Computer-aided approach for the development and characterisation of bioplastic from orange peels incorporated with rice husk

by

Nurul Syafiqah binti Ismail

F18A0199

A thesis submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Technology Product Development) with Honours

Faculty of Agro-Based Industry
Universiti Malaysia Kelantan

2022

DECLARATION

I hereby declare that the work embodied here is the result of my own research except for the excerpt as cited in the references.

Signature

Student's Name : Nurul Syafiqah binti Ismail

Matric No : F18A0199

Date : 28th February 2022

Verified by :

TS. DR. SITI NUURUL HUDA MOHAMMAD AZMIN
Senior Lecturer
Faculty of Agro-Based Industry
Universiti Malaysia Kelantan

Supervisor Signature

Supervisor's Name:

Stamp :

Date : 28th February 2022

ACKNOWLEDGEMENT

Firstly, I would like to thank to Allah S.W.T, for His love, giving me all His astonishing provisions, strength, and patience for the successful accomplishment of this study. I would like to thank to my supervisor, Dr. Siti Nuurul Huda binti Mohammad Azmin for her appreciable guidance and encouragement during every stage of the project journey.

A special word to thanks to all laboratory assistance in Faculty of Agrobased Industry, especially Mr. Suhaimi bin Omar, Mr. Khalid bin Zakaria, Mr. Abdul Rohimi bin Mohd Jari, Mr. Nik Ahmad Fakhrudin bin Nik Dzulkefli, and Mrs. Nor Hidayah binti Hamzah for their guidance and support during laboratory work.

Also, I would like to extend my acknowledgement to all my friend who have been always beside me, helping me physically, emotional, and psychologically to overcome all the obstacle I faced during finish my study.

ABSTRACT

Nowadays, people use plastic bags as packaging materials because it is lightweight, cheap, and convenient. However, the use of petroleum-based plastics can have a major environmental impact. Based on statistical data obtained from the Solid Waste and Public Cleansing Management Corporation (SWCorp), the daily waste production among Malaysians increased by 100.75 percent to 38,142 tons in 2018, compared to 19,000 tons, in 2005. This research focuses on developing and characterizing pectin and cellulose-based bioplastic formulation. Development of pectin and cellulose-based bioplastic was carried out at difference ratio pectin and cellulose (6:4, 7:3, 8:2, 9:1) % and mass of plasticizer (0.1, 0.2, 0.3, 0.4) ml/g whereas cellulose dissolved in a 3% (w/v) of NaOH, and pectin dissolved in distilled water with pectin: distilled water = 1:10 (w/v) ratio. The films were prepared by casting technique using a film-formation solution. In order to obtain the optimal condition in developing the bioplastic, an optimization process has been carried out. A Central Composite Design (CCD) technique from Response Surface Methodology (RSM) was used to investigate the effects of independent variables on bioplastic developed properties. Moisture content, thickness, and density of bioplastic developed were analyzed. From the analysis of experimental results, the highest and lowest value of moisture content (39.98% and 6.84%), film thickness (0.232 mm and 0.040 mm), and film density (0.049 g/ml and 0.027 g/ml) was obtained, respectively. The coefficient of determination (R^2) for moisture content, film thickness, and film density was 0.8568, 0.9473, and 0.7296, respectively. The optimized condition for production bioplastic was 6.001% concentration pectin, 0.408 g of plasticizer, and 26.184 ml volume film-formation solution with a high value of desirability (1.000), which was giving film with moisture content 41.654%, film thickness 0.230 mm. and film density 0.049 g/ml.

Keywords: Central Composite Design, optimization, Analysis of variance, bioplastic Response Surface Methodology.

ABSTRAK

Kini, orang ramai menggunakan beg plastik sebagai bahan pembungkusan kerana ia ringan, murah dan mudah. Walau bagaimanapun, penggunaan plastik berasaskan petroleum boleh memberi kesan alam sekitar yang besar. Berdasarkan data statistik yang diperoleh daripada Perbadanan Pengurusan Sisa Pepejal dan Pembersihan Awam (SWCorp), pengeluaran sisa harian dalam kalangan rakyat Malaysia meningkat sebanyak 100.75 peratus kepada 38,142 tan pada tahun 2018, berbanding 19,000 tan, pada tahun 2005. Penyelidikan ini memfokuskan kepada pembangunan dan pencirian pektin dan formulasi bioplastik berasaskan selulosa. Pembangunan bioplastik berasaskan pektin dan selulosa telah dijalankan pada nisbah perbezaan pektin dan selulosa (6:4, 7:3, 8:2, 9:1) % dan jisim plasticizer (0.1, 0.2, 0.3, 0.4) ml/ g manakala selulosa dilarutkan dalam 3% (b/v) NaOH, dan pektin dilarutkan dalam air suling dengan pektin: air suling = nisbah 1:10 (w/v). Filem-filem itu disediakan dengan teknik tuangan menggunakan larutan pembentukan filem. Bagi mendapatkan keadaan optimum dalam membangunkan bioplastik, proses pengoptimuman telah dijalankan. Teknik Reka Bentuk Komposit Pusat (CCD) daripada metodologi permukaan tindak balas (RSM) telah digunakan untuk menyiasat kesan pembolehubah tidak bersandar pada sifat dibangunkan bioplastik. Kandungan lembapan, ketebalan, dan ketumpatan bioplastik yang dibangunkan telah dianalisis. Daripada analisis keputusan eksperimen, nilai kandungan lembapan tertinggi dan terendah (39.98% dan 6.84%), ketebalan filem (0.232 mm dan 0.040 mm), dan ketumpatan filem (0.049 g/ml dan 0.027 g/ml) maing-masing diperolehi. Pekali penentuan (R^2) untuk kandungan lembapan, ketebalan filem, dan ketumpatan filem masing-masing ialah 0.8568, 0.9473, dan 0.7296. Keadaan optimum untuk bioplastik pengeluaran ialah 6.001% kepekatan pektin, 0.408 g pemplastik, dan larutan filem isipadu 26.184 ml dengan nilai kebolehinginan yang tinggi (1.000), yang memberikan filem dengan kandungan lembapan 41.654%, ketebalan filem 0.230 mm. dan ketumpatan filem 0.049 g/ml.

Kata kunci: Reka Bentuk Komposit Pusat, pengoptimuman, Analisis varians, bioplastik, Metodologi Permukaan Respons

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

		Page
CH_4	Methane	2
CO_2	Carbon dioxide	2
N_2	Nitrogen	9
NaOH	Sodium hydroxide	13
H_2SO_4	Sulfuric acid	13
CS_2	Carbon disulphide	13
$C_3H_5(OH)_3$	Glycerol	29
R^2	Coefficient of determination	49

LIST OF ABREVIATIONS

		Page
EBO	European Bioplastic Organization	2
SWCorp	Solid Waste and Public Cleansing Management Corporation	3
CCD	Central Composite Design	5
RSM	Response Surface Methodology	5
PLA	Polylactic acid	7
PET	Polyethylene terephthalate	7
PE	Polyethylene	7
PP	Polypropylene	7
PS	Polystyrene	7
PVC	Polyvinylchloride	7
PA	Polyamide	7
LDPE	Low-density polyethylene	7
HDPE	High-density polyethylene	7
GHG	Green House Gas	9
PCBs	Polychlorinated biphenyls	10

DDT	Dichlorodiphenyltrichloroethane	10
PHA	Polyhydroxyalkanoates	14
PHB	Polyhydroxy butyrate	14
EPS	Exopolysaccharides	14
RHA	Rice husk ash	21
MCC	Microcrystalline cellulose	22
PPS	Pineapple peel extract solution	23
CNC	Cellulose nanocrystal	24
CSG	Cress seed gum	25
TGA	Thermogravimetric analysis	25
SEM	Scanning electron microscopy	26
FTIR	Fourier Transform Infrared	27
WVTR	Water vapor transmission rate	27
PBD	Plackett-Burman design	27
PEG	Polyethylene glycol	29
CV	Coefficient of variance	50
AP	Adequate precision	50
PRESS	Predicted sum of square	51
ANOVA	Analysis of variance	53
OTR	Oxygen transmission rate	81

CHAPTER 1

INTRODUCTION

1.1 Background of research

Plastic is a growing packaging material widely used compared to paper, aluminium foil, and glass. Plastic shows many advantages such as lightweight, odorless, unbreakable, easy to shape, and easy to carry anywhere. Based on Global Plastic Production statistics, the production of plastic worldwide reached 368 million metric tons in 2019 (Tiseo, 2022). However, it also presents disadvantages, which are detrimental to the environment. One of the disadvantages of plastic does not completely decompose. Plastic takes up to hundreds of years to decompose and remains in nature as waste, thus impacting the environment, including marine life.

Recently, numerous studies have been conducted to find new alternatives to help in reducing the problem related to environmental pollution. One of the inventions is by developing bioplastics from waste to replace synthetic plastics. Bioplastic is a kind of plastic manufactured from recycled materials, including corn starch, vegetable oils, and other agricultural waste. According to the European Bioplastics Organization (EBO), " plastics made and processed from renewable sources or biodegradable plastics and/or compostable." Bioplastics are environmentally friendly plastics because they can be disposed of in the environment and can decompose quickly with the help of enzyme action of microorganisms. Microorganisms can break down biodegradable plastics into the air, and gases such as methane (CH_4) and carbon dioxide (CO_2) will occur (van den Oever et al., 2017). Temperature, presence of microorganisms, oxygen, and water are all factors that influence biodegradability (van den Oever et al., 2017). In addition, according to Muhammad Shamsuddin (2017), bioplastics will biodegrade. Carbon dioxide gas, water, methane, and various other natural materials are produced naturally.

Although there are many types of starch to make bioplastics, the most common type of starch used is corn starch. In the current situation, many researchers have concentrated their hard work on other industrial, agricultural wastes such as orange peel and rice husk that are cheaper and more suitable for cellulose-based bioplastics. With an annual harvest of over a billion pounds, citrus is the world's biggest fruit crop. More than 124.3 million tonnes have been produced worldwide (Mahato et al., 2020). In the industrial processing of orange juice production, after the orange fruit extracts into juice, about 50-60% of the orange peel residue from the juice intake will be discarded as waste consisting of peels, seeds, and membranes (Wilkins et al., 2007). In addition, orange peel also has other compounds such as pectin, starch, protein, lignin, soluble sugar,

hemicellulose, cellulose, ash, fat, and flavonoids. These compounds are suitable and exciting for bioplastic applications (Bátori et al., 2019).

Besides, rice (*Oryza sativa L.*) is a major crop and a significant food source for billions of people worldwide. Paddy farms in Malaysia, each year, with a land area of around 680,000 hectares, a total of 840,000 tonnes of rice husk are produced (Arjmandi et al., 2015). Furthermore, rice husk is a waste that has not been completely used in the environment (Jannah et al., 2019). Rice husk is one of the sources of biomass that can be used as a raw material for the production of bioplastics. Moreover, rice husk contains silica, which has been used to improve the composite materials' mechanical properties (Arjmandi et al., 2015). Apart from having a high silica content, rice husk also has a high cellulose content of around 76% and can be used as a bioplastic raw material (Jannah et al., 2019).

1.2 Problem statement

Nowadays, people use plastic bags as packaging materials because it is lightweight, cheap, and convenient. However, the use of petroleum-based plastics can have a significant environmental impact. One of the leading causes of garbage collection is the irresponsible attitude of human beings who throw garbage without caring about the adverse environmental effects and quality of life. Based on statistical data obtained from the Solid Waste and Public Cleansing Management Corporation (SWCorp), daily waste generation among the people in Malaysia was recorded an increase of 100.75 percent to 38,142 tonnes in 2018, compared to 19,000 tonnes, in 2005. Furthermore, waste

management standards in Malaysia are still poor, and improvements are needed. These include low generation and composition rates, inefficient waste storage and collection systems, and landfills with toxic and hazardous wastes (Sreenivasan et al., 2012). Therefore, this research is one of the efforts to overcome the problem of the use of plastics that cannot decompose naturally by producing bioplastics from orange peel incorporated with rice husk.

1.3 Research objective

This study composes of three objectives which are:

1. To develop a systematic generic computer-aided approach for designing bioplastic added with orange peels and rice husk by considering economic and environmental sustainability.
2. To obtain the optimum condition of the independent variables for the development of bioplastic pectin and cellulose-based film formulation by using a response surface methodology.
3. To validate the bioplastic formulation (result from the developed framework) through an experimental study.

1.4 Scope of research

In this research, the preparation of biodegradable plastics based on orange peel and rice husk was conducted based on the experimental design generated by Design-Expert software. Bioplastic properties, including moisture content, film thickness, and film density, were investigated. The optimization process of the pectin and cellulose-based bioplastic was determined using a Response Surface Methodology (RSM). Central Composite Design (CCD) was used in Response Surface Methodology (RSM), resulting in 10 experimental in this study. The optimal film was developed according to the optimum result generated by the Design Expert. This research also covers the thermogravimetric analysis for optimal film development.

1.5 Significant of research

The objectives for this research were to produce bioplastics from orange peel incorporated with rice husk, test the moisture content, thickness, and density of the bioplastics produced, and investigate the thermogravimetric of optimal bioplastics developed. The results of this research have a positive impact on the economy, especially on the environment. This research can also be used as a guide to the researchers to conduct other studies. Waste disposal can be reduced for the environment if synthetic plastics are replaced with bioplastic. Lastly, it also has a positive impact on the economy through producing and using biodegradable plastics.

CHAPTER 2

LITERATURE REVIEW

2.1 Plastic

Plastics are polymeric molecules with long chains that have unique properties such as being not expensive, lightweight, strength, high thermal, electrical insulation, and corrosion resistance. Plastics can be an excellent packaging material because of these characteristics. The versatility of their properties can be used in natural polymers, thermoplastics, thermosetting plastics, and modified natural polymers (Andrady & Neal, 2009). We can see that we have used materials made of plastic in our daily lives. Plastics are used in transports, in the food industry, in household products (such as jars, beverages bottles), in personal products (such as shampoo and conditioner bottles), and other various industrial applications (Andrady & Neal, 2009).

2.1.1 Plastic as a material for packaging

The most widely used plastics in the food processing industry are petrochemical-based plastics. These plastics are not biodegradable because they have the main chemical structure of covalently bonded hydrocarbon molecules (Jariyasakoolroj et al., 2020). These plastics can be categorized into two which are thermosets and thermoplastic. Thermosets cannot be reprocessed by heat and are not recyclable, therefore they are not used in food packaging industry.

While for thermoplastics, they can be reprocessed using heat, easy to be moulded, and recyclable. Other than that, these plastics also cheap and have unique properties, such as good barriers against carbon dioxide, oxygen, and water vapor, and have good tensile properties. Polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA), low-density polyethylene (LDPE), and high-density polyethylene (HDPE), are types of petrochemical-based plastics that have become increasingly commonly used as flexible and rigid packaging materials. Table 2.1 show the types of plastics and example of their applications (Habtemichael & Groterath, 2019).

Table 2.1: Types of plastics and their applications

Plastics	Application
Polyethylene (PE)	Films, tubes, plastic parts, laminates
Polyvinylchloride (PVC)	Cables, plumbing pipes
Polypropylene (PP)	Bottle caps, bottles, car parts, plastics containers
Polyethylene terephthalate (PET)	Water drink bottles, containers, and packaging application

Polyamide (PA) / Nylon	Fishing nets, clothing, ropes
Polystyrene (PS)	Disposal cups, refrigerator liners, electronic housings
Low-density polyethylene (LDPE)	Grocery bags, shrink-wrap, coatings for cartons
High-density polyethylene (HDPE)	Shampoo, conditioner, laundry bottles

Source: (Habtemichael & Groterath, 2019)

2.1.2 Disadvantages of plastics

Petrochemical-based plastics are still available exclusively in a variety of manufacturing application industries. Oil-based polymers are not biodegradable, and to recycle and reuse them is difficult (Kumar & Thakur, 2017). Although plastic recycling is a beneficial technology for reducing global plastic waste, but there are several issues that occur during the recycling process. The cost of recycled plastic is frequently higher than the cost of production new plastic. Petroleum-based plastics are non-biodegradable and can last hundreds of years. They are made from non-renewable materials such as petroleum, coal, and natural gas that take decades to decay in nature or in the environment. Microorganisms, ultraviolet light, moisture, and water do not affect plastics much (Faris et al., 2014). Non-biodegradable synthetic polymer materials have caused serious environmental problems such as land pollution, water pollution, and air pollution.

2.2 Environmental pollution by plastic wastes

2.2.1 Land pollution

Disposal of this plastic can contaminate the soil because the plastic will release substances into the soil that are poisonous, which then the toxic flows underground and can affect the condition of water resources in the environment. Disposal of plastic to land or landfills will lead plastic chemicals such as stabilizers, poisonous coloring components, plasticizers, and heavy metals degrade and ultimately seep into different facets of the atmosphere, causing soil and water contamination (Okunola A et al., 2019).

Furthermore, landfills contain heavy metals such as lead, cadmium, asbestos, as well as pesticides, disinfectants, pharmaceutical waste, organics, and chemicals, that can pollute underground water. With a combination of toxic substances and decomposition of organic matter from landfills can change that soil structure and texture, thus affecting agricultural activities and in turn, have a negative impact on biodiversity (Kehinde et al., 2020). Other than that, Verma et al. (2016) reportedly at landfills, about 20% of Green House Gas (GHG) such as methane gas (CH_4), carbon dioxide (CO^2), nitrogen (N_2), fossil fuels, and sulphur are released during microbial biodegradation of plastics, which will contribute to global warming.

2.2.2 Water pollution

Disposal of plastic waste into the water has a negative impact on water pollution, especially on aquatic animals. When plastic is thrown into the water, more harmful substances will form automatically. These pollutants and toxins will enter tissues and skins or can be eaten by aquatic animals. Human health will be at risk because humans will eat this contaminated seafood. As we know that plastic are releases toxic chemicals like polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) (Kehinde et al., 2020).

Disposal of plastic into rivers and seas will cause aquatic life such as fish, seabirds, turtles, shellfish, crustaceans, and marine mammals to be swallowed or entangled with plastic particles, can cause suffocation, and can be fatal. In addition, the large quantity of garbage in the sea will make it very difficult for aquatic life to get food and escape from danger.

2.2.3 Air pollution

Most people will burn solid waste especially plastic materials because it is counted as a method of waste management. However, this method is highly discouraged because it can affect the environment that is air pollution. Carbon dioxide gas will be released into the environment during the combustion of plastic. Greenhouse gases such as nitric oxide, carbon dioxide, methane, water vapor, and synthetic fluorination gases will trap heat and cause the planet to become hotter and global warming will occur.

Pollutants such as heavy metals, PCBs, dioxins, and furan are released during open burning which can cause health issues, including respiratory problems. During dechlorinated biphenyls (PCBs), dioxins, and furan are released into the atmosphere through the combustion of plastics which in turn will result in the possibility of excessive environmental pollution (Kehinde et al., 2020). In addition, toxins released from burning plastic and food waste can cause respiratory disorders, heart disease, affect the kidneys, liver, nervous system, and skin, as well as cause cancer and death. (Kehinde et al., 2020).

2.3 Bioplastic

According to the European Bioplastics Organization (EBO), bioplastics are described as "plastics made and processed from renewable sources or biodegradable plastics and/or compost,". On the other hand, Muhammad Shamsuddin (2017) stated that bioplastics are materials produced in part or whole from polymers extracted from renewable sources such as cassava starch, corn starch, or cellulose that comes from trees, bark, and other plant-based material.

2.3.1 Development of bioplastics

Bioplastics have recently emerged as one of the most advanced bio-based and biodegradable materials produced from waste, wood, and other renewable sources. In the current scenario, many researchers are focusing their hard work on creating bioplastics from various renewable sources such as jackfruit (Lothfy et al., 2018), waste banana peels (Naing & Shwe, 2020), organic waste (Goswami et al., 2015), agriculture waste (Chan et al., 2021), newspaper waste (Sudhanshu Joshi, Ujjawal Sharm, 2016), oil palm empty

fruit bunch (Isroi et al., 2017), corn starch (Keziah et al., 2018), potato starch (Arikan & Bilgen, 2019), rice straw (Agustin et al., 2014), rapeseed oil (Delgado et al., 2018), and bacteria (Ali et al., 2017)

2.3.2 Types of bioplastics

a. Starch-based bioplastic

Starch is a biodegradable natural resource and the most commonly used organic raw material. Plants synthesize and accumulate starch as a form of energy storage in their structures. Corn, wheat, rice, potatoes, cassava, peas, and a variety of other botanical forms are high in starch. Standard technologies such as blow moulding, blown film, injection moulding, extrusion, and thermoforming to extract starch that can be made into bioplastics (Faris et al., 2014).

Because of its low expense, availability, and biodegradability, starch is widely used as a replacement for polystyrene (PS) and as a thermoplastic. Starch is an appealing commodity for packaging applications. However, starch has low moisture tolerance and poor mechanical properties that limit its use. Therefore, to enhance the properties of starch, starch can be combined with certain additives and various biopolymers (Yadav et al., 2018).

b. Cellulose-based bioplastics

Cellulose based bioplastics are made from cellulose, which is derived from softwood wood as the primary raw material. Wood pulp, hemp, and cotton are examples of widely used cellulose sources (Faris et al., 2014). It is vital for researchers to investigate the mechanical properties and stability of cellulose for packaging materials. There are a few biodegradable cellulose-based packaging products on the market, but their use is restricted due to high manufacturing costs.

Furthermore, cellulose is very challenging to use in packaging materials due to its hydrophilic and crystalline properties, which have bad mechanical properties. Therefore, it must be treated and prepared with chemicals such as sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), carbon disulphide (CS_2), and others to create cellophane that has excellent mechanical properties (Majid et al., 2018).

c. Protein-based bioplastics

Protein is a complex structure made up of amino acids that can be found in plants like wheat and corn gluten. Protein-based materials can be used in a variety of industries because they are reusable, biodegradable, and have strong gas-barrier properties. In contrast, proteins have hydrophilic properties similar to starch-based polymers. Therefore, proteins must be combined with other polymers or chemically or microbiologically modified (Majid et al., 2018).

To prepare edible and biodegradable packaging films, soy protein can be used. Soybeans are high in protein and low in fats and oils. Soybeans have a protein content ranging from 40 to 55% (Faris et al., 2014). Thus, soy can be processed into plastics and

films because of its high protein content. Films derived from soy protein show excessive fragility so that their appearance is limited. To enhance them, soy protein must be enhanced with the addition of glycerol which acts as a plasticizer (Kokoszka et al., 2010).

d. Microbial polymers

This polymer is made by the fermentation of polysaccharides by bacteria. It includes polyhydroxyalkanoates (PHA), polyhydroxy butyrate (PHB), and several microbial polysaccharides, such as pullulan, curdlan, and xanthan. The polyhydroxyalkanoates (PHAs) are biodegradable, thermoplastic, biocompatible, and thermostable with having a melting point of about 180°C. These polymers provide excellent packaging films whether used alone or in combination with starch or synthetic plastic (Tharanathan, 2003). PHAs have potential as an alternative to conventional polymers, as they have similar physical and chemical properties. PHA also demonstrates the ability to print, taste and odor protection, heat sealing, oil resistance, and temperature stability, which can enhance its application in the food industry (Rahman, 2019).

Pullulans are produced by yeasts such as the fungus *Aureobasidium pullulans* from substrates containing sugars, soluble in water, and exopolysaccharides (EPS). Pullulan-based films are tasteless, odourless, non-toxic, and naturally biodegradable, edible, homogeneous, translucent, printable, heat-blocked, versatile, and have a strong oxygen barrier. Pullulan membranes inhibit fungal growth, thus making them ideal for use in the food applications (Freitas et al., 2014).

