most common example of plants are Lotus leaves and rose petals. They use this property to clean themselves from dust particles. Fish and sharks have this property in their skin to protect themselves from pollution (oil). Also, insects such as mayflies, dragonflies, stoneflies, scorpion flies, butterflies, and flies got this property in their wings and eyes. Whenever water droplets encounter wings, makes it hard for these insects to fly and when water meets eyes, visibility will be blocked. So, to overcome this problem, nature has provided this property to them. Finally, water striders, mosquitoes, and spiders have this property in their legs, which helps them to walk easily on water surfaces(Darmanin & Guittard, 2015). Some of the examples of superhydrophobicity in nature are shown in Figure 3.

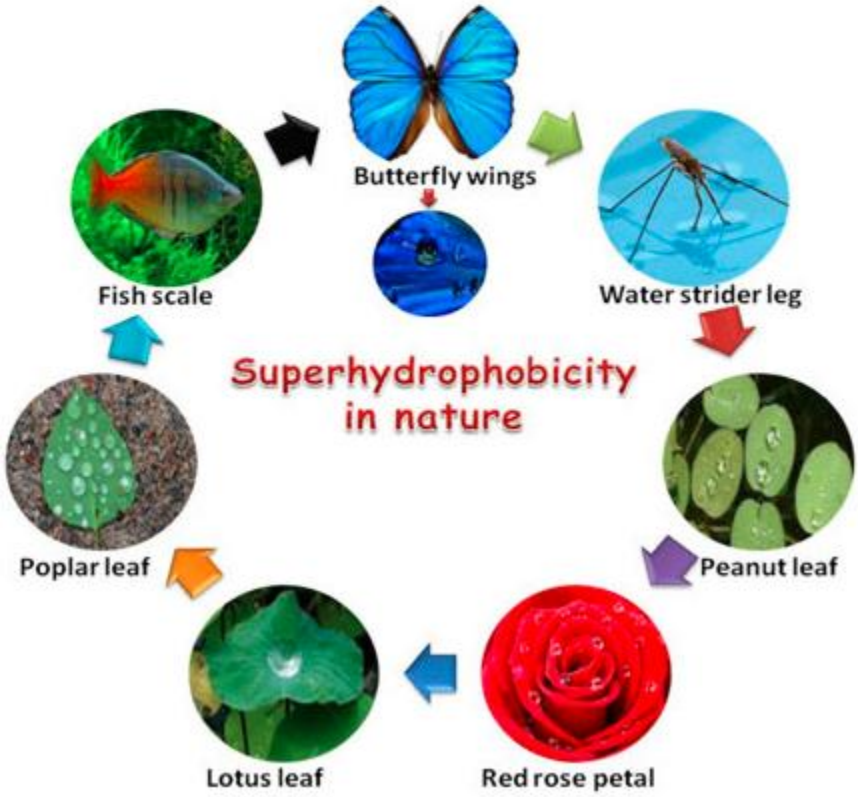


*Figure 3: Superhydrophobicity in nature*(Shaikh, 2020)

## 2.2 Applications of superhydrophobic surfaces

Superhydrophobic surfaces possess numerous benefits and some of them will be discussed below.

### 2.2.1 Anti-icing properties

In recent years, the superhydrophobic coating has emerged as an effective anti-icing solution. By creating air pockets on the surface, these coatings enable water droplets to slide off easily, preventing freezing and minimizing frost-related damage. Traditional methods like local warming and chemical additives, to fight against frost, have limitations, but superhydrophobicity offers a practical approach without special requirements. This is particularly valuable for protecting equipment from ice storms, including electrical transmission lines. Insulators on transmission lines can benefit from anti-icing surfaces to prevent ice formation in cold areas(Khodaei, 2020).

### 2.2.2 Self-cleaning property

The surface of the lotus leaf remains clean regardless of any surrounding contamination due to its unique structure and superhydrophobic properties. The leaf is coated with wax, and its surface has a low sliding angle, allowing water to easily slide and remove any dust. These properties are known as self-cleaning properties of superhydrophobic surfaces and coatings. Various methods have been used to synthesize superhydrophobic coatings for industrial applications. It is important to know that a true self-cleaning surface includes both super hydrophilicity and photocatalytic behavior to clean dirt. Using the term “self-cleaning surface” for superhydrophobic surfaces, which repeal water and remain dry, is not appropriate. While these surfaces do not clean themselves, they facilitate the removal of dirt as water drops roll over their surface(Jeevahan et al., 2018; Khodaei, 2020).

### 2.2.3 Anticorrosion resistance coatings

Superhydrophobic surfaces are being studied as a potential solution to reduce corrosion on metal surfaces, including aluminum, copper, magnesium, and steel alloys. Corrosion of metal layers occurs in two conditions: when immersed in a corrosive solution or when exposed to humid air. However, the superhydrophobic coating can trap air, acting as a natural insulator that protects direct contact between the corrosive medium and the metal material. This air-trapped barrier provides some level of corrosion resistance on these surfaces(Vazirinasab et al., 2018).

### 2.2.4 Drag force reduction

The drag force is a significant challenge encountered by objects, like ships and submarines, moving through water. It emerges from the friction between the water and the surface of the object. Taking inspiration from shark skin, researchers have developed a superhydrophobic coating to minimize drag. These coatings feature hierarchical micro and nanoscale surface structures with air pockets, like those found on shark skin. By reducing the contact between the solid surface and the surrounding liquid, superhydrophobic coatings effectively decrease the drag force(Khodaei, 2020).

### 2.2.5 Anti-fouling

Biofouling, also known as biological fouling, refers to the growth of organic substances on a solid surface. Alongside organic matter, inorganic substances like corrosion deposits, ice, oil, and suspended particles may also grow, collectively known as fouling. Biofouling especially occurs in the marine environment, where significant aquatic growth is observed on structures such as ships, submarines, and marine infrastructure. The growth of organisms on these surfaces leads to increased drag, reducing the speed of ships and placing additional stress on engines, resulting in higher fuel consumption. However, the use of superhydrophobic surfaces can minimize this issue by reducing the contact area between water and the solid surface, by minimizing the growth of biological matter on the surface(Jeevahan et al., 2018).

