ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

Production, Characterization and Optimization of Starch Based Biodegradable Bioplastic from Waste Potato Peel with the Reinforcement of False banana (*Ensete ventricosum*) Fiber

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By Tarekegn Yohannes Chaffa

A thesis Submitted to School of Chemical and Bio Engineering in partial fulfilment of the requirements for the Degree of Master of Science in Chemical Engineering (Process Engineering)

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This is to certify that a thesis work entitled **"Production, Characterization and Optimization of Starch Based Biodegradable Bioplastic from Waste Potato Peel with the Reinforcement of False banana *(Ensete ventricosum)* Fiber"** prepared by Tarekegn Yohannis Chaffa and submitted in partial fulfillment of the requirement for the Degree of Master of Science in chemical engineering (Process engineering) is compiled according with the regulation of Addis Ababa university and meets the acceptable standards with respect to originality and quality.

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DECLARATION

I declare this thesis work entitled **"Production, Characterization and Optimization of Starch Based Biodegradable Bioplastic from Waste Potato Peel with the Reinforcement of False banana *(Ensete ventricosum)* Fiber"** submitted for Master of Science in Chemical Engineering (Process engineering) at Addis Ababa Institute of Technology, Addis Ababa University is my own original work. I assure that there is no research work done and has been presented with such a title for an award of diploma, degree or any other in any college, university or institution. All the work obtainable in this research study is original and credit has been given by citation for every piece of information taken from journals, books and websites.

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ABSTRACT

Recently, environmental pollution from petroleum-based synthetic plastics become the headache of the world. Their greatest problem is that they are not biodegradable. To combat this serious issue producing biodegradable bioplastics is becoming the best solution ever. In this study, potato peel starch (PPS) based bioplastic film was developed. The proximate composition of potato peel was found to be 8.13, 4.89, 10.13, 0.27, 9.52 and 51.16 % by dry weight basis for moisture, ash, crude protein, crude fat, crude fiber and starch content, respectively. PPS contained 18.6, 0.01, 0.53, 0.00 and 27.93 % for moisture, ash, protein, fat and amylose, correspondingly. A bioplastic film was prepared by a method of gelatin casting, in which the effect of 13.33, 23.33 and 33.33 % v/w (HCl or glycerol to PPS ratio) concentration of HCl and glycerol as well as drying temperature (45^{o}C, 55^{o}C and 65^{o}C) was studied. The experiment was designed and optimized by a three-variable, three-level Box–Behnken design using Response Surface Methodology (Design Expert® software version 13.0.1). The ANOVA analysis showed that the model is significant with P<0.0001. The optimum values of the influencing factors were found to be HCl concentration 23.33 %v/w, glycerol 20 %v/w and drying temperature of 48^{o}C. Under optimal conditions a bioplastic film with tensile strength of 6.449MPa, percent elongation at break of 19.87%, biodegradability of 83.92 and water absorption of 59.94 % was synthesized with insignificant difference from model predicted result. A bioplastic film reinforced with 6% w/w False banana fiber has shown remarkably enhanced maximum tensile strength recorded to be 8.878MPa. Furthermore, analysis of FTIR, XRD and surface morphology were conducted. The produced bioplastic film had sufficient physicochemical properties confirming the usage of waste potato peel as a source of starch for the synthesis of biodegradable bioplastics that can substitute conventional plastics.

***Keywords*:** Potato peel, Starch-based, bioplastic, biodegradable, False banana fiber

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LISTS OF ACRONYMS AND ABBREVIATIONS

Abbreviations

a.u	arbitrary unit
Adeq	Adequate
BioD	Biodegradability
cm	centimeter
conc.	Concentration
df	degrees of freedom
dw	dry weight
eq	equation
et al	and others
Fig.	figure
g	gram
hrs.	hours
kV	kilovolt
mA	milliampere
min	minute
mL	milliliters
mm	millimeter
MPa	megapascals
Std. Dev.	Standard deviation
v/v	volume by volume ratio
v/w	volume by weight ratio
w/w	weight by weight ratio
wt.	weight
µm	micrometer

Acronyms

ABS	Acrylonitrile Butadiene Styrene
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
BBD	Box–Behnken design
Bio-PET30	Polyethylene containing 30% biobased
CCD	Central composite design
CEN	European Committee for Standardization
CV	coefficient of variation
DOE	Design of experiment
EB	Elongation at break
EN	European standards
FAO	Food Agriculture Organization
FTIR	Fourier transform infrared
HARC	Holetta Agricultural Research Center
IfBB	Institute for Bioplastics and Bio-composites
IR	Infrared
MEG	Monoethylene glycol
PE	Polyethylene
PET	Polyethylene Terephthalate
PHA	Poly Hydroxyalkanoate
PLA	Poly Lactic Acid
PP	Polypropylene
PPS	Potato peel starch
PTA	Purified terephthalic acid
RSM	Response surface methodology
TPS	Terephthalate Polystyrene
TS	Tensile strength
WA	Water absorption
XRD	X-ray diffraction

CHAPTER 1: INTRODUCTION

1.1. Background

The original meaning of the word plastic was "flexible and easy to form." In the past century and a half, humans have learned to make synthetic polymers, sometimes using natural substances such as cellulose, but more often using the abundant carbon atoms provided by petroleum and other fossil fuels. The development of plastics began with the use of natural materials with inherent plastic properties, such as shellac and chewing gum. However, all kinds of fully synthetic materials that we think are modern plastics began to develop about 100 years ago. (Rajendran et al. 2012; Science History Institute 2020; PlasticsEurope 2020)

The world's first-ever man-made cellulose-based plastic was "Parkesine", which was introduced by Alexander Parkes in 1862. The modified form of Parkesine was conceived in 1869 by John Wesley Hyatt, who was stimulated by a company in New York who offered a $10,000 quotation to anyone who could provide a substitute for ivory. By treating cellulose derived from cotton fibers with camphor (waxy transparent solid with a strong aroma which is found in the every green wood of the Camphor laurel), Hyatt discovered a plastic that can be made into various shapes and imitate natural materials such as tortoise shell, horn, linen and ivory (Barrett 2018; Science History Institute 2020).

Because of the expanded utilization of fossil-based fuel, resource limitation, continuous change of price, and effect on the climate and the environment, there has been a significant shift toward utilizing biodegradable materials (Syed 2016). In the 1970s and 1980s, the pollution caused by plastic packages considered "indestructible" had become a solemn subject creating anxiety about waste increased. The indestructible nature of conventional plastics in green way urged scientists and researchers to start discussions about conceivable solutions based on the search for materials that can degrade in landfills, henceforward research on biodegradable polymers became an imperative topic (Antonio J.F. Carvalho 2013; Science History Institute 2020). Bioplastics help in the reduction of reliance on petroleum-based polymers, the reduction of persistent plastic waste, and the better control of CO_2 emissions in the environment. (K.M. Gupta 2011).

Sustainable plastic products are biobased plastics which are produced with minimum environmental and social impacts. Sustainable plastic products can be defined as being produced

with lower carbon footprint, lower waste generation and lower pollution generation than virgin petroleum-based plastics and end-of-life using composting processes rather than being sent to landfill (Greene 2014).

Polysaccharides represent by far the most abundant biopolymers on earth, with cellulose, chitin, and starch dominating. Starch has been one of the choice materials since the inception of human technology and it is certainly one of the most versatile materials for potential use in polymer technology. The development of novel uses for starch-based products, in the context of the growing demand for renewable-resource-based materials, has surely raised demand for starch production. When starch granules are heated in an excess of water (90% w/w) or of another solvent able to form hydrogen bonding starch undergoes an irreversible order-disorder transition known as gelatinization or destructuration (Antonio J.F. Carvalho 2013).

The production technologies employed in the case of film forming technique for bioplastic includes casting, molding (Compression and injection molding) and extrusion (Single-screw and twin-screw extruders) methods (Gurram et al. 2018; Abe et al. 2021). From waste potato peel one can extract starch and synthesize starch-based biodegradable bioplastic using one of the listed films forming technology.

1.2. Statement of the problem

Plastic pollution is the accumulation of synthetic products in the environment to the point where they pose a threat to wildlife and their habitats, as well as human populations. Plastics have been discovered to be persistent pollutants of numerous environmental niches, from Mount Everest to the bottom of the sea, by the end of the twentieth century. In conjunction with this, worldwide annual plastic production increased from 1.5 million tonnes in 1950 to 275 million tonnes in 2010 and 359 million tonnes in 2018 (Moore 2020). The production of plastics has come to at 550 million tons in 2019 (K. Kumar, Kannan, and Senthilvelan 2020). By projecting current global waste management trends to 2050, it is assumed that the primary plastic waste generated will be around 26,000 Mt (Dobo et al. 2019). In 2015, Ethiopians consumed roughly 172,000 tons of plastic. By 2020, this figure was predicted to rise to 308,000 tons of plastic (Statista 2021). The main problem with these abundantly produced synthetic plastics is that they are not biodegradable or fully breakdown in landfills. In line with this, their incineration is one of the main inputs for the increase of emission of pollutant gases to the environment where the environmental pollution is

the main concern of the whole world currently. That is why production of biodegradable bioplastics become the best way to combat the problem.

The estimation of FAO showed that in 2018 more than 368 million metric tons of potatoes were produced globally, a substantial increase from 333.6 million tons in 2010 (Shahbandeh 2020). Ethiopia has the biggest potential for potato production among African countries. About 70% of its arable land is thought to be suitable for potato production, mostly in highland areas above 1 500 m (PotatoPRO 2020). Recently, processed products of potatoes such as French fries, chips, and puree have experienced growing popularity (Arapoglou et al. 2010). Potato chips are the most popular food-processed potato product, and they generate a lot of potato peel as a by-product (Gebrechristos and Chen 2018). Industrial processing generates between 70 and 140 thousand tons of potato peels worldwide annually (D. Wu 2016). Waste potato peels are generated in bulk from small scale chips processors and from Ethiopian universities student's cafeteria. The potato peel wastes from these source end primarily as waste causing environmental pollution and in some cases as animal feed. In this way, the country is losing high amount of starch with dumped peels due to inefficiency of peeling. But it is better to use the waste potato peels, which contains high amount of starch, as a raw material for the production of starch based biodegradable bioplastics.

The use of potato peels as a raw material for the production of starch-based bioplastic is not new for researchers. But their works were limited on the investigation of the effect of drying temperature on bioplastic film properties. Thus, in this study investigation of drying temperature as well as the analysis of the effect of plasticizer and the gelatinization assisting chemicals were conducted.

The application of bioplastics has been limited because of their low mechanical strength. Even though numerous studies have showed the use of synthetic fibers as reinforcements in bioplastics due to their strong mechanical properties, they are problems to the environment by themselves possessing non-biodegradability characteristics. In comparison to synthetic fibers, application of lignocellulosic fiber is biodegradable, renewable, low cost and widely available. Compared to cellulose reinforced bioplastics, there were much fewer studies on lignocellulosic fibers and lignin reinforced bioplastics (Yang, Ching, and Chuah 2019). Among the lignocellulosic fibers, False banana *(Ensete ventricosum)* fiber is abundantly found in Ethiopia. To our knowledge, in a search for done researches on False banana *(Ensete ventricosum)* fiber as reinforcement for bioplastic

there were only limited documents. This shows using this fiber as new source for the reinforced bioplastic product introduces mechanically enhanced bioplastic film to the world. Hence, in this study the False banana *(Ensete ventricosum)* fiber was used as reinforcement for the synthesis of reinforced starch-based bioplastic to assess its input on tensile strength.

1.3. Research questions

- Is it possible to produce starch-based biodegradable bioplastic from waste potato peel?
- What are the compositions of waste potato peel?
- What are the acceptable physiochemical properties to characterize the synthesized bioplastic?
- Does the concentration of glycerol, HCl and drying temperature affect the physiochemical properties of synthesized biodegradable-bioplastic?
- Can one enhance the tensile strength of bioplastic film by reinforcing with False banana (*Ensete ventricosum*) fiber?

1.4. Research objective

1.4.1. General objective

The general objective of this research was producing a starch based biodegradable bioplastic from waste potato peel starch and investigation of False banana fiber reinforcement.

1.4.2. Specific objectives

The specific objectives were:

- To analyze the proximate composition of the raw material potato peel and starch extracted from fresh potato peel.
- To investigate the effect of glycerol concentration, HCl concentration and drying temperature on mechanical properties, biodegradability and water absorption of PPS based bioplastic; and to optimize the process variables.
- To characterize the physiochemical properties of the synthesized biodegradable bioplastic.
- To examine the effect of using False banana *(Ensete ventricosum)* fiber reinforcement at different loading ratio on tensile strength of PPS based bioplastic film.

1.5. Significance of the study

This study will play a great role by showing a way that waste peels containing starch can be utilized as a raw material for the production of valuable products like bioplastics and will motivate researchers to identify the composition of peels from agrofood processing industries to use them for the production of valuable products as per their composition. In another word, this study has its own part in knowledge generation. Moreover, it has outstanding importance on awareness creation for Environmental Protection Agencies, policy makers and community on the valorization of potato peel waste. Again, this study will be striking example for a usage of False banana (*Ensete ventricosum*) fiber as a reinforcement in bioplastic film products to enhance their mechanical properties. Finally, the data generated in this study can be used as the steppingstones for other researchers whose concern matches the area of this study.

1.6. Scope of the study

The depth, width and length of this research was the production of starch-based biodegradable bioplastic from a waste potato peel starch. The collected fresh potato peel was washed and used for the extraction of starch from it. The extracted starch was dried and ready to be used for the synthesis of bioplastic. In this study HCl was used to break-down the branched amylopectin found in the starch and glycerol to plasticize. Enset fiber was used as reinforcement to produce starch-based reinforced biodegradable bioplastic to analyze its effect on tensile strength. Investigation of the effect of plasticizer concentration, HCl acid concentration and drying temperature were part of this research, and also characterization of the bioplastic product by biodegradation with enzyme action, tensile strength, elongation at break, Morphological analysis, XRD analysis and FTIR analysis were incorporated.

CHAPTER 2: LITERATURE REVIEW

2.1. Plastic

A plastic is a synthetic or man-made polymer that is similar in many respects to the natural resins originated from trees and other plants. Plastics are a by-product of the petroleum refining process. Plastics are defined by Webster's Dictionary as "any of a variety of complex organic compounds produced by polymerization that can be molded, extruded, cast into various shapes and films, or formed into filaments and used as textile fibers" (S. Pathak, Sneha, and Mathew 2014). These petroleum-based plastics are synthetic plastics and most commonly called plastic or conventional plastic. Conventional plastics are made from fossil fuels and are non-biodegradable. They include Polyethylene (PE), Polypropylene (PP), Polyethylene Terephthalate (PET), Polystyrene (PS), Acrylonitrile Butadiene Styrene (ABS) etc. (Kripalani 2020).

2.1.1. Beginning History of Plastic

At the London International Exhibition in 1862, Alexander Parkes unveiled the world's first man-made plastic. The plastic was named "Parkesine" by him. Parks found this plastic while trying to make a synthetic substitute for shellac for water proofing, and it was promoted as a replacement for ivory and horn. Parkesine was a biobased plastic made from cellulose and hence can be called bioplastic. Despite the fact that Parkesine was not a commercial success, it was a significant step forward in the development of man-made plastic. In Albany, New York, in 1869, John Wesley Hyatt developed a means to manufacture a better form of Parkesine by treating cellulose obtained from cotton fiber with camphor. Celluloid is the most frequent name for the improved plastic. (Science History Institute 2020; Barrett 2018; Plastics Industry Association 2020). From this information it can be said that the first man-made plastic in the era of plastic development was bioplastic.

2.1.2. Problems related to plastic

Today's average consumer comes into everyday touch with a variety of plastic materials, the majority of which are produced from petroleum and were developed specifically to resist natural degradation processes. Synthetic plastics tend to persist in natural environments because they are mainly nonbiodegradable. Many lightweight single-use plastic products and packaging materials, which account for roughly half of all plastics produced, are discarded improperly at or near the

point where they no longer serve a purpose for the consumer. They pollute the environment as soon as they are dropped on the ground, thrown out of a car window, heaped onto an already full trash bin, or inadvertently carried away by a gust of wind. Plastic pollution isn't just a problem on land. In 2014, the first oceanographic research to analyze the amount of near-surface plastic waste in the world's ocean indicated that at least 5.25 trillion individual plastic particles weighing around 244,000 tonnes(269,000 tons) were floating on or near the surface. (Moore 2020).

Fig. 2.1. Plastic pollution by (A)Plastic bags and bottles littered on a beach. (B)plastic bottles and other garbage in a lake (Moore 2020)

2.2. Bioplastics

A bioplastic is a plastic manufactured in partly or entirely from polymers generated from biological sources such as sugarcane, potato starch, or tree and straw cellulose. Some bioplastics breakdown naturally in the atmosphere, while others are designed to compost in an industrial composting plant with the help of fungi, bacteria, and enzymes (Bioplastics Guide 2016b). Bioplastics have gotten a lot of attention as a possible substitute for conventional fossil-based plastics because of the reason that they are produced from renewable resources (Brodin et al. 2017). The work of (Siakeng et al. 2019) has also stated that the development of most bioplastic is assumed to reduce fossil fuel usage, and plastic waste, as well as carbon dioxide emissions. Furthermore, they stated that the

biodegradability properties of these plastics have a positive impact on society, and so that the increased awareness of biodegradable packaging attracts researchers and industries.

2.2.1. Beginning History of bioplastics

In 1862 Alexander Parkes (United Kingdom, London) created Parkesine, the first man-made plastic made from cellulose. Parkesine was a biobased plastic, a bioplastic. Galalith is invented by German chemists in 1897. It's a biodegradable plastic made from casein (milk). Commercial breakthrough was limited for several reasons. Galalith could not be moulded. Milk was scarce and the development of oil-based plastics were boosted during WWI. Galalith is still used today to make buttons. In 1907 Leo Baekeland invented Bakelite that was coined a 'National Historic Chemical Landmark' due to its importance. Bakelite was a synthetic plastic that was revolutionary for its electrical non-conductivity and heat-resistant properties in electrical insulators, radio and telephone casings and other products such as kitchenware, jewelry, toys and firearms (Barrett 2018).

2.2.2. Classification of bioplastic

Bioplastics are classified as biodegradable and non-biodegradable based on biodegradability (Kripalani 2020; Bioplastics Guide 2016b).

- **Biodegradable bioplastics:** These are usually materials made from cellulose acetate (plant based) or lactic acid-based blends (or pure starch blends) and are used for primarily single use plastics like cutlery or some applications across pens / toys etc. Examples: Poly Lactic Acid (PLA), Poly Hydroxyalkanoate (PHA), Polyhydroxybutyrate (PHB), Starch-based and Cellulose-based bioplastics.
- **Non-biodegradable bioplastics:** Plastics are generally comprised of carbon, hydrogen, oxygen, etc. If the source of carbon is completely/partly from petrochemicals, then the plastic is said to be non-biodegradable. Example: Bio-PE (which are made of regular PE and plant-based PE, mixed in a proportion), Bio-PET (70/30 blend of PTA and MEG), etc. Bio-PE is also called as Green- PE.