Curdlan, a bacterial polysaccharide produced by *Agrobacterium biobar* and *Agrobacterium tumefaciens*, is primarily used in the food industry as a gel-forming agent, but it has significant potential in the production of the packaging films industry (Rahman, 2019). Xanthan is an extremely viscous, water-soluble, and non-toxic material produced by *Xanthomonas campestris* fermentation using sucrose or glucose as the main carbon source. Xanthan is not widely used in the packaging industry due to its high manufacturing costs (Rahman, 2019).

e. Bioplastics chemically synthesized from renewable sources

Polylactic acid (PLA) is a biodegradable polyester that is highly durable and derived from 100 percent renewable products such as corn starch. It has great potential as a replacement for high-density polyethylene (HDPE) and low-density polyethylene (LDPE), polystyrene (PS), and polyethylene terephthalate (PET) in a variety of commodity applications and commercial uses (Drumright et al., 2000). PLA is becoming an advanced alternative as a food packaging material because it has been found that it is better than synthetic plastic materials (Auras et al., 2005). PLA is a relatively inexpensive biopolymer to produce and can be produced in large quantities. PLA can be used in thermoformed cups, films and trays, pots, and coatings for paper and paperboards, and others.

2.3.3 Application of bioplastics

Bioplastic production is getting greater attention in various industrial applications. This is because the production of bioplastic materials is a safe way to minimize the amount of inert materials disposed of in landfills and can help an environment that is free from pollution and important for consumers and industry. Table 2.2 shows the overview of the application of bioplastics in food packaging (Rahman, 2019).

Table 2.2**:** Application of bioplastic in food packaging

Packaging Applications	Biopolymer	Company
STARCH BASED		
Milk chocolates	Corn starch trays	Cadbury Schweppes food Group, Marksand Spencer
Organic tomatoes	Corn based packaging	Iper supermarkets (Italy), Coop Italia
CELLULOSE		
Kiwi	Biobased trays wrapped with cellulose film	Wal-Mart
Potatoes chips	Metalized cellulose film	Boulder Canyon
Organic Pasta	Cellulose based packaging	Birkel
Sweets	Metalized cellulose film	Quality street, Thornton
POLYLACTIC ACID (PLA)		
Beverages	PLA Cups	Mosburger (Japan)
Fresh salads	PLA Bowls	McDonald's

Coffee and tea	Cardboard cups coated with PLA	KLM
Fresh cut fruits and vegetables, bakery goods, salads	Rigid PLA trays and packs	Asda (retailer)
Yogurt	PLA jars	Stonyfield (Danone)
Organic fruit and vegetables	PLA packaging	Mont Blanc Primeurs
Pasta	PLA packaging	Biorigin
Herbs	PLA packaging	Asda (retailer)

Source: (Rahman, 2019)

2.4 Raw material

2.4.1 Orange peel

Figure 2.1: Orange peel

In most citrus fruits, the sweet orange is the most common kind of commercial citrus fruit grown in tropical and subtropical climates worldwide (Boukroufa et al., 2015). They consist mainly of peels (flavedo and albedo), pulp, and seeds. In the processing of orange industry, as a result of juice extraction, approximately 50-60% of the initial mass residue, which consists of orange peels, pulps, and seeds (Ángel Siles López et al., 2010). Orange peel also includes soluble sugars, starch, fibre (cellulose, hemicellulose, lignin, and pectin), ash, fat, and protein (Ángel Siles López et al., 2010).

Oranges have a wide variety of uses in a wide range of industries. Orange essential oil derived from juice processing waste can now be considered a high-value commodity. This essential oil can be extracted from orange peel and flavedo using various extraction methods (McKay et al., 2021). For example, orange essential oil is a flavoring agent that can be found in beverages, ice cream, and other food items, as well as in the

pharmaceutical industry. Since orange fruit has anti-inflammatory and antibacterial properties, essential oil from orange fruit can be used in the pharmaceutical industry. Furthermore, orange oil is used in the production of toilet soaps, perfumes, cosmetics, and home care products (Boukroufa et al., 2015).

Apart from that, other compounds contained in orange peel include protein, lignin, soluble sugar, hemicellulose, cellulose, ash, fat, lignin, and flavonoids. Bátori et al. (2017) found that compounds are ideal and interesting for use in bioplastic applications. Orange peel is rich in pectin, containing 20-30% dried pectin, as well as dietary fibre, essential oils, and bioactive substances that are beneficial to human health and include antioxidant and antimicrobial effects. Furthermore, as described in the article Venkatesh & Sutariya (2019), in the preparation of edible film, the pectin content of orange peel serves as a biopolymer. According to McKay et al. (2021), sweet orange peel essential oil (*Citrus sinensis L.*) has antimicrobial properties against bacteria and fungi in vapor and liquid form. Nowadays, packaging with antimicrobial properties is very much needed in packaging systems because the antimicrobial properties can reduce, delay, or inhibit the growth of microorganisms for a desired period of time (Almenar, 2020).

Several researchers have conducted studies to produce bioplastics from Citrus peels. Marsi et al. (2019) have conducted a study on the orange peel used as a bioplastic. The purpose of the study was to figure out the best concentration of orange peel in making biodegradable plastics and to test their mechanical and physical properties including tensile strength, SEM microstructure analysis, water droplet test and biodegradable plastic biodegradation test based on orange peel for packaging applications.

Besides, bioplastic from orange peel incorporated with pomegranate peel has been conducted by Venkatesh & Sutariya (2019). This study also evaluates bioplastic properties, including thickness and density, color of biofilm, film opacity, moisture

content, solubility film, weight loss, and microbial study. The microbial study was investigated by using bread sample as followed by the procedure in (Venkatesh & Sutariya, 2019).

Furthermore, Bátori et al. (2017) also have created bioplastic from orange peel. In this study, they have analysed the characteristics of orange peel, such as moisture content of orange peel powders, carbohydrate content, and pectin content. For bioplastic production, they analyse bioplastic properties such as morphology, mechanical and thermal analysis, antimicrobial activity test, and biodegradability test of bioplastic development. The antimicrobial test results are negative, reporting that films have no antimicrobial property. In this test, findings reveal that the microorganism's lag phase duration and exponential phase are the same in both the blank and the sample containing the film. Bátori et al. (2017) state that one of the reasons is that orange oil has been removed during the preventive measure of sugar removal, which is also done as acold pressing method.

2.4.2 Rice husk

Figure 2.2: Rice husk

Rice husk is a widely available agricultural by-product. Paddy farms in Malaysia, each year, a total of 840,000 tons of rice husk is produced with a land area of around 680,000 hectares (Arjmandi et al., 2015). Furthermore, rice husk is a waste that has not yet been completely used in the world (Jannah et al., 2019). Rice husk has been widely used as a new energy source in various farm areas. Various uses have been proposed due to its high capacity, low bulk density (90-150 kgm^{-3}), hardness, deep roughness, weather tolerance, and special composition (Arjmandi et al., 2015). Rice husk has been used as a bio-fertilizer, animal feed, absorbent and construction material, and pesticide (Battegazzore et al., 2014). Furthermore, Because of its high caloric content (4012 Kcal kg^{-1}), rice husk is also used as a renewable fuel in manufacturing plants.

Around 20–25 percent by weight of rice husk ash (RHA), which comprises more than 90% silica, is produced during combustion, as well as other metal oxides. The main components of rice husk, according to Battegazzore et al. (2014), are 38.3% of cellulose, 31.6% of hemicellulose, 11.8% of lignin and 18.3% of silica. Therefore, rice husk is one of the sources of biomass that can be developed as a bioplastic raw material. Rice husk also contain silica, which has been used to effectively boost the mechanical properties of

composites (Arjmandi et al., 2015). Rice husk, in addition to having a high silica content, also has a high cellulose content of around 76 %, making it excellent raw material for bioplastics (Jannah et al., 2019).

The use of RHA and silica has been applied in a variety of applications such as processors in cement, fertilizers, and catalyst carriers. Furthermore, silica has been shown to be effective for different polymer matrices (Battegazzore et al., 2014). For example, Fuad et al. (1995) have conducted research into the properties of polypropylene-based composites incorporating silica from RHA.

2.5 Properties of bioplastic

2.5.1 Moisture content

The moisture content determination provides information about the total water present in the film (Cerqueira et al., 2012). Thus, understanding the films' moisture content and the total soluble matter is essential for food packaging applications (Singh et al., 2015). The amount of water present in films indicates their hydrophobicity, and hence, hydrophilic films have a higher moisture content (Bourbon et al., 2011). Sobral et al. (2001) discovered that increasing the plasticizer concentration raises the film's moisture content due to its high hygroscopicity, which also contributes to the reduction of forces between adjacent macromolecules.

According to a study from Abdullah et al. (2020), with the increase in microcrystalline cellulose (MCC) concentration, the moisture content of bioplastics decreased. Bioplastic without MCC had a moisture content of 20.33 % while adding 20 % MCC reduced the moisture content to 9.16 %. The addition of MCC to bioplastic matrices decreased moisture content (Abdullah et al., 2020). As Rodsamran & Sothornvit discovered in 2019 study of pectin film from pineapple peel, moisture content increased from 19.64 to 33.51% when the pectin to water ratio increased from 10:90 to 100:0. The neutral sugars found in pineapple peel pectin extract solution (PPS) can effectively retain water molecules in the film matrix because they are based on the organic compounds of pineapple peel (Rodsamran & Sothornvit, 2019).

2.5.2 Thickness

Thickness is an important parameter that influences the use of film in the formation of the packaged product. Other films' mechanical properties, such as tensile strength and elongation, can also be affected by thickness (Khairunnisa et al., 2018). A study from Venkatesh and Sutariya (2019), developed biofilms from pectin fruit peel waste. It was discovered that as the glycerol concentration increased, the film's thickness also increased. This could be due to the hydrophilic nature of glycerol, which adsorbed more moisture with increasing concentration, resulting in swelling of the film and thus increased film thickness. In addition, according to Singh et al. (2015), film thickness depends on the nature and composition of the films. According to study from Chodijah et al. (2019), at the addition of 5 g pectic banana peels gave the highest thickness value of

0.00387 cm, while the addition of 1 g pectin banana peel gave the lowest value of 0.00311 cm. Hence, the addition of pectin causes an increasing the thickness of the film.

The thickness value of edible films increases as glycerol concentration increases (Khairunnisa et al., 2018). The effect of plasticizer concentration on film thickness was reported by Nordin et al. (2020), who found that adding glycerol to the film matrix increased the film thickness from 79 µm to 85 µm. This is due to the fact that glycerol is a clear compound that dissolves easily in water, increases the viscosity of the solution, and binds water. As a result, the higher the concentration of glycerol added, the lower the water evaporation rate will be because some of the water in the edible film solution is bound by glycerol, affecting the thickness of the edible film produced (Khairunnisa et al., 2018). This could be attributed to the role of plasticizers in disrupting and restructuring intermolecular polymer chain networks, resulting in more free volumes and thicker film thickness (Muhammed Lamin Sanyang et al., 2015).

2.5.3 Density

The density of a chitosan film decreases as the concentration of a plasticizer increases (Singh et al., 2015). Study from Abdullah et al. (2019), the density of bioplastics was in the range of 1.2 - 1.3 g/cm3, which is suitable for light bioplastic. The density of bioplastics decreased as the cellulose nanocrystal (CNC) content increased, but this study found a variable result. This is due to the fact that the preparation of the bioplastic can affect the interaction of the fillers and the bioplastic matrix (Muhammad et al., 2019). According to study from Abdullah et al. (2020), the density of bioplastic increased as

cellulose concentration increased. It could be attributed to an increase in bioplastic thickness, which corresponded to a higher content of MCC as a filler in the starch matrix.

However, it is undeniable that increasing the proportion of plasticizers from 15 to 45 % reduced the density of the films slightly (Muhammed Lamin Sanyang et al., 2015). Jouki et al. (2013) reported the density effects of glycerol incorporation into cress seed gum films (CSG). Whereas Nordin et al. (2020) reported that adding glycerol to corn starch film made no difference in density. This can be attributed to a change in film formulation, which caused a simultaneous increase in the volume of the film, which increased the thickness of the film, resulting in no significant difference in the density of the films.

2.5.4 Thermal stability

Thermal analysis is a test that evaluates chemical, physical, and structural changes in a material due to temperature change. Temperature is a fundamental state variable that influences the majority of chemical reactions, physical properties, and structural transformations. As a broad concept, thermal analysis is any scientific or technological characterization of a material in which temperature is varied as an experimental parameter (Nurazzi et al., 2021). The thermal stability of a polymer is defined as its ability to withstand the action of heat. Thermogravimetric analysis (TGA) of polymers is commonly used to determine their thermal stability. Polymer thermal stability is heavily influenced by its chemical structure, degree of crystallinity, and molecular weight. Thermogravimetric analysis determines how the weight of the film samples changes as the temperature rises (Cerqueira et al., 2012).

According study from (Bátori et al., 2017), production of pectin-cellulose biofilms from citrus waste, the thermogravimetric analysis results from this study consist of three stages of thermal degradation. The first degradation of biofilm occurs at an average temperature of 80.38 – 80.61°C, which is caused primarily by the evaporation of water in the samples. At a maximum temperature of 251.86 – 253.20 °C, the second degradation occurred. This stage's process results in major weight loss related to the depolymerization of the pectin present in the films. The final stage of thermal decomposition occurred at temperatures ranging from 351.89 °C to 354.34 °C; the decomposition of the films could be referred to as cellulose decomposition. The remaining ash of the films after thermal treatment is 21.97-23.20%.

2.6 Combination of pectin and cellulose based bioplastic from previous study

In previous study from Zannini et al. (2021), developed biofilm from pectin and lignocellulose fibers derived from citrus pomace biomass. In this study, thermogravimetric analysis, scanning electron microscopy (SEM), and mechanical tests such as tensile and puncture tests of biofilms were performed. (González Moreno et al., 2021) have also done research on developing bioplastics from citrus fruit pectin and cellulose nanocrystals. Pectin-cellulose bio composites demonstrated good biodegradability in seawater in their study.

In addition, study from the solution casting method is used to create biofilms from orange waste. This study aimed to improve the properties of pectin-cellulose bioplastic

to create a uniform film structure with no holes, which was lacking in a previous study by Bátori et al. (2017) had some holes in its structure. Another study from Fath et al. (2019), created a bioplastic from pectin and starch with the addition of nanocrystalline cellulose. This study aims to determine the effect of adding nanocrystalline cellulose and glycerol to a pectin-starch bioplastic. The density, scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR), and water vapor transmission rate (WVTR) of bioplastics were investigated.

2.7 Optimization process

Optimization is a technique for searching for variable values deemed optimal, effective, and efficient to achieve the desired result (Riza et al., 2019). The Plackett–Burman design (PBD) and the central composite design (CCD), as well as response surface analysis, are the most popular options (Aramvash et al., 2015). The primary goal of RSM is to select factors in order to achieve the best possible response. Design-Expert is used in numeral optimization to analyze the factor and response. The software can use each goal to maximize, minimize and target in the time period under study (Shojaei et al., 2020).

The response surface methodology (RSM) is a statistical technique used to design experiments, evaluate the relative significance of several independent variables, and determine the optimum conditions for desirable responses. The response surface method has an advantage over the other techniques in that it requires fewer experiments, lowers experimental costs, and can be used for multiple responses (Novianti et al., 2021). Central composite design (CCD) is the most widely used design among the various RSM classes.

It provides extensive information while revealing overall experiment error in the fewest number of runs (Ramadas et al., 2010). Organized statistical approaches, such as response surface methodology (RSM), which employ sequential experimental methods, are more reliable than unplanned experiments (Ramadas et al., 2010).

2.8 Reaction Mechanism in Bioplastic Formulation

2.8.1 Reaction pectin in water

Pectin is a structural heteropolysaccharide found in most plant primary cell walls. Its provides mechanical strength and flexibility due to interaction with other cell wall components (Khamsucharit et al., 2018). Pectin is highly soluble in water and, as a result of its aqueous solubility, it can be used in various applications (Ray, 2019). The ability of pectin to form spreadable gels is its most important physical property. When water was added to pectin, the solution formed a gel. According to (Smith, 2003), pectin is a class of substances that form gels when dissolved in water under certain conditions. A gel is formed when polymer chains interact along a portion of their length to create a three-dimensional network (BeMiller, 1986).

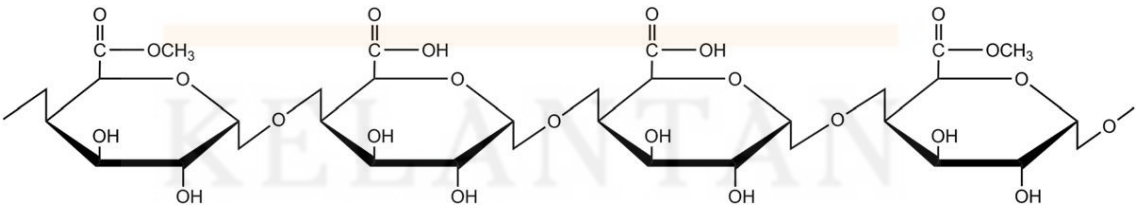


Figure 2.3: Chemical structure of pectin (Source: Medina & Dzalto, 2017)

2.8.2 Glycerol as a plasticizer

A plasticizer is a substance that softens or makes it more flexible when added to another substance (Godwin, 2000). Plasticizers not only make the films more manageable, but they also impart and improve properties that are weak or absent in the native polymer (Tyagi & Bhattacharya, 2019). Sorbitol, polyethylene glycol (PEG), and glycerol are plasticizers capable of reducing internal hydrogen bonds and thus increasing intermolecular distances (Lusiana et al., 2019).

Glycerol is a simple trihydric alcohol that appears as a clear, odourless, viscous liquid with a sweet taste (Yeong et al., 2012). Glycerol is hygroscopic and water soluble, with a low toxicity. $C_3H_5(OH)_3$ is the chemical formula for glycerol (Lazar, 2018). Glycerol, as a plasticizer, is hydrophilic. More glycerol in the bioplastic increased its water swelling and influenced hydrogen's strength and force bond between the hydroxyl groups (Inayati et al., 2019).

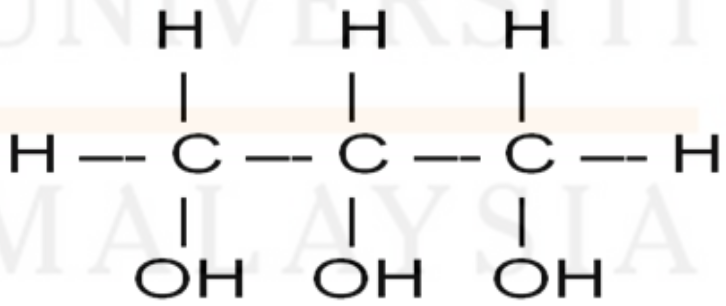


Figure 2.4: Chemical structure of glycerol (Source: Lazar, 2018)

2.8.3 Additional of Sodium Hydroxide (NaOH) and cellulose

In the experiment, sodium hydroxide is simply used to neutralize the pH of the medium (Gaonkar. M.R, Palaskar. P, 2017). NaOH can cause cellulose to swell and even dissolve cellulose (Wang, 2008). When cellulose is dissolved in NaOH, NaOH breaks the hydrogen bonds between the chains and interacts with the hydrophilic hydroxyl group of cellulose (Xiong et al., 2014).

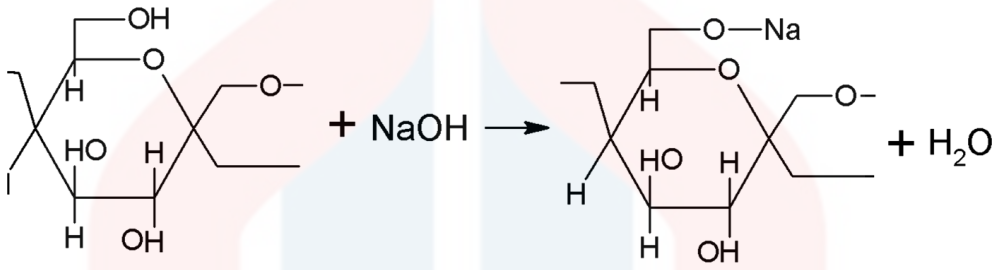


Figure 2.5: Reaction of cellulose fiber with NaOH (Source: Masłowski et al., 2018)

2.8.4 Reaction between pectin and cellulose

Pectin forms their own network within which the cellulose/xyloglucan network is embedded. However, according to Gawkowska et al. (2018), there are some suggestions regarding the interactions of cellulose with pectin. The adsorption of commercial citrus pectin to cellulose was investigated by Zykwinska et al. (2005), and it was discovered that these pectin did not bind to cellulose. This could be because this pectin was extracted in highly acidic conditions, which have a low content of side chains. Meanwhile, in a study conducted by Agoda-Tandjawa et al. (2012), the addition of citrus pectin to cellulose had no effect on the rheological properties of cellulose.

CHAPTER 3

METHODOLOGY

3.1 Chemicals and reagents

The chemical and reagents that were used in this study were glycerol, sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), ethanol (C_2H_5OH), hydrochloric acid (HCL), and distilled water.

3.2 Apparatus

The apparatus used in conducting this study includes an aluminium foil, magnetic stirrer, beaker, medium bottle, funnel, conical flask measuring cylinder, polystyrene petri dish, spatula, centrifuge tubes, glass rod, filter paper (Smith 180mm), and pestle and mortar.

3.3 Equipment

The equipment used in conducting this experiment includes an electric grinder (Philips HR2056/01), sieve (Impact BS410), hot plate (Stuart US152), dehydrator (BioChef 6 Tray), digital micrometer (Alx SR44), centrifuge (Eppendorf Centrifuge 5810R), desiccator, electronic weight balance (Sartorius BSA 4202S-CW), pH meter (Hanna HI2211), drying oven (Memmert UF110), thermogravimetry (Mettler Toledo TGA/DSC 2 simultaneous analyzer).

3.4 Sample

Orange peels were obtained from a local grocery store located at Jeli, Kelantan, while rice husk was obtained from e-commercial platform Shopee.

3.5 Experimental procedures

3.5.1 Preparation of orange peel powder

Orange peels were washed to eliminate any unwanted particles. The washed orange peel was dried in a dehydrator (BioChef 6 Tray) at 65°C for 4 hours (Listyarini et al., 2020). After drying, the orange peels were ground into a fine powder with an electric grinder (Philips HR2056/01). The powder was sieved into 600 µm using a sieve (Impact BS410) to remove undesirable particles and form uniform smooth film. Then, the peel

powder was stored in an airtight container and kept out of direct sunlight until used (Venkatesh & Sutariya, 2019). The preparation of orange peel powder is shown in Figure 3.1.