## 2.3 Edible superhydrophobic films

In the 21$^{st}$ century, the world food crisis has become a bigger problem with food waste. This has led to growing concern among scientists and society regarding the effective reduction of food waste resulting from food waste during the preservation process, such as lipid oxidation, enzymatic browning, and microbial contamination. Additionally, residual food left in containers, especially liquid food products after pouring, creates a problem. One commonly adopted solution to this problem is to use new packaging materials, which include antimicrobial, antioxidant, and moisture/flavor loss prevention properties, which control the exchange of oxygen, carbon dioxide, and water to prevent food oxidation, moisture absorption, and spoilage. (Zhang et al., 2021)

There is a growing demand for such packaging materials in the food industry that not only meet above mentioned requirements but are also derived from inexpensive and renewable raw materials. Bio-based packaging materials have been developed by collecting selected components into film packaging models. Edible films, which can be consumed along with the food they contain, offer improved nutritional quality, and preservation of food color, texture, and moisture by acting as a physical barrier for gases and water vapor. These films can also be enhanced with functional activities like antioxidants, antimicrobials, and vitamins(Manrich et al., 2017). Superhydrophobic films, inspired by lotus leaf (self-cleaning ability), have found some applications, such as antifogging coatings, corrosion prevention, fog harvesting, and oil-spill cleanup(Zhang et al., 2021). Figure 4 shows the self-cleaning ability of the lotus leaf.

Edible films are made from bio-waste, which helps to reduce the food waste produced daily by the food processing industries. Using bio-waste to produce such films helps in sustainability and circular economy. Inspired by biowaste production, research done by Wang et al. was used because it was done by using tomato peel and beeswax. Tomato peel was used for the cutin extraction and beeswax was used to modify the surface to superhydrophobic. So, the upcoming sections from 2.4 – 2.7 describe tomato peels as raw materials, beeswax, cutin, and pectin.

*Figure 4: self-cleaning property of lotus leaf*(Liu et al., 2016)

## 2.4 Tomato peel as a renewable resource for cutin extraction

Approximately, one-third of the food produced for human consumption is lost as waste, according to the Food and Agricultural Organization (FAO) of the United Nations. This significant fraction of food loss occurs throughout the entire value chain, from crop residue during production to processing, sales, and consumption because food production is a wasteful process. The fruit and vegetable processing industries generate a vast amount of waste annually. These residues, obtained from various vegetables and fruits, include beneficial resources such as proteins, polysaccharides, dietary fiber, antioxidants, and natural pigments. The tomato processing industry fits perfectly in the example(Simões et al., 2023). After potatoes, tomato is the second most produced and consumed crop with worldwide production of 180 million tons, 183 million tons, and 186 million tons in 2018, 2019, and 2020 respectively(Simões et al., 2023). Figure 5 illustrates the production of fresh and processed tomatoes.

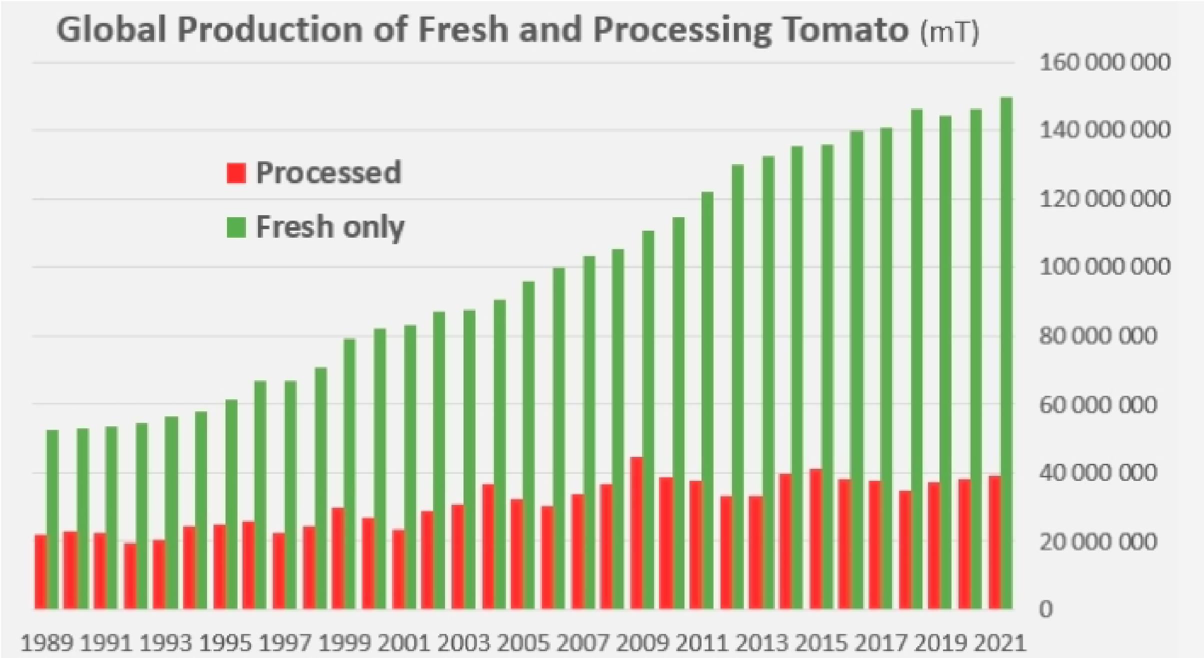


*Figure 5: Global Production of Fresh and Processed Tomatoes*(Branthôme, 2023)

Tomatoes are highly popular among vegetables for their extensive health advantages and the wide variety of products they offer in the market. Consuming tomatoes has numerous benefits such as a decrease in the risk of cancer, cardiovascular illness, and chronic diseases. Recent research has additionally found that the consumption of tomatoes and tomato products helps to improve skin, bone, and brain health(Bhatkar et al., 2021). Despite these benefits, every year, 5-13% of the whole tomato production represents the by-products produced by the tomato processing industries. Annually, these processing industries generate around 18 million tons of tomato residues, which include peels and seeds, to produce various tomato goods including peeled tomatoes, tomato puree, crushed tomato, tomato juice, and tomato concentrate. The disposal of tomato industrial solid waste, particularly tomato peels, involves either disposing them or using them as animals feed in the zoo or using them for biogas generation. Also, when these peels are dried, can serve as a substrate for fertilizer production. However, in recent years, good management and reutilization of argo-industrial waste have become a priority. These by-products present increasing disposal challenges and potential pollution issues, resulting in the loss of valuable biomass and nutrients. Therefore, new methods and policies have been introduced to address the handling and treatment of by-products, focusing on their recovery, bioconversion, and utilization to obtain valuable ingredients. The reutilization of these by-products is an important technology that contributes to sustainable development, the generation of value-added products, and the advancement of a circular economy. Circular economy represents the entire life cycle from production and consumption to waste management and the secondary raw materials market, representing a promising approach to achieving environmental sustainability.

The tissue of tomato peel, which is a highly structured material, can be described as a complex polyester wax, with a minimum hydrophobic nature reactivity and contains components such as cutin, cuticular waxes or soluble lipids, polysaccharides (mainly cellulose and pectin), polypeptides, phenolic compounds, and lycopene. Additionally, it contains minerals (potassium, calcium, magnesium, and sodium), and fatty acids(Cifarelli et al., n.d.).