2.2.3. Biodegradation of Bioplastics

The first step of biodegradation mechanism of biodegradable polymers involves oxidation or hydrolysis by respective enzymes to create functional groups that improve its hydrophobicity.

Thereby, high molecular weight plastic polymers are degraded into low molecular weight compounds having delicate mechanical properties, thus, making it more accessible for further microbial assimilation (Roohi et al. 2017). Enzymes play a major role in microbial degradation of plastics where microbial exoenzymes degrade complex polymers into small molecules of short chain length as monomers, dimers and oligomers, which are capable of passing through the semi-permeable bacterial/fungal membranes where they can be utilized as carbon and energy sources and further mineralized (Shah et al. 2008). If the size of these cleaved polymers is too large, then they are first depolymerized to pass through the cellular membrane, where they can be absorbed and biodegraded by intracellular enzymes within microbial cell (Roohi et al. 2017).

The previous work of (Tokiwa et al. 2009) has cited that Takagi *et al.* developed polycaprolactone/gelatinized starch blends using corn starch and assessed their biodegradability by an enzyme called α-amylase. The same researchers have given an emphasis on the two-step process enzymatic degradation of bioplastics by hydrolysis. Their paper explains that, first, the enzyme binds to the polymer substrate then subsequently catalyzes a hydrolytic cleavage. Furthermore, polymers are degraded into low molecular weight oligomers, dimers and monomers and finally mineralized to carbon dioxide gas and water. This shows that the of use α-amylase enzyme for the test of biodegradation of starch-based bioplastic is suitable.

2.2.4. Statistics related to bioplastic globally

The data compiled in cooperation with respected scientific partners of IfBB-Institute for Bioplastics and Bio-composites (University of Applied Sciences and Arts Hannover, Germany) and the nova-Institute (Hürth, Germany) shows that bioplastics manufacturing capacity was expected to rise from around 1.6 million tonnes in 2013 to around 6.7 million tonnes by 2018.(European Bioplastics 2014).

Asia's significance as a key production hub has grown with a view to regional capacity development. The majority of the projects currently being implemented are in Thailand, India, and China. By 2018, Asia had produced over 75% of all bioplastics. In comparison, Europe, which has been at the forefront of research and development, has only around 8% of the production capacity. (European Bioplastics 2014).

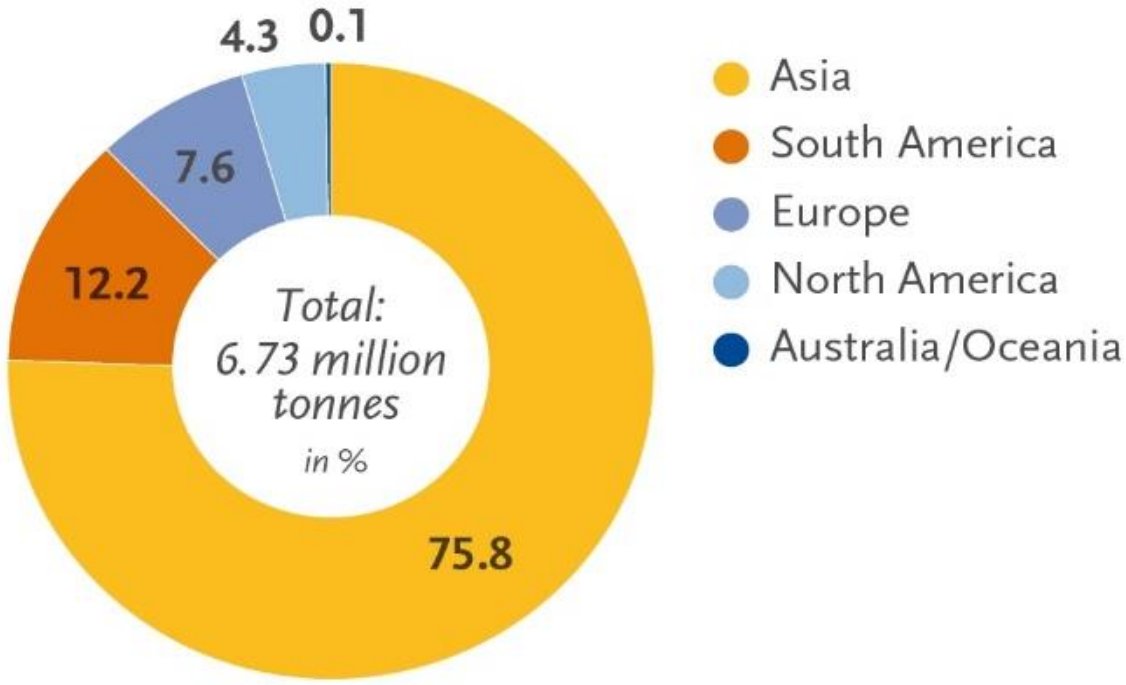


Fig. 2.2. Global production capacities of bioplastics by region in 2018

Global production capacities of bioplastics by material type in 2019 was dominantly hold by biobased non-biodegradable bioplastic so called Bio-PET30 which is 76.5% from the total production of 7.85 million tonnes as presented in Fig. 2.3. This shows that the production of Bio-PET30 in amount was about 6 million tonnes. Biodegradable polyesters take 6.6% and PLA takes 5.6% share of the total production. Biobased non-biodegradable bioplastic takes 83.6% and biodegradable take the rest.

The pie chart depicted in Fig. 2.3 has exposed that the global production capacity of biodegradable bioplastics is still less (approximately 16%) when compared with that of non-biodegradable. This shows that industries of different country should give attention to increase their production capacities of biodegradable bioplastics by taking in consideration the environmental problem issues related with non-biodegradable plastics.

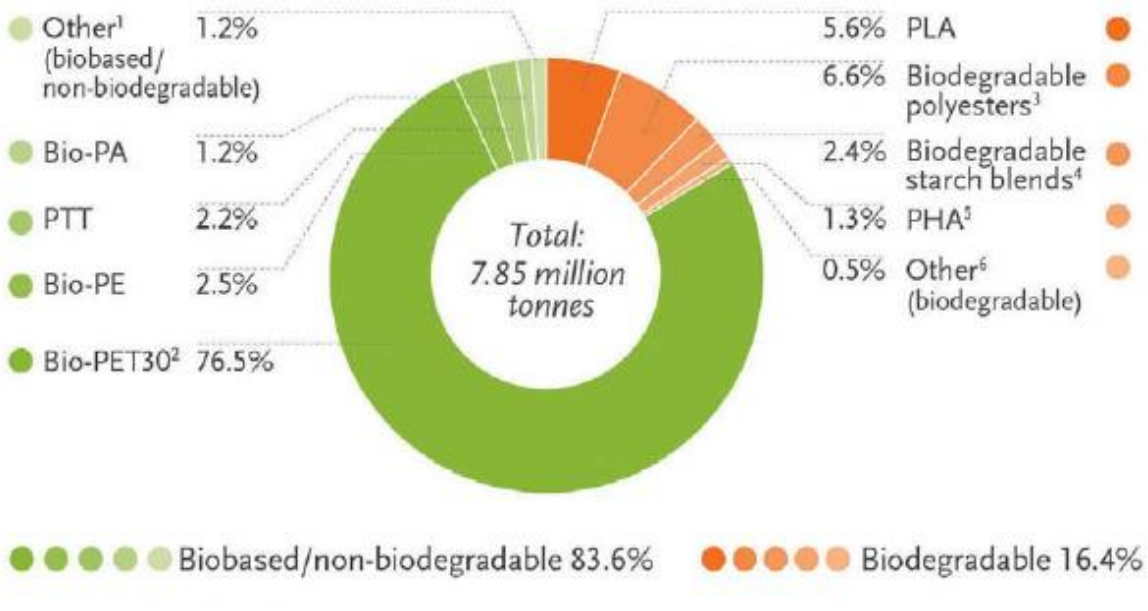


Fig. 2.3. Global production capacities of bioplastics by material type in 2019 (Source: European Bioplastics, nova-Institute, 2019)

As shown in Fig. 2.4 flexible packaging has the biggest market segment within the bioplastics industry in the year 2019. It takes 43% from the total production followed by rigid packaging 16%, agriculture and horticulture 14%, and coatings and adhesives 9%.

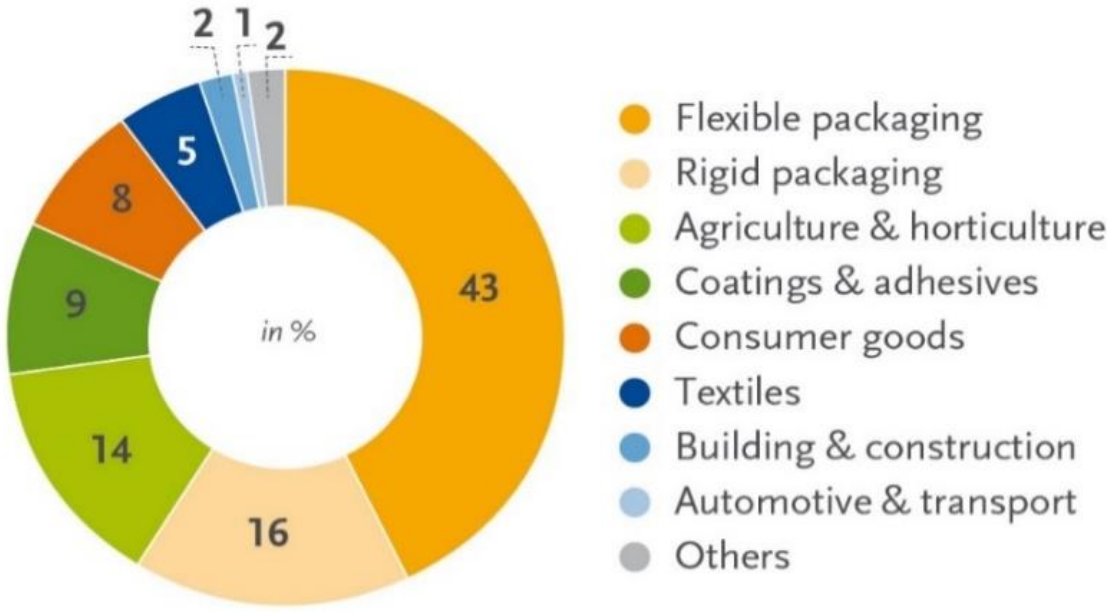


Fig. 2.4. Biodegradable plastics by market segment in 2019 (Source: European Bioplastics, nova-Institute, 2019)

2.2.5. Application of bioplastic

In search of new material solutions and proceeding an eye on the goal of sustainable production and consumption, bioplastics have various (potential) advantages. Advances in how they are produced is letting bioplastics with manageable physical properties to be produced enabling uses which mimic those of oil-based plastics. The properties of some bioplastics allow novel functions to be carried out. Injection molding, extrusion, and thermoforming are just a few of the ways bioplastics can be processed in a similar manner to petrochemical plastics. Bioplastic polymers can be combined with co-polymers or other polymers to boost their tensile strength (Bioplastics Guide 2016a).

Current end-use segments of bioplastics can be fall under biodegradable(short-lived) products and non-biodegradable (durable) products. Biodegradable(short-lived) bioplastic products can be used in packaging (Shopping bags, compostable waste collection bags, trays and Punnets for vegetables, fruits, meat and eggs), disposable catering service wares and medical applications (Implants such as screws, pins or plates, and material for pills and capsules). In another side, non-biodegradable (durable) bioplastic products can be applied in automotive interiors like seats, head rests or arm rests, and Mobile phone cases. There are also emerging application areas of bioplastics which includes 3D printing, metalized biaxial oriented (PLA films for food packaging), baby products (Toys and Teethers), modified PLA for durable applications (Interiors and under the hood automotive parts) (Bioplastics Guide 2016a).

In relation to the production of packaging, conventional plastics are being replaced by bioplastics at a rapid pace. There's a big demand for bioplastic packaging and it is the largest segment of the European bioplastic market – estimated at around 44% of 2.05 million tonnes in 2017. In 2019, global manufacturing capacities of bioplastics amounted approximately 2.11 million tonnes with almost 53 % (1.14 million tonnes) of the volume destined for the packaging market – the biggest market segment within the bioplastics industry. Bioplastic packaging options include bags for compost, agricultural foils, horticultural products, nursery products, toys and textiles. They are also often used for disposable cups, salad bowls, plates, clingfilm and food containers. Food shelf life has been substantially enhanced because of the introduction of sophisticated bioplastic packaging for food products (Key Plastics 2018; European Bioplastics 2020).

Fig. 2.5. Application areas of bioplastics in everyday life (European Bioplastics 2020)

2.3. Raw material and Chemicals for production of bioplastic

2.3.1. Waste Potato Peel

Potato (*S. tuberosum*) is the world's fourth most widely cultivated food crop after wheat, rice and maize. It is one of the fastest expanding food crops in Sub-Saharan Africa. Ethiopia has considerable potential for potato cultivation (P. D. Pathak et al. 2018; Brasesco, Asgedom, and Casari 2019). The climatic and edaphic conditions in Ethiopia's agro-ecologies were suitable for producing high yielding and high-quality potatoes. Since the registration of the first potato variety (AL-624) in 1987, more than 36 potato varieties were released by the research centers and higher educational institutions. Researchers has done different studies on more than eighteen potato varieties of the total (Gebreselassie, Wahassu, and Shimelis 2016; Tessema, Mohammed, and Abebe 2020).

In Ethiopia, the amount of potato supplied to the market in the years 2015/16 and 2016/17 was 338,163, 200 kilograms and 270,721, 880 kilograms, respectively. As literatures done shows, the production and demand of potato increased from year to year (Worku 2019). The information gathered from Holetta Agricultural Research Center (HARC) during the month March of this year indicated that the potato varieties like Gudene and Jalene would be the most commonly supplied varieties to central Addis Ababa in that season from different parts of the country. The intensive use of potato is now generating potato peel wastes all around the corner of Ethiopia.

Potato peel, a byproduct of the food processing industry, has the potential to be a low-cost, high-value, and affordable starting material for the synthesis of commercially important substances, value addition, and product extraction, including dietary fiber and biopolymers. The knowledge of physical and chemical composition of potato peel would help in devising an environmentally friendly approach for the utilization of potato peel. Potato peel is rich in starch consisting 52% by dry weight (Javed et al. 2019).

2.3.2. Starch

Plastics that can degrade biologically are made from starch, cellulose, chitosan, and protein extracted from renewable biomass (Marichelvam, Jawaid, and Asim 2019). Starch is an abundant naturally occurring material essentially comprising two kinds of glucose macro molecules namely amylose and amylopectin. Amylose is a polymer consisting of a linear structure, while amylopectin is a polymer consisting of branched structure. These two polysaccharides constitute 98-99% by weight of the starch. Of them amylose makes up roughly 20-30% by weight of starch, while amylopectin makes up 70-80% (S. Kumar and Thakur 2017; Shafqat et al. 2020). The higher the amylose content, the stronger the film; however, the branching structure of amylopectin often results in a film with poor mechanical properties which can be improved by using plasticizers such as sorbitol and glycerol (S. Kumar and Thakur 2017).

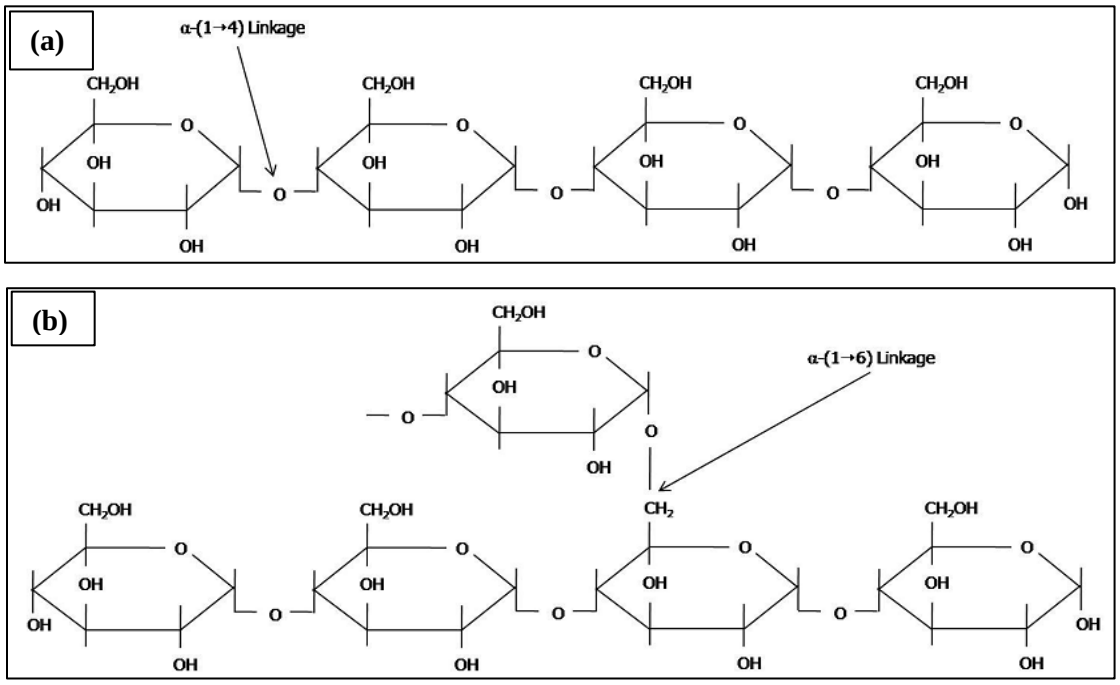


Fig. 2.6. Major Components of Starch. (a). Linear Amylose Chain (b). Branched Amylopectin chain (Grace and Liew 2016)

Because starch granules are insoluble in cold water, they absorb water and swell-up only when heated. The heating of starch solution leads it to gelatinize. Gelatinization is basically the structural change of starch granules that takes place during heating with a specific starch-water ratio. It is the breakdown of molecular order inside the starch granule, as well as any associated and irreversible alterations resulting in change of the properties (Grace and Liew 2016). Starch gelatinization is a complicated process in which starch granules inflate and become disrupted as a result of the action of a plasticizer, and the crystallinity of starch macromolecules is partially or totally lost (Lara and Salcedo 2016).

2.3.3. Hydrochloric acid

Starches are modified to make them suitable for specific applications. They are treated with acid to function properly during processing. Chemical modifications to starch are usually done by reacting with the hydroxyl groups in the starch molecule (K.M. Gupta 2011). In the production of starch based biodegradable bioplastic, we need to break the complex branched amylopectin chains of starch. When starch is blended with acids like HCl or acetic acid (Vinegar) and water while heating on a hot plate they assist easily formation of gelatin and plasticization.

2.3.4. Plasticizer

An appropriate plasticizer selection in the formulation of plastic materials is a critical step in achieving the desired physical and thermomechanical properties for each ultimate application. Polyols, particularly glycerol, have been widely employed as plasticizers in the manufacturing of numerous bioplastics (J.M.Aguilar et al. 2020).