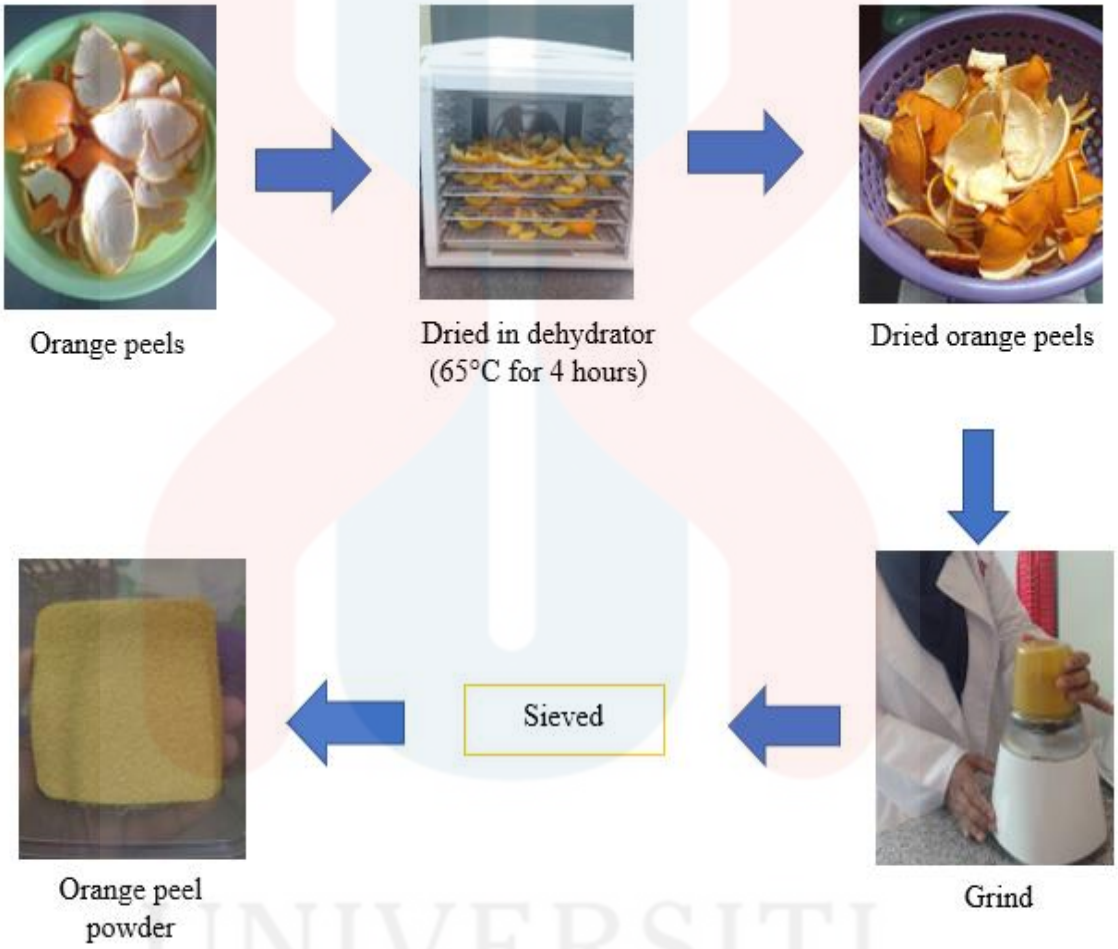


Figure 3.1: Preparation of orange peel powder

3.5.2 Extraction of pectin from orange peel

A 150 mL of distilled water was added in 150 mL with 10 g of orange peel powder, and 2 M HCl was added to get a pH of 2.0. Next, the mixture was mixed and heated using a hot plate (Stuart US152) for 60 min at 50 ° C until homogeneous. After filtering the mixture, 150 mL of ethanol were added to the clear filtrate and allowed to keep at room temperature for 60 min. Then, pectin was precipitated and centrifuged by using a centrifuge machine (Eppendorf Centrifuge 5810R) for 20 min at 1500 rpm. The precipitated pectin was then rinsed with ethanol in composition 1: 2 (pectin: ethanol). The obtained pectin was then dried at 50 ° C for 24 hours in an oven (Memmert UF110). The dried pectin is mashed using pestle and mortar and sieved into 600 µm using a sieve (Impact BS410) (Listyarini et al., 2020). The pectin extraction from the orange peel is illustrated in Figure 3.2.

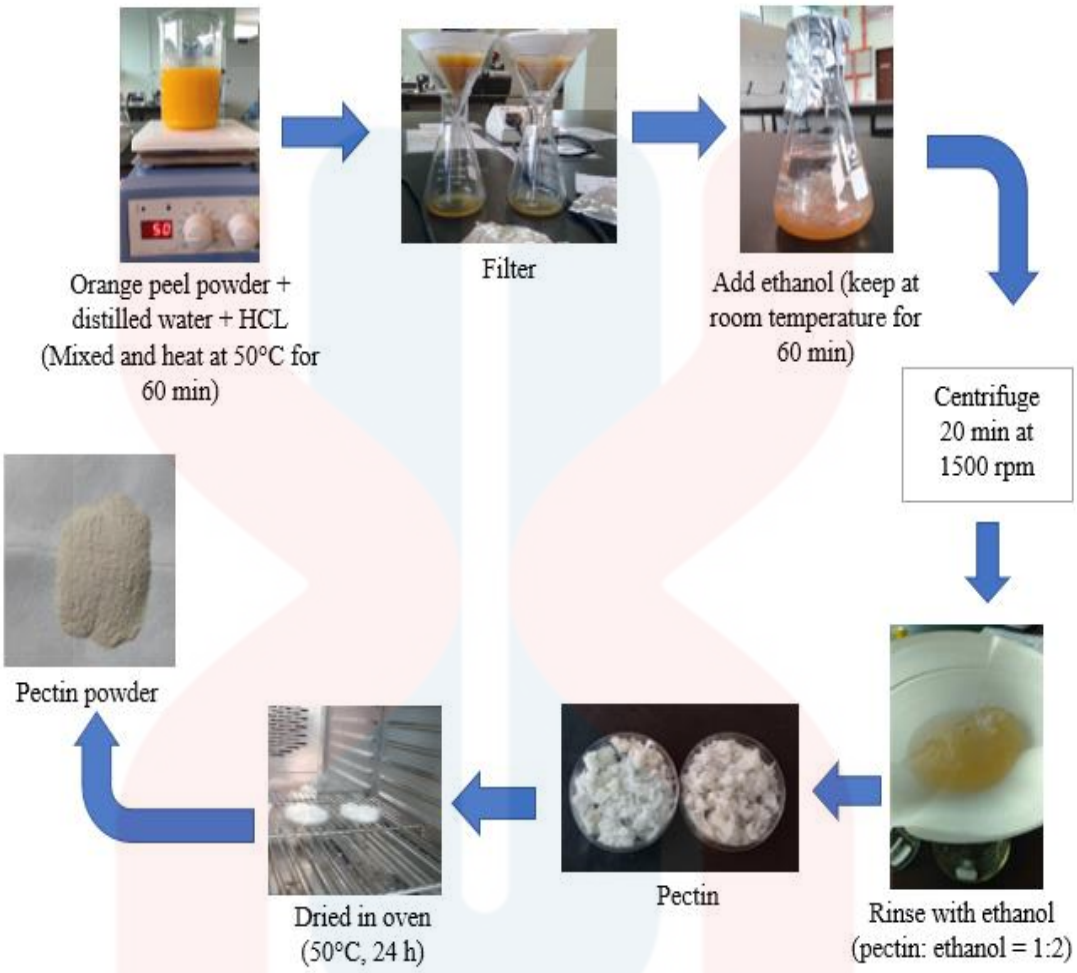


Figure 3.2: Pectin extraction from the orange peel

3.5.3 Preparation of rice husk powder

Rice husk was washed and dried in a dehydrator (BioChef 6 Tray) at 55°C for 4 hours. Next, the rice husk was ground using an electric grinder (Philips HR2056/01) to obtain a smaller size of rice husk. After that, the rice husk was sieved into 600 µm with a sieve (Impact BS410) to get a more delicate rice husk powder. The preparation of rice husk powder is presented in Figure 3.3.

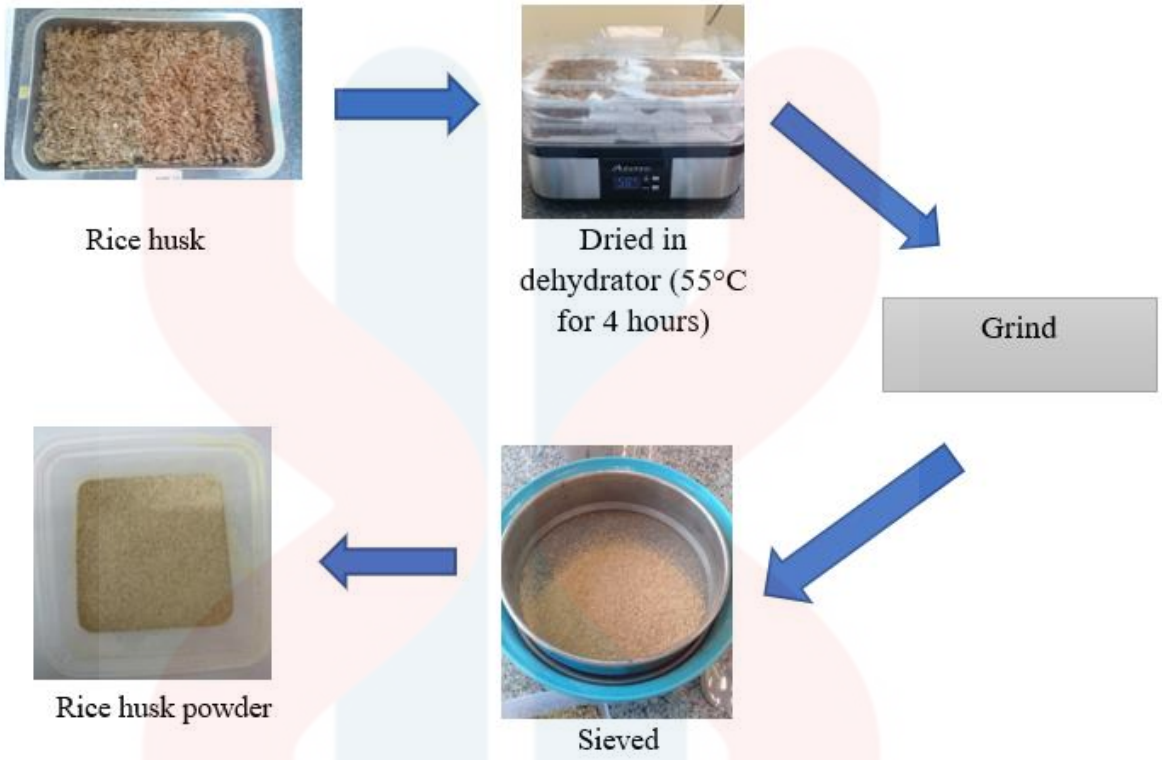


Figure 3.3: Preparation of rice husk powder

3.5.4 Extraction of cellulose from rice husk

A 250 mL beaker was filled with 10 g of rice husk powder, and then added with 100 mL of 12% NaOH. The mixture was stirred with a magnetic stirrer and then heated using a hot plate (Stuart US152) for 3 hours at 80°C. The mixture was then filtered, and the residue was rinsed with distilled water until neutral pH was obtained. The residue was placed in a 250 mL beaker before adding with 100 mL of 2.5% NaOCl solution. The mixture then was heated using a hot plate (Stuart US152) and stirred for one hour at 80°C. Next, the precipitate was filtered using filter paper (Smith 180mm) and rinsed with distilled water until it reached a neutral pH value (Yunus et al., 2019). The obtained cellulose was then dried at 50°C for 24 hours in an oven (Memmert UF110). The cellulose extraction from rice husk is shown in Figure 3.4.

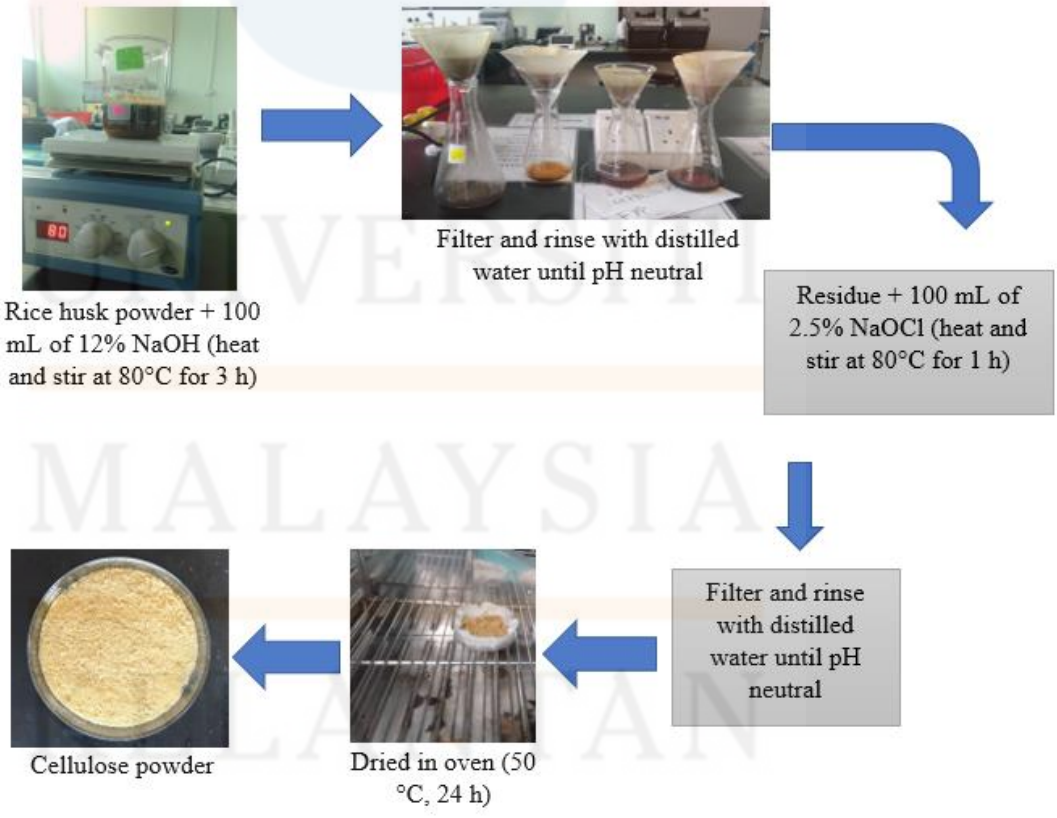


Figure 3.4: Cellulose extraction from rice husk

3.5.5 Production of bioplastic

Pectin and cellulose were prepared in 10 g at various ratios (6: 4, 7: 3, 8:2, and 9:1). The cellulose was then dissolved in a 3% (w/v) solution of NaOH, and the pectin was dissolved in distilled water with the ratio of pectin to distilled water is 1: 10 (w/v). Pectin solution was heated and stirred on a hotplate (Stuart US152) for 10 minutes. Glycerol was added to the pectin solution as a plasticizer in concentrations of 0.1, 0.2, 0.3, and 0.4 mL/g of pectin mass, respectively. The mixture of glycerol and pectin was heated using a hot plate (Stuart US152). Cellulose was added to the mixture at 70°C and heated until it reached 85°C (Lubis & Harahap, 2018). The mixture was then cooled and poured into a Petri plate and air dry at room temperature for 4-5 days. The film was peeled from the Petri dish after drying. The schematic diagram that represents the formation of film development is shown in Figure 3.5. The film was stored in a desiccator until further analysis of the bioplastic properties was performed (Venkatesh & Sutariya, 2019).

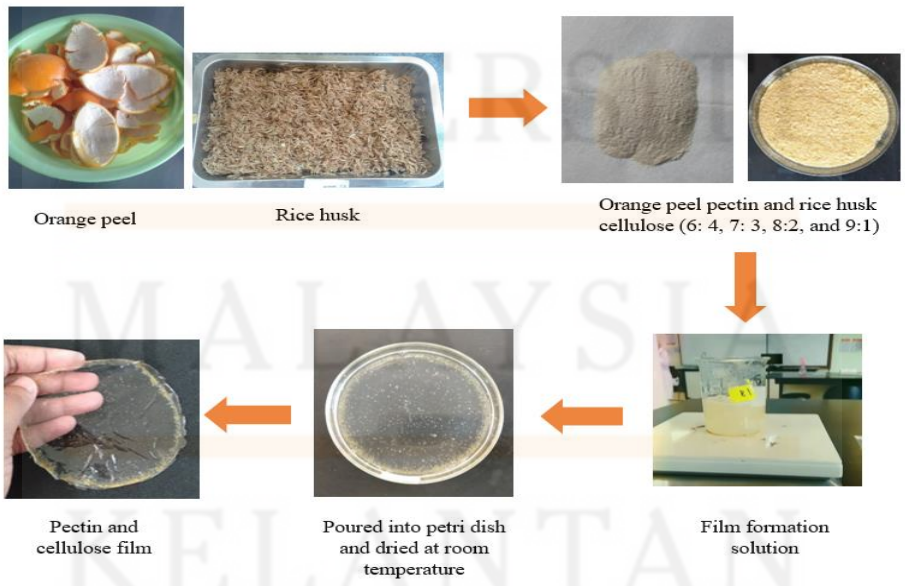


Figure 3.5: Schematic represent the formation of film development from orange peel and rice husk

3.6 Experimental Design by using Design Expert Software Version 13

RSM-Design Expert was chosen to identify the optimum conditions for the essential elements of a process in order to amplify and maximize the response. Three experimental factor design was employed by Central Composite Design. The factors used were the ratio of pectin to cellulose (A), the mass of the plasticizer (B), and the volume to pour into a petri dish (C). The lowest and the highest levels of factor were fixed based on a preliminary study. Table 3.1 presents the list of factors and the range of levels used. The responses measured were moisture content, thickness, and density. Table 3.2 present the 10 experimental trials that were generated by Central Composite Design (CCD) in Response Surface Methodology (RSM) to reach maximum results with the minimal number of possible experiments. The experiment was conducted according to the design. The results from this study were analyzed using Design Expert Software Version 13 to determine the best value for the biodegradable film made from a combination of orange peel pectin and rice husk cellulose.

Table 3.1: Factors and their level used in design

Factor	Name	Unit	Level	
			Low level	High level
A	Ratio	%	6	10
B	Mass of plasticizer	g	0.1	0.5
C	Volume	mL	20	30

Table 3.2: Experimental trial generated by the Design-Expert software

Run	Factor A (ratio, %)	Factor B (mass of plasticizer, g)	Factor C (volume, mL)
1	7.5	0.25	25
2	7.5	0.25	17
3	6	0.4	30
4	6	0.1	30
5	9	0.4	20
6	7.5	0.5	25
7	6	0.1	20
8	9	0.1	30
9	10	0.25	25
10	9	0.1	20

3.7 Sensory evaluation

The sensory analysis smell, color, texture, and appearance for bioplastic development were evaluated by visual and textural evaluation using the eyes and hand.

3.8 Characterization of bioplastic developed

3.8.1 Moisture content of bioplastic

The moisture content of the film sample was determined by following the procedure described by (Jouki, Tabatabaei Yazdi, et al., 2013). The initial weight of the film sample to be prepared in (3 cm × 3 cm) as shown in Figure 3.6 and was measured using an accurate analytical guess balance of 0.01 g. Next, the sample was dried at 105°C for 24 h in an oven (Memmert UF110) until the sample reached constant weight and store

in a desiccator to cool and weight again (Sanyang et al., 2015). The moisture content in bioplastic was calculated according to Equation 3.1.

$$\text{Moisture content}, \% = \frac{\text{inital weight (g)} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100 \quad (3.1)$$

Figure 3.6: Bioplastic sample (3 cm x 3 cm)

3.8.2 Thickness of bioplastic

The thickness (T) of the resulting film was measured using manual digital micrometre (Alx SR44) to the nearest 0.01 mm as shown in Figure 3.7. According to the procedure of Venkatesh & Sutariya, (2019), readings were taken randomly on five different locations of film and an average value of the film thickness was calculated.

Figure 3.7: Digital micrometre

3.8.3 Density of bioplastic

Film density is calculated by dividing the weight of the film by the volume of the film where the volume of the film is taken from the volume of the film poured into the petri dish (Jouki, Tabatabaei Yazdi, et al., 2013).

3.9 Optimization of the formulation bioplastic using Design Expert Software Version 13

The ratio of pectin to cellulose, the mass of plasticizer, and volume film-formation solution are knowns independent variables that affect the moisture content, film thickness, and density of bioplastic. The central composite design (CCD) of responses surface methodology was used to obtain the optimum value of an independent variable. The bioplastic was optimized, taking all the responses for their desired optimum value (Jancy et al., 2020).

3.10 Thermogravimetric Analysis (TGA) of development of optimal bioplastic

Thermal degradation of bioplastic was investigated using thermogravimetry (Mettler Toledo TGA/DSC 2 simultaneous analyzer) as shown in Figure 3.8. In the presence of nitrogen at a flow rate of 40 mL/min, the 10 mg sample was heated using thermogravimetry (Mettler Toledo TGA/DSC 2 simultaneous analyzer) from 40°C to 500°C at a heating rate of 10°C/min. The thermogravimetric (TG) and derivative

thermogravimetric (DTG), can identify the thermal decomposition in bioplastic by weight loss (Abdullah, Putri, et al., 2019).

Figure 3.8: Mettler Toledo TGA/DSC 2 simultaneous analyzer

CHAPTER 4

RESULT AND DISCUSSION

4.1 Statistical analysis using Design-Expert version 13

4.1.1 Factors and Responses

Table 4.1 present the data of ten experimental runs performed in accordance with the Central Composite Design (CCD). The experimental data was generated using the response surface method. In this study, Expert Design Software 13.0.5.0 was used in the least square regression ANOVA. The statistical software program was used to generate the model equation, interaction effects of the three independent variables (ratio of pectin to cellulose, mass of plasticizer, and volume film-formation solution) on the corresponding quality of the bioplastic, and surface plots using the fitted equation obtained from the regression analysis and one of the independent variable constants.

From Table 4.1, it can be concluded that the ratio of pectin to cellulose, the mass of plasticizer, and volume film-formation solution influence the values of moisture content, thickness, and density of the developed bioplastic. Run 7 shows the highest moisture content value, which is 39.98% using the 6:4 ratio of pectin to cellulose, 0.1 g of plasticizer, and 20 mL of film-forming solution. Meanwhile, the lowest moisture content value is obtained from Run 10 with the value of, 6.84% using the formation of 9:1 of pectin ratio to cellulose, 0.1 g of plasticizer, and 20 mL of film-forming solution.

The highest value for film thickness was obtained from Run 3, which is 0.232 mm with 6:4 of the ratio of pectin to cellulose, 0.4 g of plasticizer, and 30 mL volume film-forming solution. Meanwhile, the lowest film thickness value is obtained from the experiment Run 10, which is 0.040 mm with 9:1 of the ratio of pectin to cellulose, 0.1 g of plasticizer, and 20 mL film-formation solution.

For the density of bioplastic, the highest value is 0.049 g/mL, which is obtained from Run 5 with 9:1 of the ratio of pectin to cellulose, 0.4 g of plasticizer, and 20 mL of film-formation solution. Meanwhile, the lowest value of density of bioplastic is 0.027 g/mL, which is obtained from Run 9 with 10:0 of the ratio of pectin to cellulose, 0.25 g of plasticizer, and 25 mL film-formation solution.

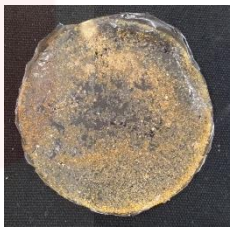
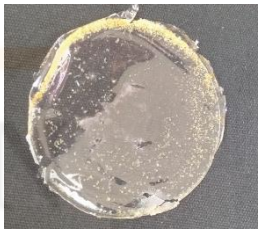
Table 4.1: Experimental runs with responses

Std	Run	Factor A (Ratio, %)	Factor B (Mass of plasticizer, g)	Factor C (Volume, mL)	Response 1 (moisture content, %)	Response 2 (film Thickness, mm)	Response 3 (Density, g/mL)
10	1	7.5	0.25	25	34.24	0.190	0.039
9	2	7.5	0.25	17	38.57	0.094	0.045
6	3	6	0.4	30	37.54	0.232	0.039
4	4	6	0.1	30	25.75	0.180	0.034
3	5	9	0.4	20	39.74	0.066	0.049
8	6	7.5	0.5	25	39.62	0.174	0.047
1	7	6	0.1	20	39.98	0.152	0.040
5	8	9	0.1	30	32.45	0.148	0.035
7	9	10	0.25	25	30.44	0.062	0.027
2	10	9	0.1	20	6.84	0.040	0.045

4.2 Sensory analysis

Table 4.2 represents the sensory evaluation of each bioplastic formed in a different ratio of orange peel pectin and rice husk cellulose, including color, texture, smell, and physical appearance. The states formulation are following the ratio calculated by Design Expert software version 13.