## 2.5 Beeswax as a hydrophobic agent

Beeswax is a naturally derived wax that is created by honeybees within their hives. It is produced by specialized glands located under the abdomen of worker bees and is utilized in the construction of honeycombs. Female worker bees possess eight glands in their abdominal segment responsible for wax production. When honey is harvested and processed, beeswax is obtained as a by-product(Tinto et al., 2017).

Beeswax is a chemically complex substance that is produced by the wax glands located in the abdomen of honeybees. It is secreted by bees that are approximately 12 to 18 days old, and initially, it is in a liquid state that solidifies upon exposure to air(Coppock, 2021). These glands produce small wax platelets when the bees consume royal jelly and participate in constructing hives. The bees scrape off these wax platelets and then chew and crush them, combining them with saliva and various enzymes to transform them into flexible and workable pieces for the construction of honeycombs, which provide shelter to bees and larvae also, the storage for honey and pollen(Tinto et al., 2017).

Beeswax, when prepared for the first time, is a food-grade wax with a white color. However, the presence of propolis and colorants from pollen causes a color change to yellow. The specific odor of beeswax is influenced by factors such as the types of honey, bees, propolis, and pollen involved. In terms of its physical characteristics, beeswax has a crystalline structure, which is influenced by storage conditions. Also, during storage, the wax not only crystallizes but also becomes more elastic and rigid. Notably, beeswax’s hardness is an important quality, and it shows greater elasticity at lower temperatures. When it is heated, the physical properties of beeswax change. Cooling heated beeswax results in around 10%

shrinkage. Furthermore, when beeswax is heated to temperatures ranging from 30 to 35 degrees Celsius, it acquires plastic-like properties(Menezes & Athmaselvi, 2018). The melting point of beeswax is not constant and slightly differs depending on its origin, typically ranging from 61 to 66 degrees Celsius. Its relative density at 15 °C falls between 0.958 and 0.970 g/cm$^3$, while its electrical resistance ranges from $5\times10^{12}$ to $20\times10^{12}$ Ωm. The thermal conductivity coefficient of beeswax is $2.5\times10^{-3}$ Jcm/s °C cm$^2$. The saponification value lies between 85 to 100(Tinto et al., 2017). Beeswax is insoluble in water but soluble in organic solvents (ether, acetone, xylol, benzene, chloroform, and tetrachloromethane)(Menezes & Athmaselvi, 2018), and after warming, in alcohol and fatty oils. Depending on the place of production, species of bees, and age of wax, the composition of beeswax differs. Normally, beeswax contains 15% of hydrocarbons, 71% of esters, 8% of free acids, and 6% of other compounds(Tinto et al., 2017).

Beeswax has a rich historical importance and offers a huge range of applications compared to other beeswax products. In the past, it was used for candle making due to its higher melting point, which allows candles to remain in the same structure even in hot weather conditions. Beeswax was also used for modeling and casting purposes. Using the lost-wax process, some of the world's finest bronze statues and gold ornaments have been made. This process involved creating a beeswax model that was wrapped with mud or plaster. After drying, the heating process comes into action, which allows it to melt and escape the wax leaving the cavity inside the model into which molten metal could be poured. When melted metal solidifies, it takes the exact shape of the original beeswax model and then the casing material can be removed.

In recent times, one of the important applications of beeswax is in the production of ointments, skin creams, and lotions. Also, it can be used in polishes, protective coatings, and as a lubricant in military weapons and equipment(Crane, 2009). Also, when melted, beeswax can be used as a hydrophobic agent because of its insoluble properties in water(Wang et al., 2020).

## 2.6 Cutin

Cutin is a main component of barrier tissue of fruit and vegetables, working as a high molecular weight bio-polyester. The outer layer of vegetables is composed of a cell membrane known as the "epidermal cell". The epidermal cell is a collection of cells with numerous shapes and functions that cover different parts of the plant, including the leaves, flowers, roots, and steam. These cells generate substances that provide waterproofing properties, resulting in the formation of a protective film called the “cuticle”. Cutin, the main component of the cuticle, is responsible for its structure and function. Throughout the plant’s growth and development, the thickness and composition of the cuticle experience significant changes. These modifications are closely linked with variations in the composition of cutin. As a result, cutin serves as a protective interface between the plant and its environment, minimizing the effect of pathogens and environmental factors(Bueno et al., 2022). Additionally, cutin regulates nutrient flow between different plant cells and organs and helps protect against pathogens. The primary elements of cutin are C16-C18 hydroxy-fatty acid moieties. This bio-polyester acts as a barrier against microbial attacks and contributes to the leaf’s ability to retain water and prevent air penetration.

Various methods, including alkaline hydrolysis and transesterification, can be used to depolymerize cutin by breaking its ester bonds. The amount of plant cuticle material in the agricultural sector has been estimated to range from 180 to 1500 kg/ha, and the overall volume of cutin biomass can reach 109-1010 tons. Notably, tomatoes are known to possess a significant amount of cutin(Ibrahim et al., 2019).

## 2.7 Pectin

Pectin is a form of structural fiber present in the primary cell wall and the intracellular layer of plant cells. Pectin can be found in various fruits such as apples, oranges, lemons, and other citrus fruits. Citrus fruits contain 0.5% to 3.5% pectin, especially found in the peel part of the fruit. As fruits ripen, pectin goes through a transformation from an insoluble substance (in unripe fruits) to water-soluble material (in ripened fruits). Pectin is a polymer with a linear structure, where several hundred to thousand galacturonic acid monomer units are linked together through α-(1-4)-glycosidic bonds, forming a backbone. The average molecular weight of pectin ranges from 50 to 150 kDa. The pectin molecule’s backbone is substituted for a specific reason with α-(1-2) rhamnopyranose unit, which can have side chains of galactose, mannose, glucose, and xylose. Galacturonic acid in pectin undergoes methyl esterification. Based on the degree of methyl esterification, there are two types of pectin, high methoxyl pectin, and low methoxyl pectin. High methoxyl pectins are defined as having more than 50% esterified galacturonic residues, while low methoxyl pectins have less than 50% esterified galacturonic acid residues.

Pectin is mostly used in food production industries and households as a thickener. Also, it is used to produce jams and jellies. Pectins are also considered to have some unique abilities for the prevention or treatment of diseases such as intestinal infections, atherosclerosis, cancer, and obesity(Mudgil, 2017).

# 3 Experimental Part

The experimental part was carried out in four parts.