The most common plasticizers for starch are water and glycerol (Greene 2014). In addition to water, glycerol and citric acid, there are other complex plasticizers like 1-ethyl-3-methylimidazolium acetate, sorbitol or triethylene glycol that have been successfully used. Among the conventional plasticizers, glycerol is much more effective than water. However, glycerol has shown lower tensile strength and water barrier properties. (Syed 2016; Abera et al. 2020). Glycerol has a lesser molar mass (nearly half) than sorbitol, which accounts for its superior plasticization ability, and thus allowing the molecules of glycerol and starch to interact more efficiently (Shafqat et al. 2021).

2.4. Lignocellulosic fiber reinforcement in bioplastic

With the growing environmental impact related to the production, disposal, and recycling of synthetic fiber based polymeric matrix composites, environmentally friendly composites have been developed using materials based on natural resources. Disposal of agro-waste from industrial crops has recently become a major concern in developing countries. The use of agro-waste for the development of sustainable composite materials is increasing as a result of its availability. A high fiber loading is generally desired for bio-composites to achieve good mechanical performances. (Nur Syafiqaz, Chen, and Ahmad 2021).

Natural fiber reinforced polymer composites are lightweight, economical and available in a variety of forms. They have low densities, comparable material properties, high molding flexibility and are environmentally friendly, making them a conceivable substitute to traditional fillers like mica, calcium carbonate and glass (Pandey et al. 2010). According to (ASTM D883−17 2017) the definition of reinforced plastic was stated that it is a plastic with high strength fillers imbedded in the composition, resulting in some mechanical properties superior to those of the base resin.

2.4.1. False banana fiber

In Ethiopia, over 80% of False banana (*Ensete ventricosum)* production is intense in south and south western part of the country. Enset foods used as primary food for about 20% of the entire Ethiopian population. The plant is harvested and huge amounts of biomass residues are originated, principally from the pseudo stem (i.e., fiber bundles obtained from the leaf sheaths after being scrapped to produce starchy food) and the inflorescence stalk. (Getachew, Desta, and Bekele 2016; Berhanu, Kiflie, Miranda, et al. 2018).

The interest in using natural fibers has increased significantly in the last few years, especially because of its use as an agent of reinforcement. The abundance in nature combined with the ease of its processing is an attractive feature, which makes it an important substitute for synthetic fibers which are potentially toxic. Low cost, low density, biological degradability, renewability, strong mechanical properties, and non-toxicity are just a few characteristics of lignocellulosic fibers which make their usage advantageous (Getu 2012).

False banana fibers have a comparatively low content of extractives (4.4 percent), of which more than 90 percent were polar compounds that are soluble in ethanol and water. It also contains lignin

10.5%, holocellulose 87.5% and α-cellulose 59.6%. It was found that holocellulose and α-cellulose contents were high (Berhanu, Kiflie, Miranda, et al. 2018). As (Brodin et al. 2017) stated, lignocellulosic materials from agricultural residues have the chemical composition of 41-52% cellulose, 25-27% of hemicelluloses and 18-25% lignin, 0.5-10% of other components as extractives.

2.5. Bioplastic production technologies

According to (Rajeev and Katrin 2020) bioplastic synthesizing means can be classified into five main groups based on raw material origin and corresponding polymer production technology.

1) Extracting directly from biomass;

2) Producing by natural or genetically modified organisms;

3) Synthesizing from bio-based monomers;

4) Synthesizing from petrochemicals;

5) Producing by combining the above-mentioned technologies and polymers derived therefrom.

Bioplastics could be produced by biomass extraction from naturally occurring biopolymers such as polysaccharides (e.g. starch, cellulose) and proteins (Rajeev and Katrin 2020). The work of (Gurram et al. 2018; Abe et al. 2021) exposed technologies used in bioplastic production such as casting, molding and extrusion technology on the basis of film formation technique. Rendering this, the technology of direct extraction from biomass and solution casting technique for film formation was employed in this research work.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study location

The experimental work for this thesis paper was conducted at AAU Process Engineering laboratories of School of Chemical and Bio-Engineering at Addis Ababa institute of Technology and in College of Natural Science of Addis Ababa University at the Center for Food Science and Nutrition. The compositional analysis of potato peel was partly done at Ethiopian Public Health Institute, Food and nutrition laboratory. Some of the characterization of synthesized bioplastic product was carried-on at Adama Science and Technology University and Addis Ababa Science and Technology University

3.2. Materials, Chemicals and Equipments

3.2.1. Materials

The main starting raw material used for this study was waste fresh potato peel. The fresh potato peel was collected from local chips processing shop located around Addis Ababa institute of Technology, Addis Ababa. The part of collected fresh potato peel was dried for four days in sunlight for compositional analysis of the potato peel and the rest was freshly used for the extraction of starch. Whereas, False banana fiber which was used for reinforcement, was collected from Amaro Kelle special woreda which is found in South Nation and Nationalities People Region.

3.2.2. Chemicals and Reagents

There were different chemicals and reagents used when doing the experiment. All the chemicals used were analytical grade. Some of the chemicals used were liquid and others were solid in state when bought from the chemical selling shops. The major chemicals used were presented in Table 3.1.

Table 3.1. Lists of major chemicals and reagents used in this study with their purpose

Chemical Name	Sourced	State	Purpose in this study
Hydrochloric Acid (HCl, 35% w/v)	Hill Mark Trading PLC, Addisu Gebeya, NEW/DG-005	Liquid	To break down the branched chains of Amylopectin part of starch to assist gelatinization

Sodium hydroxide (NaOH, assay:99.8%)	RANCHEM Industry and Trading, Kirkos Shopping Center C-322	Solid Pellets	Used for neutralization after gelatinization and used in amylose determination.
Glycerol ($C_3H_5(OH)_3$, 99.8% purity)	Hill Mark Trading PLC, Addisu Gebeya, NEW/DG-005	Viscous liquid	Used as plasticizer in the production of bioplastic film
Ethanol (CH_3CH_2OH, 99.9 %)	Fine Chemical General Trading, Addis Ababa	liquid	Used in amylose determination
α-amylase Enzyme	BGI Ethiopia, Beer factory, Addis Ababa	Liquid	Used for the test of biodegradability by Enzymatic action.
Distilled water	Center for Food Science and Nutrition, College of natural science, AAU	Liquid	For solution preparation, to wash instruments and for mixing all the ingredients during bioplastic film preparation

3.2.3. Equipments and Instruments

Various equipments and instruments were used to extract starch, to analyze proximate composition and to analyze the physicochemical properties. Instruments like plastic bag, mixing blender, muslin cloth, chopping board, measuring cylinders, beakers, glass stirring rod, pipette, crucibles, desiccator, funnel, scissor and ruler were used. In addition to these instruments cutting mill, weighing balance (OHAUS CORP-ARA520), drying oven (DHG-9055A), hot air oven (OV/125/SS/F/DIG/A), temperature adjustable electric hot plate (STUART UC125), Muffle furnace (CSF12/13-Astone Lane, Hope Sheffield, S30 2RR, England), Optical Microscopy (B-150 OPTIKA, Italy), Attenuated Total Reflection Fourier Transform Infrared Spectroscope (Thermo SCIENTIFIC iS50 ABX), Spectrophotometer (SPECTRO UV-VIS DOUBLE BEAM PC, 8 SCANNING AUTO CELL, UVD-3200. LABOMED.INC.), Vortex mixer (MS 3 D, IKA®, USA), Texture analyzer (Model TA Plus, LLOYDЖ Instruments, England), Soxtec unit (Soxtec™ 2055 Fat analyzer, Soxtec, Sweden) and Kjeldhal analyzer (Kjeltec™ 2300 analyzer, Wagtech, Sweden) were used.

3.3. Experimental Framework

The general experimental framework included in this study ranges from the sample collection to the production of biodegradable bioplastic film and its characterization. Preparation of False banana (*Ensete ventricosum*) fiber preparation and production of False banana fiber reinforced bioplastic was also part of this general framework. The general experimental framework is depicted in the figure below.

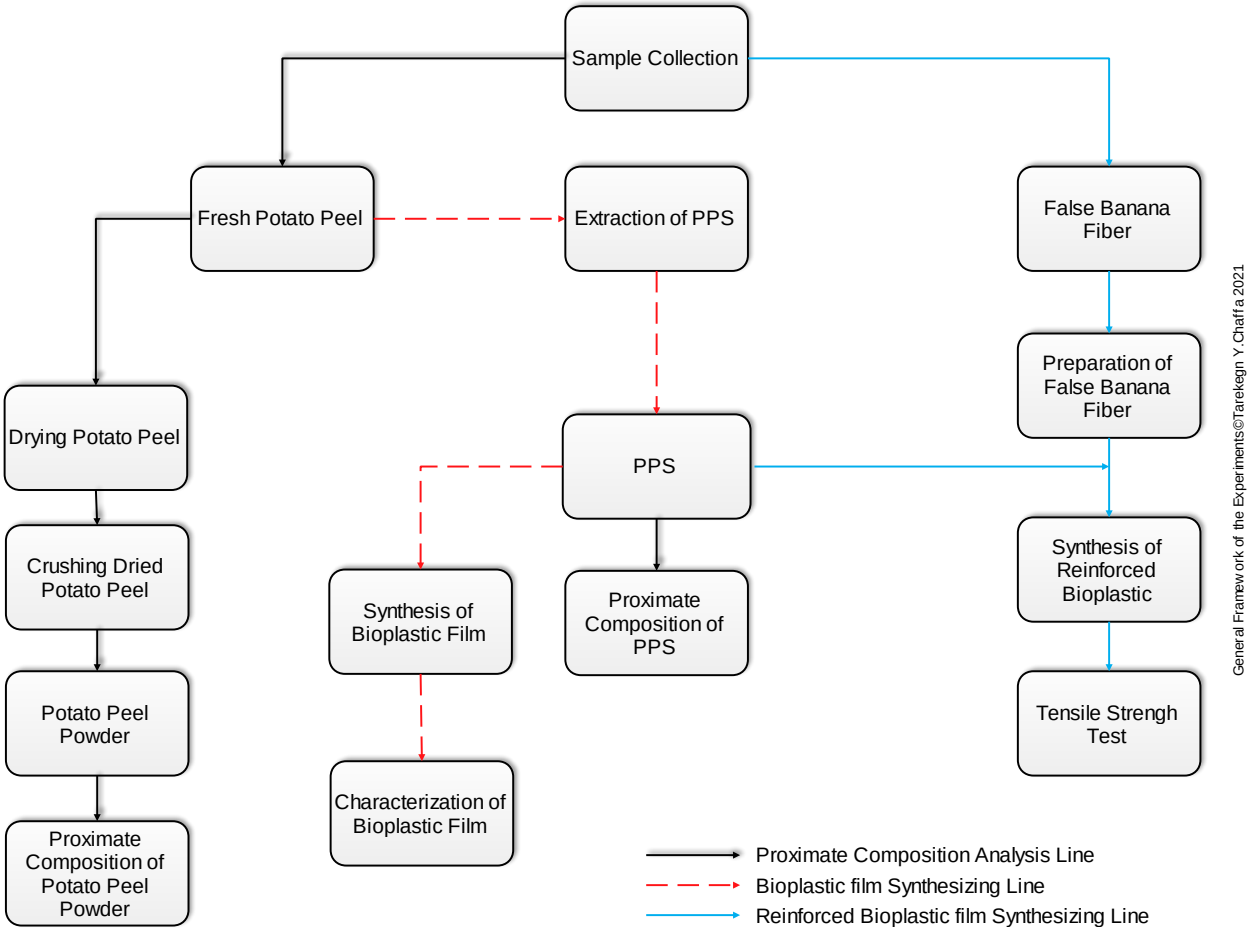


Fig. 3.1. General experimental framework of the study

3.4. Proximate Compositional Analysis

The compositional analysis like moisture content, ash content, fat content and protein content were done by method of oven drying, muffle furnace burning, Soxhlet and Kjeldahl, respectively, for both the potato peel and PPS. In addition to these, total carbohydrate content, starch content and

crude fiber contents were done for the potato peel; and amylose and amylopectin contents were done for PPS. Furthermore, ash and moisture contents were also done for the False banana fiber.

3.4.1. Moisture Content Determination

The moisture content determination was performed by oven drying method according to the official method 930.15 (AOAC 2016). First, crucible preparation was done. The aluminum made dishes were washed with distilled water and dried properly in drying oven (DHG-9055A) at 105^{o}C for 20minutes to remove the moisture. After 20 minutes the aluminum dishes were taken out from the oven and were allowed to cool in desiccator for 30 minutes. Now then, the aluminum dishes were fully ready to use in the analysis of moisture content. Aluminum dish preparation was followed by sample preparation. The sample weighing stage was the most important part of the sample preparation stage. Before weighting the sample, the calibration status of the balance machine was checked. Then, by placing the cooled aluminum dishes on the weighing machine its weight was noted as weight of blank aluminum dishes, W_{bd}. After the weight of the aluminum dish was made zero, 5 g of the sample was transferred into the dish using dry clean spatula and the sample was spread on the surface of the dish by shaking. Now, by opening the door of hot air oven (OV/125/SS/F/DIG/A) carefully the dishes with the sample were put inside and its door was tightly closed. The power of the oven was turned on and the temperature was set at 105^{o}C for 3 hours. After 3 hours the power of the hot air oven was turned off and the aluminum dishes were carefully taken out of the oven and allowed to cool for 30 minutes in desiccator. After cooling, it was weighed and the final weight was noted as weight of the petri dish containing the dried sample, W_{ds}. Finally, the moisture content was determined using the following equation.

$$Moisture\ \% = \frac{W_s - (W_{ds} - W_{bd})}{W_s} \times 100 \qquad Eq(3.1)$$

Where: W_{bd}=Weight of the blank aluminum dish, W_s= Weight of the Sample and W_{ds}= Weight of the aluminum dish with dried sample.

3.4.2. Ash Content Determination

The ash content determination was done according to the official method 942.05 (AOAC 2016). First, after the crucibles were washed with 6N HCL by heating on hot plate they were washed again 3 times with distilled water. The washed crucibles were dried properly in drying oven at

105^{o}C for 20minutes. After 20 minutes the crucibles were taken out from the oven and were allowed to cool in desiccator for 30 minutes. Crucible preparation was followed by sample preparation. The sample weighing and charring stage was the most important part of the sample preparation stage. Before weighting the sample, the calibration status of the balance machine was checked. Then, by placing the cooled crucible on the weighing machine its weight was noted as weight of blank crucibles, W_{bc}. After the weight of the crucible was made zero, 2.5 g of the sample was transferred into the crucibles using dry clean spatula and the sample was charred on temperature adjustable electric hot plate (STUART UC125) starting from low temperature to 400^{o}C under a hood till the fume stopes. Next to this, the crucibles with the charred sample were carefully put inside the Muffle furnace (Type CSF12/13-Astone Lane, Hope Sheffield, S30 2RR, England) and its door was tightly closed. The power of the Muffle furnace was turned on and the temperature was set at 550^{o}C for 5 hours. After 5 hours, the power of the Muffle furnace was turned off and waited for the drop of furnace temperature below 250^{o}C. When Muffle furnace temperature was dropped below 250^{o}C, the crucibles were carefully taken out of the oven and allowed to cool for 1 hour in desiccator. After cooling, it was weighed and the final weight was noted as weight of the crucible with ash, W_{ca}. Finally, the ash content was determined using the following equation.

$$Ash\ \% = \frac{W_{ca} - W_{bc}}{W_s} \times 100 \qquad Eq(3.2)$$

Where: W_{bc}=Weight of the blank crucible,

W_s=Weight of the sample and

W_{ca}=Weight of the crucible with ash.

3.4.3. Protein Content Determination

The protein content was determined according to the official method 2001.11 (AOAC 2016). First, 1 g of a sample was transferred to a test tube containing 12 ml of H_2SO_4 acid and 6 g of catalyst (the catalyst is mixture of 7 g K_2SO_4 and 0.8 g $CuSO_4$). This mixture was held overnight. Then, the test tube with the mixture was allowed for digestion at 420^{o}C for 2 hours until clear solution appeared. The digestion was performed under fume hood. After digestion was completed, the digested sample was let to cool in fume hood. Before starting distillation and titration analysis the

running water for condensation was checked. The sample tube with the cold digested sample was put in Kjeltec analyzer unit (Model Kjeltec^{TM} 2300 analyzer, Wagtech, Sweden). The distillation took place there in Kjeltec using 25 mL of 40% NaOH and 25mL of boric acid with methyl red indicator solution. Finally, the distillate was titrated with standard 0.1 M HCl to a reddish color and the analyzed result was recorded. The crude protein calculation was performed by using Eq.3.3 and Eq.3.4.

$$\%Nitrogen = \frac{(V_S - V_B) \times M \times 14.01}{W_s \times 10} \qquad Eq(3.3)$$

Where: Vs= volume (ml) of standard HCl used to titrate a sample,

V_B= volume (ml) of standard HCl used to titrate a blank,

M= Molarity of standard HCl,

14.01= atomic weight of nitrogen,

W_s= weight (g) of test portion or sample,

10= factor to convert mg/g to percent and

The result found for nitrogen was then multiplied by a factor to get the protein content of the sample. According to early determinations of researches, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content (FAO 2003).

$$\%Protein = \%Nitrogen \times 6.25 \qquad Eq(3.4)$$

3.4.4. Fat Content Determination

The fat content was determined by Soxhlet technique according to the official method 2003.06 (AOAC 2016). First, the extraction flask was washed with hot distilled water thoroughly and dried in drying oven at $103^o \pm 2^oC$ for 30 minutes. The extraction flask was then transferred to desiccator to cool to room temperature. The cooled extraction flask was weighed and recorded as mass of dried flask, W_f. 50 mL of petroleum ether was added in the extraction flask and set-in flask holder of Soxtec unit (Soxtec^{TM} 2055 Fat analyzer, Soxtec, Sweden). The bottom of an extraction thimble was covered with a 2 cm layer of fat free cotton. Next to this, 2 g of test sample was weighed (recorded as W_s) and transferred into the thimble. After the top of the sample in the thimble was

covered with a defatted cotton, the thimble was hanged to the condenser and the condenser was fitted to the mouth of extraction flask. After all the setup was finished, extraction was conducted at 70^{o}C for 4 hours. After finishing the extraction process the extraction flasks were remove from the extractor. The extraction flask with extracted fat was dried in oven at 105^{o}C for 30 min to remove moisture. Finally, the extraction flask was cooled in desiccator for 30 minutes to room temperature and weighed immediately after it was taken out of the desiccator recording its weight as weight of dried flask with fat, W_{ff}. The analysis was done in triplicate and the average value was taken after calculating the value of crude fat by Eq (3.5).

$$\%\ Crude\ fat = \frac{W_{ff} - W_f}{W_s} \times 100 \qquad Eq(3.5)$$

3.4.5. Determination of Total Carbohydrate

The total carbohydrate was determined by the method so called total carbohydrate by difference (FAO 2003). The other constituents in the sample like protein, fat, moisture and ash which were determined individually were summed and subtracted from 100 to present the result in percent. The value of protein, fat, moisture and ash were in percent.

$$Total\ Carbohydrate\ (\%) = 100 - (Moisture + Ash + Protein + Fat) \qquad Eq(3.6)$$

3.4.6. Starch Content of potato peel

Determination of starch content of the potato peel was performed by polarimetric method according to (EN 1999/79/EC 1999). A portion of 2.5 g of crushed potato peel was weighed and put into a 100 mL volumetric flask; and then its content was mixed with 25 mL of 1.128% HCl solution. After addition of another 25 mL of 1.128% HCl solution, the suspension was heated on a boiling water bath for 15 min. The flask was shaken well continuous for the first 3 minutes to avoid formation of agglomerates. Once the hydrolysis was finished, exactly after 15 minutes the flask has to be taken from the water bath, 30 mL of cold distilled water was added and cooled immediately down to 20°C. After fast cooling, clarification using 5 mL of Carrez I (a solution made from 21.9 g zinc acetate [Zn $(CH_3COOH)_2 \cdot 2H_2O$], 3 g glacial acetic acid make up with a distilled water to 100 ml volume) and 5 mL of Carrez II (a solution prepared from 10.6 g $K_4[Fe(CN)_6] \cdot 3H_2O$ and distilled water made to 100 mL) solutions was performed. Finally, a volumetric flask was filled up by distilled water, its content was properly mixed, and filtrated using

a filtration funnel. The obtained filtrate was then transferred to a sample cell of 200 mm optical path length and total optical rotation was measured (the reading was recorded for *P*) using a polarimetry at 20°C. Next to this determination of optical rotation of substances soluble in ethanol was performed.