Table 4.2: Sensory analysis of produced bioplastics

Run/ Ratio	Sensory evaluation			
	Color	**Texture**	**Smell**	**Physical appearance**
1 (7.5:2.5)	Slightly yellowish with little bit transparent	Slightly rough	Sweet-sour smell	
2 (7.5:2.5)	Slightly yellowish with little bit transparent	Slightly smooth	Sweet-sour smell	
3 (6:4)	Yellowish	Rough	Sweet-sour smell	
4 (6:4)	Yellowish	Rough	Sweet-sour smell	
5 (9:1)	Slightly yellowish with little bit transparent	Slightly smooth	Sweet-sour smell	

6 (7.5:2.5)	Slightly yellowish with little bit transparent	Slightly rough	Sweet-sour smell	
7 (6:4)	Yellowish	Slightly rough	Sweet-sour smell	
8 (9:1)	Slightly yellowish with little bit transparent	Slightly smooth	Sweet-sour smell	
9 (10:0)	Transparent	Smooth	Sweet-sour smell	
10 (9:1)	Slightly yellowish with little bit transparent	Slightly smooth	Sweet-sour smell	

Color analysis revealed the role of pectin and cellulose concentrations in the formation of bioplastics. The bioplastic color indicates the percentage ratio of pectin to cellulose, where the pectin content results in bioplastic transparency. Oliva-Moreno & Encinas (2021) also mention in their study that the pectin bioplastic film produced is transparent before adding Rosin powder. The yellowish color of the bioplastic comes from the cellulose rice husk, while the pectin provides the color of transparency. The results obtained agree with the Truong & Kobayashi (2020) bioplastic films generated from pectin from dragon fruit peels had a transparent yellowish. The bioplastic color of the pectin to cellulose ratios, 7.5:2.5 and 9:1 is slightly yellowish with a bit of transparency. While the ratio of pectin to cellulose, 6:4 is yellowish, and for the bioplastic ratio of pectin to cellulose, 10:0 is transparent.

For the texture analysis, the texture of bioplastic is determined by the amount of pectin and cellulose present. The bioplastic becomes rougher as the cellulose concentration increases, while higher pectin concentration gives the smooth surface. It is noticed that the pectin can dissolve in the solution that makes the bioplastic develop are smooth texture. On the other hand, all the bioplastic developed to have a sweet-sour smell. This is because the source of pectin is from orange peel, which has a sweet-sour smell.

4.3 Model fit summary

To assess the quality of the fit of the polynomial model, the coefficients of determination R^2, adjusted coefficient (Adjusted R^2), and predicted coefficient (Predicted R^2) were used. The coefficient of determination, R^2 indicated the model's adequacy and fitness (Halim et al., 2009). The values of the R^2 coefficients were ranged between 0 and 1 (Noordin et al., 2004). The coefficient of determinations, R^2 in this study for moisture content, film thickness, and film density were 0.8568, 0.9473, and 0.7296, respectively.

When comparing models with a different number of terms, the adjusted R^2 value comes in useful. The predicted R^2 measures how well the model estimates the response, and the difference between predicted R^2 and adjusted R^2 should be around 0.20 of adjusted R^2 (Irianto et al., 2019). In the case of moisture content, the adjusted R^2 was 0.5703, and the predicted R^2 was -1.4507. The adjusted R^2 and predicted R^2 for film thickness were 0.8420 and 0.6692, respectively. While, for film density, the adjusted R^2 is 0.1887, and the predicted R^2 is -4.4929.

The coefficient of variance (CV) is the ratio of the standard error of estimation to the observed mean value (Irianto et al., 2019). A low coefficient of variation (less than 10%) indicates that the research data is excellently suitable (Ghafari et al., 2009). The CV for moisture content is 20.52%, for film thickness is 19.06%, and for film, density is 15.23%. Thus, CV values for all responses are not excellently suitable for the model.

An adequate precision (AP) represents the signal-to-noise ratio, and it is a criterion for determining whether the model is adequate for navigating the design space, with the desired value greater than 4. An adequate precision of 6.5021 was obtained for moisture content, and a ratio of 6.5021 indicates a sufficient signal. This model can help to navigate the design space. Similarly, adequate precision for film thickness was 9.0710,

and a signal-to-noise ratio of 9.0710 indicates a sufficient signal. This model can help to navigate the design space. Furthermore, an adequate precision for film density was obtained of 3.5751, and a ratio of 3.5751 indicates an insufficient signal, and this model cannot be used to navigate the design space. Hence, it is concluded that the chosen model fits well for moisture content and film thickness.

The predicted sum of squares (PRESS) is a measure of how well a particular model fits a given point in the design, and small PRESS values are desired (Irianto et al., 2019). The PRESS value for moisture content, film thickness, and density were 2285.47, 0.0102, 0.0023, respectively. The result of the model fit summary is shown in Table 4.3.

Table 4.3: Model Fit Summary

	Response		
	Moisture content	**Thickness**	**Density**
Std. Dev	6.67	0.0253	0.0061
Mean	32.52	0.1330	0.0399
C.V.%	20.52	19.06	15.23
R^2	0.8568	0.9473	0.7296
Adjusted R^2	0.5703	0.8420	0.1887
Predicted R^2	-1.4507	0.6692	-4.4929
Adequate Precious	6.5021	9.0710	3.5751
Press	2285.47	0.0102	0.0023

4.3.1 Final equation in terms of actual factor

In terms of actual factors, the equation can be used to predict the response for different levels of each factor. For each factor, the levels should be specified in the original units. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the centre of the design space. In terms of actual values, the quadratic model equations for the three responses, moisture content, film thickness, and fill density, can be written as Equations 4.1, 4.2, and 4.3:

Moisture content

$$= (234.28119) - (31.22347 * \text{Ratio}) + (85.25874 * \text{Mass of plasticizer}) - (6.96830 * \text{Volume}) + (9.35194 * \text{Ratio} * \text{Mass of plasticizer}) + (1.04833 * \text{Ratio} * \text{Volume}) - (4.23108 * \text{Mass of plasticizer} * \text{Volume}) \quad (4.1)$$

Film thickness

$$= (0.545432) - (0.083964 * \text{Ratio}) + (0.709757 * \text{Mass of plasticizer}) - (0.011072 * \text{Volume}) - (0.050751 * \text{Ratio} * \text{Mass of plasticizer}) + (0.002576 * \text{Ratio} * \text{Volume}) - (0.008559 * \text{Mass of plasticizer} * \text{Volume}) \quad (4.2)$$

Film density

$$= (-0.017396) + (0.006958 * \text{Ratio}) + (0.303389 * \text{Mass of plasticizer}) + (0.001403 * \text{Volume}) - (0.020048 * \text{Ratio} * \text{Mass of plasticizer}) - (0.000186 * \text{Ratio} * \text{Volume}) - (0.005415 * \text{Mass of plasticizer} * \text{Volume}) \quad (4.3)$$

4.3.2 Final equation in terms of coded factor

The CCD provided the model equation in terms of the coded factor for moisture content, film thickness, and density. Factor such as ratio pectin to cellulose, the mass of plasticizers, and volume film-formation solution were coded as A, B, and C, respectively. In model equations in terms of coded factors for moisture content, film thickness, and film density are given as Equations 4.4, 4.5, and 4.6.

Moisture content

$$= +34.86 - 4.02 * A + 7.44 * B - 0.8181 * C + 2.10 * AB + 7.86 * AC - 3.17 * BC \quad (4.4)$$

Film thickness

$$= +0.1507 - 0.0484 * A + 0.0173 * B + 0.0305 * C - 0.0114 * AB + 0.0193 * AC - 0.0064 * BC \quad (4.5)$$

Film density

$$= +0.0393 - 0.0041 * A + 0.0026 * B - 0.0067 * C - 0.0045 * AB - 0.0014 * AC - 0.0041 * BC \quad (4.6)$$

4.4 Analysis of moisture content bioplastic

ANOVA of response for moisture content of bioplastic is shown in Table 4.4. The F value is 2.99, and the prob > F value for the model is greater than 0.05, which is 0.1985. The Model F-value of 2.99 indicates that the model is not significant in comparison to the noise. An F-value of this magnitude has a 19.85% chance of occurring due to noise. Model terms are significant if the P-value is less than 0.0500. A, B, C, AB, AC, and BC are not significant model terms in this case. Values greater than 0.1000 indicate that the model terms are insignificant. If there are many insignificant model terms (aside from those required to support hierarchy), these insignificant model terms can be removed, potentially resulting in an improved model. The independent variables (ratio, volume film-formation solution, the interaction between ratio and mass of plasticizer, and interaction between mass of plasticizer and volume film-formation solution) are insignificant terms which means they have no effect on the moisture content.

Table 4.4 Analysis of variance for moisture content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	799.01	6	133.17	2.99	0.1985	not significant
A-Ratio	71.45	1	71.45	1.60	0.2946	
B-Mass of plasticizer	446.07	1	446.07	10.02	0.0507	
C-Volume	2.97	1	2.97	0.0666	0.8130	
AB	12.15	1	12.15	0.2730	0.6375	
AC	314.86	1	314.86	7.07	0.0764	
BC	27.64	1	27.64	0.6209	0.4882	
Residual	133.57	3	44.52			
Cor Total	932.58	9				

Effect of the independent variable on moisture content

The moisture content of the film describes the percentage of water in the film, which affects the shelf life of the products stored in the film (Hema Prabha & Ranganathan, 2017). The amount of water present in films indicates how hydrophobic the films are. As a result, hydrophilic films have a higher moisture content (Bourbon et al., 2011). Table 4.1 displays the moisture content values, while Figure 4.1 depicts the effect of the independent factor on the bioplastic moisture content.

According to the results from Table 4.1, the highest percentage of moisture content for bioplastic produced is 39.98% on 6:4 of the ratio of pectin to cellulose. On the other hand, the lowest percentage of moisture content for bioplastic produced is 6.84%, with 9:1 of pectin to cellulose ratio. Based on the result, 9:1 of pectin to cellulose ratio in the bioplastic is the best bioplastic for moisture content property, as it displays the lowest moisture content, which is 6.84%. The effect of pectin to cellulose ratio on the moisture content of bioplastic is shown in Figure 4.1(a) below.

From the graph, it can be observed that moisture content decrease as the pectin to cellulose ratio starts to increase from low level up to centre point, until higher level. According to Abdullah et al. (2020), the moisture content of cassava starch-based bioplastic decreases when the concentration of cellulose is added. This is consistent with the finding from Mendes et al. (2020) observed the values of the moisture content of the films decreased with the addition of bagasse fiber which 15% of malt bagasse present a lower moisture content (5.2 %) than the pectin film without adding bagasse fiber (7.5%). As Rodsamran & Sothornvit, discovered in a 2019 study of pectin film from pineapple

peel, moisture content increased from 19.64 to 33.51% when the pectin to water ratio increased from 10:90 to 100:0.

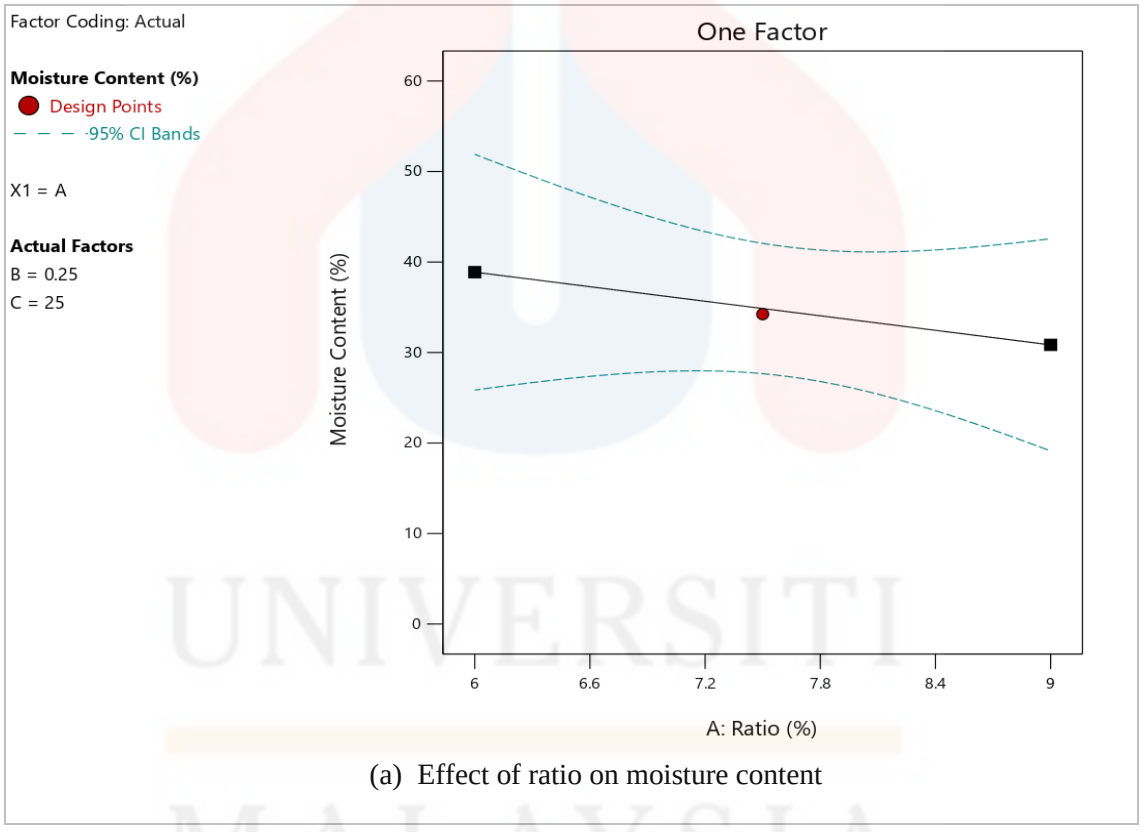
Low moisture content assists the bioplastic in reducing the possibility of mold growth, which can affect the bioplastic's appearance and mechanical properties. Microorganisms with a high moisture content have a faster metabolic activity (Borah et al., 2019). In this study, 9% pectin bioplastic was found to be the most suitable for use as a food packaging material. The result was similar to studies on bioplastic from pectin of dragon fruit by Listyarini et al. (2020) that showed the moisture content of bioplastic of 0.23 g pectin is in the range of 5.71% - 12 %. Bioplastic is preferable for use as a plastic container because of its high pectin content. The emulsifier function of pectin increases the intermolecular bond in the film, making the bioplastic film more elastic (Chodijah et al., 2019).

As depicted in Figure 4.1(b), the effect of the mass of plasticizer on moisture content can be observed that the mass of plasticizer increases the response of moisture content increase up to higher level. This result is in agreement with that of Hirpara et al. (2021), who found a significant increase in the moisture content with increasing in plasticizers. A similar result was reported by Seixas et al. (2013) in pectin bioplastic from citrus peel, which resulted in increased concentration plasticizer increased the moisture content of biofilm.

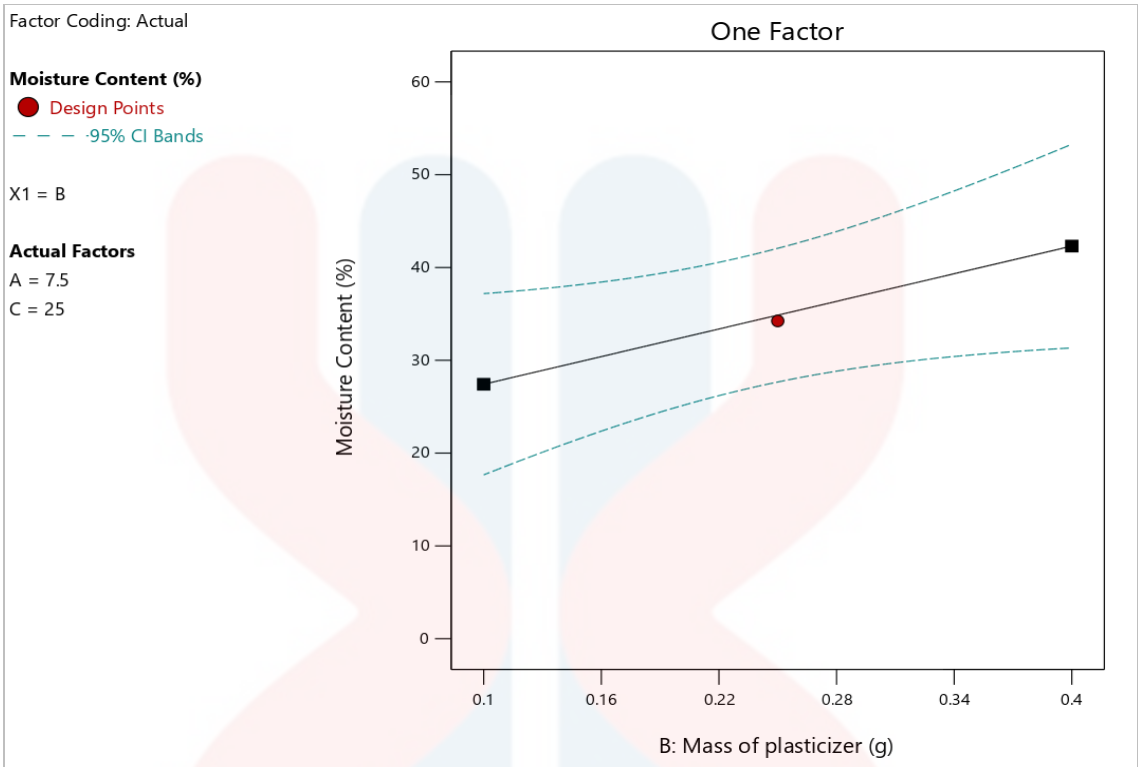
On the other hand, high concentrations of plasticizers, promote water molecule adsorption due to their hydrophilic nature, which retains water in the film matrix and forms hydrogen bonds (O–H) (Cerqueira et al., 2012). Singh et al. (2015) discovered that increasing the plasticizer concentration increases the moisture content of the film because of its high hygroscopicity. Besides, several studies have found that adding more

plasticizers increases the moisture content of hydrocolloid films (Ghasemlou et al., 2011; Hernández-Muñoz et al., 2003; (Kristo & Biliaderis, 2006).

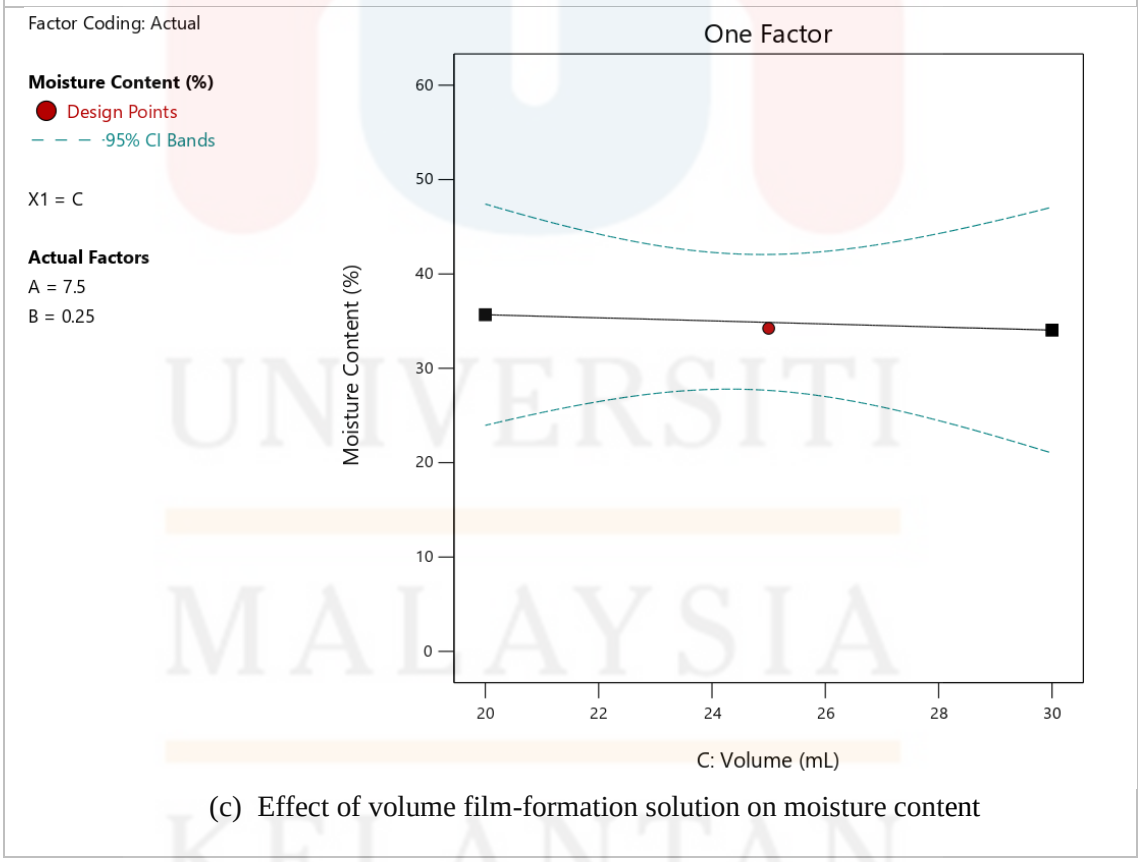
The effect of volume film-formation solution on the moisture content of bioplastic is shown in Figure 4.1(c). As shown in the figure, the value of moisture content slightly decreases when increasing in volume. The 20 mL film-forming solution gives the highest and lowest percentage of bioplastic moisture content obtained from this study.



(a) Effect of ratio on moisture content



(b) Effect of mass of plasticizer on moisture content



(c) Effect of volume film-formation solution on moisture content

Figure 4.1: Effect of the independent variable on moisture content

Interaction effect of factors on the moisture content of bioplastic.

Figures 4.2 (a), (b) show the effect of the interaction of pectin-cellulose ratio and mass of plasticizer where the maximum value of moisture content (39.98%) was at 6:4 of pectin to cellulose ratio and 0.1 g of plasticizer. A high ratio of pectin to cellulose (9:1) and 0.1 g of plasticizer showed the lowest value of moisture content (6.84%). The contour plot and 3D surface plot for moisture content of bioplastics as a function of ratio pectin to cellulose (A) and mass of plasticizers (B) are presented in Figure 4.2 (a) and (b).

Besides, the contour plot and 3D surface plot show the influence of ratio pectin to cellulose (A) and volume film-formation solution (C) on moisture content is shown in Figure 4.2 (c) and (d). The maximum value of moisture content (39.98%) was at 6:4 of the ratio of pectin to cellulose, and volume film-formation solution is 20 mL, while for a minimum value of moisture content (6.84%) which used the same volume film-formation solution which is 20 mL at the addition of 9:1 of the ratio of pectin to cellulose.

The effect of the mass of plasticizer (B) and volume film-formation solution (C) on the moisture content of bioplastic is presented in Figure 4.2 (e) and (f). The maximum and minimum values of moisture content which is 39.98% and 6.84%, respectively, were obtained in the same condition factor with is 0.1 g of plasticizer and 20 mL film-formation solution. It can conclude that the mass of plasticizer and volume film-formation solution does not influence the value of moisture content of bioplastic.