(i) Extraction of cutin from tomato peel
(ii) Development of the film using cutin and pectin
(iii) Applying beeswax in the film
(iv) Testing

The experimental process was conducted by following the article done by Wang et al. but there were some changes made according to the requirement of our equipment and materials available in the Arcada UAS lab. In place of an autoclave hot plate was used for the cooking process and gravity separation was done in place of a centrifuge process for the extraction of pure cutin.

The extraction process of cutin was done twice to get the required results. Also, the separation process of tomato peels was done at home, to reuse the tomato.

## 3.1 First Batch (Extraction of Cutin)

First of all, 500 grams of “Cherry Tomatoes” was bought from the local market. Then, the tomatoes were put into the boiling water for two minutes, so that the skin and fruit of the tomatoes separates easily. Then the tomatoes skin and pulp were separated manually. Changes between before and after boiling can be seen easily in Figure 6. From 500 grams of tomatoes, 38.79 grams of peeled skin was obtained.

*Figure 6:Before boiling and after boiling*

Then, all the peeled skin was transferred to the beaker with a magnetic stirrer on it. After that, 155ml of 3% NaOH solution was added to it and the beaker was placed on the hot plate with a watch glass on the top of the beaker to control the evaporation of NaOH solution. At around 90 degrees Celsius, the mixture started boiling. Then the temperature was kept constant, and it was cooked for two hours.

After two hours, the mixture was taken out from the hot plate and was left for a while to cool down. Later, when it was cooled down, it was first filtered with the sieve to remove the large particles then, again following the Gravity filtration method, it was filtered using “Qualitative filter paper, 401” with particle retention 12-15 μm.

Figure 7 illustrates the process of cooking and filtration,


*Figure 7:cooking, filtration using a sieve, filter paper, and gravity filtration*

The obtained mixture was treated with 6 mol/L HCl solution until its pH level reaches 5.485. The main objective of this process was to acidify the liquid phase sample until pH level 5-6 and to obtain the cutin from the precipitation of the sample.

Then the mixture was transferred to the six tubes for the centrifugation process, which was done at 6500 rpm for 15 minutes. After the centrifugation, obtained cutin was washed with distilled water and centrifuged thrice in the same manner. In every wash, the color of the sample was different, from dark to light, as seen in Figure 8. When the cutin was extracted after the third wash, it was transferred to the dish and was left to freeze dry.


*Figure 8:sample after cutin recovery*

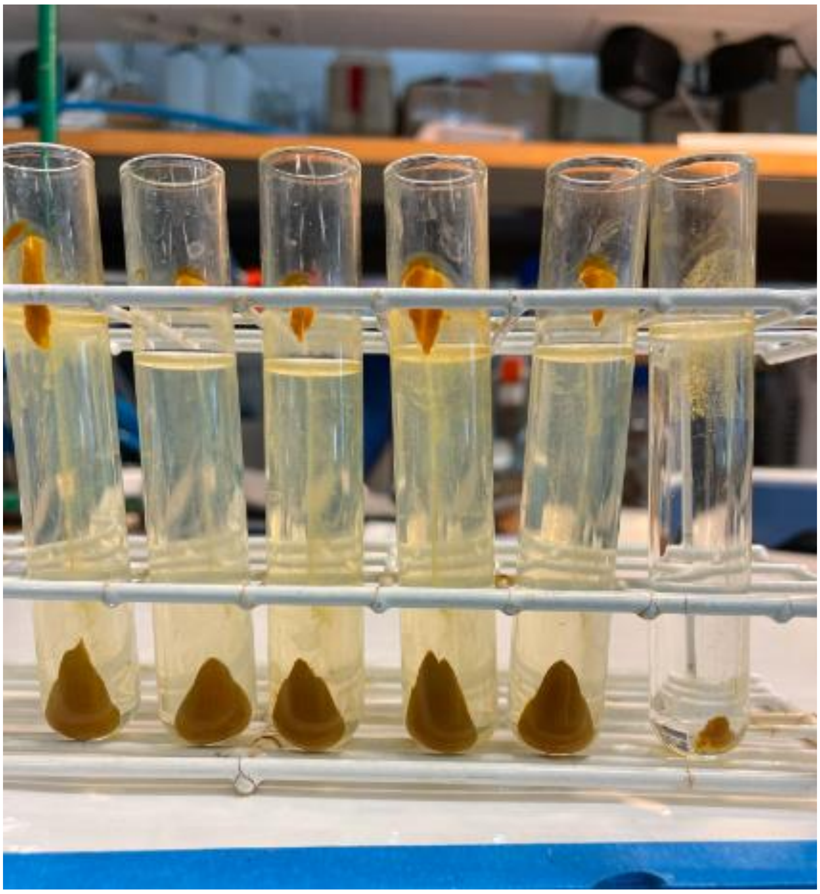

*Figure 9:Obtained cutin from the centrifuge process*

*Figure 10: Obtained cutin after drying*

The obtained cutin was so sticky and insoluble in water, so a second batch of cutin extraction was done.

## 3.2 Second batch of cutin extraction

Two kg of cherry tomatoes were purchased from the local market (Lidel). Then all the tomatoes were boiled for one minute in boiling water for one minute. Then the skin and pulp were separated. From 2 kg of tomatoes, 123.86 g of skin was obtained.

Then 100 g of tomato peels was transferred to the beaker with a mechanical stirrer, mixed with 400 ml of 3% NaOH, and placed on a hot plate for cooking. A mechanical stirrer was used because the quantity of peels was more, and the magnetic stirrer was not mixing well. At around 90 degrees Celsius, it started boiling and the temperature was kept constant throughout the process.

After 30 minutes, the quantity of NaOH was decreased because of the vapor, so 32 ml of NaOH was added to the mixture to maintain the original quantity of NaOH present in the beaker, which was marked already. Every minute, the quantity of solution was decreasing, and it was becoming a big deal. So, after another 30 minutes, 48 ml of NaOH was added and the solution was transferred to the conical flask with a magnetic stirrer, to use the “air cooling reflux” for the remaining cooking process. Reflux was used to condense down the vapor and to control the loss from the boiling mixture. After that, there was not any loss in the quantity of mixture, and cooking was done for 3 hours in total.

After cooking, the mixture was filtered using a vacuum filtration process. In place of filter paper, a fine strainer was used to separate the peels remaining and the solution mixture. Then the mixture was treated with 6 mol/L HCl until the pH of the sample reached 5.19.so the cutin can be obtained through precipitation by acidifying the sample.

Then the sample was left in a beaker for the settling down of the cutin through the precipitation process. Once the cutin was precipitated, the solid phase and liquid phase were separated using a syringe. After that, the residue was washed with distilled water and let the cutin settle down. The same process was repeated two more times and the cutin was extracted.