5 g of crushed potato peel was put into a 100 mL volumetric flask and 80 mL ethanol (40%) was added. Now the flask was left for 1 hour at room temperature. During that time, the flask was shaken vigorously six times to make sure, that the sample was mixed with the ethanol. Afterwards, the volumetric flask was filled up to the mark with 40% v/v ethanol, shaken and filtered. Then after, 50 ml of the filtrate was transferred into a 250 mL Erlenmeyer flask and 2.1 mL 25% HCl was added. The flask was shaken well, connected to a reflux condenser and put into a boiling water bath. Exactly after 15 minutes the flask was taken from the water bath, transferred to 100 ml graduated flask, and rinsed with some cold water. Subsequently, it was cooled down to a temperature of 20°C. After that the sample was clarified with 5mL of Carrez I solution and 5mL of Carrez II solution, distilled water was added up to volume. After shaking, the sample was filtrated and optical rotation of the filtrate was measured (recorded as *P′*) at 20°C. Finally, the starch content in percent was calculated by using the following equation:

$$Starch\ Content\ (\%) = \frac{2000(P - P')}{[\alpha]_D^{20^o}} \qquad Eq(3.7)$$

Where: *P* = Total optical rotation in angular degrees.

P′ = Optical rotation in angular degrees of the substances soluble in 40% (V/V) ethanol.

$[\alpha]_D^{20^o}$=Specific rotation of pure starch at 20°, measured in a polarimeter with the wavelength of 589nm (sodium D-line), which is + 185.4° for potato starch.

3.4.7. Crude fiber Determination of Potato Peel

Crude fiber analysis was done according to official method 962.09 (AOAC, 2000). The crude fiber was analyzed using the FiberCape systems (Model FC221 Fiber Cap) consist of a hotplate and an extraction beaker. 1.5 g weighed sample was transferred into a 600 mL extraction beaker and about 200 mL of 1.25% Sulfuric acid was added. The mixture was then boiled on hot plate (Model 2022 Hot Plate) for 30 minutes by gently keeping the level constant with distilled water. After the 30 minutes heating, 20 mL 28% KOH was added and boiled it again gently for another 30 minutes.

Then, washing was performed with 1% sulfuric acid and NaOH solution. Next, the sample was filtered and dried in drying oven (Memmert 854 Schwabach, Germany) at 130^{o}C for 2 hours. Then it was cooled at room temperature for 30 min in a desiccator and weighed recording the weight as weight of dried sample. The dried sample was then moved to muffle furnace (GALLENKAMP, Model FSL 340-0100, UK) for 30 min ashing at 550^{o}C. Finally, after ashing, the sample was cooled in a desiccator and weighed (recorded as weight after ashing). The crude fiber content was determined using Eq (3.8).

$$\%\ Crude\ fiber = \frac{W_{ds} - W_{as}}{W_s} \qquad Eq(3.8)$$

Where: Wds- weight of dried sample, g

Was- weight after ashing

Ws- weight of sample

3.4.8. Iodine Test of PPS

Iodine test is the indicator for the presence of starch in food materials. First, 10 mL starch solution of was prepared in a glass test tube using 3g of PPS. The solution was vigorously mixed for 1 minute with the help of vortex mixer (Model MS 3 D, IKA®, USA). 10 mL of distilled water was taken in another glass test tube as a control for the test. Afterwards, the two test tubes were put in test tube racks and 5 drops of iodine solution (formerly prepared using 0.2 g I_2 and 2g KI make up with distilled water to 100 mL in volumetric flask) was added to each test tubes and the color change observation by eye was recorded.

3.4.9. Determination of Amylose and Amylopectin Content of PPS

The determination of amylose content was done conferring to the procedure by (Pokhrel and Rai 2019). A PPS sample of 0.1 g was weighed and put into 100 cm^3 volumetric flask. 1 cm^3 of 99% ethanol and 9 cm^3 of 1 M sodium hydroxide (NaOH) solution were carefully added. The contents were mixed thoroughly and the sample solution was heated for 10 mins in boiling water to gelatinize the starch. After cooling the solution was made up to the mark with distilled water and shaken thoroughly. 5 cm^3 of starch solution in a 100 cm^3 volumetric flask was treated with 1 cm^3 of 1M acetic acid and 2 cm^3 of iodine solution (prepared using 0.2 g I_2 and 2g KI make up with distilled water to 100 mL in volumetric flask). The solution was finally diluted to the mark with

distilled water. The diluted solution was poured in three cubits and absorption of triplicate sample was read using a spectrophotometer (SPECTRO UV-VIS DOUBLE BEAM PC, 8 SCANNING AUTO CELL, UVD-3200. LABOMED.INC.) at 620 nm. The absorbance of a blank solution prepared accordingly was subtracted from that of the sample. The work of (Hoover and Ratnayake 2001) reported the reagent blank contains all reagents in the same amounts without the sample containing starch. The amylose content was calculated for each absorbance according to the formula below and the average value was taken.

$$Amylose\ Content\ (\%) = 3.06 \times absorbance \times 20 \qquad Eq(3.9)$$

The amylopectin content was determined with the formula presented in Eq (3.10).

$$Amylopectin\ Content\ (\%) = 100 - Amylose\ Content \qquad Eq(3.10)$$

3.5. Extraction of Starch from potato peel

Fresh potato was collected from the market by the local Chips processor. The collected potato was washed repeatedly with water to remove the dust and soil materials and peeled off using stainless steel knife. The potato peel which would be thrown as a waste was collected freshly and weighed to 11,882 grams. The fresh potato peel was then chopped using stainless steel knife on chopping board to reduce the size. For further disintegration the reduced size potato peel was finely disintegrated in cutting blender (juice mixer) and soaked with distilled water. The ratio of finely disintegrated potato peel to distilled water used for the extraction was 1:2 (g/mL). After mixing the crude slurry vigorously to assist the extraction of the starch it was filtered through a doubled tight muslin cloth and the filtrate was left for 4 hours to settle the starch. As indicated by (Izuagie et al. 2012), the supernatant was discarded after 4 hours and the settled starch was again suspended in excess 0.02% NaOH to remove any residual proteins and phenolic compounds. After standing for 4 hours, the NaOH holding supernatant was discarded and the sedimented starch was resuspended in distilled water. Soaking the settled starch with distilled water and discarding the supernatant was repeated six times until the supernatant became colorless. Researches done by (Ezgi and Bilgen 2019; Maulida, Siagian, and Tarigan 2016) presented that the starch collected after purification should be dried in hot air oven within the temperature range of 50^{o}C to 70^{o}C; and (Zoungranan et al. 2020) made known that it has to be dried in an oven at 40^{o}C for 72 hours. Accordingly, the collected wet potato peel starch (PPS) was dried in oven at 40^{o}C for 72 hours and

weighed 478 grams. Lastly, the dried PPS was kept in zip-locked bioplastic bag for further experimental process.

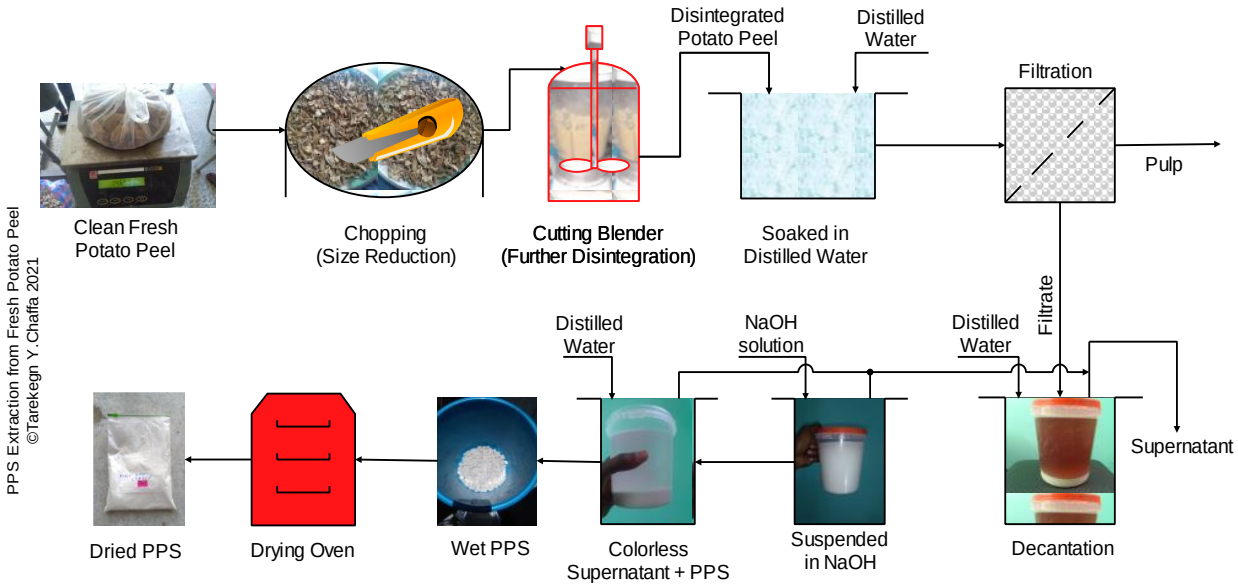


Fig. 3.2. Flow Diagram of Extraction of Starch from Fresh Potato Peel

3.6. Development of starch-based bioplastic film

First 15g of PPS was taken and dispersed in 150ml distilled water contained in 500mL glass beaker. Next 2M HCl solution prepared from 35%HCL v/v was added to the beaker at different volume (2mL, 3.5mL and 5mL) for different experimental run and stirred vigorously by stirring glass rode. Following this 99.8% glycerol by purity was added in various volume (2mL, 3.5mL and 5mL) for different experimental run as per grouped by Design Expert software (version 13.0.1) to assist plasticizing. The gelatinization was performed according to (Ezgi and Bilgen 2019) by putting the beaker, which contains the mixture of all required ingredients, on the temperature adjustable hot plate at the temperature of 100^{o}C while stirring for 20 minutes until it gelatinized. As (Nurul et al. 2016) did, after the gelation process, 2M NaOH was added to neutralize the acidity of the gelatin according to the volume of HCl added before. Then 20gm of the gelatin was taken and casted on a circular glass petri dish with diameter of 11.5 cm, and allowed to dry in oven at the set temperature of 45^{o}C, 55^{o}C and 65^{o}C for three hours with respect to the experimental run. The dried bioplastic was allowed to cool at ambient temperature. Finally, the produced bioplastics were peeled off from the petri dish; and reserved in zip-locked bioplastic bag for further investigations and characterizations (Perotto et al. 2020).

3.7. Preparation of False banana *(Ensete ventricosum)* fiber

The False banana *(Ensete ventricosum)* fiber collected from Amaro Woreda where the local women farmer has been preparing "Kocho", Ethiopians starchy staple food, was washed repeatedly with distilled water to remove any soil particles and stack pulp remained on the fiber. Then the washed False banana fiber was allowed for two days sun drying. Size reduction of the dried False banana fiber was done using cutting mill and the fibers passed through 850μm sieve was taken for further analysis.

Researchers (Yang, Ching, and Chuah 2019) stated that surface of lignocellulosic fibers had been modified widely to enhance their performance and to promote better adhesion between the natural reinforcement and the polymeric matrix. Based on this finding the fiber was treated according to (P. P. Zhang et al. 2014) by soaking in a 2 wt% NaOH solution for 4 h at 90 °C. The ratio of Ensete fiber to liquid used for the alkali treatment was 1:25 (g/mL). Such a treatment was repeated twice. Previous work (Nur Syafiqaz, Chen, and Ahmad 2021) shows the elimination of hemicellulose during NaOH treatment. This treatment led to a reduction in the hygroscopicity of the reinforced bioplastic because the eliminated hemicellulose is most hydrophilic component than cellulose and lignin. Another researcher confirms the fact that NaOH treatment and acetylation reactions makes the fiber more hydrophobic (Cyras et al. 2004).

After NaOH treatment, lignin residues were removed by bleaching. Bleaching was performed at 80 °C for 6 hours by using a solution prepared from equal volume of aqueous chlorite (1.7 wt% $NaClO_2$ in water) and an acetate buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1L using distilled water). The bleaching process was repeated twice. Finally, the bleached False banana fiber was washed with distilled water four times and allowed to dry at 50^{o}C in hot air circulating oven. The dried False banana fiber was kept ready to use it as a reinforcement.

3.8. Synthesis of False banana *(Ensete ventricosum)* fiber reinforced bioplastic

The prepared False banana fiber at different ratio (0%, 2%, 4%, 6%, 8% and 10% w/w of starch) was allowed to mix with a gelatin formed using the optimized concentration volume of HCl (3.5mL) and glycerol (3mL) by taking the same mass of starch and the same volume of distilled water as taken before for the synthesis of starch-based bioplastic without reinforcement. The synthesis of Enset fiber reinforced starch-based bioplastic was followed the procedures expressed

for the synthesis of starch based degradable bioplastic without reinforcement except the step where there was mixing of the false banana fiber occur. The casted gelatin was allowed to dry at the optimized temperature of 48^{o}C.

3.9. Bioplastic Characterization methods

3.9.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was done for the PPS, bioplastic film without fiber reinforcement and Enset fiber reinforced bioplastic film. The produced starch based degradable bioplastic was characterized with FTIR spectroscopy to identify the functional group that present in the sample to predict the type of plastic produced in this study as stated by (Nurul et al. 2016). The infrared spectra were measured using Fourier transform infrared spectroscopy (Model: Thermo SCIENTIFIC iS50 ABX) which is equipped with KBr beam splitter. The sample was placed in sample holder and the measurements were carried out at room temperature with 32 scans and 4 cm-1 resolution covering infrared spectra range of 400 to 4000 cm-1.

The previous work of (Nandiyanto, Oktiani, and Ragadhita 2019) stated that the IR spectrum is divided into three wavenumber regions named far-IR spectrum (<400 cm^{-1}), mid-IR spectrum (400-4000 cm^{-1}), and near-IR spectrum (4000-13000 cm^{-1}). Their work tells the most widely used IR spectrum in the sample analysis is mid-IR spectrum. The mid-IR spectrum is divided into four regions of the single bond region (2500-4000 cm^{-1}), the triple bond region (2000-2500 cm^{-1}), the double bond region (1500-2000 cm^{-1}), and the fingerprint region (600-1500 cm^{-1}). Therefore, the result was discussed accordingly.

3.9.2. Water absorption

Water absorption was investigated according to standard method (ASTM D570-98 2000). The bioplastic films were cut with size approximately 2x2 cm^2 and the weighed mass were recorded for dry weight. The bioplastic films were put into a Petri dish filled with distilled water for 24 hours. After 24 hrs., the films were removed from the distilled water and weighed to measure the wet weight. Water absorption was calculated using the following formula (Maulida, Siagian, and Tarigan 2016; Agustin et al. 2014):

$$\%\, Water\ absorption = \frac{W_w - W_d}{W_w} \times 100\% \qquad Eq(3.11)$$

Where, W_w= Wet weight and W_d= Dry weight

3.9.3. Tensile strength and percent elongation at break

Tensile strength and elongation at break are very important mechanical properties of the produced bioplastic. The test was done using the standard method (ASTM D882-18 2018). These properties were performed using the equipment so-called Texture analyzer (Model TA Plus, LLOYDЖ Instruments, England). The test was done after conditioning the test sample according to Procedure A of (ASTM D618-13 2013). The test specimens were conditioned at a temperature of 23^{o}C and 50% relative humidity for a period of 40 hours. Afterwards, the bioplastic film specimens of 100 mm by 10 mm size were prepared. Then, the bioplastic film strips were positioned in the pneumatic grips of the texture analyzer. The crosshead speed was set at 50 mm/min. After feeding the thickness and width of the test film to the software installed on personal computer that was coupled with the texture analyzer, the analyzer was let to run. The result of tensile strength was generated in MPa and elongation at break in percent.

3.9.3.1 Tensile Strength

The tensile Strength of the bioplastic was calculated by dividing the maximum force by the original average cross-sectional area of the specimen. The result was articulated in megapascals (MPa) which is force per unit area and reported to three significant figures. The maximum force can occur at the breaking point. The calculation was done according to the formulae below.

$$Tensile\ Strength\ (MPa) = \frac{Maximum\ Force\ (N)}{Crossectional\ Area\ (mm^2)} \qquad Eq(3.12)$$

3.9.3.2 Percent Elongation at Break

Elongation values are quantitatively relevant and appropriate for engineering design (ASTM D570-98 2000). Percent Elongation at Break was calculated by dividing the extension at the moment of rupture of the bioplastic film by the initial gage length of the bioplastic and multiplying by 100. This can be written in formula form as follow.

$$\%Elongation\ at\ Break = \frac{Extension\ at\ rapture\ (mm)}{Initial\ Gage\ Length\ (mm)} \times 100 \qquad Eq(3.13)$$

3.9.4. Biodegradability in Enzyme by Weight Loss

According to European Committee for Standardization (CEN), biodegradation is a degradation caused by biological activity, especially by enzymatic action, leading to a significant change in

the chemical structure of a material (Ezgi and Bilgen 2019). Microorganisms are the primary source of alpha-amylase producing a high volume of the enzyme (Far et al. 2020). These researches show that alpha-amylase produced by microorganisms fractures the polymer chain thus cause the biodegradation of starch-based bioplastic. The biodegradability of produced bioplastic was tested, first, enzyme solution was prepared by adding 1mL of alpha-amylase enzyme in a 10mL distilled water in seventeen different conical glass test tubes. Next, a 2cm by 3cm cut bioplastic films were weighed and recorded as initial sample weight. Then after the cut pieces of bioplastic films were soaked in a solution prepared with Alpha-amylase enzyme and let to stand for 24hours. After 24hours the solution was gently removed and the disintegrated bioplastic left in the test tube was allowed to dry in the drying oven for 30min. After drying, the samples were weighed and the results were recorded as the final weight of the samples. The amount of biodegradation occurred was calculated by the following equation adopted from the work of (Nurul et al. 2016).

$$\%\ Biodegradation\ by\ Enzyme\ = \frac{W_o - W_f}{W_o} \times 100 \qquad Eq(3.14)$$

Where, W_f = Final weight of sample and W_o=Initial weight of samples

3.9.5. Morphological Analysis

The morphology and surface structure of the bioplastic with and without reinforcement of the False banana fiber was observed clearly by imaging with the utilization of Optical Microscopy (B-150 OPTIKA, Italy) coupled with personal computer.