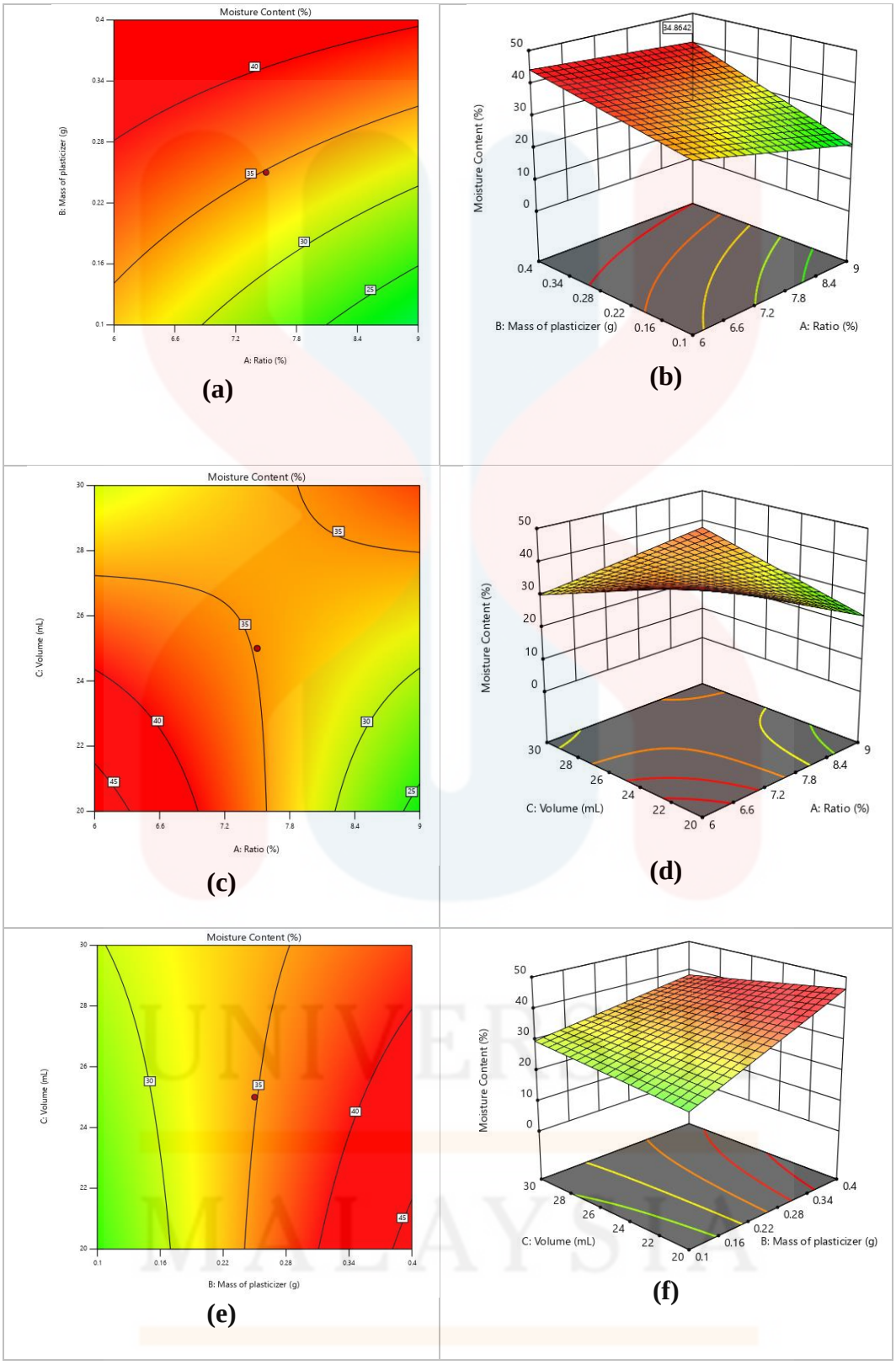


Figure 4.2: Contour and a 3D surface plot showing the effect of (a), (b) ratio and mass of plasticizer, (c) (d) ratio, and volume, (e) (f) volume and mass of plasticizer on the moisture content of bioplastic

4.5 Analysis of thickness of bioplastic

Table 4.5 shows the ANOVA of response for bioplastic film thickness. In Table 4.5, the value of Prob > F is less than 0.05, indicating that the model is significant, which is desirable because it indicates that the model's terms have a significant effect on the response. The model's F-value of 9.00 indicates that it is significant. This large F-value has a 4.96% chance of occurring due to noise. Model terms are significant if the P-value is less than 0.0500. A, B, C, AB, AC, and BC are ideal model terms in this case. Values greater than 0.1000 indicate that the model terms are insignificant. If there are a large number of insignificant model terms (not including those required to support hierarchy), model reduction may improve the model. The independent variables (mass of plasticizer, the interaction between ratio and mass of plasticizer, the interaction between ratio and volume film-formation solution, and interaction between mass of plasticizer and volume film-formation solution) are insignificant terms which means it has no effect on the film thickness.

Table 4.5: Analysis of variance for film thickness

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0347	6	0.0058	9.00	0.0496	significant
A-Ratio	0.0104	1	0.0104	16.14	0.0277	
B-Mass of plasticizer	0.0024	1	0.0024	3.74	0.1487	
C-Volume	0.0041	1	0.0041	6.43	0.0850	
AB	0.0004	1	0.0004	0.5570	0.5096	
AC	0.0019	1	0.0019	2.96	0.1839	
BC	0.0001	1	0.0001	0.1760	0.7030	
Residual	0.0019	3	0.0006			
Cor Total	0.0366	9				

Effect of the independent variable on film thickness

The thickness of the film is an important factor that affects the drying rate and the structure of the film, as well as the physical and barrier properties of the film. Because of different combinations of the independent variables, the thickness value ranged from 0.040 to 0.232 mm. As depicted in Figure 4.3(a), the effect of increased pectin to cellulose ratio on film thickness decreases the film thickness. However, based on the result from Table 4.1, the addition of a 6:4 ratio of pectin to cellulose gave the highest thickness value of 0.232 mm. In contrast, the addition of the 9:1 ratio of pectin to cellulose showed the lowest thickness value of 0.040 mm.

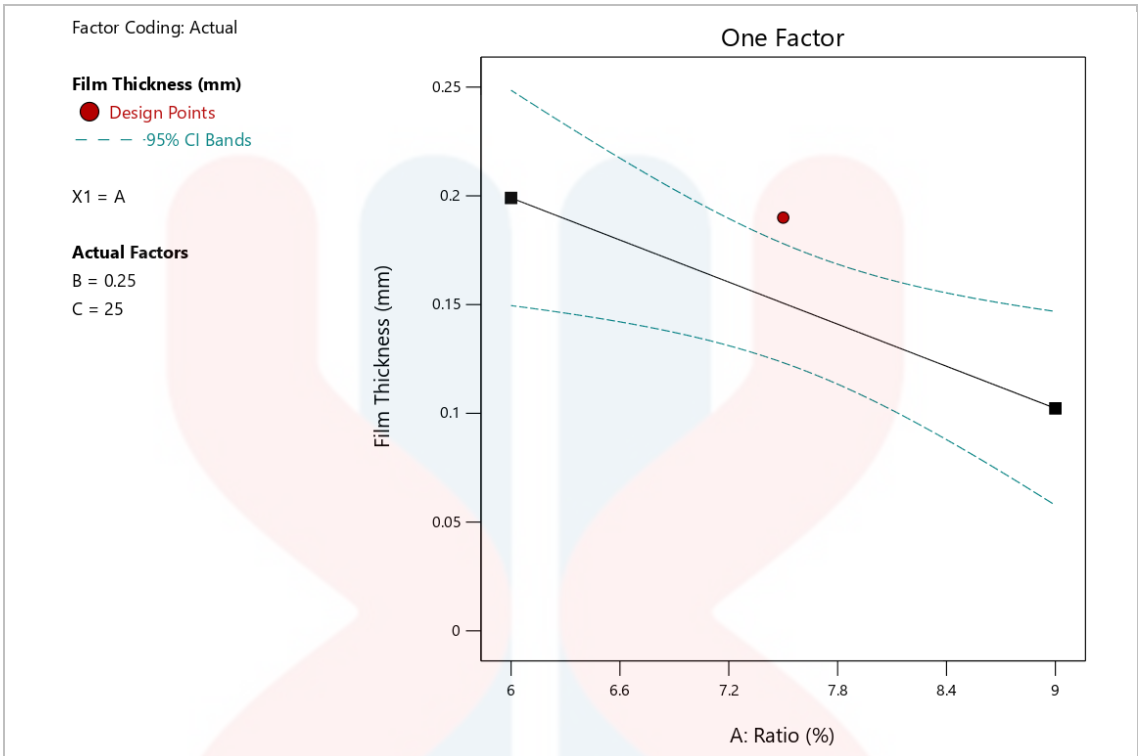
The addition of pectin increases the total dissolved solids in the film-formation solution, resulting in an increase in film thickness (Chodijah et al., 2019). According to Zannini et al. in a 2021 study, the higher the concentration of pectin-cellulose ratio from pomace biomass, the thicker the film. The results are also in agreement with the studies of Galus et al. (2012) the films with higher contents of pectin from apple had a higher film thickness. Mendes et al. (2020) developed pectin film incorporated with bagasse fiber showed an increased film thickness range 0.04 – 0.10 mm with increased concentration pectin and bagasse fiber.

The effect of the mass of plasticizer on film thickness is shown in Figure 4.3(b). As shown in the figure, it can be observed that as the mass of the plasticizer increases, the film thickness also increases. The result obtained agree with Tarique et al. (2021) that an increase in glycerol concentration from 15 to 30% increased the film thickness from 156 to 163 μm. According to studies from Khairunnisa et al. (2018), an increase in the addition of glycerol causes the film thickness to increase. Furthermore, Tarique et al.

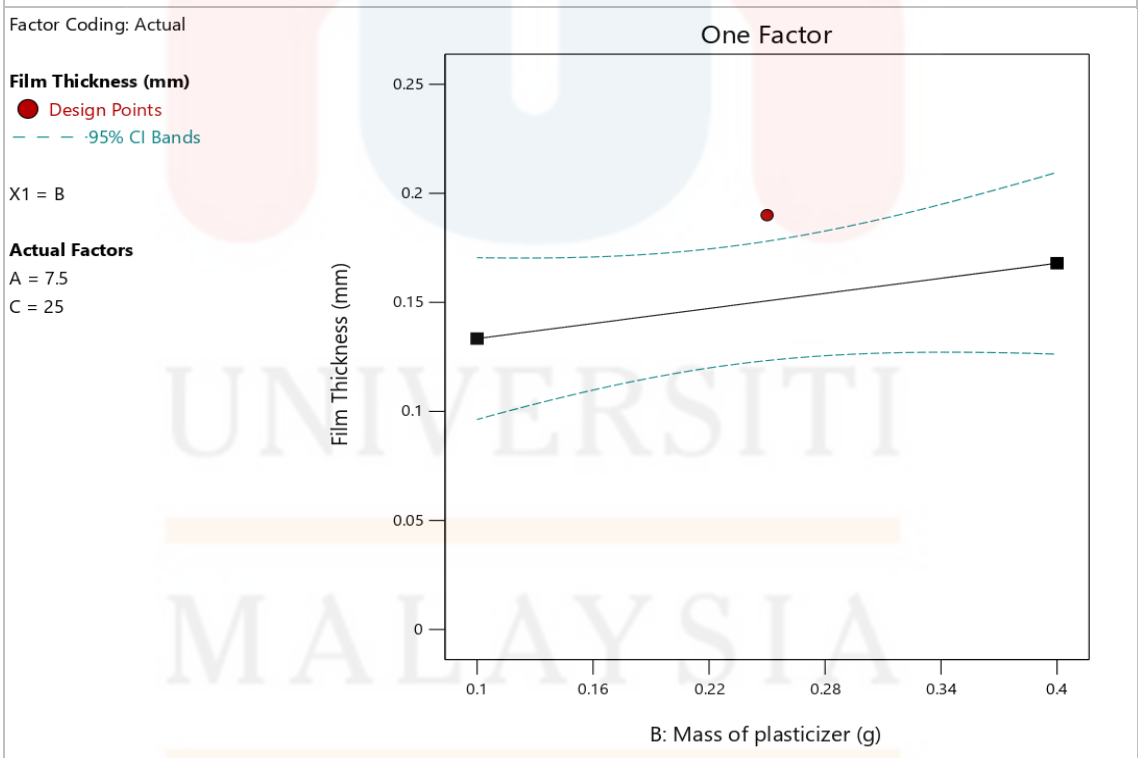
(2021) stated the role of plasticizers in upsetting and restructuring intermolecular polymer chain networks, converting all free volumes into a thicker film.

However, based on the results from table 4.1, the addition of 0.1 g of plasticizer resulted in the greatest thickness of 0.232 mm. In comparison, the addition of 0.4 g of plasticizer resulted in the thinnest layer of 0.040 mm. Seixas et al. (2013) also mentioned in their study that increasing plasticizer (glycerol) concentration in films of pectin was decreased the thickness of biofilm.

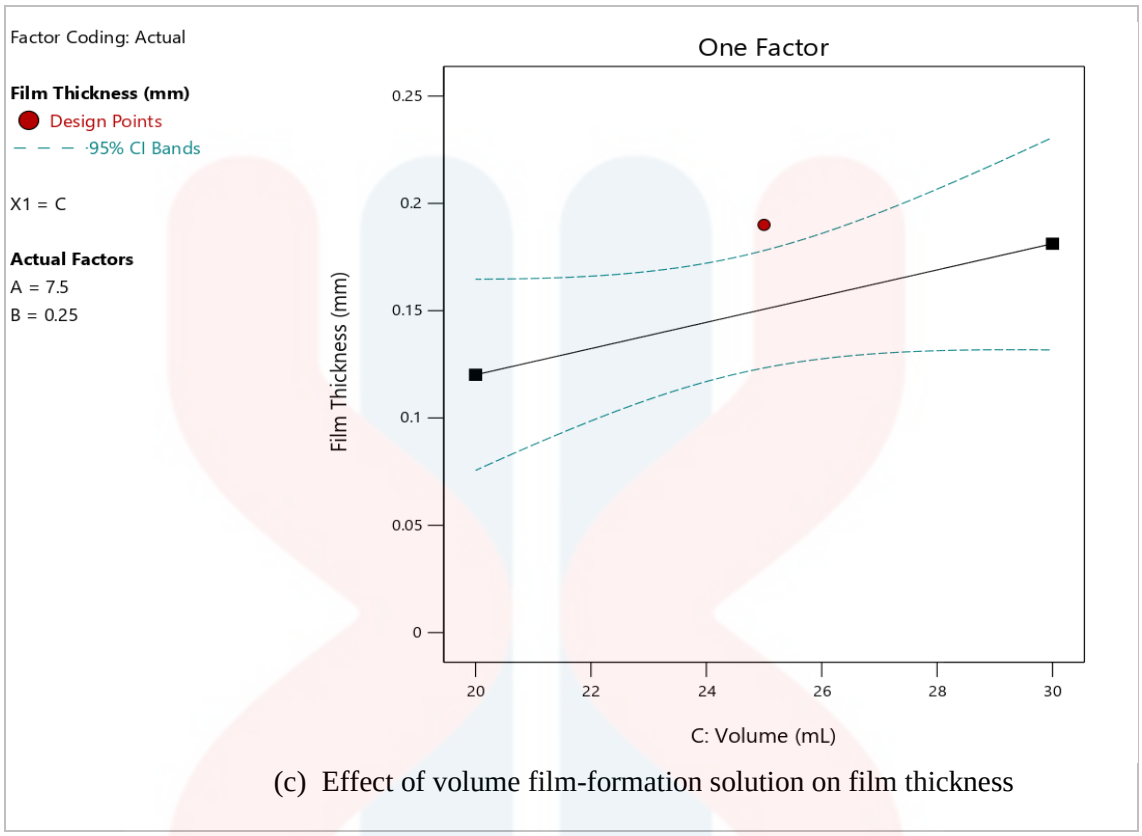
Figure 4.3(c) illustrates that increasing the volume film-formation solution causes the value of film thickness to increase. Based on Table 4.1, 30 mL of film-formation solution gives the higher film thickness value, and the lowest value of film thickness is 20 mL of film-formation solution. It can be concluded that the volume of the film-formation solution affects the film thickness. According to Ulyarti (2021), the amount of film-forming solution (either volume or mass) poured per area of the molds influences the thickness of bioplastic. The higher the amount of film-forming solution that is poured, the thicker the bioplastic produced.



(a) Effect of ratio on film thickness



(b) Effect of mass of plasticizer on film thickness



(c) Effect of volume film-formation solution on film thickness

Figure 4.3: Effect of the independent variable on film thickness

Interaction effect of factors on the film thickness of bioplastic.

Figure 4.4 (a), (b) show the effect of the interaction of pectin-cellulose ratio and mass of plasticizer where 0.232 mm is the highest film thickness at 6:4 ratio of pectin to cellulose and 0.4 g plasticizer. High ratio pectin to cellulose (9:1) and with 0.1 g of plasticizer showed the lowest value of film thickness (0.040 mm). The contour plot and 3D surface plot for the film thickness of bioplastics as a function of ratio pectin to cellulose (A) and mass of plasticizers (B) are presented in Figures 4.4 (a) and (b).

Further, Figure 4.4 (c) and (d) represent the contour and a 3D surface plot showing the influence of ratio pectin to cellulose (A) and volume (C) on film thickness. The highest film thickness (0.232 mm) was at 6:4 ratio of pectin to cellulose, and 30 mL film-forming solution, while the lowest value of film thickness (0.040 mm) used 20 mL volume film-formation solution and 9:1 of the ratio of pectin to cellulose.

The contour and 3D surface plots in Figure 4.4 (e) and (f) indicate the effect of the mass of plasticizer (B) and volume film-formation solution (C) on film thickness. The highest and lowest values of moisture content, which is 0.232 mm and 0.040 mm, respectively, were obtained with 0.4 g and 0.1 g of plasticizer and 30 mL and 20 mL volume film-forming solution, respectively.

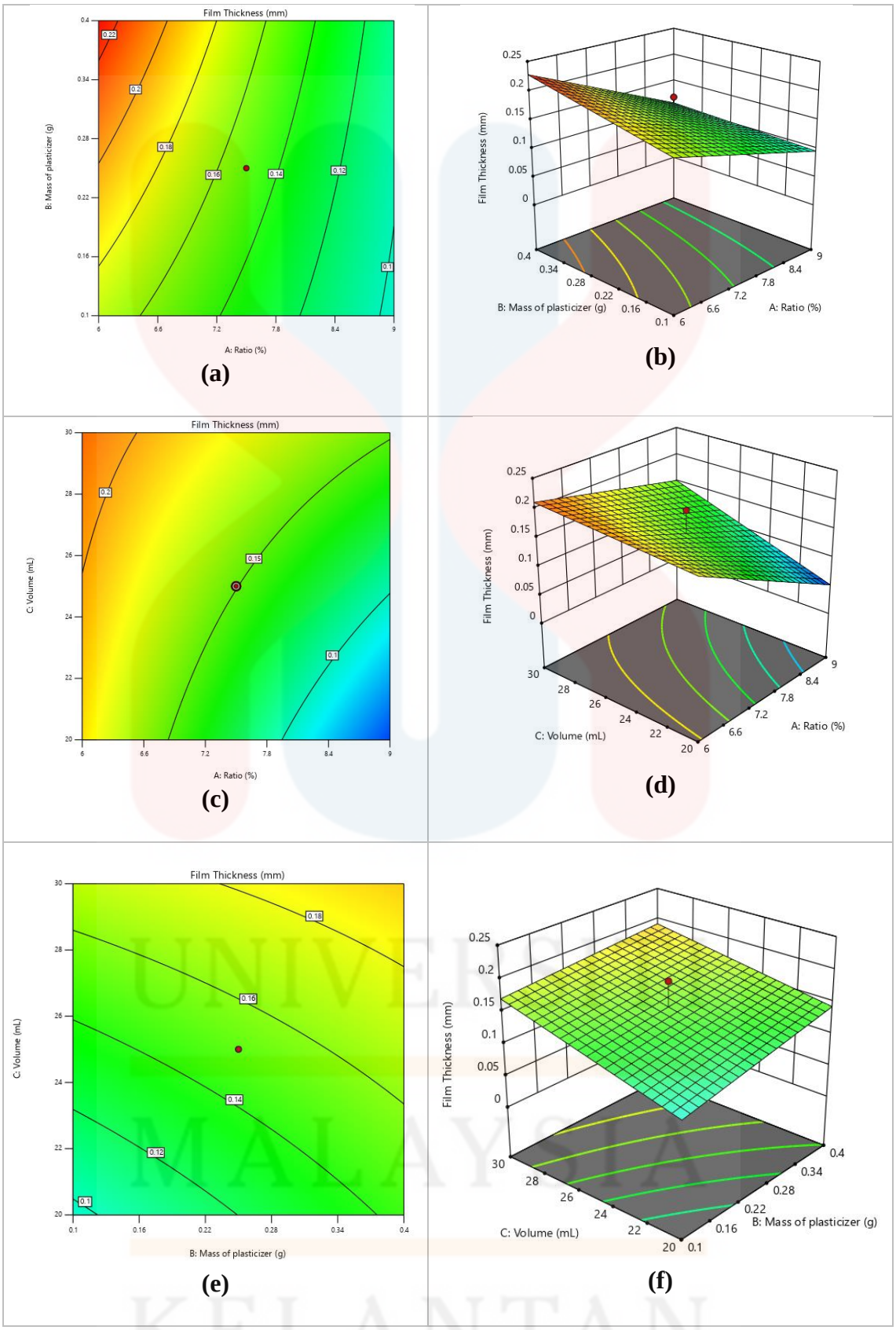


Figure 4.4: Contour and a 3D surface plot showing the effect of (a) (b) ratio and mass of plasticizer, (c) (d) ratio and volume, (e) (f) volume and mass of plasticizer on the film thickness of bioplastic

4.6 Analysis of density of bioplastic

Table 4.5 shows the ANOVA of response for density of bioplastic. The F value is 1.35, and the prob > F value for the model is greater than 0.05. The Model F-value of 1.35 indicates that the model is not significant in comparison to the noise. An F-value of this magnitude has a 43.49 percent chance of occurring due to noise. Model terms are significant if the P-value is less than 0.0500. A, B, C, AB, AC, and BC are not significant model terms in this case. Values greater than 0.1000 indicate that the model terms are insignificant. If there are many insignificant model terms (other than those required to support hierarchy), these insignificant model terms can be removed, resulting in a better model. The independent variables (ratio, mass of plasticizer, volume film-formation, the interaction between ratio and mass of plasticizer, the interaction between ratio and volume film-formation solution, and interaction between mass of plasticizer and volume film-formation solution) are insignificant terms which means it has no effect on the film density.

Table 4.6: Analysis of variance for film density

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0003	6	0.0000	1.35	0.4349	not significant
A-Ratio	0.0001	1	0.0001	1.99	0.2535	
B-Mass of plasticizer	0.0001	1	0.0001	1.53	0.3046	
C-Volume	0.0002	1	0.0002	5.45	0.1018	
AB	0.0001	1	0.0001	1.51	0.3069	
AC	9.962E-06	1	9.962E-06	0.2691	0.6398	
BC	0.0000	1	0.0000	1.22	0.3495	
Residual	0.0001	3	0.0000			
Cor Total	0.0004	9				

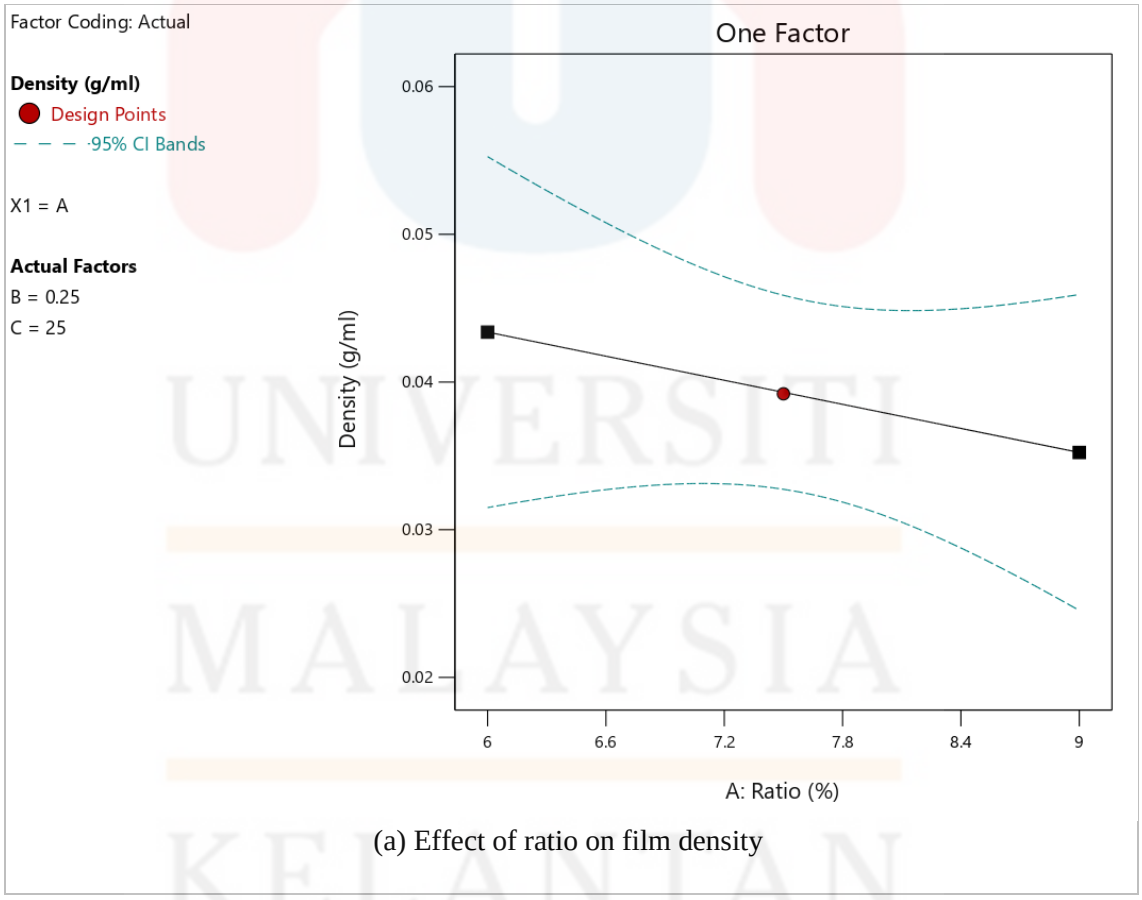
Effect of the independent variable on film density

The effect of pectin to cellulose ratio on film density of developed bioplastic was shown in Figure 4.5(a). The figure below shows that increasing the pectin ratio to cellulose decreases response film density from low level coded to high level coded. The higher value of film density (0.049 g/mL) with the 9% pectin and 1% cellulose. The lower value of film thickness (0.027 g/mL) with 10% pectin content and 0% cellulose content. The cellulose content affects the film density of bioplastic in this study. A study from Mosisa & K. (2021) found that the film density of bioplastic produced increases when the cellulose concentration is added. Muhammad et al. (2019) developed starch film incorporated with cellulose from mangosteen peel, showing the density of bioplastic decreases as cellulose content increases. According to González Moreno et al. (2021), cellulose is used as a filler to produce bioplastic due to its low density, good mechanical properties, and complete biodegradability.