While conducting the cutin and washed liquid separation, cutin present in the liquid was recovered through centrifugation at 6500 rpm for 10 minutes. Cutin obtained from the process was washed and the centrifugation process was repeated twice in the same manner. Figures 11-18, shows all the process of cutin extraction.

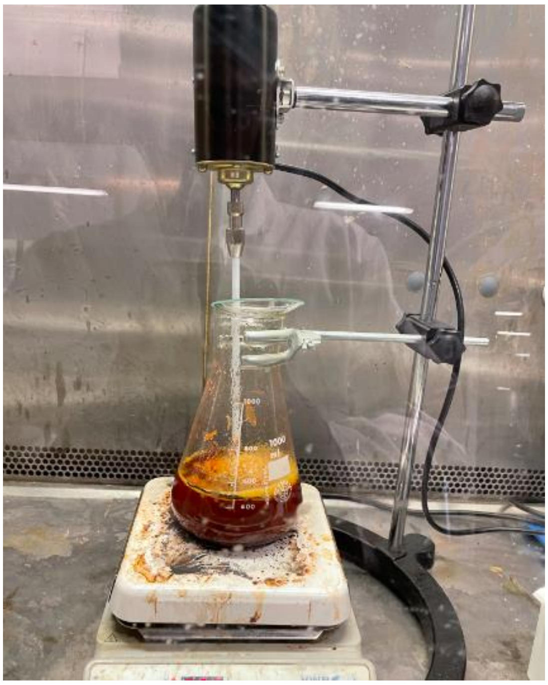

*Figure 11: cooking using mechanical stirrer.*

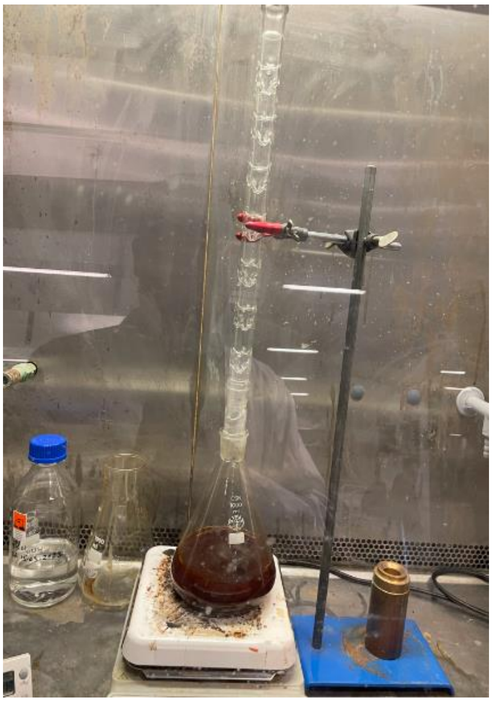

*Figure 12:cooking using flux*

*Figure 14: Residue after filtration*

*Figure 13: Cutin precipitation*

*Figure 15: After first wash*

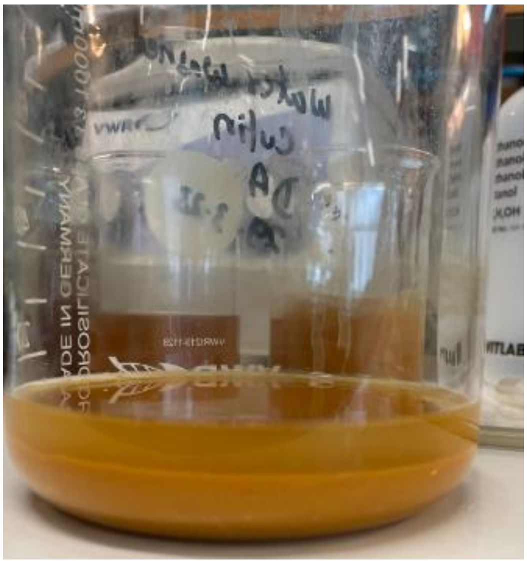

*Figure 16:After second wash*

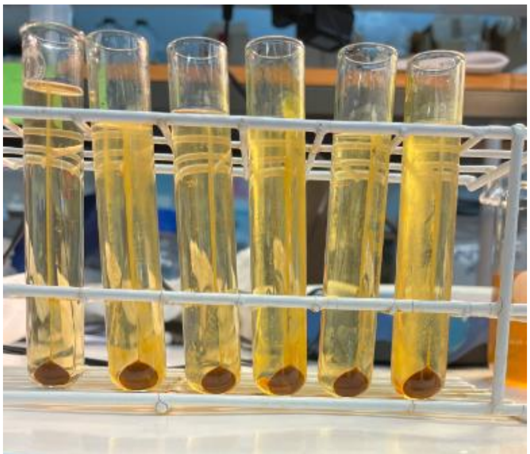

*Figure 17:Cutin recovered using centrifuge.*

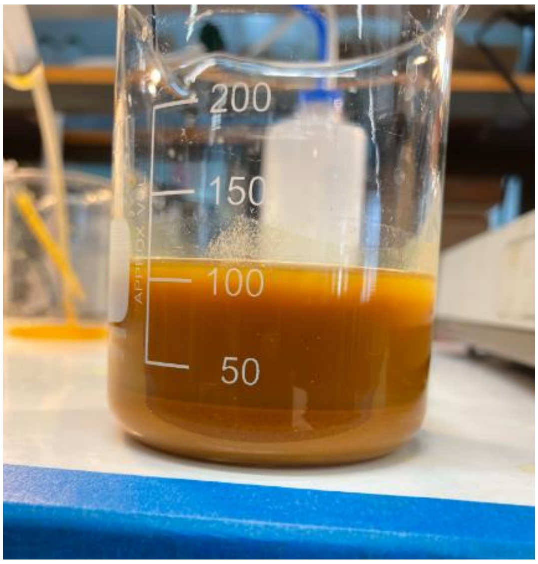


*Figure 18: After the final wash*

## 3.3 Preparation of cutin pectin membrane (CPM)

### 3.3.1 From the first batch

Dried cutin was supposed to be mixed with distilled water to make the cutin solution but as already talked about it in the above chapter, it was not soluble in water, so the leftover cutin was mixed with 5 ml of ethanol but it did not mix completely. Still, the mixture was transferred to a petri dish and then pectin, which was mixed with distilled water at room temperature (commercially made pectin was used), was mixed with cutin solution. Then the mixture was put into a vacuum dryer at 45 degrees Celsius overnight to obtain the CPM. The next day, after 18 hours, the membrane was taken out.

*Figure 19: CP membrane in petri dish*

*Figure 20: CP membrane*

The obtained CPM was so brittle, and, in less amount, the further process was not possible.