3.9.6. Crystallinity analysis (XRD analysis)

The crystallinity of PPS, pure bioplastic film and False banana fiber reinforced bioplastic film were investigated with the aid of X-ray diffraction (XRD) instrument (Model DW-XRD-Y7000) set with Cu radiation source. The XRD was operated at 40kV voltage and 30mA current supplied at room temperature. The sampling pitch, preset time and 2θ scanning angle were set at 0.0200º, 0.40 sec and 10.000 to 90.000 degrees, respectively with a mode of continuous scanning and 3º min^{-1} scan speed. Percent of crystallinity index was calculated with Eq (3.15) by the aid of OriginPro 2015 software.

$$Crystallinity\ Index(\%) = \frac{Area\ of\ all\ Crystalline\ Peaks}{Area\ of\ all\ Crystalline\ \&\ amorphous\ peaks} \qquad Eq(3.15)$$

3.10. Experimental Design

In recent years, design of experiment (DOE) has been frequently applied to optimize analytical methods due to its advantages, such as a reduction in the number of experiments that need to be executed, which results in lower consumption and considerably less laboratory work. In the year 1951, Box and Wilson used RSM to explore the relationships between explanatory and response variables in statistics. RSM is an effective tool for optimizing the process when multi factors and interactions affect the desired response. The BBD is one of the special experimental designs in RSM. It was proposed to be used only for three levels of each three factors and at the same time it results in fewer reasonable number of experimental trials to evaluate multiple variables and their interactions, and is more convenient and less expensive to run than the CCD with the same number of factors. Additionally, BBD is rotatable, meaning that the model would possess a reasonably stable distribution of scaled prediction variance throughout the experimental design region (L. Wu et al. 2012).

In this study, the three-level, three-factor Box–Behnken experimental design was used to investigate and validate process parameters affecting the synthesized bioplastic film. The experimental runs, analysis technique, optimization and ANOVA were ensured by using a software called Design – Expert ® (Version 13.0.1). The 2M concentration HCl 2-5mL, glycerol 2-5mL and drying temperature 45-65^{o}C were the input independent variables (factors). The factors were successively coded as +1, 0 and -1 for high, intermediate and low values, respectively. The intermediate value was the mean of lower and higher values. The responses were tensile strength, elongation at break, water absorption and biodegradability.

Table 3.2. Independent factors and their coded levels used for experimental run and optimization

Factors	**Coded symbol**	**Range and Coded levels**		
		-1	0	+1
HCl, mL	A	2	3.5	5
Glycerol, mL	B	2	3.5	5
Drying Temperature, ^{o}C	C	45	55	65

Table 3.3. Box–Behnken design matrix showing the factors and responses of experimental run

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
	A: HCl Conc. (mL)	B: Glycerol Conc. (mL)	C: Drying temperature (°C)	Tensile Strength (MPa)	Elongation at break (%)	Biodegradability (%)	Water Absorption (%)
1	5	3.5	65				
2	3.5	3.5	55				
3	3.5	3.5	55				
4	5	3.5	45				
5	3.5	3.5	55				
6	3.5	3.5	55				
7	2	3.5	65				
8	2	3.5	45				
9	3.5	3.5	55				
10	2	5	55				
11	2	2	55				
12	3.5	2	45				
13	5	5	55				
14	3.5	5	65				
15	3.5	5	45				
16	3.5	2	65				
17	5	2	55				

Considering all the linear terms, square terms and by linear interaction items, data from the BBD were analyzed by multiple regression to fit the following quadratic polynomial model:

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j \qquad Eq(3.16)$$

Where Y is the response (that is tensile strength or elongation at break or biodegradability or water absorption), k is the number of factors, x_i and x_j are the coded factors, β_o, β_i, β_{ii}, and β_{ij} are the regressions coefficients for intercept, linear, quadratic and interaction terms, respectively. The model coefficients were obtained using Design Expert (Version 13.0.1.) software (L. Wu et al. 2012; Sharma, Singh, and Dilbaghi 2009; Berhanu, Kiflie, Neiva, et al. 2018).

CHAPTER 4: RESULT AND DISCUSSION

4.1. Proximate composition of potato peel

The proximate analysis of potato peel of the present study was displayed in Table 4.1. The moisture content of dry potato peel was found to be 8.133% in this study. This result is higher, approximately in two-fold, than those reported by (Elkahoui et al. 2018) as 3.67% and 4.46% for the variety of Organic Russet and Red potato respectively, and the reported value by (Kumari et al. 2017) as 4.08% for Lady Claire potato variety of Ireland. The moisture content of potato peel for the variety Spunta and Agria as reported by (Jeddou et al. 2016) and (Amado et al. 2014) respectively, shown the same value (7.3%) which is not far this much from the result found in this study.

Table 4.1. Proximate Compositional values of *Solanum tuberosum* peels of this study with previous literature report

Potato peel variety and origin	**Proximate Composition, % dry weight**							**References**
	moisture	Ash	Protein	Starch	Fat	fiber	carbohydrate	
Unknown variety, Ethiopia	8.13	4.89	10.13	51.16	0.27	9.52	76.58	Results from this study
Organic Russet, United States	3.67	7.32	11.98	---	1.12	21.4	76	(Elkahoui et al. 2018)
Non-organic Russet, US	3.78	7.34	17.19	---	1.1	22.4	71	(Elkahoui et al. 2018)
Red potato, United States	4.46	6.69	15.99	---	0.81	---	72	(Elkahoui et al. 2018)
Gold potato, United States	5.66	9.12	14.17	---	1.17	---	70	(Elkahoui et al. 2018)
Lady Rosetta, Ireland	6.98	7.24	11.17	---	2.09	---	72.53	(Kumari et al. 2017)
Lady Claire, Ireland	4.08	4.83	12.44	---	1.27	51	77.38	(Kumari et al. 2017)
Spunta, Tunisia	7.3	0.91	2.11	---	0.73	---	88	(Jeddou et al. 2016)
Agria, Spain	7.3	5.46	6.47	---	0.00	---	86.97	(Amado et al. 2014)
Unknown variety, Greece	---	6.34	8	52.14	2.6	---	68.7	(Arapoglou et al. 2010)
Unknown variety, Egypt	4.35	7.83	12.68	50.55	1.03	16.3	62.14	(Badr and El-waseif 2018)

The result also shown 4.89% ash content which is higher than 0.91% those reported for Spunta by (Jeddou et al. 2016) and 4.83% reported for Lady Claire by (Kumari et al. 2017). The ash content of potato peel of Gold potato variety shows 9.12% which is the highest value as reported by (Elkahoui et al. 2018).

The most abundant macronutrients in potato peel are carbohydrates as shown by the researchers previous study (Sampaio et al. 2020). In the present finding, the amount of total carbohydrate in the potato peel was 76.58% dry weight. This approximately agrees with the result reported by (Elkahoui et al. 2018) for potato peel variety of Organic Russet(76% dw) and those reported by (Kumari et al. 2017) as 77.38 percent dry weight for the potato peel variety of Lady Claire. The second highest macronutrient of potato peel, as it can be observed from Table 4.1., is starch, which made it valuable raw material for the synthesis of starch-based bioplastic film. Starch was found to be 51.16% dry weight which is less than the one reported (52.14%) by (Arapoglou et al. 2010) and higher than that reported (50.55%) by (Badr and El-waseif 2018).

The previous researches presented in Table 4.1. has shown that protein is the third most abundant macronutrient in potato peel next to starch, although the content fluctuates extensively among studies from variety to variety, ranging from 2 to 17% dry weight. The protein content of potato peel in this study was 10.13% which is higher than the values reported by (Jeddou et al. 2016; Amado et al. 2014; Arapoglou et al. 2010) and less than that reported (Kumari et al. 2017; Elkahoui et al. 2018). The review paper of (Sampaio et al. 2020) has cited the work of Choi et al.(2016), who studied the nutritional protein composition of potato peel and reported a total crude protein of 9.52–10.58 % dry weight. This result agrees with the result found in this study. The Nitrogen content of the potato peel was determined and results 1.62% dry weight, which is 0.32% higher than the result reported by (Arapoglou et al. 2010).

The amount of fat in potato peel was found 0.27 % dry weight, in this research, which is very less compared to the previous reports presented in Table 4.1 except the zero fat for potato peel of Agria variety reported by (Amado et al. 2014). The highest fat content report for potato peel is 2.6% dw report of (Arapoglou et al. 2010) for unknown variety from Greece.

Fiber content of potato peel in this study was 9.518 % dw. Research study done by (Elkahoui et al. 2018) on the total dietary fibre content of organic and non-organic potato peel of the Russet variety has found 21.4 and 22.4 % dw respectively. This reported result is higher than result of this

study, approximately in double. Potato peel of Spunta and the commercial variety has 10.14% and 9.21% of soluble dietary fibre, respectively (Sampaio et al. 2020). The total dietary fiber study on potato peel from the Lady Claire variety done by (Kumari et al. 2017) had reported 51% dw, which is even high result.

Generally, the composition of potato peel has shown variation in different research studies even though there were agreements with some researches. According to (Kumari et al. 2017), these variations in potato peel composition may be attributed to numerous influences including varietal alterations, peeling techniques, agronomic and other environmental factors.

4.2. Proximate composition of PPS

The iodine test for PPS solution in this study has shown positive result. The white color suspension of PPS solution before the addition of iodine solution was changed to deep blue or blue-black color when iodine solution was added. This shows that the white powder, PPS, extracted from potato peel was starch. The control test tube with starch free distilled water didn't show any color change rather than captivating yellowish color, which is the color of iodine solution. This result agrees with the previously reported results. Researchers (Brumm, Hebeda, and Teague 1989) reported that, after the addition of iodine solution, the starch-free samples appeared yellow and solutions containing granular or soluble starch, amylose or amylose-complexes gave a green or blue color which is an indicative of a positive starch test. Researcher (Fleischer 2019) explained iodine test for starch in converse way. He wrote that the presence of iodine in a weakly alkaline solution can be indicated by adding a few drops of a diluted solution of amylose ("starch"), leading to the typical blue colour.

The proximate composition of PPS was presented in Table 4.2. The PPS had moisture content of approximately 18.6%, which is acceptable, given that up to 20% of moisture content is allowed in starches used commercially as raw materials as reported by (Martínez et al. 2019). The moisture content obtained for PPS had higher than those reported for starches extracted from different varieties. The highest moisture content is related with the method used, extraction by water, to extract the starch (Abera et al. 2019). The work of (Tester, Karkalas, and Qi 2004) had exposed that moisture content of air-equilibrated starches ranges from about 10-12% for cereals and to about 14-18% for some roots and tubers, which is in agreement with the result of this research.

Table 4.2. Proximate Compositional values of Solanum tuberosum PPS in comparison with literature reports

Starch source and variety	Proximate composition, % dry weight					References
	Moisture	Ash	Crude protein	Crude fat	Amylose	
PPS, unknown	18.6	0.01	0.53	0.00	27.93	Results from this study
Potato, Imilla blanca	9.2	0.26	0.58	0.00	28.2	(Martínez et al. 2019)
Potato, Imilla negra	9.3	0.29	0.58	0.00	30	(Martínez et al. 2019)
Potato, Loc'ka	10.2	0.22	0.74	0.07	24.9	(Martínez et al. 2019)
Potato, unknown	4.03	0.33	0.2	0.23	---	(Abera et al. 2019)

The ash content is the leftover after the combustion process into carbon-free product that comes from minerals and inorganic salts in the fresh tuber, fertilizer use, and can also come from the soil and air contamination during processing (Abera et al. 2019). The ash content of PPS had the lowest value at around 0.01%, while the starch from unknown variety of potato had the highest value (0.33%) as reported by (Abera et al. 2019). The results 0.26, 0.29 and 0.22% reported by (Martínez et al. 2019) for potato varieties of Imilla blanca, Imilla negra and Loc'ca, respectively, had also greater values. The ash content value of PPS agrees with the value reported by (Izuagie et al. 2012) for Christ Thorn seed. The low ash content is an indication of the good quality of the starch (Tejavathi, Sujatha, and Karigar 2020). Therefore, the low ash content of PPS implies less containment of minerals, inorganic salts and soil contamination showing its good quality for bioplastic production.

The starch extracted from potato peel (PPS) had no fat content (0.00%), which is in agreement with the reported values of starch obtained from potato varieties of Imilla blanca and Imilla negra as presented in Table 4.2. The protein content of PPS was 0.525%. This value almost matches the results reported (0.58%) in the work of (Martínez et al. 2019) for potato starch of Imilla blanca and Imilla negra variety. The earlier research study of (Tester, Karkalas, and Qi 2004) reported purified starches contains less than 0.6% protein. The potato peel had 0.27% fat and 10.13% protein. The zero fat and lowered protein (0.525%) content of PPS is an indicative of the effectiveness of NaOH solution to remove these macronutrients which were showed up in the

peels. The soaking of wet starch obtained from the potato peel in NaOH solution had removed 94.768% protein and the fat completely.

The amylose content was found to be 27.93% and hence 72.07% was amylopectin. Researchers (Subroto et al. 2020; Tejavathi, Sujatha, and Karigar 2020) reported that, in most foodstuffs, amylose is a component of starch which amounts to around 20-30% and amylopectin around 70-80%. Strong gelation properties and a linear helical polymer structure of amylose make a high amylose starch as a useful film forming material (Tejavathi, Sujatha, and Karigar 2020). The obtained amylose value in this work matches that of Imilla blanca starch.

4.3. Functional group analysis

The result of FTIR analysis of the sample showed that FTIR spectrum of the sample was obtained at the wavenumbers in the range of 400-4000 cm^{-1} (Rizwana Beevi et al. 2020). The results of this study showed broader peaks at 3255.26cm^{-1}, 3270.38cm^{-1} and 3270.95cm^{-1} for PPS, pure bioplastic film and reinforced bioplastic film, respectively (Fig. 4.1). The sharp band at 2930.70 cm^{-1} for pure bioplastic film and 2928.20 cm^{-1} for reinforced bioplastic film are characteristic of C-H stretches associated with the ring hydrogen atoms of CH_2 (Babu et al. 2014; Abera et al. 2020). The previous work revealed a broad transmittance band in the range of between 3650 and 3250 cm^{-1}, indicates hydrogen bond confirming the existence of either hydrate (H_2O), hydroxyl (-OH), ammonium, or amino (N-H) functional groups. The ambiguity of differentiating these functional groups can be avoided with the specification that for hydroxyl compound, it should be followed by the presence of spectra at frequencies of 1600-1300, 1200-1000 and 800-600 cm^{-1}. (Nandiyanto, Oktiani, and Ragadhita 2019). The research of (Perotto et al. 2020) explained the significance of analysis of the -OH band of the FTIR spectra that it can be very informative on the status of the water molecules and their interaction with the macromolecules in the bioplastic film. According to the summative findings of these former researchers, the broader band results of this study at 3255.26 cm^{-1}, 3270.38 cm^{-1} and 3270.95 cm^{-1} for PPS, pure bioplastic film and reinforced bioplastic film, respectively, can be the result of -OH stretching. This decision is solid as they were followed by the presence of spectra at frequencies of 1334.35 cm^{-1}, 1148.13 cm^{-1}, 1077.09 cm^{-1} and 762.06 cm^{-1} for PPS; 1339.60 cm^{-1}, 1149.80 cm^{-1}, 1078.50 cm^{-1} and 1003.09 cm^{-1} for pure bioplastic film; and 1337.24 cm^{-1}, 1150.13 cm^{-1}, 1077.92 cm^{-1} and 1003.63 cm^{-1} for reinforced bioplastic film.

The characteristic band at 3255.26 cm^{-1} for PPS was originally from the O–H stretching vibration, and the band transmittance intensity was later stretched to 3270.38 cm^{-1} for pure bioplastic. This was due to the gelation process and incorporation of glycerol as plasticizing agent. Also, the characteristic peak at 1638.30 cm^{-1} for PPS was slightly shifted to 1643.22 cm^{-1} and 1643.87 cm^{-1} band of pure bioplastic film and reinforced bioplastic film, respectively. This phenomenon indicated the formation of hydrogen bonds (Niu et al. 2021). According to (Moreno et al. 2017) the absorption around 1640 cm^{-1} is a typical band residing in the spectra of starch and its derivative, related to the tightly bound water. The research done by (Niu et al. 2021) has also reported the peaks around 1636 cm^{-1} represent the C=O stretching vibrations of glycoside bonds in the starch branch that confirms the peaks at 1638.30 $cm^{-1}$for PPS.