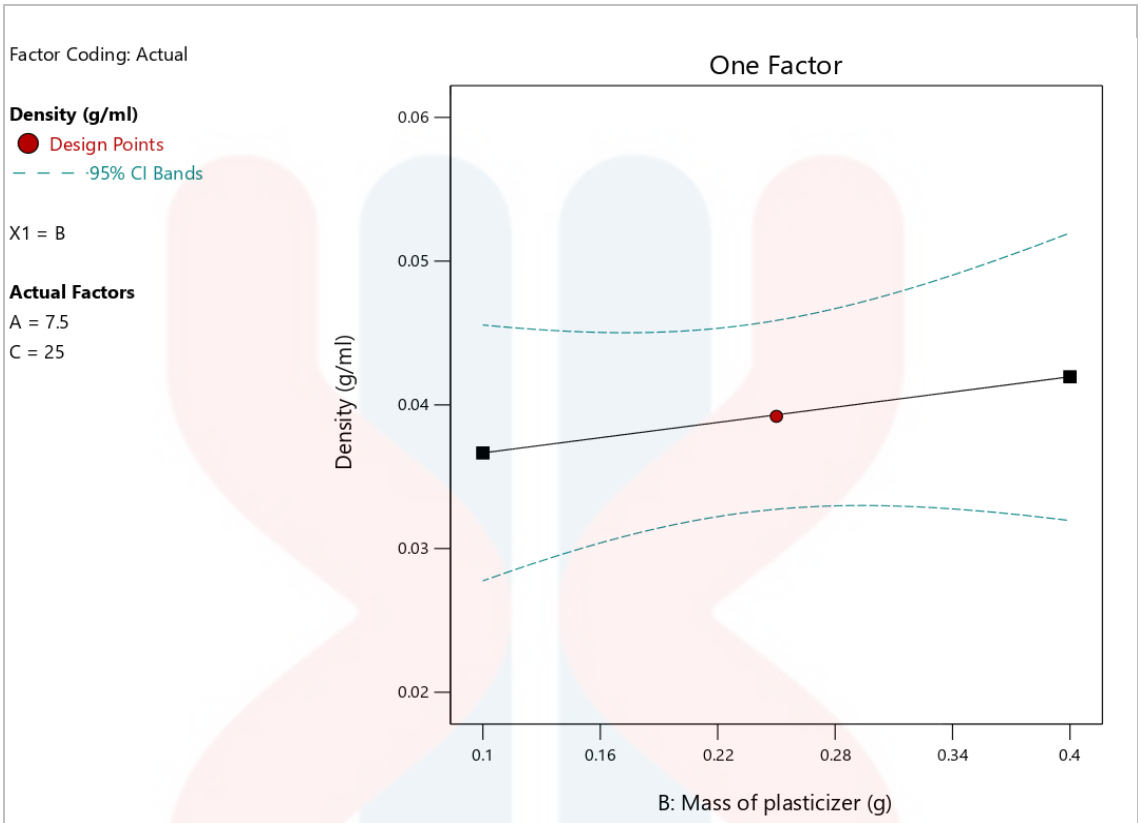
The effect of the mass of plasticizer on film density of bioplastic is shown in Figure 4.5 (b) below. From the graph, it can be observed that increasing the mass of plasticizer increases response film density from low level coded up to higher level. The addition of a plasticizer by 0.4 g gave a higher density (0.049 g/mL). While 0.027 g/mL lowers film density value by adding 0.25 g of plasticizers. Fath et al. (2019) developed pectin and starch film incorporated with nanocrystalline cellulose, showed the result higher value of density biofilms 0.068 gram/cm^3 with 30 wt.% of glycerol contents. Furthermore, according to Fath et al. (2019), when the glycerol content was increased to 40 wt.% glycerol disrupted the intermolecular and intramolecular bond in starch and pectin, reducing the density of the bioplastic. At this point, glycerol tends to increase the strong interaction between glycerol itself. According to Tarique et al. (2021) with the increasing

plasticizer concentration, the film density slightly decreased. In addition, evidence from Nordin et al. (2020), reported that adding glycerol to corn starch film did not result in a significant difference in density. Furthermore, Sanyang et al. (2016) discovered that the differences in film density between the various bioplastics were not statistically significant.

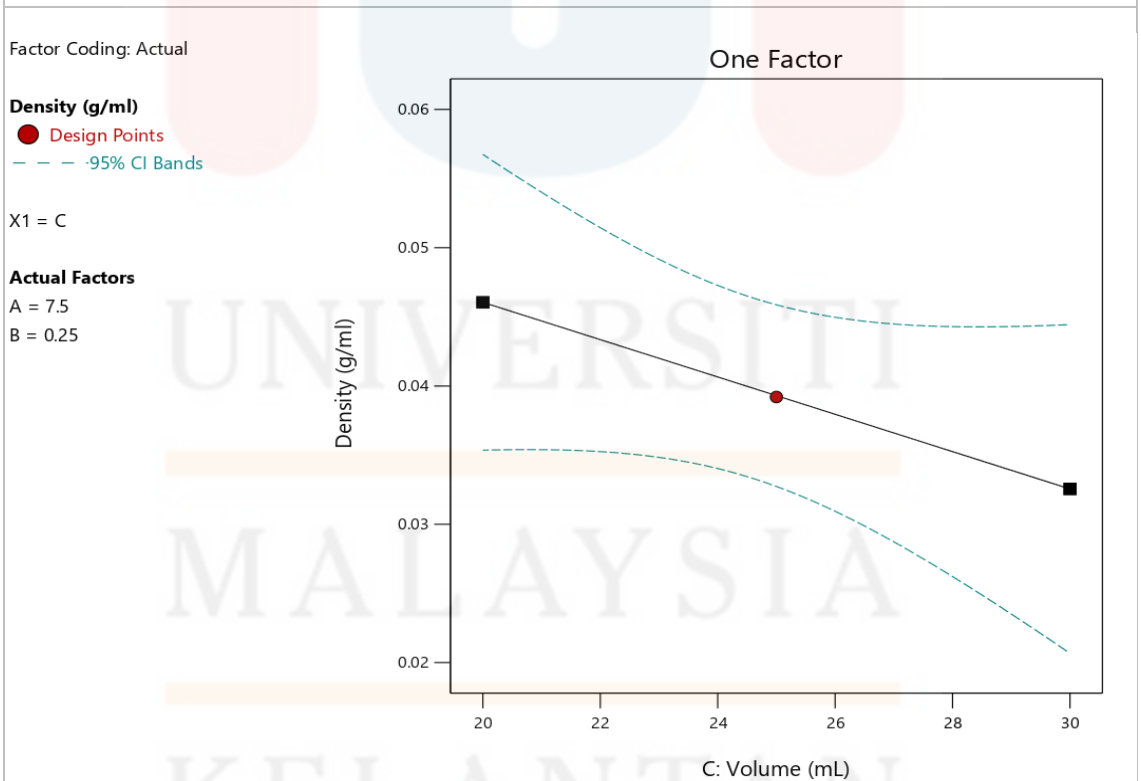
The effect of volume film-formation solution on the film density of bioplastic is shown in Figure 4.5(c). As shown in the figure, the film density value decreases when the volume film-formation solution increases. The volume of 20 mL film-formation solution gives the highest value of film density, and the higher volume film-formation solution, which is 30 mL, provides the lowest value in film density.



(a) Effect of ratio on film density



(b) Effect of mass of plasticizer on film density



(c) Effect of volume film-formation solution on film density

Figure 4.5: Effect of the independent variable on film density

Interaction effect of factors on film density of bioplastic.

The contour and 3D surface plot, as shown in Figure 4.6 (a) and (b), indicates the interaction of ratio (A) and mass of plasticizer (B) on film density. The interaction of pectin-cellulose ratio and mass of plasticizer gives the result to the highest value of film density, 0.049 g/mL at 9:1 of pectin to cellulose ratio and 0.4 g of plasticizer. 10:0 of the ratio of pectin to cellulose and 0.25 g of plasticizer showed the lowest film density value, 0.027 g/mL. The contour plot and 3D surface plot for film density of bioplastics as a function of ratio pectin to cellulose (A) and mass of plasticizers (B) are presented in Figures 4.6 (a) and (b).

Further, the contour and 3D surface plots in Figures 4.6 (c) and (d) illustrate the interaction effect of ratio (A) and volume (C) on film density. The maximum value of film density, 0.049 g/mL was at 9:1 ratio of pectin to cellulose, and volume film-forming solution is 20 mL, while for the minimum value of film density, 0.027 g/mL, using 25 mL volume film-forming solution and 10:0 ratio pectin to cellulose.

The contour and 3D surface plots in Figure 4.6 (e) and (f) indicate the effect of the mass of plasticizer (B) and volume film-formation solution (C) on film density. The maximum value of film density, 0.049 g/mL was at 9:1 ratio of pectin to cellulose, and volume film-formation solution is 20 mL, while for the minimum value of film density, 0.027 g/mL with the addition of 10:0 ratio pectin to cellulose and 25 mL volume film-formation solution.

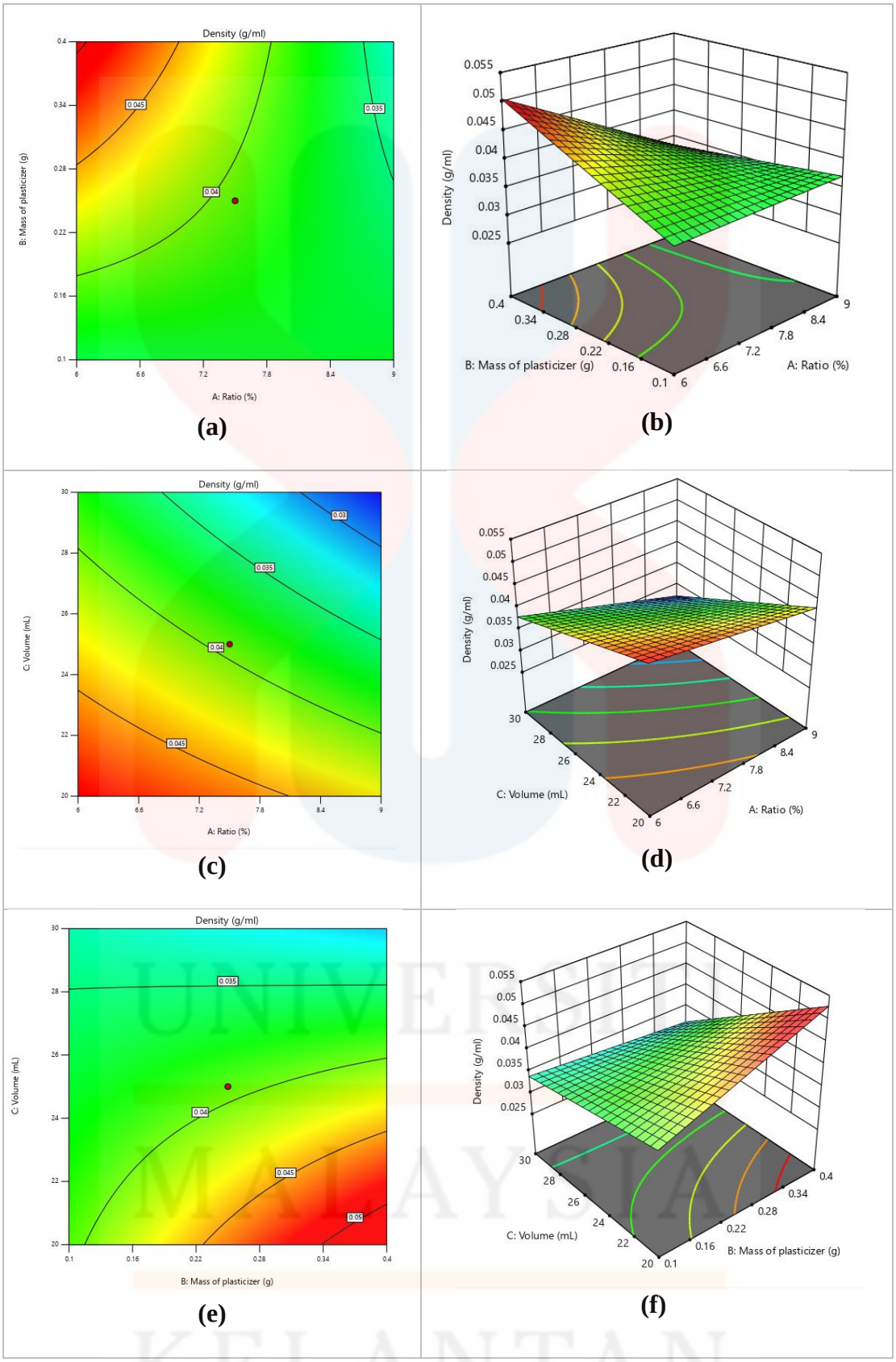


Figure 4.6: Contour and a 3D surface plot showing the effect of (a), (b) ratio and mass of plasticizer, (c), (d) ratio and volume, (e) (f) volume, and mass of plasticizer on the density of bioplastic

4.7 Optimization of the formulation bioplastic using Response Surface Methodology (RSM).

Numerical and graphical optimizations were done for the design to determine the optimal conditions adapted to the constraints as presented in Table 4.7. An optimal processing condition of the bioplastic developed from orange peel pectin and rice husk cellulose was selected for further characterization based on ratio pectin to cellulose, the mass of plasticizer, and volume film-formation solution set in the range and as well as maximizing moisture content, film thickness, and film density of the bioplastic.

Table 4.7: Response Constraints for Optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Ratio	is in range	6	10	1	1	3
B: Mass of plasticizer	is in range	0.1	0.5	1	1	3
C: Volume	is in range	20	30	1	1	3
Moisture Content	maximize	6.84	39.98	1	1	3
Film Thickness	maximize	0.04	0.23	1	1	3
Density	maximize	0.0268	0.0485	1	1	3

The desirability profile for the optimum conditions suggested by the RSM is shown in Table 4.8. The selected optimization formulation depends on the desirability value of each solution suggested. The desirability value showed that the selected conditions were suitable for optimum responses for moisture content, film thickness, and density of bioplastic. The desirability of the suggested optimized formulation may depend on the data obtained from 10 experimental designs and from the analysis of each response. The parameters have been reported by numerical optimization, and the process variable has been optimized; 100 solutions have been found. Only one solution is selected out of 100 solutions. Table 4.8 shows the optimized solution predicted by the numerical optimization procedure.

The desirability value is used to determine the solution's outcome. The desirability value 1 indicates that the response is perfect, while the desirability value 0 indicates that the response must be discarded. The desirability value of the optimization performed is 1.000, which means that the process conditions have a 100% chance of producing the film with the characteristics that correspond to the optimization target. The desirability value is used to determine the degree of accuracy of the optimal solution results (Singh et al., 2015). The optimized set of values obtained through RSM is shown as a ramp function for minimum moisture content, film thickness, and density in Figure 4.7.

Table 4.8: Optimized solution predicted by numerical optimization procedure

No.	Ratio	Mass of plasticizer	Volume	Moisture Content	Film Thickness	Density	Desirability	
1	6.001	0.408	26.184	41.654	0.230	0.049	1.000	Selected

Figure 4.7 depicts the optimal factor settings as red points, and the optimal response prediction values as blue. The desirability of 1.000 indicates that the goals were easily attained, and better results may be available. Out of the 100 solutions, the optimum values with the highest desirability (100%) and the one that meets the desired criteria were chosen. The primary goal of optimization is to identify a good set of process conditions (parameters) that meet all of the goals and produce improved results. Accordingly, a bioplastic with 6:4 of ratio pectin to cellulose, 0.408 g of plasticizer, and 26 mL film-forming solution could be obtained 41.654% moisture content, 2.230 mm film thickness, and 0.049 g/mL density. Figure 4.8 represents the contour plots for the optimized parameter.

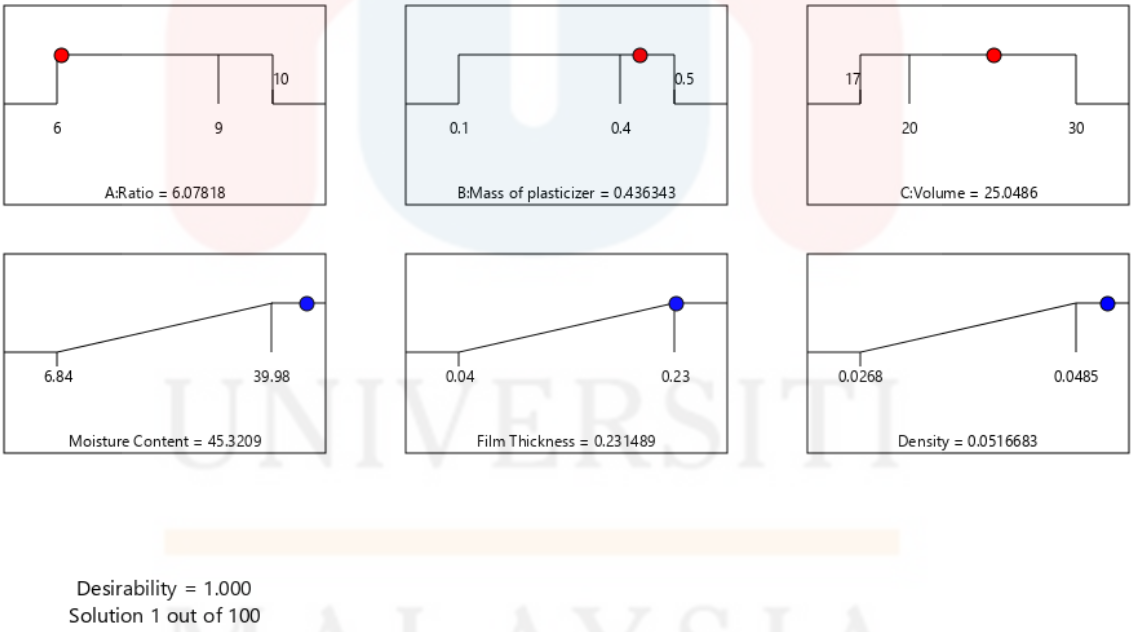


Figure 4.7: Ramp plots for optimized parameters

Factor Coding: Actual

All Responses

0 1

X1 = A

X2 = B

Actual Factor

C = 26.5782

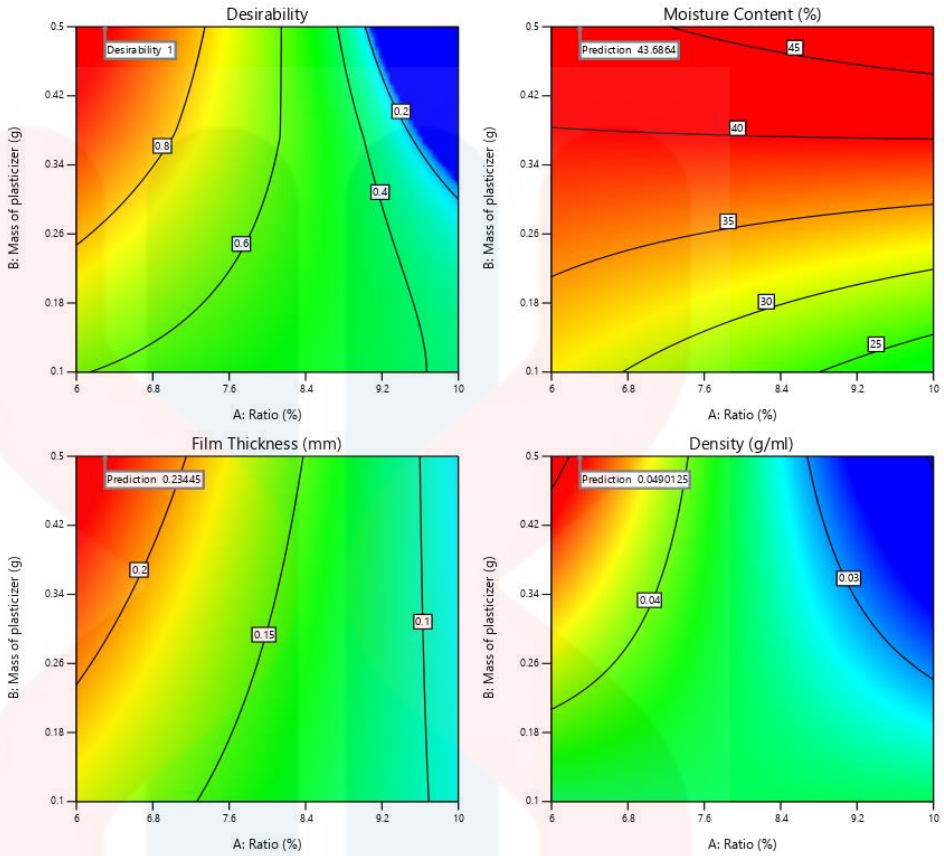


Figure 4.8: Contour plots for optimized parameter

4.8 Thermogravimetric analysis of development optimal bioplastic

Thermogravimetric analysis is a technique used to investigate the reaction of thermal decomposition between weight change and temperature, which is lost because of temperature on the material. The result of the thermal analysis is a curve known as a thermogram. Thermal decomposition is the process of converting a sample's form into a simpler form, which is influenced by a variety of factors such as heating rate, material composition, pressure, temperature, moisture, particle size, and residence time. Thermogravimetric analysis was performed to determine the thermal stability of the bioplastic films produced. Figure 4.9 depicts the mechanism of thermal decomposition of a pectin cellulose-based bioplastic.

From the TG graph, it was concluded that the film started to melt around 43.09ºC, and the complete degradation of the film was around 495.99ºC. The initial degradation of bioplastic starts at temperature 43.09ºC, which is primarily caused by the evaporation of the moisture content of bioplastic films, and glycerol is known to be a hydrophilic substance that holds moisture (Hii et al., 2016). According to studies on pectin-cellulose biofilms from citrus wastes by Bátori et al. (2017), initial degradation starts at an average temperature of 80.38-80.61°C.