### 3.3.2 From the second batch

Development of CPM from the second batch does not include the dried cutin. Cutin solution extracted from multiple washes was used. If dried cutin was taken, then it had to be mixed

with distilled water so, already washed cutin was used. Also, pectin was prepared following the same process as the first batch.
Then the obtained solution was mixed following the 50/50 ratio (as shown in Figure 21) creating eight samples. Then the solution was transferred to Teflon, so the membrane would be easily taken out. Then the samples were placed into the vacuum dryer overnight at 48 degrees Celsius to obtain CPM. Figure 22 represents the obtained CPM.
The table below shows the weight taken for all the samples to create CPM.

| Samples | Cutin (g) | Pectin (g) | CPM (g) |
|---|---|---|---|
| 1 | 5.2 | 5.2 | 0.4349 |
| 2 | 5.0 | 5.02 | 0.4159 |
| 3 | 5.01 | 5.01 | 0.4013 |
| 4 | 5.0 | 5.03 | 0.4222 |
| 5 | 5.0 | 5.0 | 0.4120 |
| 6 | 5.0 | 5.07 | 0.4128 |
| 7 | 5.03 | 5.02 | 0.4194 |
| 8 | 5.64 | 5.64 | 0.485 |


*Figure 21: 50/50 ratio of cutin and pectin*

*Figure 22: cutin-pectin membrane*

After the membranes were extracted, some of them were treated with beeswax solution for further testing of contact angles. Also, one treated and one untreated membrane was given to the colleague (Sapana Ghimire) for the biodegradability test, and the rest were used by another colleague (Deepa Shrestha) for the contact angle test and wettability test.

# 4 RESULTS

## 4.1 Membrane

The obtained membrane from the cutin and pectin mixture was dark brown in color in the middle and dark color in the edges as shown in Figure 23. Teflon was used to place the mixture and when the membrane was ready, they were obtained in asymmetrical shape. Some parts of the membrane were rising, and some parts were plain. The Cutin and pectin mixture seems to be evenly mixed, but the dark part of the membrane was quite thin and more brittle. Also, the membrane had lots of air bubbles in it, and it is seen in Figure 23. Obtained membranes were brittle but after applying beeswax, they turned out to be flexible.

*Figure 23:CPM with air bubbles on it*

## 4.2 FTIR Test

The FTIR test was done after preparing the membrane and the results were compared with the results from the article followed. All the results are discussed below.