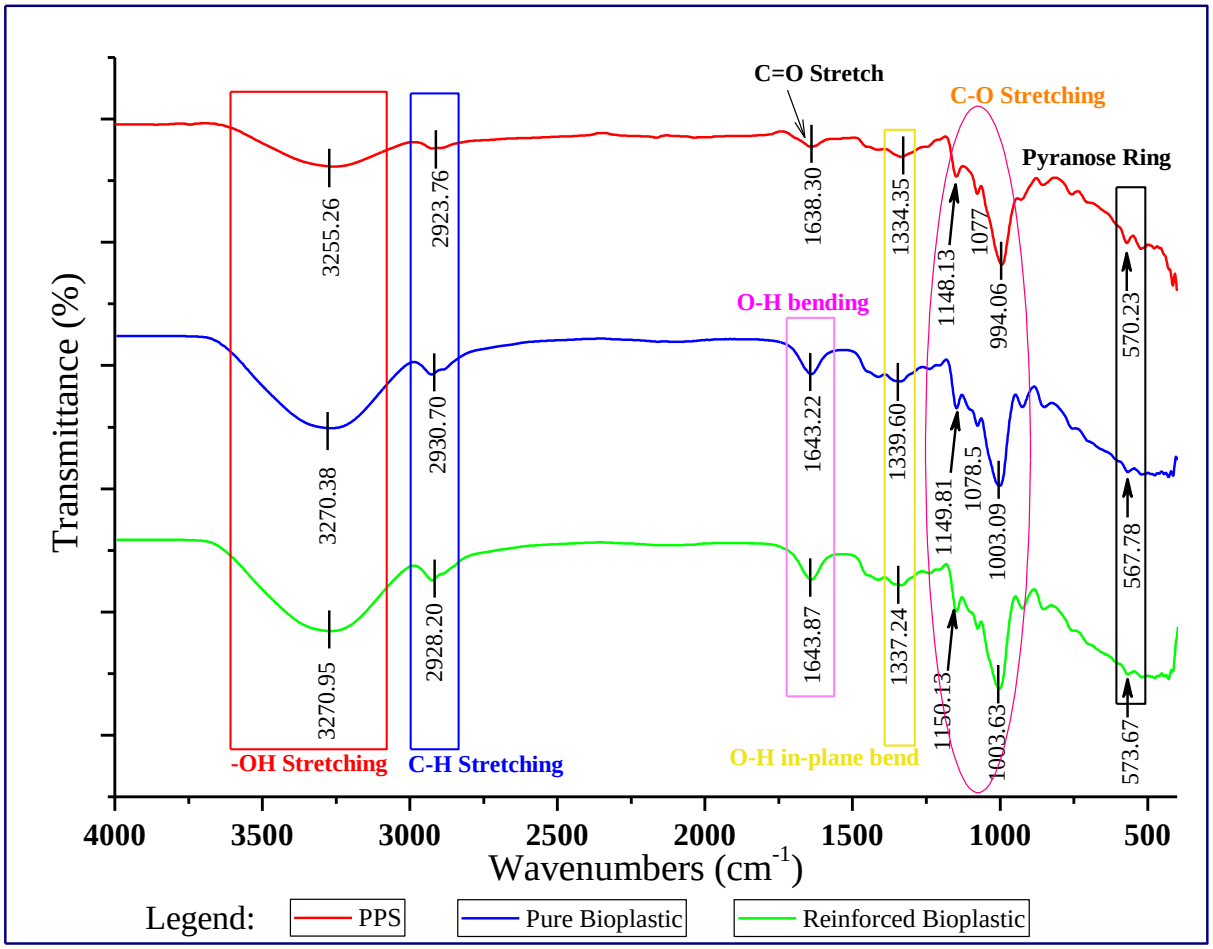


Fig. 4.1. The FTIR peaks for PPS, pure bioplastic and reinforced bioplastic

The characteristic peak at 1078.5 and 1003 $cm^{-1}$for the bioplastic film are related to C-O bond stretching of PPS starch and skeletal modes of pyranose ring was indicated at 570 cm^{-1}, while the

peak around 1643 cm^{-1} was associated with O-H bending (Abera et al. 2020). The addition of glycerol has affected peaks around 1148.13 cm^{-1} for PPS is associated to C-O-C antisymmetric bridge stretching. This peak, initially observed at 1148.13 cm^{-1} for the PPS, was displaced to 1149.81 cm^{-1} in pure bioplastic film and to 1150.13 cm^{-1} in reinforced bioplastic film, when glycerol was added (Bergo et al. 2008). *Nandiyanto et al.* has provided that 1350–1260 cm^{-1} indicates primary or secondary hydroxyl compound OH in-plane bend. The peaks found at 1334.35, 1339.60 and 1337.24 cm^{-1} showed OH in-plane bend for PPS, pure bioplastic and reinforced bioplastic, respectively.

In conclusion, the FTIR results confirmed that the compatibility between False banana (*Ensete ventricosum*) fiber and PPS was attributed to intermolecular hydrogen bonding and uniform dispersion of the fiber during the blending and film formation. The enhancement in the -OH stretching vibration at approximately 3270 cm^{-1} of pure bioplastic to 3270.95 cm^{-1} of reinforced bioplastic suggested the occurrence of association (Niu et al. 2021). The insignificant shift of IR spectrum of the pure bioplastic film when reinforced with False banana (*Ensete ventricosum*) fiber gave the finding of absence of chemical reactions.

4.4. Morphological Analysis of the bioplastic film

The morphological features of bioplastic film with out and with reinforcement of False banana fiber were evaluated using Optical Microscopy (Fig. 4.2). The Optical Microscopy images in Fig.4.2. depict that the pure bioplastic film (a film without fiber) had different morphological characteristics from the False banana fiber reinforced bioplastic. The micrograph for pure bioplastic film showed uniform and dense matrix except the irregular dimpled surface which was resulted from the foam formed during gelatinization process. In the same way, the reinforced one was also dense matrix but irregular dimples were not seen. The work of (Yang, Ching, and Chuah 2019) revealed the reinforcement of lignocellulosic fiber had affected the morphology of the biopolymer. It has also shown the fiber dispersion, length and concentration influenced the strength of bioplastic film.

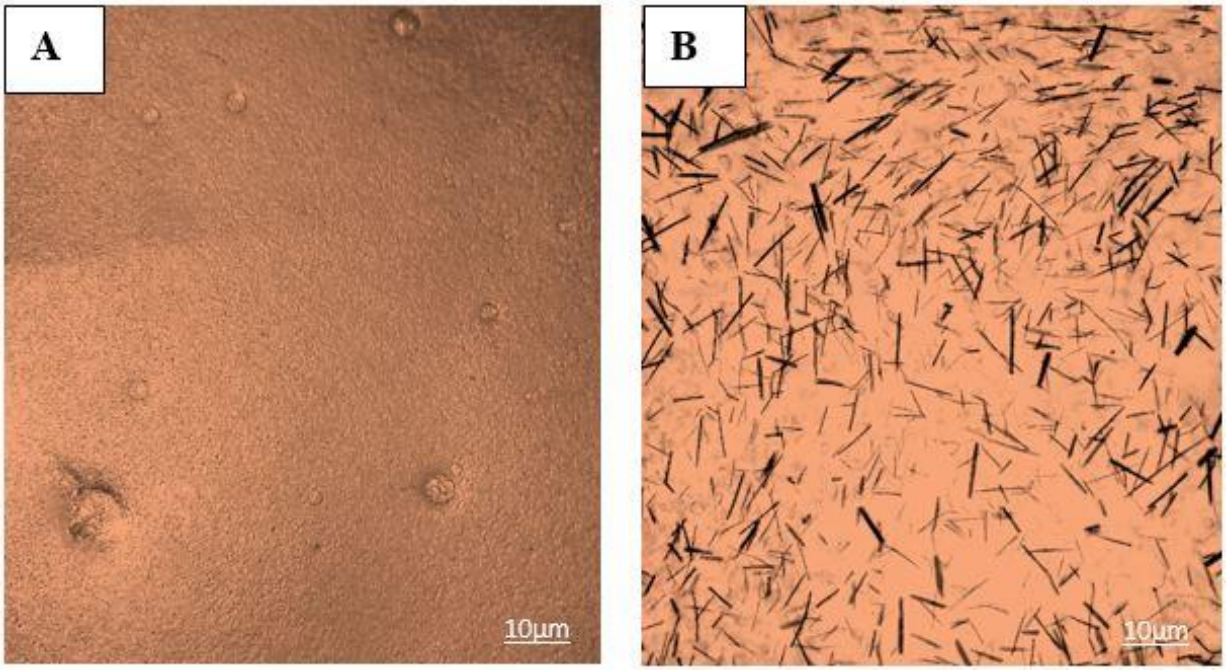


Fig. 4.2. Optical microscopy images of PPS based bioplastic films: (A) Pure bioplastic film; (B) False banana fiber reinforced bioplastic film

4.5. XRD Analysis (Crystallinity)

X-ray diffraction patterns have been widely used to determine the degree of crystallinity of starch granules (Tejavathi, Sujatha, and Karigar 2020) and also to characterize the compatibility of biofilms (R. Zhang, Wang, and Cheng 2018).

The crystal structures of the starch granules are categorized as A-type, B-type, or C-type. A-type starches have a doublet at around 17° and 18° of 2θ and present strong diffraction peaks about 15° and 23° of 2θ. B-type patterns show a strong peak at 17° and small peaks around 15°, 22°, and 24° of 2Theta. C-type patterns are a combination of A- and B-type. As explained previously Type-A, type-B and type-C are characteristic of cereal, tuber and legumes starches, respectively. PPS demonstrated peaks at 15.19°, 17.31°, 22.96° and 24.1^{o} of 2Theta. This result confirms that PPS had type-B crystal structures, which agrees with previously reported result for potato starch (Abera et al. 2019; Tejavathi, Sujatha, and Karigar 2020).

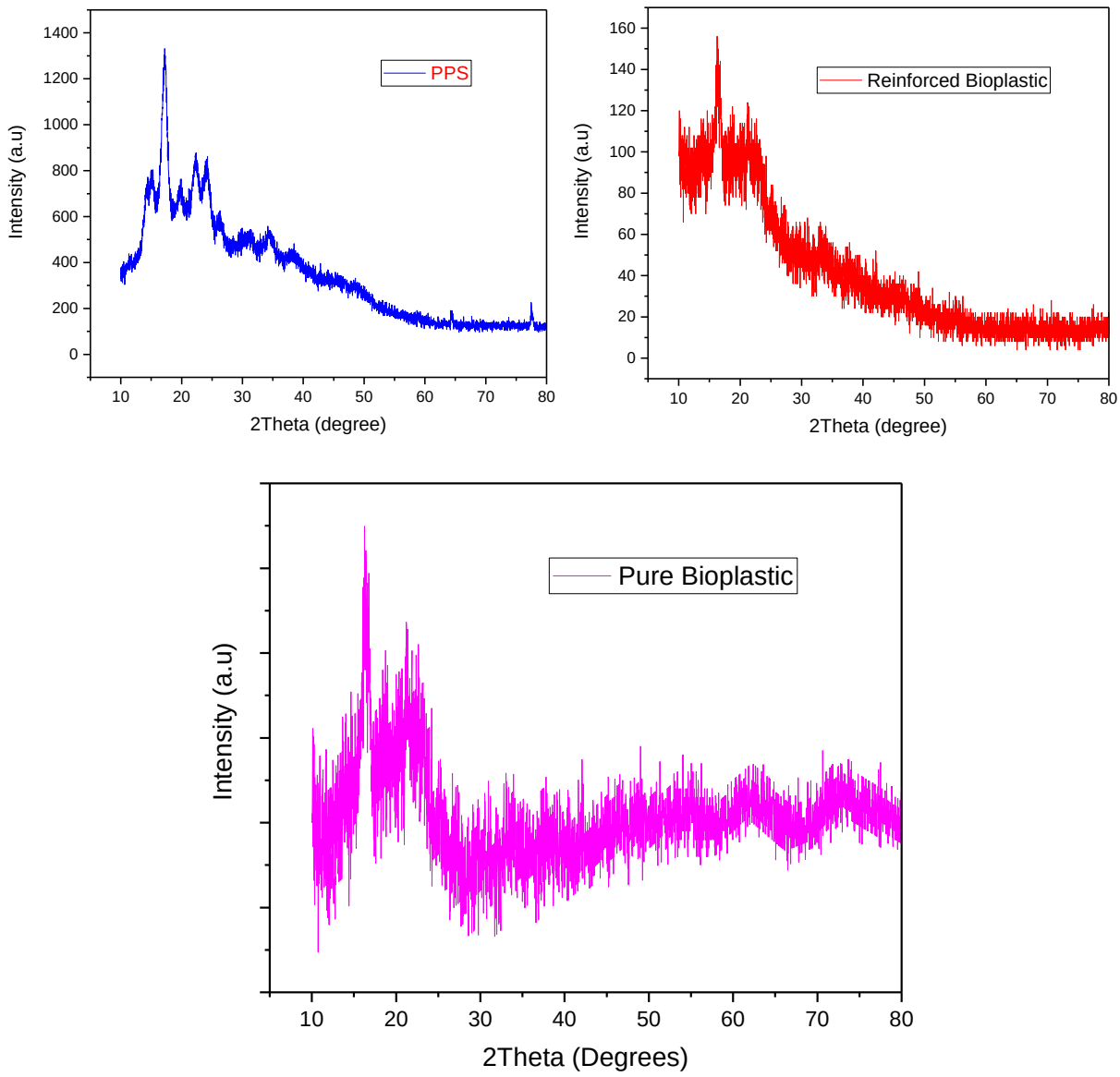


Fig. 4.3. X-ray diffraction patterns of PPS, pure bioplastic film and 6% w/w False banana fiber reinforced bioplastic film

The crystallinity indexes were found to be 19.87%, 8.52% and 10.26% for PPS, pure bioplastic and reinforced bioplastic, respectively. The study of (dos Santos et al. 2016) revealed, the crystallinity index of the potato starches ranged from 20.02% to 21.59% which is approximately in agreement with the finding for PPS in this study. The crystallinity index decrement for the bioplastic films in this work was the indication that bioplastic films become more amorphous because of gelation process. Starch films could have an amorphous character because of starch gelatinization with heat source for initiation (Bergo et al. 2008). Previously done researches had

exposed the complete or partial destruction of the crystallinity of native starch granules due to melting of amylopectin crystalline domains when it gelatinized and the chance of residual crystallinity (caused by the incomplete dissolution or melting of starch) to occur after processing (Lara and Salcedo 2016). Accordingly, 16.32^{o} and 21.28^{o} of 2Theta crystalline peaks were showed up for the PPS based bioplastic films. Researcher (Bergo et al. 2008) had observed some tendency to crystalline peaks, at 2θ≈20°, for films with 30 and 45 g glycerol/100 g starch. In the same way, another researcher reported the clear appearance of characteristic diffraction peak of the pure potato starch films at around 20° of 2Theta, which could be attributed to the crystalline and amorphous fractions of the potato starch (R. Zhang, Wang, and Cheng 2018). They also revealed exhibition of good miscibility of the crystalline and non-crystalline ingredients in the films urges to have lower crystallinity than that of the single-crystalline ingredient.

4.6. Tensile Strength Investigation of False Banana Fiber Reinforced Bioplastic

The False banana (*Ensete ventricosum*) fiber reinforcement in production of PPS based bioplastic had an increasing trend of the tensile strength of the bioplastics as depicted in Fig.4.2. The results show significant enhancement of increment on tensile strength with the fiber loading up to 6%. However, the tensile strength decreased above 6% w/w fiber loading. The pure bioplastic with tensile strength of 6.449MPa was increased to 8.247, 8.619 and 8.878MPa by the incorporation of 2%, 4% and 6% fiber loading respectively. According to the paper of (Yang, Ching, and Chuah 2019), the incorporation of lignocellulosic fiber had increasing impact on tensile strength of bioplastic polymers which agrees with the present work.

Table 4.3. The measured values of tensile strength and elongation at break for Ensete fiber reinforced PPS based bioplastic film

Fiber loading, % w/w of starch	Tensile strength, MPa	Elongation at break, %
0%	6.449	19.87
2%	8.247	18.46
4%	8.619	17.74
6%	8.878	17.71
8%	7.174	16.94
10%	6.837	16.96

The tensile strength of reinforced bioplastic film showed decreased value of 7.174 and 6.837 MPa for 8 and 10% w/w fiber loading, respectively. According to (Buzarovska et al. 2008) the reason for this decrement in tensile strength was the reduction of effective cross-sectional area of continuous phase due to the increment of dispersed phase fiber loading. The same paper reason outs such a result can be also due to the imperfect distribution of the reinforcing fibers through the polymer matrix, as well as very poor adhesion between the polymer resin and the fiber. Another research done by (Yang, Ching, and Chuah 2019) reported that higher filler content may lead to poor distribution of filler across the matrix causing the stress in the continuous phase to increase and promote poor tensile strength.

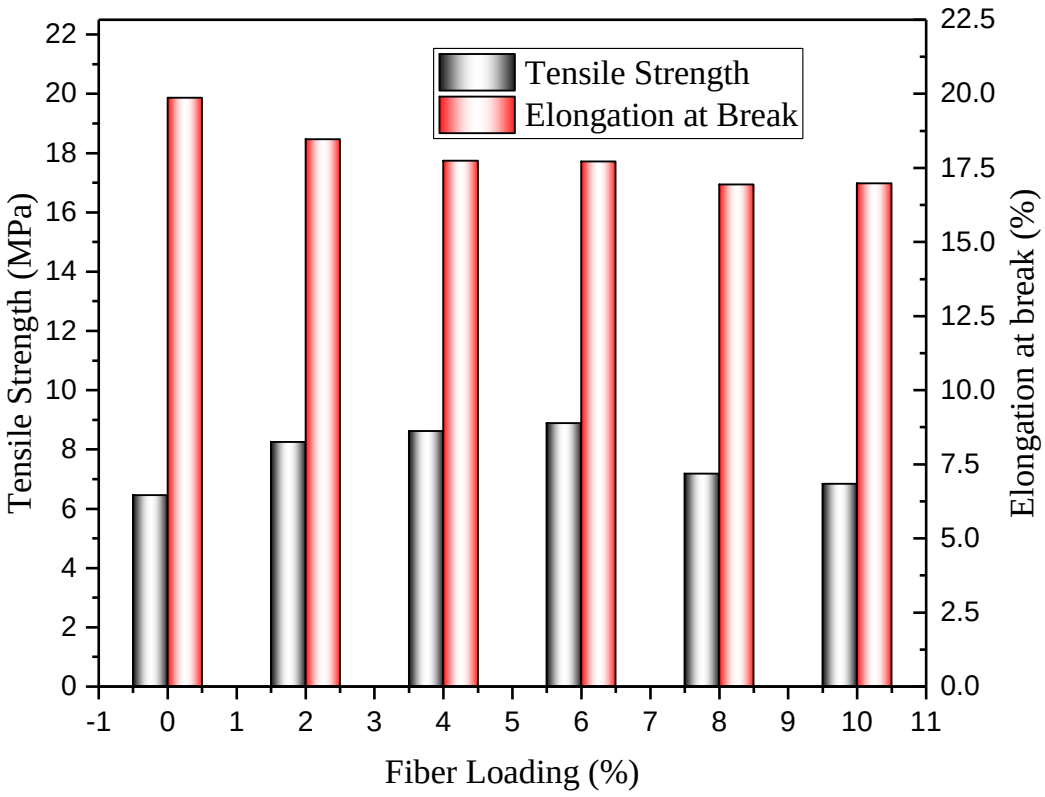


Fig. 4.4. The effect of Ensete fiber loading on tensile strength and elongation at break of PPS based bioplastic

The elongation at break had decreasing trend with the increase of fiber loading. The recorded results for percent elongation at break were 19.87, 18.46, 17.74, 17.71, 16.94 and 16.96% when the fiber loading was 0, 2, 4, 6, 8and 10% w/w respectively. This decrement elongation at break was because of the fibre loading which promote brittleness and increase rigidity of the reinforced bioplastic film (Yang, Ching, and Chuah 2019).

4.7. Experimental Design Analysis

4.7.1. Box-Behnken Design Matrix of Factors and Responses

Response surface method of specific design called Box-Behnken design was used to carry out 17 experimental runs. Laboratory experiment was conducted for the seventeen experimental runs of different combinations of factors. The results generated for the responses were put in Table 4.4.