Major weight loss of bioplastic occurred between 220°C and 350°C, which could be attributed to the thermal decomposition of the bioplastic film's components. Hii et al. (2016) reported that glycerol (plasticizer) decomposed at a temperature around 260°C and the thermal decomposition of pectin at a temperature around 200°C (Ruano et al., 2020) and for cellulose starts to decompose at a temperature between 275°C and 350°C (Mansaray & Ghaly, 1998). The process of this stage causes rapid thermal decomposition with a large mass loss and runs quickly because of the large amount of nitrogen present.

The charcoal is flammable at temperatures ranging from 350°C to 480°C because it is surrounded by volatile matter and oxygen diffused on its surface, which simultaneously burns the charcoal and volatile matter. This stage follows the release of volatile matter, which either leaves or forms carbon (Wahyuningtyas et al., 2017). The final degradation of the thermal decomposition process in bioplastic occurred at 496.99°C and it was recorded a residual mass of 35.1113%. The result obtained from Bátori et al. (2017) shows that the remaining ash of the films after thermal treatment is 21.97-23.20%. The residual mass of optimal film was observed from the TGA graph in Figure 4.9.

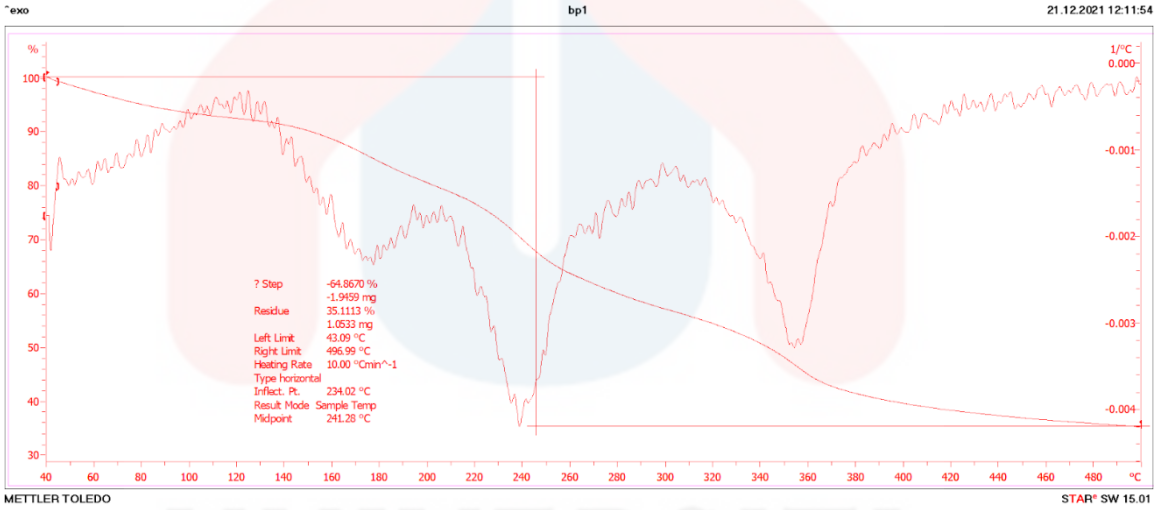


Figure 4.9: Thermogravimetric analysis of optimal bioplastic

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, the development of bioplastic from orange peel pectin and cellulose from rice husk was investigated. Response surface methodology using Central Composite Design was employed to analyse the moisture content, thickness, and density of bioplastic developed by optimizing the independent variables ratio pectin to cellulose, mass of plasticizer, and volume film-formation solution. Response surface plots were acquired to evaluate the significance of the independent variables on the response.

A statistically significant model was developed to describe the relationship between moisture content, film thickness, film density with the independent variable. Both ratio and volume film-formation solution were found not significant factors ($p > 0.05$) influencing the responses. Meanwhile, the mass of the plasticizer was found to be a significant factor ($p < 0.05$) influencing the responses.

The optimum result was obtained after conducting the experiment at the selected optimum condition, which are ratio (6.001%), the mass of plasticizer (0.408 g), and volume film-formation solution (26.184 mL) with a high value of desirability (1.000). These values were selected from 100 alternative optimal solutions set by a design expert. The optimized parameters resulted in bioplastic in moisture content, thickness, and density values of 41.654%, 0.230 mm, and 0.049 g/mL, respectively. The optimal bioplastic has been validated and characterized by thermogravimetric analysis (TGA).

5.2 Recommendation

From this study, it is clear that bioplastic can produce using pectin orange peel incorporation with rice husk cellulose using glycerol as a plasticizer for the present study. Other commonly available pectin sources can be explored. Fruit wastes like pomegranate peel and jackfruit peel also have high pectin content. The future study should focus on characterization to improve pectin's and cellulose-based bioplastic quality, mechanical and physicochemical properties. The properties of bioplastic such as tensile strength, modulus of elasticity, elongational at break, water vapor transmission rate, biodegradability, morphology, and oxygen transmission rate (OTR) should be investigated in further research.

REFERENCES

Abdullah, A. H. D., Fikriyyah, A. K., Putri, O. D., & Puspa Asri, P. P. (2019). Fabrication and Characterization of Poly Lactic Acid (PLA)-Starch Based Bioplastic Composites. *IOP Conference Series: Materials Science and Engineering*, *553*(1). https://doi.org/10.1088/1757-899X/553/1/012052

Abdullah, A. H. D., Putri, O. D., Fikriyyah, A. K., Nissa, R. C., & Intadiana, S. (2020). Effect of microcrystalline cellulose on characteristics of cassava starch-based bioplastic. *Polymer-Plastics Technology and Materials*, *59*(12), 1250–1258. https://doi.org/10.1080/25740881.2020.1738465

Abdullah, A. H. D., Putri, O. D., & Sugandi, W. W. (2019). Effects of Starch-Glycerol Concentration Ratio on Mechanical and Thermal Properties of Cassava Starch-Based Bioplastics. *Jurnal Sains Materi Indonesia*, *20*(4), 162. https://doi.org/10.17146/jsmi.2019.20.4.5505

Agoda-Tandjawa, G., Durand, S., Gaillard, C., Garnier, C., & Doublier, J. L. (2012). Properties of cellulose/pectins composites: Implication for structural and mechanical properties of cell wall. *Carbohydrate Polymers*, *90*(2), 1081–1091. https://doi.org/10.1016/j.carbpol.2012.06.047

Agustin, M. B., Ahmmad, B., Alonzo, S. M. M., & Patriana, F. M. (2014). Bioplastic based on starch and cellulose nanocrystals from rice straw. *Journal of Reinforced Plastics and Composites*, *33*(24), 2205–2213. https://doi.org/10.1177/0731684414558325

Ali, W. S., Zaki, N. H., & Obiad, S. Y. N. (2017). Prouction of bioplastic by bacteria isolated from local soil and organic wastes. *Current Research in Microbiology and Biotechnology*, *5*(2), 1012–1017. https://www.researchgate.net/publication/315375411_Production_of_bioplastic_by_bacteria_isolated_from_local_soil_and_organic_wastes

Almenar, E. (2020). Innovations in packaging technologies for produce. In *Controlled and Modified Atmospheres for Fresh and Fresh-Cut Produce*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-804599-2.00012-0

Andrady, A. L., & Neal, M. A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1977–1984. https://doi.org/10.1098/rstb.2008.0304

Ángel Siles López, J., Li, Q., & Thompson, I. P. (2010). Biorefinery of waste orange peel. *Critical Reviews in Biotechnology*, *30*(1), 63–69. https://doi.org/10.3109/07388550903425201

Aramvash, A., Akbari Shahabi, Z., Dashti Aghjeh, S., & Ghafari, M. D. (2015). Statistical physical and nutrient optimization of bioplastic polyhydroxybutyrate production by Cupriavidus necator. *International Journal of Environmental Science and*

Technology, *12*(7), 2307–2316. https://doi.org/10.1007/s13762-015-0768-3

Arikan, E. B., & Bilgen, H. D. (2019). Production of bioplastic from potato peel waste and investigation of its biodegradability. *International Advanced Researches and Engineering Journal*, *December*, 93–97. https://doi.org/10.35860/iarej.420633

Arjmandi, R., Hassan, A., Majeed, K., & Zakaria, Z. (2015). Rice Husk Filled Polymer Composites. *International Journal of Polymer Science*, *2015*. https://doi.org/10.1155/2015/501471

Auras, R. A., Singh, S. P., & Singh, J. J. (2005). Evaluation of oriented poly(lactide) polymers vs. existing PET and oriented PS for fresh food service containers. *Packaging Technology and Science*, *18*(4), 207–216. https://doi.org/10.1002/pts.692

Bátori, V., Jabbari, M., Åkesson, D., Lennartsson, P. R., Taherzadeh, M. J., & Zamani, A. (2017). Production of Pectin-Cellulose Biofilms: A New Approach for Citrus Waste Recycling. *International Journal of Polymer Science*, *2017*. https://doi.org/10.1155/2017/9732329

Bátori, V., Lundin, M., Åkesson, D., Lennartsson, P. R., Taherzadeh, M. J., & Zamani, A. (2019). The effect of glycerol, sugar, and maleic anhydride on pectin-cellulose thin films prepared from orange waste. *Polymers*, *11*(3). https://doi.org/10.3390/polym11030392

Battegazzore, D., Bocchini, S., Alongi, J., & Frache, A. (2014). Rice husk as bio-source of silica: Preparation and characterization of PLA-silica bio-composites. *RSC Advances*, *4*(97), 54703–54712. https://doi.org/10.1039/c4ra05991c

BeMiller, J. N. (1986). *An Introduction to Pectins: Structure and Properties*. 2–12. https://doi.org/10.1021/bk-1986-0310.ch001

Borah, A., Balasubramanian, S., Kaur, A., Kaur, J., Sukhija, S., & Balasubramanian, S. (2019). Thermal and microbial characteristics of barley pasta as affected by moisture content. *Our Herit*, *67*(May 2020), 300–314.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., & Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, *24*, 72–79. https://doi.org/10.1016/j.ultsonch.2014.11.015

Bourbon, A. I., Pinheiro, A. C., Cerqueira, M. A., Rocha, C. M. R., Avides, M. C., Quintas, M. A. C., & Vicente, A. A. (2011). Physico-chemical characterization of chitosan-based edible films incorporating bioactive compounds of different molecular weight. *Journal of Food Engineering*, *106*(2), 111–118. https://doi.org/10.1016/j.jfoodeng.2011.03.024

Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., & Vicente, A. A. (2012). Effect of glycerol and corn oil on physicochemical properties of polysaccharide films - A comparative study. *Food Hydrocolloids*, *27*(1), 175–184. https://doi.org/10.1016/j.foodhyd.2011.07.007

Chan, J. X., Wong, J. F., Hassan, A., & Zakaria, Z. (2021). Bioplastics from agricultural waste. *Biopolymers and Biocomposites from Agro-Waste for Packaging Applications*, *December*, 141–169. https://doi.org/10.1016/b978-0-12-819953-4.00005-7

Chodijah, S., Husaini, A., Zaman, M., & Hilwatulisan. (2019). Extraction of Pectin from Banana Peels (Musa Paradiasica Fomatypica) for Biodegradable Plastic Films. *Journal of Physics: Conference Series*, *1167*(1), 1–7. https://doi.org/10.1088/1742-6596/1167/1/012061

Delgado, M., Felix, M., & Bengoechea, C. (2018). Development of bioplastic materials: From rapeseed oil industry by products to added-value biodegradable biocomposite materials. *Industrial Crops and Products*, *125*(September), 401–407. https://doi.org/10.1016/j.indcrop.2018.09.013

Faris, N. A., Noriman, N. Z., Sam, S. T., Ruzaidi, C. M., Omar, M. F., & Kahar, A. (2014). Current research in biodegradable plastics. *Applied Mechanics and Materials*, *679*(January 2016), 273–280. https://doi.org/10.4028/www.scientific.net/AMM.679.273

Fath, M. T. Al, Nasution, H., Harahap, H., & Ayu, G. E. (2019). Biocomposite of pectin and starch filled with nanocrystalline cellulose (NCC): The effect of filler loading and glycerol addition. *AIP Conference Proceedings*, *2175*(November). https://doi.org/10.1063/1.5134576

Freitas, F., Alves, V. D., Reis, M. A., Crespo, J. G., & Coelhoso, I. M. (2014). Microbial polysaccharide-based membranes: Current and future applications. *Journal of Applied Polymer Science*, *131*(6), 1–11. https://doi.org/10.1002/app.40047

Fuad, M. Y. A., Mustafah, J., Mansor, M. S., Ishak, Z. A. M., & Omar, A. K. M. (1995). Thermal properties of polypropylene/rice husk ash composites. *Polymer International*, *38*(1), 33–43. https://doi.org/10.1002/pi.1995.210380104

Galus, S., Turska, A., & Lenart, A. (2012). Sorption and wetting properties of pectin edible films. *Czech Journal of Food Sciences*, *30*(5), 446–455. https://doi.org/10.17221/444/2011-cjfs

Gaonkar. M.R, Palaskar. P, N. R. (2017). Production of bioplastic from banana peels. *I*, *6*(1), 526–536.

Gawkowska, D., Cybulska, J., & Zdunek, A. (2018). Structure-related gelling of pectins and linking with other natural compounds: A review. *Polymers*, *10*(7). https://doi.org/10.3390/polym10070762

Ghafari, S., Aziz, H. A., Isa, M. H., & Zinatizadeh, A. A. (2009). Application of response surface methodology (RSM) to optimize coagulation-flocculation treatment of leachate using poly-aluminum chloride (PAC) and alum. *Journal of Hazardous Materials*, *163*(2–3), 650–656. https://doi.org/10.1016/j.jhazmat.2008.07.090

Ghasemlou, M., Khodaiyan, F., & Oromiehie, A. (2011). Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. *Carbohydrate Polymers*, *84*(1), 477–483.

https://doi.org/10.1016/j.carbpol.2010.12.010

Godwin, A. D. (2000). Plasticizer selection for specific applications. *Applied Polymer Science*, 157–175.

González Moreno, A., Guzman-Puyol, S., Domínguez, E., Benítez, J. J., Segado, P., Lauciello, S., Ceseracciu, L., Porras-Vázquez, J. M., Leon-Reina, L., Heredia, A., & Heredia-Guerrero, J. A. (2021). Pectin-cellulose nanocrystal biocomposites: Tuning of physical properties and biodegradability. *International Journal of Biological Macromolecules*, *180*, 709–717. https://doi.org/10.1016/j.ijbiomac.2021.03.126

Goswami, G., Goswami, M. G., & Purohit, P. (2015). Bioplastics from Organic Waste. *International Journal of Engineering Research & Technology*, *3*(23), 1–3.

Habtemichael, K., & Groterath, A. (2019). Challenges and Opportunities. *Migration and Refugees: Global Patterns and Local Contexts*, *June*, 141–147. https://doi.org/10.4324/9781315813363-10

Halim, S. F. A., Kamaruddin, A. H., & Fernando, W. J. N. (2009). Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer studies. *Bioresource Technology*, *100*(2), 710–716. https://doi.org/10.1016/j.biortech.2008.07.031

Hema Prabha, P., & Ranganathan, T. V. (2017). Modelling and optimization of cassava starch based film for food packaging applications Modelling and Optimization of cassava starch based film for food. *Pakistan Journal of Biotechnology*, *14*(4), 571–586.

Hii, S., Lim, J., Ong, W., & Wong, C. (2016). Agar From Malaysian Red Seaweed As. *Engineering Science and Technology*, *7*, 1–15.

Hirpara, N. J., Dabhi, M. N., & Rathod, P. J. (2021). Development of Potato Starch Based Biodegradable Packaging Film. *Biological Forum-An International Journal*, *13*(1), 529. www.researchtrend.net

Inayati, Pamungkas, D. J., & Matovanni, M. P. N. (2019). Effect of glycerol concentration on mechanical characteristics of biodegradable plastic from rice straw cellulose. *AIP Conference Proceedings*, *2097*(April). https://doi.org/10.1063/1.5098285

Irianto, H. E., Agusman, Fransiska, D., & Musfira. (2019). Mechanical and barrier properties of composite films based on kappa-carrageenan-polyvinyl alcohol. *International Journal of Engineering and Advanced Technology*, *8*(6 Special Issue 3), 489–499. https://doi.org/10.35940/ijeat.F1090.0986S319

Isroi, Cifriadi, A., Panji, T., Wibowo, N. A., & Syamsu, K. (2017). Bioplastic production from cellulose of oil palm empty fruit bunch. *IOP Conference Series: Earth and Environmental Science*, *65*(1). https://doi.org/10.1088/1755-1315/65/1/012011

Jancy, S., Shruthy, R., & Preetha, R. (2020). Fabrication of packaging film reinforced with cellulose nanoparticles synthesised from jack fruit non-edible part using response surface methodology. *International Journal of Biological*

Macromolecules, *142*, 63–72. https://doi.org/10.1016/j.ijbiomac.2019.09.066

Jannah, M., Ahmad, A., Hayatun, A., Taba, P., & Chadijah, S. (2019). Effect of filler and plastisizer on the mechanical properties of bioplastic cellulose from rice husk. *Journal of Physics: Conference Series*, *1341*(3). https://doi.org/10.1088/1742-6596/1341/3/032019

Jariyasakoolroj, P., Leelaphiwat, P., & Harnkarnsujarit, N. (2020). Advances in research and development of bioplastic for food packaging. *Journal of the Science of Food and Agriculture*, *100*(14), 5032–5045. https://doi.org/10.1002/jsfa.9497

Jouki, M., Khazaei, N., Ghasemlou, M., & Hadinezhad, M. (2013). Effect of glycerol concentration on edible film production from cress seed carbohydrate gum. *Carbohydrate Polymers*, *96*(1), 39–46. https://doi.org/10.1016/j.carbpol.2013.03.077

Jouki, M., Tabatabaei Yazdi, F., Mortazavi, S. A., & Koocheki, A. (2013). Physical, barrier and antioxidant properties of a novel plasticized edible film from quince seed mucilage. *International Journal of Biological Macromolecules*, *62*, 500–507. https://doi.org/10.1016/j.ijbiomac.2013.09.031

Kehinde, O., Ramonu, O. J., Babaremu, K. O., & Justin, L. D. (2020). Plastic wastes: environmental hazard and instrument for wealth creation in Nigeria. *Heliyon*, *6*(10), e05131. https://doi.org/10.1016/j.heliyon.2020.e05131

Keziah, V. S., Gayathri, R., & Priya, V. V. (2018). Biodegradable plastic production from corn starch. *Drug Invention Today*, *10*(7), 1315–1317.

Khairunnisa, S., Junianto, Zahidah, & Rostini, I. (2018). The effect of glycerol concentration as a plasticizer on edible films made from alginate towards its physical characteristic. *World Scientific News*, *112*(September), 130–141.

Khamsucharit, P., Laohaphatanalert, K., Gavinlertvatana, P., Sriroth, K., & Sangseethong, K. (2018). Characterization of pectin extracted from banana peels of different varieties. *Food Science and Biotechnology*, *27*(3), 623–629. https://doi.org/10.1007/s10068-017-0302-0

Kokoszka, S., Debeaufort, F., Hambleton, A., Lenart, A., & Voilley, A. (2010). Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films. *Innovative Food Science and Emerging Technologies*, *11*(3), 503–510. https://doi.org/10.1016/j.ifset.2010.01.006

Kristo, E., & Biliaderis, C. G. (2006). Water sorption and thermo-mechanical properties of water/sorbitol-plasticized composite biopolymer films: Caseinate-pullulan bilayers and blends. *Food Hydrocolloids*, *20*(7), 1057–1071. https://doi.org/10.1016/j.foodhyd.2005.11.008

Kumar, S., & Thakur, K. (2017). Bioplastics - classification, production and their potential food applications. *Journal of Hill Agriculture*, *8*(2), 118. https://doi.org/10.5958/2230-7338.2017.00024.6

Lazar, V. R. (2018). *Quantification of Bone Using a 3 . 0 Tesla Clinical Magnetic*

Resonance Scanner. *April*.

Listyarini, R. V., Susilawati, P. R., Nukung, E. N., & Yua, M. A. T. (2020). Bioplastic from Pectin of Dragon Fruit (Hylocereus polyrhizus) Peel. *Jurnal Kimia Sains Dan Aplikasi*, *23*(6), 203–208. https://doi.org/10.14710/jksa.23.6.203-208

Lothfy, F. A., Haron, M. F., & Rafaie, H. A. (2018). Fabrication and Characterization of Jackfruit Seed Powder and Polyvinyl Alcohol Blend as Biodegradable Plastic. *Journal of Polymer Science and Technology*, *3*(2), 1–5.

Lubis, M., & Harahap, M. B. (2018). *PRODUCTION OF BIOPLASTIC FROM AVOCADO SEED STARCH REINFORCED WITH MICROCRYSTALLINE CELLULOSE FROM SUGAR PALM FIBERS*. *13*(2), 381–393.

Lusiana, S. W., Putri, D., Nurazizah, I. Z., & Bahruddin. (2019). Bioplastic Properties of Sago-PVA Starch with Glycerol and Sorbitol Plasticizers. *Journal of Physics: Conference Series*, *1351*(1). https://doi.org/10.1088/1742-6596/1351/1/012102

Mahato, N., Sharma, K., Sinha, M., Baral, E. R., Koteswararao, R., Dhyani, A., Hwan Cho, M., & Cho, S. (2020). Bio-sorbents, industrially important chemicals and novel materials from citrus processing waste as a sustainable and renewable bioresource: A review. *Journal of Advanced Research*, *23*, 61–82. https://doi.org/10.1016/j.jare.2020.01.007

Majid, I., Thakur, M., & Nanda, V. (2018). Biodegradable Packaging Materials. *Encyclopedia of Renewable and Sustainable Materials*, *1993*(2000), 688–697. https://doi.org/10.1016/b978-0-12-803581-8.10356-x

Mansaray, K. G., & Ghaly, A. E. (1998). Thermal degradation of rice husks in nitrogen atmosphere. *Bioresource Technology*, *65*(1–2), 13–20. https://doi.org/10.1016/S0960-8524(98)00031-5

Marsi, N., Huzaisham, N. A., Hamzah, A. A., Zainudin, A. Z., & Zafiah, A. (2019). *Biodegradable Plastic based on Orange Peel for Packaging Application*. *1*(2), 1–6.

Masłowski, M., Miedzianowska, J., & Strzelec, K. (2018). Influence of peroxide modifications on the properties of cereal straw and natural rubber composites. *Cellulose*, *25*(8), 4711–4728. https://doi.org/10.1007/s10570-018-1880-6

McKay, S., Sawant, P., Fehlberg, J., & Almenar, E. (2021). Antimicrobial activity of orange juice processing waste in powder form and its suitability to produce antimicrobial packaging. *Waste Management*, *120*, 230–239. https://doi.org/10.1016/j.wasman.2020.11.048

Medina, L. A., & Dzalto, J. (2017). Natural fibers. *Comprehensive Composite Materials II*, *1*, 269–294. https://doi.org/10.1016/B978-0-12-803581-8.09877-5

Mendes, J. F., Norcino, L. B., Manrich, A., Pinheiro, A. C. M., Oliveira, J. E., & Mattoso, L. H. C. (2020). Development, physical-chemical properties, and photodegradation of pectin film reinforced with malt bagasse fibers by continuous casting. *Journal of Applied Polymer Science*, *137*(39), 1–13. https://doi.org/10.1002/app.49178

Mosisa, G., & K, V. R. (2021). *Production , Characterization and Optimization of Biodegradable Plastic from Corn starch and Sugarcane Bagasse*. *17*(4), 39–50.