### 4.2.1 Pure Cutin, Pectin, and Cutin-Pectin Membrane

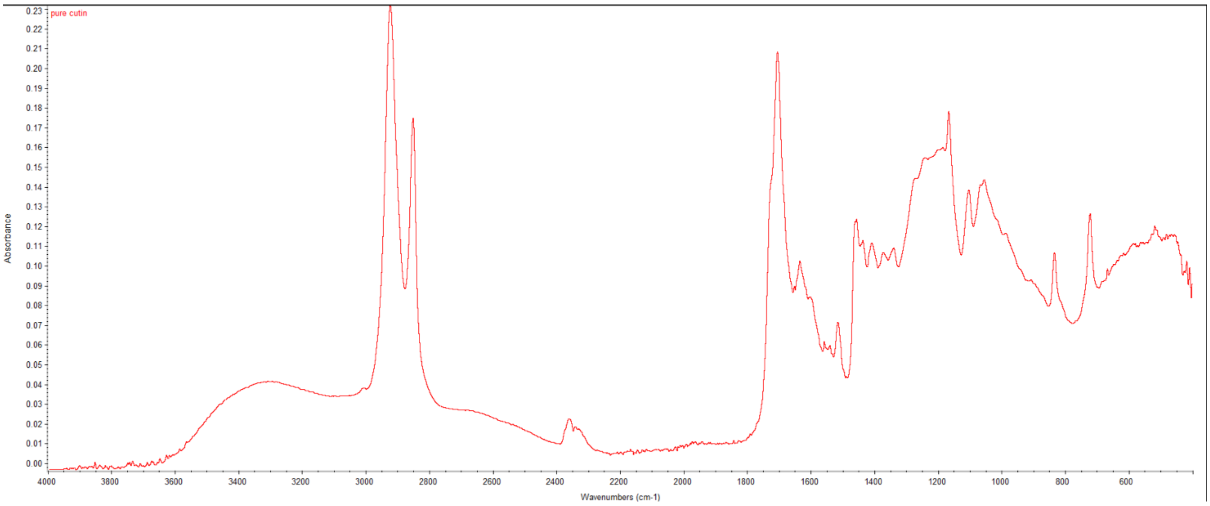


*Figure 24: Pure Cutin*

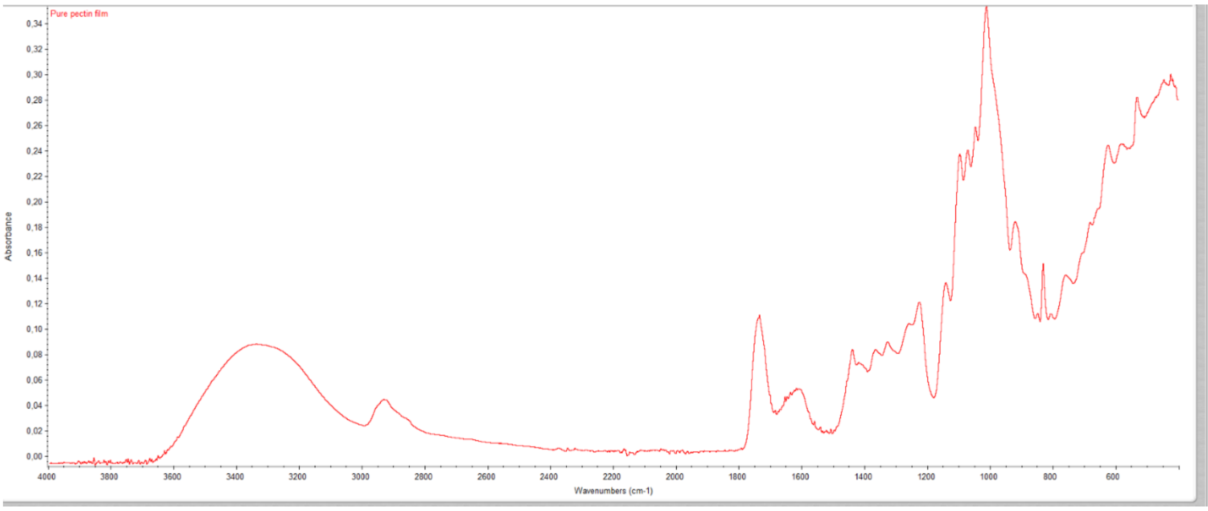


*Figure 25: Pure Pectin*

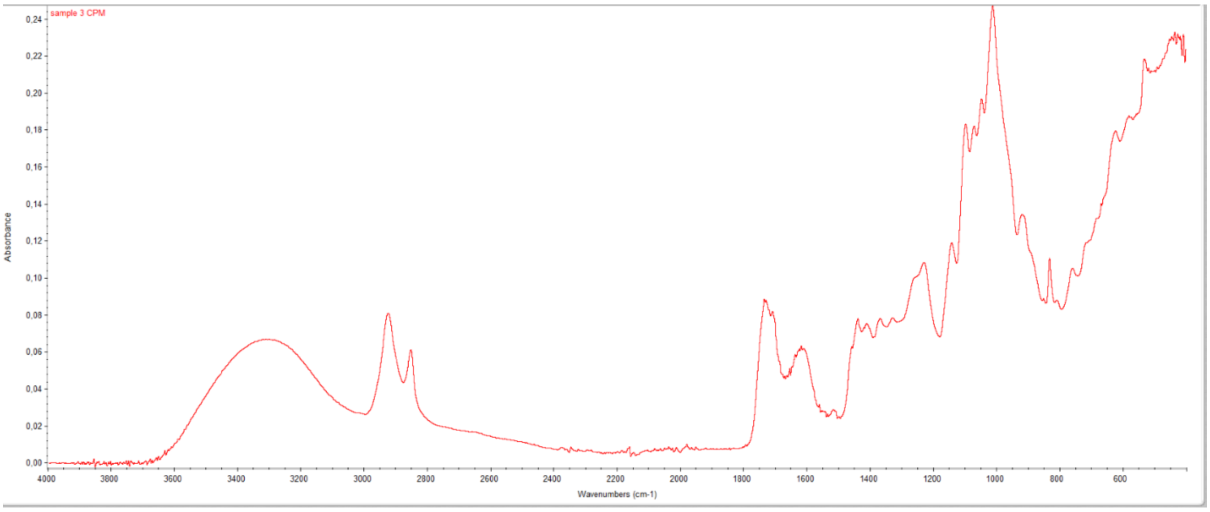


*Figure 26: Cutin-Pectin Membrane*

In all three figures, the peak at 3400-3500 $cm^{-1}$, stretched to $-NH_2$ and -OH, indicating the presence of hydrogen bonds in the obtained cutin, pectin, and cutin-pectin membrane. Moreover, spectra around 2900 - 2800 $cm^{-1}$ of cutin and membrane, show the stretching vibration of $-CH_3$ and $-CH_2$ groups, which indicates the existence of cutin. But, at the same point, pectin spectra do not show any obvious peaks, which proves the cutin-pectin membrane gains $-CH_3$ and $-CH_2$ group from the cutin. Also, this peak indicates the membrane acquires hydrophobic nature from cutin as pectin spectra do not show any peak.  Peaks at 1700 $cm^{-1}$of cutin spectra, reach the ester group, which proves the cutin existence. Finally, the membrane spectra show similar spectra with cutin and pectin, indicating it acquires the properties of both cutin and pectin(Wang et al., 2020).