Table 4.4. The Box-Behnken design matrix values for factors and responses

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
	A:HCl Conc. mL	B:Glycerol Conc. mL	C:Drying Temperature oC	Tensile Strength MPa	Elongation at break %	Biodegradability %	Water Absorption %
1	5	3.5	65	7.619	18.04	81.34	52.34
2	3.5	3.5	55	6.681	19.71	82.63	57.64
3	3.5	3.5	55	6.676	19.78	82.65	57.67
4	5	3.5	45	6.468	21.58	84.94	60.42
5	3.5	3.5	55	6.674	19.79	82.65	57.66
6	3.5	3.5	55	6.678	19.74	82.67	57.65
7	2	3.5	65	7.269	16.86	81.37	52.61
8	2	3.5	45	6.637	20.39	85.4	60.67
9	3.5	3.5	55	6.671	19.82	82.68	57.69
10	2	5	55	6.388	21.08	83.75	59.29
11	2	2	55	6.438	17.02	82.41	56.35
12	3.5	2	45	6.208	18.84	84.66	59
13	5	5	55	6.349	22.22	83.6	58.89
14	3.5	5	65	6.927	19.32	82.24	53.82
15	3.5	5	45	6.029	22.9	85.94	62.36
16	3.5	2	65	7.108	15.37	80.66	51.58
17	5	2	55	6.647	18.24	82	56.32

4.7.2. Analysis of variance (ANOVA) for tensile strength

The suggested model for tensile strength in this study was a quadratic model for which analysis of variance was used. The F-value of 17104.74 for the model indicates that it is significant. An F-value of this magnitude has a 0.01 percent chance of occurring due to noise. Model terms with P-values less than 0.0500 are significant. In this case A, B, C, AB, AC, BC, B^2, C^2 are significant model terms. The model terms are not significant if the value is bigger than 0.1000. Model reduction may improve your model if there are many insignificant model terms (not counting those required to support hierarchy).

Table 4.5. ANOVA for quadratic model of tensile strength

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.42	9	0.2689	17104.74	< 0.0001	significant
A-HCl Conc.	0.0154	1	0.0154	979.56	< 0.0001	
B-Glycerol Conc.	0.0627	1	0.0627	3985.52	< 0.0001	
C-Drying temperature	1.60	1	1.60	1.020E+05	< 0.0001	
AB	0.0154	1	0.0154	978.03	< 0.0001	
AC	0.0673	1	0.0673	4283.34	< 0.0001	
BC	1.000E-06	1	1.000E-06	0.0636	0.8081	
A^2	0.0464	1	0.0464	2951.32	< 0.0001	
B^2	0.4455	1	0.4455	28336.47	< 0.0001	
C^2	0.1991	1	0.1991	12666.69	< 0.0001	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0001	3	0.0000	1.67	0.3086	not significant
Pure Error	0.0000	4	0.0000			
Cor Total	2.24	16				

The F-value of 1.67 for the Lack of Fit indicates that it is not significant in comparison to the pure error. There's a 30.86 percent possibility that a high Lack of Fit F-value is caused by noise. Because a non-significant lack of fit is acceptable, we want the model to fit.

4.7.3. Analysis of variance (ANOVA) for elongation at break

The suggested model for elongation at break in this study was a quadratic model for which analysis of variance was used. The F-value of 6224.12 for the model indicates that it is significant. An F-value of this magnitude has a 0.01 percent chance of occurring due to noise. Model terms with P-values less than 0.0500 are significant. In this case A, B, C, B^2, C^2 are significant model terms. The model terms are not significant if the value is bigger than 0.1000. Model reduction may improve your model if there are many insignificant model terms (not counting those required to support hierarchy).

Table 4.6. ANOVA for quadratic model of percent elongation at break

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	61.26	9	6.81	6224.12	< 0.0001	significant
A-HCl Conc.	2.80	1	2.80	2557.32	< 0.0001	
B-Glycerol Conc.	32.20	1	32.20	29445.09	< 0.0001	
C-Drying Temperature	24.92	1	24.92	22789.37	< 0.0001	
AB	0.0016	1	0.0016	1.46	0.2657	
AC	0.0000	1	0.0000	0.0229	0.8841	
BC	0.0030	1	0.0030	2.77	0.1402	
A^2	0.0003	1	0.0003	0.3119	0.5939	
B^2	0.0596	1	0.0596	54.52	0.0002	
C^2	1.23	1	1.23	1128.98	< 0.0001	
Residual	0.0077	7	0.0011			
Lack of Fit	0.0002	3	0.0001	0.0312	0.9915	not significant
Pure Error	0.0075	4	0.0019			
Cor Total	61.27	16				

The F-value of 0.03 for the Lack of Fit indicates that it is not significant in comparison to the pure error. There's a 99.15 percent possibility that a high Lack of Fit F-value is caused by noise. Because a non-significant lack of fit is acceptable, we want the model to fit.

4.7.4. Analysis of variance (ANOVA) for biodegradability

The suggested model for biodegradability in this study was a quadratic model for which analysis of variance was used. The F-value of 7729.13 for the model indicates that it is significant. An F-value of this magnitude has a 0.01 percent chance of occurring due to noise. Model terms with P-values less than 0.0500 are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve your model.

Table 4.7. ANOVA for quadratic model of biodegradability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	35.23	9	3.91	7729.13	< 0.0001	significant
A-HCl Conc.	0.1378	1	0.1378	272.13	< 0.0001	
B-Glycerol Conc.	4.21	1	4.21	8303.24	< 0.0001	
C-Drying Temperature	29.38	1	29.38	58006.43	< 0.0001	
AB	0.0169	1	0.0169	33.37	0.0007	
AC	0.0462	1	0.0462	91.28	< 0.0001	
BC	0.0225	1	0.0225	44.43	0.0003	
A^2	0.0310	1	0.0310	61.13	0.0001	
B^2	0.1655	1	0.1655	326.77	< 0.0001	
C^2	1.14	1	1.14	2254.64	< 0.0001	
Residual	0.0035	7	0.0005			
Lack of Fit	0.0020	3	0.0007	1.78	0.2906	not significant
Pure Error	0.0015	4	0.0004			
Cor Total	35.23	16				

The F-value of 1.78 for the Lack of Fit indicates that it is not significant in comparison to the pure error. There is a 29.06 percent possibility that a high Lack of Fit F-value is caused by noise. Because a non-significant lack of fit is acceptable, we want the model to fit.

4.7.5. Analysis of variance (ANOVA) for water absorption

The suggested model for water absorption in this study was a quadratic model for which analysis of variance was used. The F-value of 61091.97 for the model indicates that it is significant. An F-value of this magnitude has a 0.01 percent chance of occurring due to noise. Model terms with P-values less than 0.0500 are significant. In this case A, B, C, AB, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 4.8. ANOVA for quadratic model of water absorption

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	255.67	9	28.41	61091.97	< 0.0001	significant
A-HCl Conc.	0.8646	1	0.8646	1859.38	< 0.0001	
B-Glycerol Conc.	0.0630	1	0.0630	135.51	< 0.0001	
C-Drying Temperature	118.58	1	118.58	2.550E+05	< 0.0001	
AB	0.5041	1	0.5041	1084.09	< 0.0001	
AC	0.0000	1	0.0000	0.0538	0.8233	
BC	0.2070	1	0.2070	445.22	< 0.0001	
A^2	50.38	1	50.38	1.083E+05	< 0.0001	
B^2	62.05	1	62.05	1.335E+05	< 0.0001	
C^2	10.60	1	10.60	22791.01	< 0.0001	
Residual	0.0033	7	0.0005			
Lack of Fit	0.0018	3	0.0006	1.60	0.3227	not significant
Pure Error	0.0015	4	0.0004			
Cor Total	255.67	16				

The F-value of 1.60 for the Lack of Fit indicates that it is not significant in comparison to the pure error. There is a 32.27 percent possibility that a high Lack of Fit F-value is caused by noise. Because a non-significant lack of fit is acceptable, we want the model to fit.

4.7.6. Model Equation

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The high levels of the factors are coded as +1 and the low levels of the factors are coded as -1 by default. By comparing the factor coefficients, the coded equation can be used to determine the relative impact of the factors. When all other factors are maintained constant, the coefficient estimate represents the expected change in response per unit change in factor value. The final quadratic equations for the four responses (tensile strength, percent elongation at break, biodegradability and water absorption) in terms of coded factors which was generated based on the regression analysis are presented below.

$$TS = 6.68 + 0.0439A - 0.0885B + 0.4476C - 0.0620AB + 0.1297AC - 0.0005BC + 0.1050A^2 - 0.3253B^2 + 0.2175C^2 \qquad Eq(17)$$

$$E = 19.77 + 0.5913A + 2.01B - 1.77C - 0.0200AB - 0.0025AC - 0.0275BC - 0.0090A^2 - 0.1190B^2 - 0.5415C^2 \qquad Eq(18)$$

$$BioD = 82.66 - 0.1312A + 0.7250B - 1.92C + 0.0650AB + 0.1075AC + 0.0750BC + 0.0857A^2 + 0.1982B^2 + 0.5208C^2 \qquad Eq(19)$$

$$W = 57.66 - 0.1188A + 1.39B - 4.01C - 0.0925AB - 0.0050AC - 0.2800BC - 0.0647A^2 + 0.1153B^2 - 1.09C^2 \qquad Eq(20)$$

Where, TS is tensile strength, E is percent elongation at break, BioD is biodegradability, W is water absorption; and A, B and C are symbol for coded factors of HCl, glycerol and drying temperature, respectively.

4.7.7. Model validation and confirmation

The goodness of the model developed could be evaluated from coefficient of variation (CV) and the values of coefficient of multiple determination for multiple regression, R^2, for each response determined with the analysis of variance (Sharma, Singh, and Dilbaghi 2009). According to the work of (Manohar et al. 2013), the analysis of variance (ANOVA) technique was one of the

technique used to check the adequacy of the developed models at 95% confidence level. In this study, the regression coefficients, R^2, were found 1.0000, 0.9998, 0.9989 and 0.9999 for tensile strength, percent elongation at break, biodegradability and water absorption, respectively. The determination coefficient values for all responses are equal or closer to 1.0000, which shows the correlation between the experimental and predicted values was better. The coefficient of determination, R^2=1 for tensile strength is the measure of the goodness of fit of the model indicating the absence of not explained variation (Pujari and Chandra 2000). The coefficient of determination, R^2, 0.9998, 0.9989 and 0.9999 for percent elongation at break, biodegradability and water absorption shows 0.02, 0.11 and 0.01 % of the total variations are not explained by the model, respectively. The research paper done by (Pujari and Chandra 2000) explained that the coefficient of variation (CV) indicates the degree of precision with which the treatments are compared. Usually, higher the value of the CV, the lower is the reliability of the experiment. In this study, the lower values of 5.94%, 17%, 2.71% and 5.75% CV for tensile strength, elongation at break, biodegradability and water absorption, respectively indicates a greater reliability of the experiments performed for the selected quadratic model.

Table 4.9. Summary of Fit Statistics for all responses

	Responses			
	TS	**EB**	**BioD**	**Water absorption**
Std. Dev.	0.0040	0.0331	0.0225	0.0329
Mean	6.67	19.45	83.03	57.17
C.V.%	0.0594	0.1700	0.0271	0.0575
R^2	1.0000	0.9998	0.9989	0.9999
Adjusted R^2	0.9999	0.9997	0.9985	0.9999
Predicted R^2	0.9996	0.9994	0.9981	0.9993
Adq precision	521.9028	297.3830	306.0588	428.7267
Press	0.0011	0.0145	0.0348	0.0995

The Predicted R^2 of all responses are in reasonable agreement with the Adjusted R^2; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. Since all the ratios are greater than 4, which is desirable, the signal to noise ratio of all responses indicates an adequate signal. Hence, this model can be used to navigate the design space.

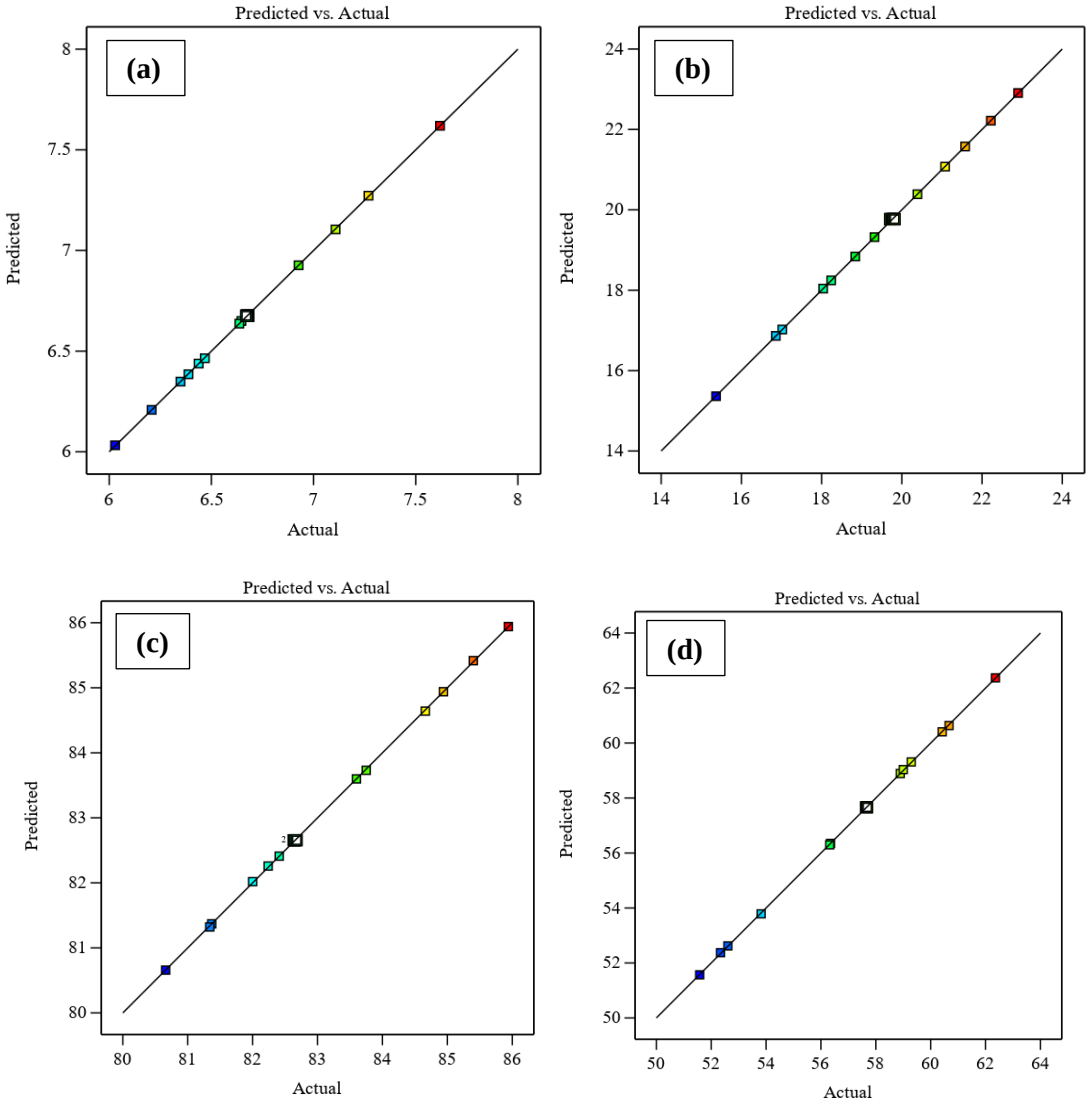


Fig. 4.5. Predicted versus actual experimental results for (a): tensile strength, (b): percent elongation at break, (c): biodegradability and (d): water absorption

The verification experiments can be also performed at the predicted conditions, indicating the validity and adequacy of the predicted models (Sharma, Singh, and Dilbaghi 2009). A normal percent probability plot of the residuals is depicted in Fig. 4.6, which exposes that the residuals generally fall on a least-square line which is used to estimate the cumulative distribution function

for the population. As it is plain from the figure, the errors are normally distributed and there are almost no serious deviation and violations of the assumptions that underlie the analysis (L. Wu et al. 2012).

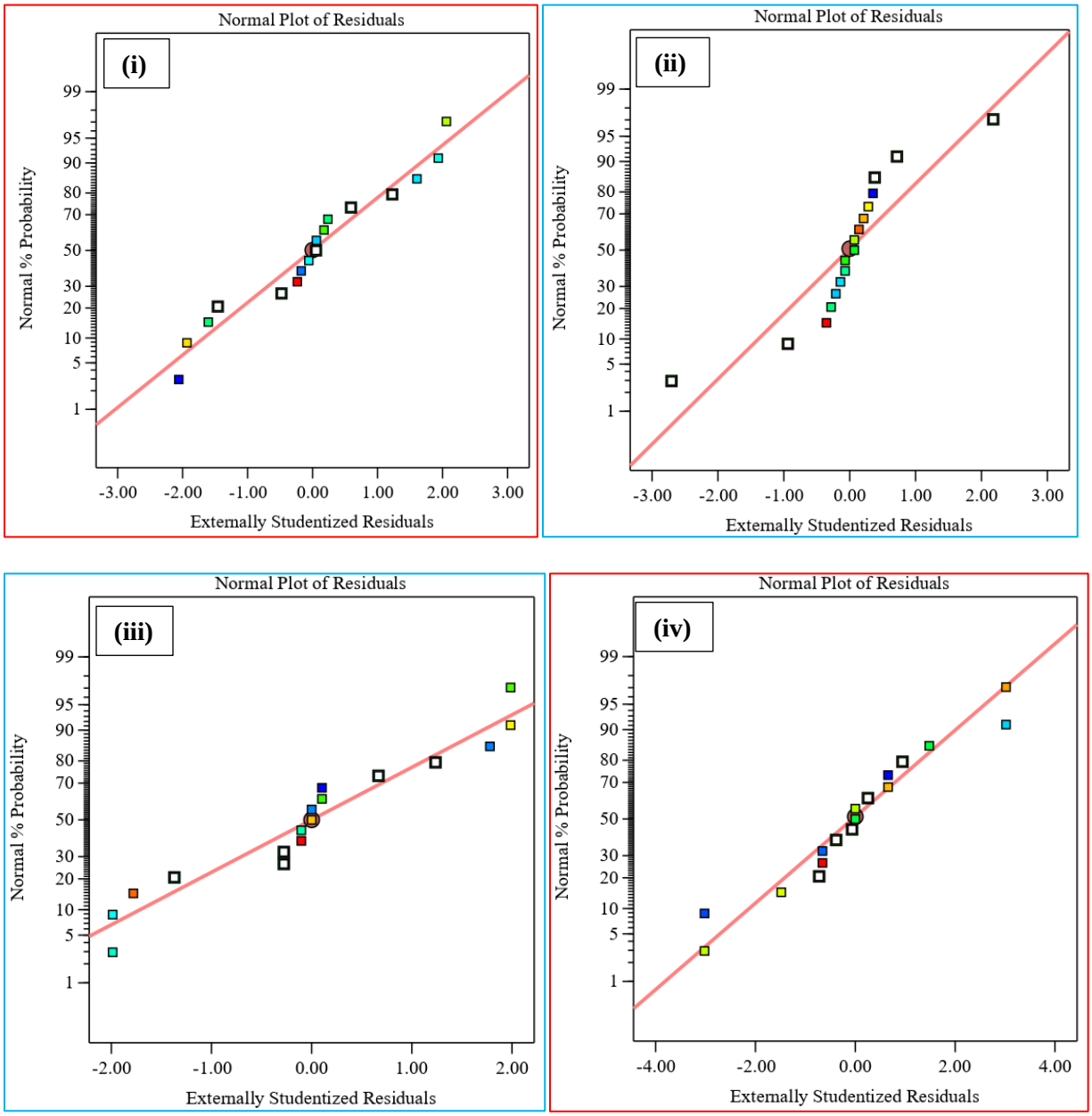


Fig. 4.6. Externally Studentized Residuals versus Normal % Probability graph of (i): tensile strength, (ii): percent elongation at break, (iii): biodegradability and (iv): water absorption

4.8. Effects of individual factors on the properties of PPS based bioplastic

4.8.1. Effects of individual factors on tensile strength

The effect of HCl concentration, glycerol concentration and drying temperature on the tensile strength of the developed PPS based bioplastic film was depicted in Fig.4.7 below. The concentration of HCl has shown a significant effect with the greater statistical significance of the observed difference ($p<0.0001$), as previously shown in Table.4.5., even though there was slight increment of tensile strength approximately beyond the usage of 23.33 % v/w acid to PPS ratio that is 3.5 mL as presented in Fig.4.7(a). This slight increment could be because of the breakdown of high amount branched chains of amylopectin molecule in PPS to linear amylose assisting the film to acquire higher tensile strength.