Muhammad, A., Roslan, A., Sanusi, S. N. A., Shahimi, M. Q., & Nazari, N. Z. (2019). Mechanical properties of bioplastic form cellulose nanocrystal (CNC) mangosteen peel using glycerol as plasticizer. *Journal of Physics: Conference Series*, *1349*(1). https://doi.org/10.1088/1742-6596/1349/1/012099

Muhammad Shamsuddin, I. (2017). Bioplastics as Better Alternative to Petroplastics and Their Role in National Sustainability: A Review. *Advances in Bioscience and Bioengineering*, *5*(4), 63. https://doi.org/10.11648/j.abb.20170504.13

Naing, H. H., & Shwe, H. H. (2020). *Utilization of Waste Banana Peel for Synthesis of Biopolymeric Film Utilization of Waste Banana Peel for Synthesis of Biopolymeric Film*. *May*.

Noordin, M. Y., Venkatesh, V. C., Sharif, S., Elting, S., & Abdullah, A. (2004). Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology*, *145*(1), 46–58. https://doi.org/10.1016/S0924-0136(03)00861-6

Nordin, N., Othman, S. H., Rashid, S. A., & Basha, R. K. (2020). Effects of glycerol and thymol on physical, mechanical, and thermal properties of corn starch films. *Food Hydrocolloids*, *106*(March), 105884. https://doi.org/10.1016/j.foodhyd.2020.105884

Novianti, T., Utami, I. D., & Ilhamsah, H. A. (2021). *Elongation Optimization of Bioplastic using Response Surface Methodology*. *Cesit 2020*, 448–453. https://doi.org/10.5220/0010313304480453

Nurazzi, N. M., Asyraf, M. R. M., Rayung, M., Norrrahim, M. N. F., Shazleen, S. S., Rani, M. S. A., Shafi, A. R., Aisyah, H. A., Radzi, M. H. M., Sabaruddin, F. A., Ilyas, R. A., Zainudin, E. S., & Abdan, K. (2021). Thermogravimetric analysis properties of cellulosic natural fiber polymer composites: A review on influence of chemical treatments. *Polymers*, *13*(16). https://doi.org/10.3390/polym13162710

Okunola A, A., Kehinde I, O., Oluwaseun, A., & Olufiropo E, A. (2019). Public and Environmental Health Effects of Plastic Wastes Disposal: A Review. *Journal of Toxicology and Risk Assessment*, *5*(2). https://doi.org/10.23937/2572-4061.1510021

Oliva-Moreno, E. E., & Encinas, A. (2021). Addition of Pine Rosin to Pectin bioplastic films for improved water resistance. *Materials Letters*, *290*, 129488. https://doi.org/10.1016/j.matlet.2021.129488

Rahman, R. (2019). Bioplastics for Food Packaging: A Review. *International Journal of Current Microbiology and Applied Sciences*, *8*(03), 2311–2321. https://doi.org/10.20546/ijcmas.2019.803.274

Ramadas, N. V., Soccol, C. R., & Pandey, A. (2010). A statistical approach for optimization of polyhydroxybutyrate production by Bacillus sphaericus NCIM 5149 under submerged fermentation using central composite design. *Applied Biochemistry and Biotechnology*, *162*(4), 996–1007.

https://doi.org/10.1007/s12010-009-8807-5

Ray, S. (2019). Advanced colon-specific delivery systems for treating local disorders. In *Polysaccharide Carriers for Drug Delivery*. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102553-6.00025-8

Riza, M., Syaubari, S., Andriansyah, A., Dewi, R., & Ernita, L. (2019). Optimization of biodegradable plastic production using response surface methodology. *IOP Conference Series: Materials Science and Engineering*, *673*(1). https://doi.org/10.1088/1757-899X/673/1/012016

Rodsamran, P., & Sothornvit, R. (2019). Preparation and characterization of pectin fraction from pineapple peel as a natural plasticizer and material for biopolymer film. *Food and Bioproducts Processing*, *118*, 198–206. https://doi.org/10.1016/j.fbp.2019.09.010

Ruano, P., Lazo Delgado, L., Picco, S., Villegas, L., Tonelli, F., Eduardo Aguilera Merlo, M., Rigau, J., Diaz, D., & Masuelli, M. (2020). Extraction and Characterization of Pectins From Peels of Criolla Oranges (Citrus sinensis): Experimental Reviews . *Pectins - Extraction, Purification, Characterization and Applications*, 1–44. https://doi.org/10.5772/intechopen.88944

Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on physical properties of biodegradable films based on sugar palm (arenga pinnata) starch for food packaging. *Journal of Food Science and Technology*, *53*(1), 326–336. https://doi.org/10.1007/s13197-015-2009-7

Sanyang, Muhammed Lamin, Sapuan, S. M., Jawaid, M., & Ishak, M. R. (2015). *Effect of plasticizer type and concentration on physical properties of biodegradable films based on sugar palm (arenga pinnata) starch for food packaging*. *September*. https://doi.org/10.1007/s13197-015-2009-7

Seixas, F. L., Turbiani, F. R. B., Salomão, P. G., Souza, R. P., & Gimenes, M. L. (2013). Biofilms composed of alginate and pectin: Effect of concentration of crosslinker and plasticizer agents. *Chemical Engineering Transactions*, *32*(August 2020), 1693–1698. https://doi.org/10.3303/CET1332283

Shojaei, S., Ardakani, M. A. H., Sodaiezadeh, H., jafari, M., & afzali, S. fakhreddin. (2020). Simultaneous optimization of parameters influencing organic mulch test using response surface methodology. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-63047-y

Singh, T. P., Chatli, M. K., & Sahoo, J. (2015). Development of chitosan based edible films: process optimization using response surface methodology. *Journal of Food Science and Technology*, *52*(5), 2530–2543. https://doi.org/10.1007/s13197-014-1318-6

Smith, D. A. (2003). Encyclopedia of Food Sciences and Nutrition. *Encyclopedia of Food Sciences and Nutrition*, 3409–3415. http://www.sciencedirect.com/science/article/pii/B012227055X00660X

Sobral P.J.A, Menegalli F.C, Hubinger M.D, & Roques M.A. (2001). Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids*, *15*(4–6), 423–432.

Sreenivasan, J., Govindan, M., Chinnasami, M., & Kadiresu, I. (2012). Solid Waste Management in Malaysia – A Move Towards Sustainability. *Waste Management - An Integrated Vision*, *2005*(April 2005). https://doi.org/10.5772/50870

Sudhanshu Joshi, Ujjawal Sharm, G. G. (2016). Bio-Plastic From Waste Newspaper. *Interntional Journal of Engineering Research and Technology*, *February*, 24–27.

Tarique, J., Sapuan, S. M., & Khalina, A. (2021). Effect of glycerol plasticizer loading on the physical, mechanical, thermal, and barrier properties of arrowroot (Maranta arundinacea) starch biopolymers. *Scientific Reports*, *11*(1), 1–17. https://doi.org/10.1038/s41598-021-93094-y

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology*, *14*(3), 71–78. https://doi.org/10.1016/S0924-2244(02)00280-7

Truong, T. C. T., & Kobayashi, T. (2020). Pectin bioplastic films regenerated from dragon fruit peels. *Vietnam Journal of Science, Technology and Engineering*, *62*(4), 18–22. https://doi.org/10.31276/vjste.62(4).18-22

Tyagi, V., & Bhattacharya, B. (2019). Role of plasticizers in bioplastics. *MOJ Food Processing & Technology*, *7*(4), 128–130. https://doi.org/10.15406/mojfpt.2019.07.00231

Ulyarti, U. (2021). *Development of Yam-Starch-Based Bioplastics with the Addition of Chitosan and Clove Oil Development of Yam-Starch-Based Bioplastics with the Addition of Chitosan and*. *25*(2). https://doi.org/10.7454/mss.v25i2.1155

van den Oever, M., Molenveld, K., van der Zee, M., & Bos, H. (2017). Bio-based and biodegradable plastics : facts and figures. In *Handbook of Ecomaterials* (Issue 1722). https://doi.org/10.18174/408350

Venkatesh, A., & Sutariya, H. (2019). Studies on Formulation and Properties of Fruit Peel Waste Incorporated Edible Film and its Effect on Quality of Bread. *Journal of Packaging Technology and Research*, *3*(2), 99–108. https://doi.org/10.1007/s41783-019-00062-z

Wahyuningtyas, N., Suryanto, H., Rudianto, E., Sukarni, S., & Puspitasari, P. (2017). Thermogravimetric and Kinetic Analysis of Cassava Starch Based Bioplastic. *Journal of Mechanical Engineering Science and Technology*, *1*(2), 69–77. https://doi.org/10.17977/um016v1i22017p069

Wang, Y. (2008). Cellulose fiber dissolution in sodium hydroxide solution at low temperature. *Institute of Natural Fibres and Medicinal Plants*, *11*(3), 133. https://smartech.gatech.edu/bitstream/handle/1853/26632/wang_ying_200812_phd.pdf

Wilkins, M. R., Suryawati, L., Maness, N. O., & Chrz, D. (2007). Ethanol production by

Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. *World Journal of Microbiology and Biotechnology*, *23*(8), 1161–1168. https://doi.org/10.1007/s11274-007-9346-2

Xiong, B., Zhao, P., Hu, K., Zhang, L., & Cheng, G. (2014). Dissolution of cellulose in aqueous NaOH/urea solution: Role of urea. *Cellulose*, *21*(3), 1183–1192. https://doi.org/10.1007/s10570-014-0221-7

Yadav, A., Mangaraj, S., Singh, R., Das, K., Kumar, N., & Arora, S. (2018). Biopolymers as packaging material in food and allied industry. *~ 2411 ~ International Journal of Chemical Studies*, *6*(2), 2411–2418. http://krishi.icar.gov.in/jspui/handle/123456789/28956

Yeong, S. K., Idris, Z., & Hassan, H. A. (2012). Palm Oleochemicals in Non-food Applications. In *Palm Oil: Production, Processing, Characterization, and Uses*. AOCS Press. https://doi.org/10.1016/B978-0-9818936-9-3.50023-X

Yunus, M. A., Raya, I., & Tuara, Z. I. (2019). Synthesis cellulose from rice husk. *Indonesia Chimica Acta*, *12*(2), 79–83.

Zannini, D., Dal Poggetto, G., Malinconico, M., Santagata, G., & Immirzi, B. (2021). Citrus pomace biomass as a source of pectin and lignocellulose fibers: From waste to upgraded biocomposites for mulching applications. *Polymers*, *13*(8). https://doi.org/10.3390/polym13081280

Zykwinska, A. W., Ralet, M. C. J., Garnier, C. D., & Thibault, J. F. J. (2005). Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiology*, *139*(1), 397–407. https://doi.org/10.1104/pp.105.065912

APPENDICES

APPENDIX A

Figure A1: Dehydrating orange peel in dehydrator

Figure A2: Dried orange peel

Figure A3: Orange peel powder

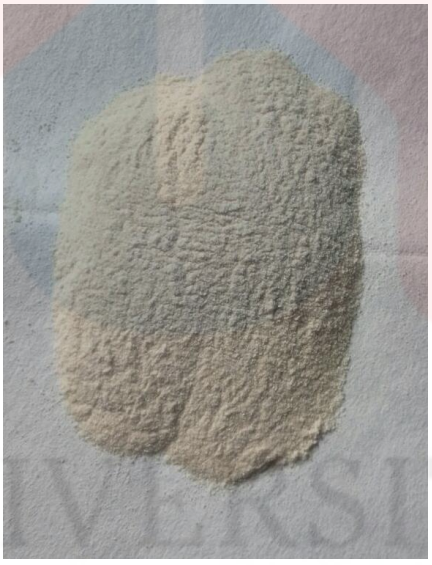


Figure A4: Pectin powder

Figure A5: Rice husk

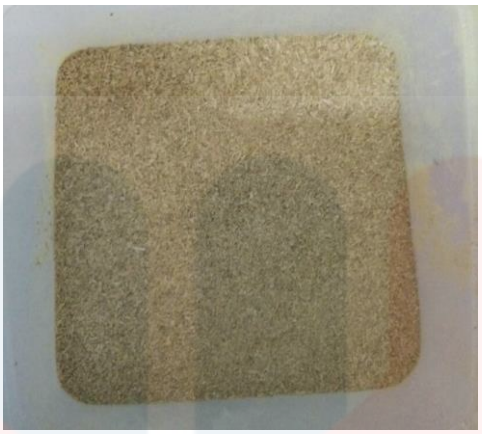


Figure A6: Rice husk powder

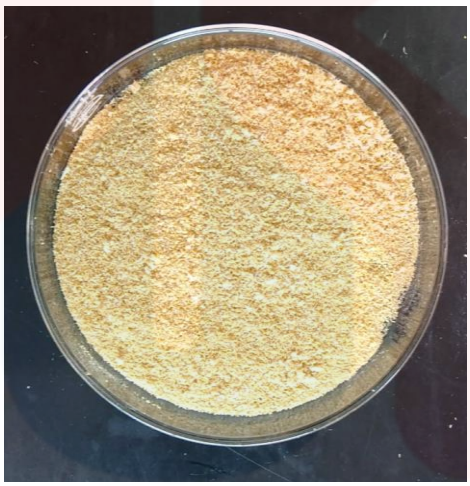


Figure A7: Cellulose powder

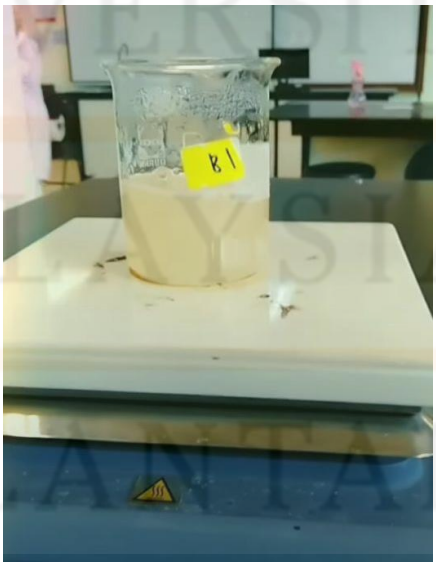


Figure A8: Film-formation solution

APPENDIX B

Table B1: Design Summary

File Version	13.0.5.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Central Composite	**Runs**	10.00
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	5.00		

Table B2: Experimental data of moisture content

Run	**Initial weight, W_i (g)**	**Final weight, W_f (g)**	**Moisture content (%) = $(W\_i - W\_f)/W\_i \times 100$**
1	0.1285 g	0.0845 g	34.24%
2	0.1286 g	0.0790 g	38.57%
3	0.2139 g	0.1336 g	37.54%
4	0.1231 g	0.0914 g	25.75%
5	0.1510 g	0.0910 g	39.74%
6	0.1623 g	0.0980 g	39.62%
7	0.1493 g	0.0911 g	39.98%
8	0.1365 g	0.0922 g	32.45%
9	0.1255 g	0.0873 g	30.44%
10	0.1082 g	0.1008 g	6.84%

Table B3: Experiment data of film thickness

Run	Reading					Average
	1	2	3	4	5	
1	0.17 mm	0.20 mm	0.15 mm	0.29 mm	0.16 mm	0.19 mm
2	0.06 mm	0.05 mm	0.18 mm	0.02 mm	0.16 mm	0.094 mm
3	0.33 mm	0.23 mm	0.21 mm	0.16 mm	0.23 mm	0.232 mm
4	0.20 mm	0.21 mm	0.10 mm	0.20 mm	0.17 mm	0.176 mm
5	0.05 mm	0.08 mm	0.09 mm	0.05 mm	0.06 mm	0.066 mm
6	0.14 mm	0.04 mm	0.26 mm	0.25 mm	0.18 mm	0.174 mm
7	0.16 mm	0.10 mm	0.27 mm	0.18 mm	0.05 mm	0.152 mm
8	0.05 mm	0.03 mm	0.25 mm	0.17 mm	0.24 mm	0.148 mm
9	0.04 mm	0.03 mm	0.06 mm	0.05 mm	0.13 mm	0.062 mm
10	0.01 mm	0.06 mm	0.04 mm	0.03 mm	0.06 mm	0.04 mm

Table B4: Experiment data of film density

Run	Mass of bioplastic	Volume film-formation solution	Film density
1	0.98 g	25 ml	0.0392 g/ml
2	0.76 g	17 ml	0.045 g/ml
3	1.18 g	30 ml	0.0393 g/ml
4	1.02 g	30 ml	0.034 g/ml
5	0.97 g	20 ml	0.0485 g/ml
6	1.18 g	25 ml	0.0472 g/ml
7	0.79 g	20 ml	0.0395 g/ml
8	1.05 g	30 ml	0.035 g/ml
9	0.67 g	25 ml	0.0268 g/ml
10	0.90 g	20 ml	0.045 g/ml

Table B5: Numerical optimization solutions (100 solutions)

No.	Ratio	Mass of plasticizer	Volume	Moisture Content	Film Thickness	Density	Desirability	
1	**6.001**	**0.408**	**26.184**	**41.654**	**0.230**	**0.049**	**1.000**	**Selected**
2	6.060	0.449	21.681	54.268	0.232	0.060	1.000	
3	6.060	0.477	23.457	50.995	0.239	0.057	1.000	
4	6.299	0.498	26.578	43.686	0.234	0.049	1.000	
5	6.076	0.460	20.918	56.696	0.233	0.062	1.000	
6	6.217	0.497	20.041	60.236	0.233	0.066	1.000	
7	6.167	0.494	24.509	48.789	0.238	0.055	1.000	
8	6.090	0.456	24.473	47.451	0.234	0.054	1.000	
9	6.086	0.454	20.574	57.175	0.231	0.062	1.000	
10	6.008	0.461	25.868	44.122	0.240	0.051	1.000	
11	6.092	0.483	23.943	49.911	0.239	0.056	1.000	
12	6.106	0.498	23.535	51.600	0.241	0.058	1.000	
13	6.012	0.448	20.665	57.016	0.234	0.062	1.000	
14	6.058	0.472	27.131	41.189	0.240	0.049	1.000	
15	6.191	0.474	21.496	55.421	0.231	0.061	1.000	
16	6.028	0.494	25.559	46.019	0.245	0.053	1.000	
17	6.024	0.416	25.438	43.709	0.230	0.050	1.000	
18	6.086	0.448	22.196	52.827	0.231	0.058	1.000	
19	6.074	0.460	24.883	46.591	0.236	0.053	1.000	
20	6.267	0.476	25.168	46.351	0.231	0.052	1.000	
21	6.043	0.461	22.736	52.217	0.236	0.058	1.000	
22	6.115	0.471	27.008	41.566	0.238	0.049	1.000	
23	6.107	0.449	23.263	50.136	0.231	0.056	1.000	
24	6.141	0.490	20.776	58.371	0.236	0.064	1.000	
25	6.068	0.443	22.550	51.792	0.231	0.057	1.000	
26	6.169	0.483	24.589	48.127	0.236	0.054	1.000	
27	6.086	0.438	26.473	41.889	0.233	0.049	1.000	
28	6.221	0.478	26.502	43.174	0.234	0.049	1.000	
29	6.290	0.500	23.956	50.262	0.233	0.056	1.000	
30	6.158	0.480	23.759	50.122	0.235	0.056	1.000	
31	6.147	0.496	20.111	60.426	0.237	0.066	1.000	
32	6.148	0.447	24.812	46.220	0.230	0.052	1.000	

33	6.030	0.431	26.213	42.297	0.233	0.049	1.000
34	6.264	0.500	26.815	43.106	0.236	0.049	1.000
35	6.001	0.431	20.014	57.772	0.230	0.062	1.000
36	6.027	0.443	20.032	58.242	0.231	0.063	1.000
37	6.024	0.495	22.985	53.108	0.244	0.060	1.000
38	6.076	0.497	21.851	56.163	0.242	0.062	1.000
39	6.204	0.491	22.546	53.625	0.234	0.059	1.000
40	6.111	0.494	24.238	49.558	0.241	0.056	1.000
41	6.181	0.475	20.404	58.266	0.231	0.063	1.000
42	6.098	0.450	21.462	54.704	0.230	0.060	1.000
43	6.011	0.433	25.739	43.551	0.234	0.051	1.000
44	6.063	0.470	21.411	55.995	0.236	0.062	1.000
45	6.192	0.486	20.642	58.230	0.233	0.064	1.000
46	6.062	0.455	23.548	49.811	0.235	0.056	1.000
47	6.060	0.483	24.648	48.070	0.241	0.055	1.000
48	6.145	0.463	23.799	49.331	0.233	0.055	1.000
49	6.108	0.461	20.059	58.723	0.231	0.064	1.000
50	6.148	0.459	26.215	43.221	0.234	0.050	1.000
51	6.066	0.476	24.329	48.639	0.239	0.055	1.000
52	6.003	0.484	21.229	57.462	0.243	0.063	1.000
53	6.098	0.459	21.417	55.271	0.232	0.061	1.000
54	6.126	0.450	25.744	44.086	0.233	0.050	1.000
55	6.140	0.462	20.712	56.980	0.230	0.062	1.000
56	6.050	0.471	21.195	56.659	0.237	0.062	1.000
57	6.093	0.483	23.724	50.490	0.239	0.057	1.000
58	6.219	0.492	20.151	59.691	0.232	0.065	1.000
59	6.008	0.435	25.209	44.934	0.234	0.052	1.000
60	6.035	0.429	26.503	41.531	0.233	0.049	1.000
61	6.033	0.446	23.992	48.381	0.235	0.055	1.000
62	6.201	0.472	23.728	49.774	0.232	0.055	1.000
63	6.255	0.488	21.227	56.588	0.230	0.062	1.000
64	6.020	0.487	20.615	59.247	0.242	0.065	1.000
65	6.041	0.448	21.867	53.820	0.233	0.059	1.000
66	6.210	0.488	20.960	57.444	0.232	0.063	1.000
67	6.129	0.453	25.579	44.590	0.233	0.051	1.000

68	6.023	0.472	20.377	59.024	0.239	0.064	1.000
69	6.295	0.489	26.109	44.564	0.233	0.050	1.000
70	6.069	0.495	21.930	55.847	0.242	0.062	1.000
71	6.009	0.478	22.043	54.962	0.241	0.061	1.000
72	6.101	0.477	24.644	47.825	0.238	0.054	1.000
73	6.043	0.477	24.096	49.340	0.240	0.056	1.000
74	6.067	0.442	23.134	50.290	0.232	0.056	1.000
75	6.019	0.471	21.509	55.985	0.239	0.062	1.000
76	6.141	0.465	26.680	42.237	0.235	0.049	1.000
77	6.066	0.482	21.068	57.500	0.239	0.063	1.000
78	6.090	0.484	25.918	44.751	0.240	0.052	1.000
79	6.247	0.475	25.204	46.260	0.232	0.052	1.000
80	6.142	0.470	20.469	58.033	0.232	0.063	1.000
81	6.107	0.450	22.554	51.961	0.231	0.058	1.000
82	6.048	0.435	23.261	49.698	0.231	0.056	1.000
83	6.094	0.456	21.023	56.134	0.232	0.061	1.000
84	6.097	0.450	21.686	54.124	0.231	0.059	1.000
85	6.066	0.480	27.044	41.637	0.241	0.049	1.000
86	6.005	0.434	20.007	57.942	0.231	0.062	1.000
87	6.033	0.463	22.330	53.381	0.237	0.059	1.000
88	6.027	0.439	24.786	46.126	0.234	0.053	1.000
89	6.240	0.488	24.843	47.618	0.234	0.053	1.000
90	6.069	0.486	22.462	54.006	0.240	0.060	1.000
91	6.011	0.498	27.587	40.514	0.246	0.049	1.000
92	6.071	0.474	24.993	46.827	0.239	0.054	1.000
93	6.058	0.495	20.231	60.514	0.242	0.066	1.000
94	6.020	0.495	27.459	40.818	0.245	0.049	1.000
95	6.103	0.494	24.927	47.699	0.241	0.054	1.000
96	6.164	0.454	25.653	44.441	0.232	0.051	1.000
97	6.000	0.398	20.001	55.942	0.222	0.060	0.987
98	6.000	0.333	20.000	52.290	0.207	0.055	0.959
99	6.000	0.328	20.000	51.985	0.206	0.055	0.956
100	6.000	0.302	20.000	50.511	0.200	0.053	0.944