### 4.2.2 Beeswax and membrane coated with beeswax

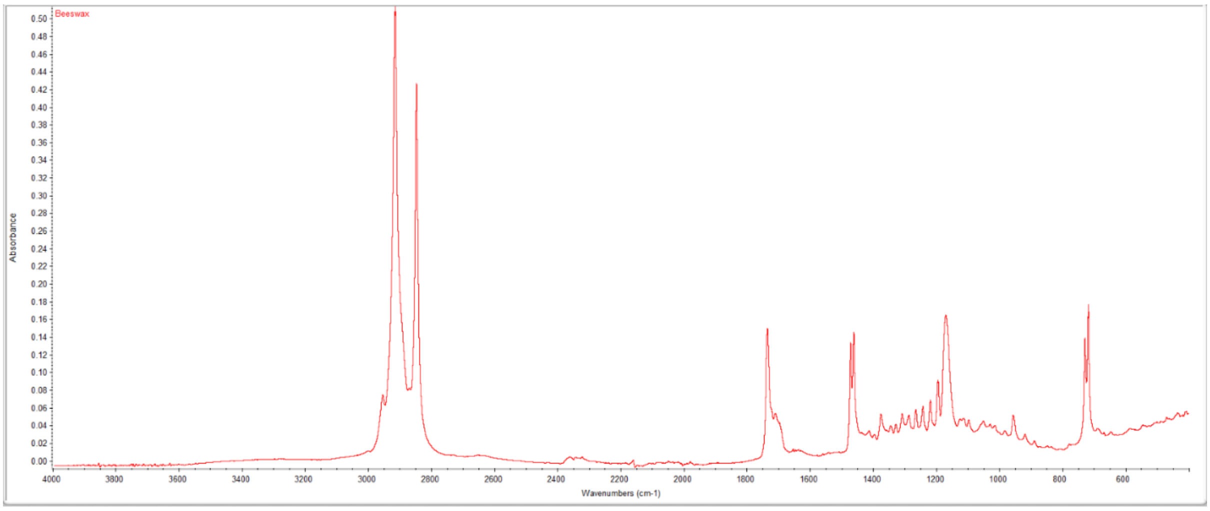


*Figure 27: Beeswax*

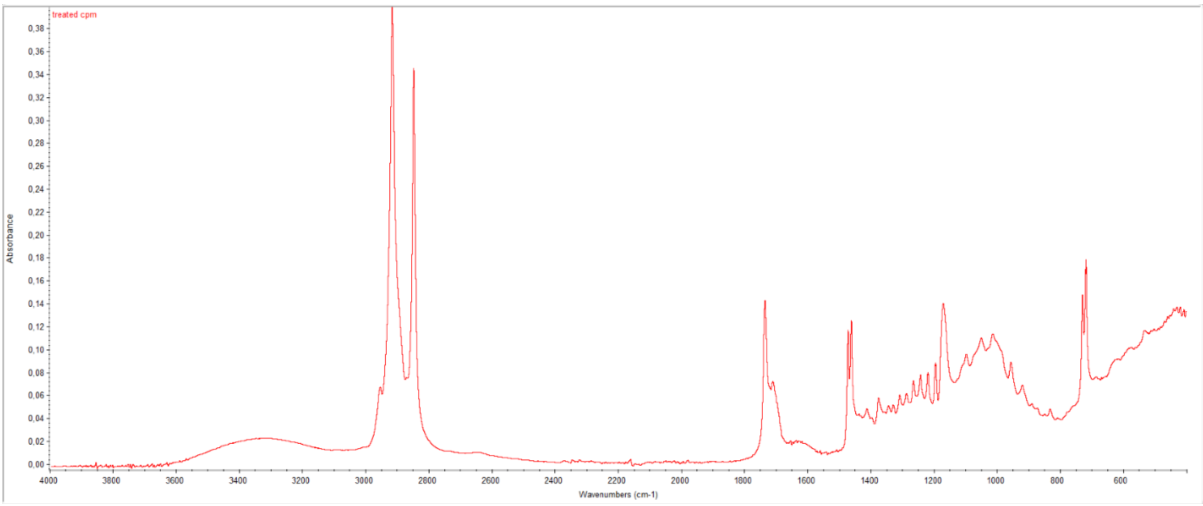


*Figure 28: Membrane coated with beeswax*

In both the figures, spectra at 2900-2800 $cm^{-1}$, shows the stretching vibration of the -$CH_3$ and -$CH_2$ group, and around 1700 $cm^{-1}$, spectra show the ester group. Both results illustrate the -$CH_3$ and -$CH_2$ group and ester group provide hydrophobic chemical properties to the membrane. Also, there is not any significant change in the beeswax and coated membrane, which illustrates the membrane acquires all the properties of beeswax after coating without any significant chemical change(Wang et al., 2020).

## 4.3 Contact Angle and Biodegradability

Contact angle and biodegradability tests were done by colleagues. The obtained contact angles of the coated and uncoated membranes were 95 and 80 degrees, respectively (as per the results obtained by Deepa Shrestha).
The biodegradability test was done by Sapana Ghimire. Two samples were used in the composter and two were used in the seawater. As per her results, all the samples vanished in the composter and seawater. It took three days for the samples to decompose in the composter and 15 minutes to decompose in the water. Samples 4 and 5 were used in the compost, with pH 5.5 and 5 and temperatures of 48 and 47.5 degrees Celsius respectively. The biodegradability results of the samples are shown in detail in Figure 29.

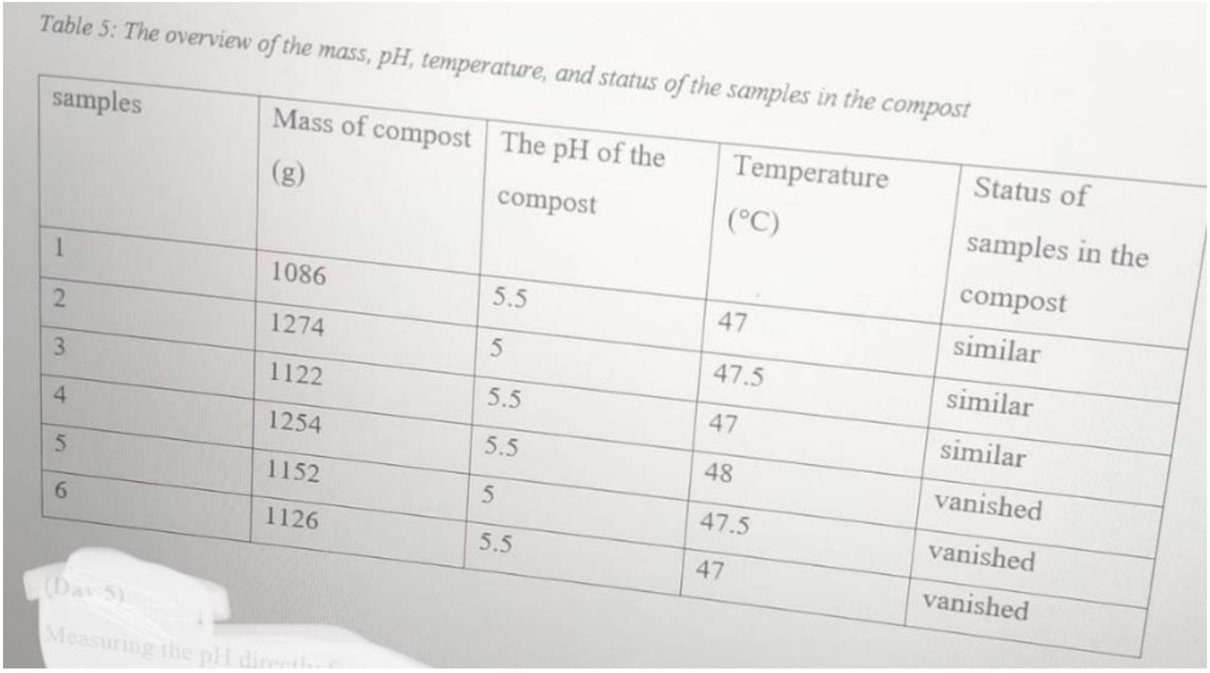
*Table 5: The overview of the mass, pH, temperature, and status of the samples in the compost*

| samples | Mass of compost (g) | The pH of the compost | Temperature (°C) | Status of samples in the compost |
|---|---|---|---|---|
| 1 | 1086 | 5.5 | 47 | similar |
| 2 | 1274 | 5 | 47.5 | similar |
| 3 | 1122 | 5.5 | 47 | similar |
| 4 | 1254 | 5.5 | 48 | vanished |
| 5 | 1152 | 5 | 47.5 | vanished |
| 6 | 1126 | 5.5 | 47 | vanished |

*Figure 29: Results of biodegradability (By Sapana Ghimire)*

# 5 DISCUSSION AND CONCLUSION

The main objective of this thesis was to create an edible superhydrophobic film using bio-waste and to do the FTIR test to check the chemical properties of the film. Extraction of the membrane was done successfully in the lab and the FTIR results show the required chemical properties in the membrane. However, the contact angle test results do not meet the required target to make the membrane superhydrophobic. When the membrane was produced, there were lots of air bubbles and small pores, created by the bubbles, present in it. I believe it is because of the air bubbles present in the pectin. Also, the obtained membranes were not symmetrical, which results in the uneven placement of beeswax spray in the membrane. Because of this, the required contact angle i.e., greater than 150 degrees was not obtained. Additionally, cutin precipitation was done using gravity separation and cutin was obtained in a liquid form (which was supposed to be obtained in a dry form) because the centrifugation process did not give the required result in the first place. The article by Wang D. used an autoclave for the cooking process and a centrifugation process was used for the cutin extraction. Whereas we used a normal hot plate for the cooking and gravity separation of cutin and liquid for the cutin extraction. Also, they used high-methanol pectin, and we used apple pectin for the construction of the membrane. These few things were different from the original article, and I think these process results in different results from the original article. However, I believe, if all the membranes were obtained symmetrically with no air bubbles and no pores, then the beeswax would have been applied evenly to the surface of the membrane, making a nice layer of beeswax on the surface, and we could have obtained the required results.

Finally, the extraction of edible superhydrophobic film using bio-waste is possible by avoiding above discussed errors and these films are suitable for the planet by helping in sustainability, less pollution, and circular economy.

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