The concentration of glycerol had also affected the tensile strength with the greater statistical significance of the observed difference ($p<0.0001$). The tensile strength of the bioplastic didn't show significant change in lower level of glycerol. But it increased very slightly up to the intermediate level, which could be because of the less glycerol to PPS %v/w ratio PPS taking advantage over it. The plot of glycerol effect on tensile strength presented by Fig.4.7 (b) plainly shows that tensile strength was decreasing immediately beyond the intermediate level of glycerol concentration. According to (Munthoub and Rahman 2011), the decreasing value in tensile strength which occurred at higher glycerol contents might be associated with the presence of free volume in the bioplastic film and excessive composition of glycerol promoting the mobility of the film rather than giving much effect on the plasticizing the film.

Fig.4.7 (c) clearly shows the increase of tensile strength from 6.029MPa to 7.619MPa with the increasing of drying temperature from 45^{o}C to 65^{o}C, correspondingly. The research done by (Sun, Song, and Zheng 2008) recorded, increasing molding temperature(studied at 25^{o}C, 65^{o}C, 85^{o}C, 105^{o}C and 125^{o}C) induced a significant improvement to increase tensile strength of wheat gluten plastics. As suggested within the same research work, the high temperatures induce cross-linkages within the gelatin matrix by the formation of intermolecular covalent bonds, which results in an increase of mechanical strength. Other researchers confirmed the fact of development of a three-dimensional network structure involving a number of different physical interactions among chain segments as a result of drying temperature (A.Jerez et al. 2005).

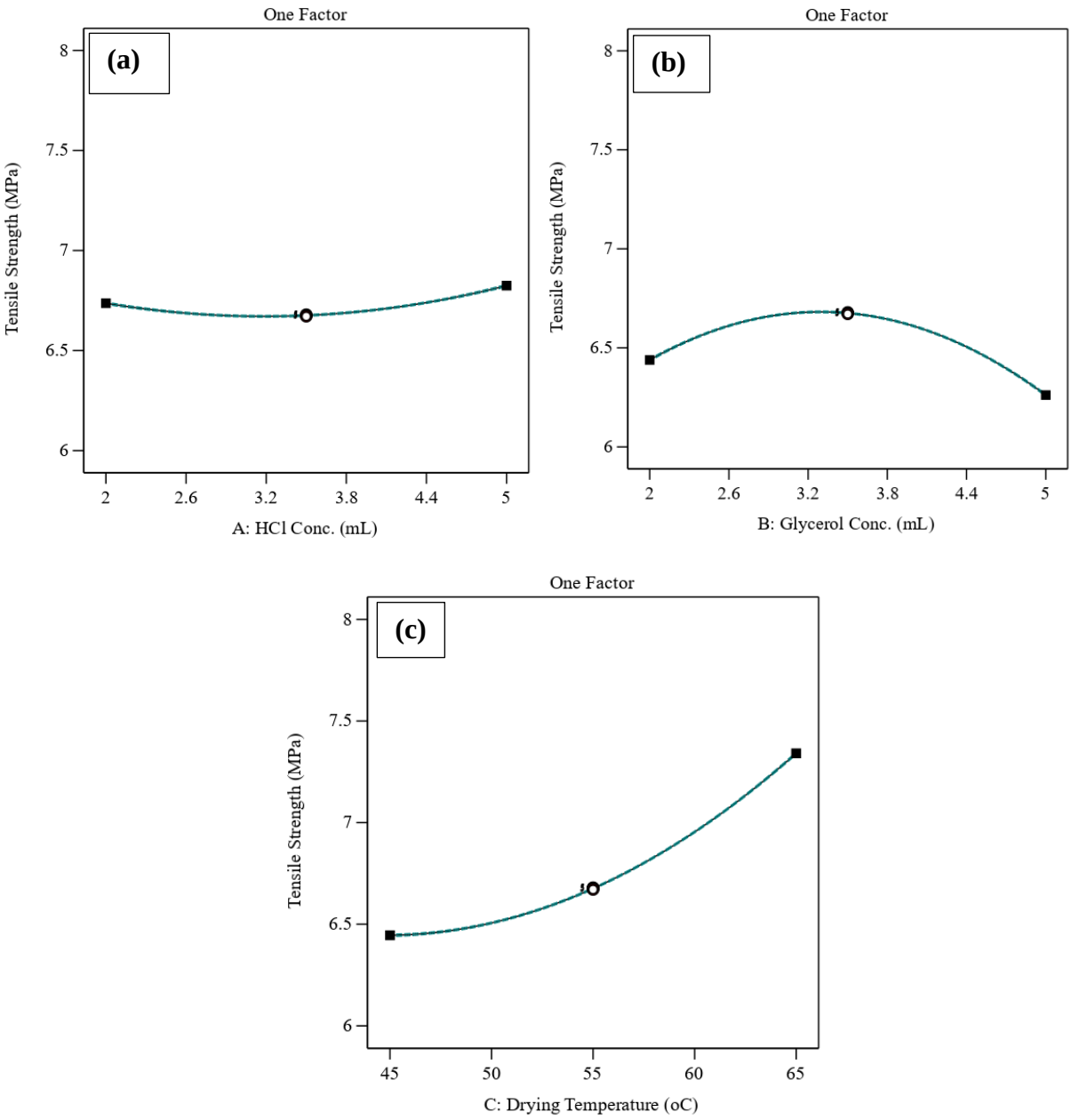


Fig. 4.7. The effect of: (a) HCl concentration, (b) Glycerol concentration and (c) Drying temperature on tensile strength of the bioplastic film

4.8.2. Effects of individual factors on percent elongation at break

The effect of HCl concentration, glycerol concentration and drying temperature on percent elongation at break of the developed PPS based bioplastic film is depicted in Fig.4.6. In this study the effect of HCl concentration has shown significant effect with the greater statistical significance of the observed difference ($p<0.0001$), as presented in Table.4.6., even though it shows slight

increment of percent elongation at break from 19.17 to 19.74 and then to 20.35% as depicted in Fig. 4.8 (a).

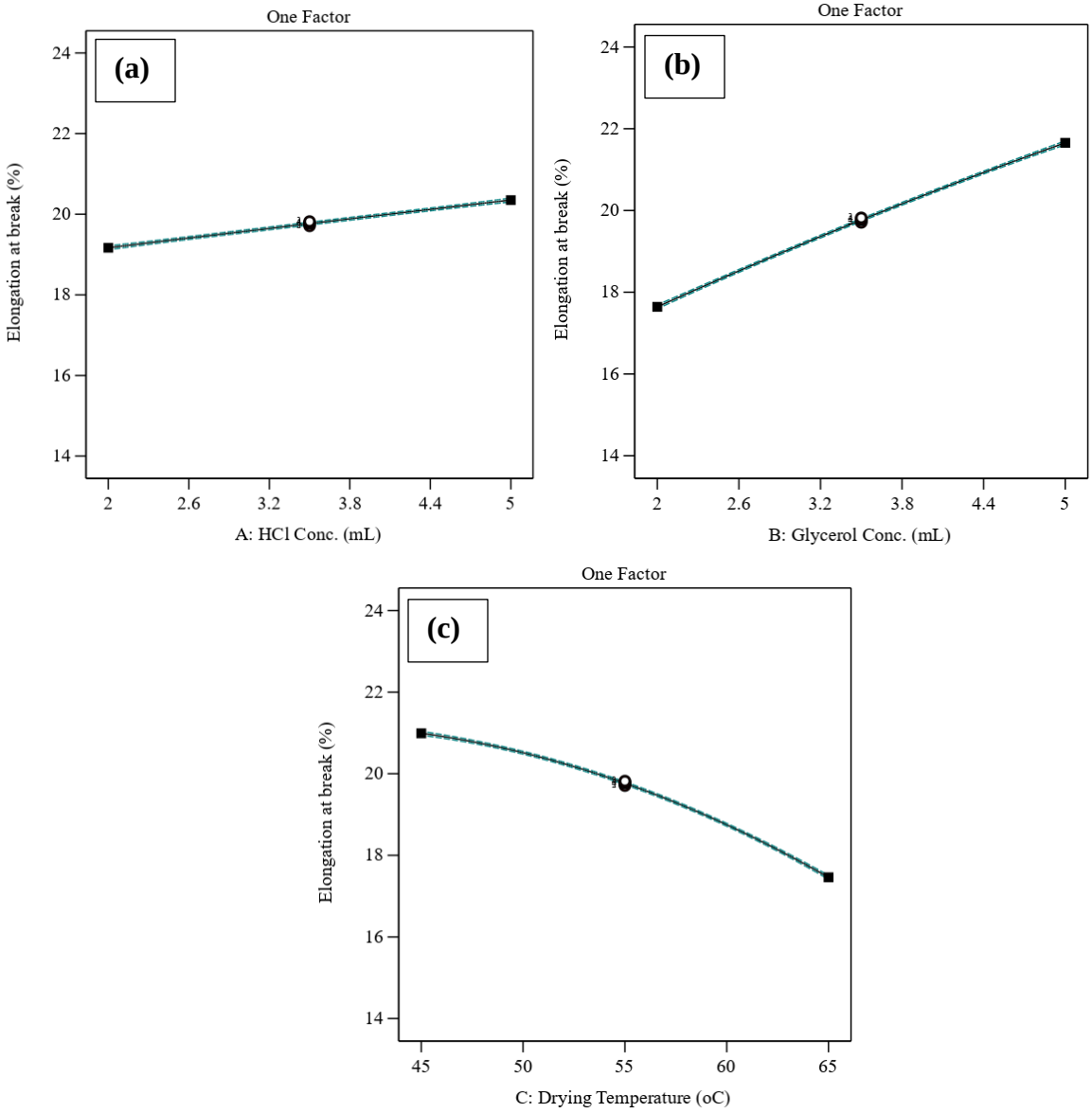


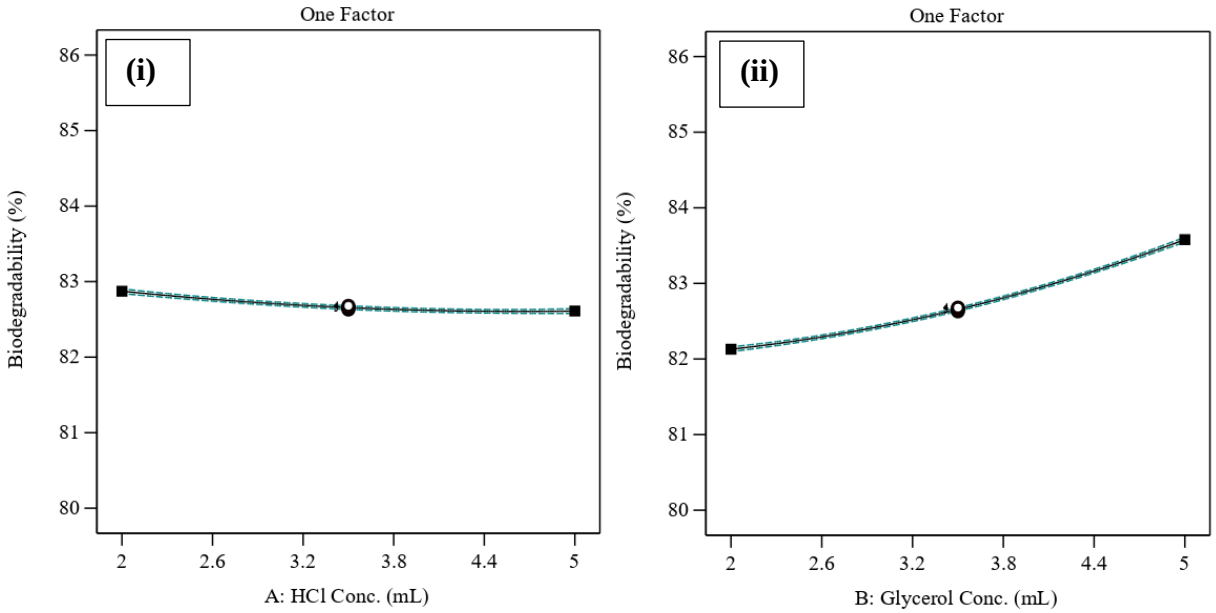
Fig. 4.8. The effect of: (a) HCl concentration, (b) glycerol concentration and (c) drying temperature on percent elongation at break of the bioplastic film

Another individual factor that affected percent elongation at break was glycerol concentration. The plot of glycerol effect on tensile strength presented by Fig.4.8 (b) plainly shows that percent elongation at break was increasing continually. According to the previous research work of (Munthoub and Rahman 2011) which disclosed that glycerol is able to adequately plasticize the

mixtures made to develop the bioplastic film and thus improved the chain flexibility and promote ductility of the starch gelatin. When the concentration of glycerol was lower the lower was percent elongation at break, which is due to insufficient plasticizer that inhibits flexibility and continuity in the matrix chain. Drying temperature had decreasing effect on percent elongation at break of the developed PPS based biodegradable bioplastic. This effect can be obviously seen from the plot presented in Fig. 4.8 (c). The research study conducted by (Sun, Song, and Zheng 2008) exposed the reason for the decrease of elongation at break was due to the fully cross-linkage of the molecule of starting material to form a dense network, which restrains the extensibility of the network strands as the drying temperature gets higher.

4.8.3. Effects of individual factors on biodegradability by enzyme action

The effect of HCl concentration, glycerol concentration and drying temperature on biodegradability was studied in this study. Their effect was graphically presented in Fig.4.9 (i)-(iii) below. Chia et al. grouped degradable plastics into four groups namely compostable plastics, photodegradable plastics, bio-based plastics and biodegradable plastics. Biodegradable plastics usually break down while interacting with water and enzymes (Chia et al. 2020). Concentration of HCl did show significant effect on the degradation of the bioplastic film produced from PPS as shown by analysis of variance with the value $p<0.0001$.



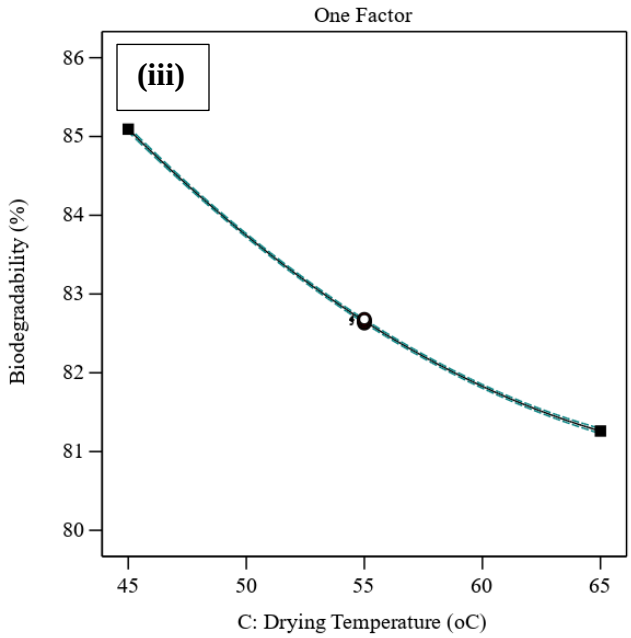


Fig. 4.9. The effect individual factors on biodegradability of the bioplastic film: (i) HCl concentration; (ii) glycerol concentration; (iii)drying temperature

The increase in concentration of glycerol had shown a significant increasing effect on biodegradability. The study done by (Tokiwa et al. 2009) explained hydrophilic property of bioplastic influences the mechanism of biodegradation. The addition of glycerol adds more hydroxyl group in the bioplastic matrix that makes it more hydrophilic and in turn made the film susceptible for biodegradation. From this study, it was observed that alpha amylase enzyme biodegradation of PPS based bioplastic synthesized with the plasticization of glycerol fall in the range of 80.66-85.94%. The starch-based bioplastic produced from banana peel plasticized with glycerol and sorbitol has shown 81.42 and 70.25% degradation in moist soil, respectively (Shafqat et al. 2021) .

Drying temperature had shown a decreasing trend of biodegradation as it tends to increase, that is illustrated in Fig.4.9 (c). Previously it was explained in this document, the formation of fully cross-linkage of the molecules of starting material forms a dense network. Therefore, it can be suggested that decreasing of biodegradability with the increasing of temperature was due to the dense network matrix of the polymer that created difficult condition for the enzyme attached to the polymer substrate to catalyze a hydrolytic cleavage.

4.8.4. Effects of individual factors on Water absorption

The effect of three independent factors namely concentration of HCl, concentration of glycerol and drying temperature on water absorption was studied in this study. The effect of HCl concentration, glycerol concentration and drying temperature had affected the property of bioplastic film with the greater statistical significance of the observed difference ($p<0.0001$). For the actual factors of 3.5mL glycerol and 55^{o}C drying temperature water absorption was slightly decreased from 57.72% to 57.65% and then to 57.48 % with the increasing of HCl usage from 2mL to 3.5mL and 5mL, respectively, as depicted in Fig.4.10 (a).

The effect of higher glycerol has shown increasing effect of water absorption of the bioplastic film and can be seen clearly from the plot of Fig.4.8 (b). For the actual factors of 2M 3.5mL HCl and 55^{o}C drying temperature water absorption was changed from 56.39% to 57.65% and then to 59.12 % with the increasing of glycerol usage from 2mL to 3.5mL and 5mL, respectively. Water absorption value of the bioplastic increased with higher content of glycerol due to the properties of glycerol, which is naturally hygroscopic. The increase in water absorption with respect to glycerol indicates that glycerol is highly hydrophilic. The presences of three hydroxyl groups in a glycerol molecule are responsible for the highest tendency for water absorption of glycerol-plasticized films (Munthoub and Rahman 2011; J.M.Aguilar et al. 2020)

Drying temperature shows remarkable decreasing of water absorption as it increases. For the actual factors of 3.5mL glycerol and 2M of 3.5mL HCl, water absorption was decreased from 60.59% to 57.65% and then to 52.56 % with the increasing of temperature from 45^{o}C to 55^{o}C and 65^{o}C, respectively. Previous studies on similar systems using Glycerol as plasticizer pointed out that mould temperature is the processing parameter with the greatest reducing influence on water uptake capacity, as higher mould temperatures lead to a greater degree of crosslinking between the polymeric chains (J.M.Aguilar et al. 2020).

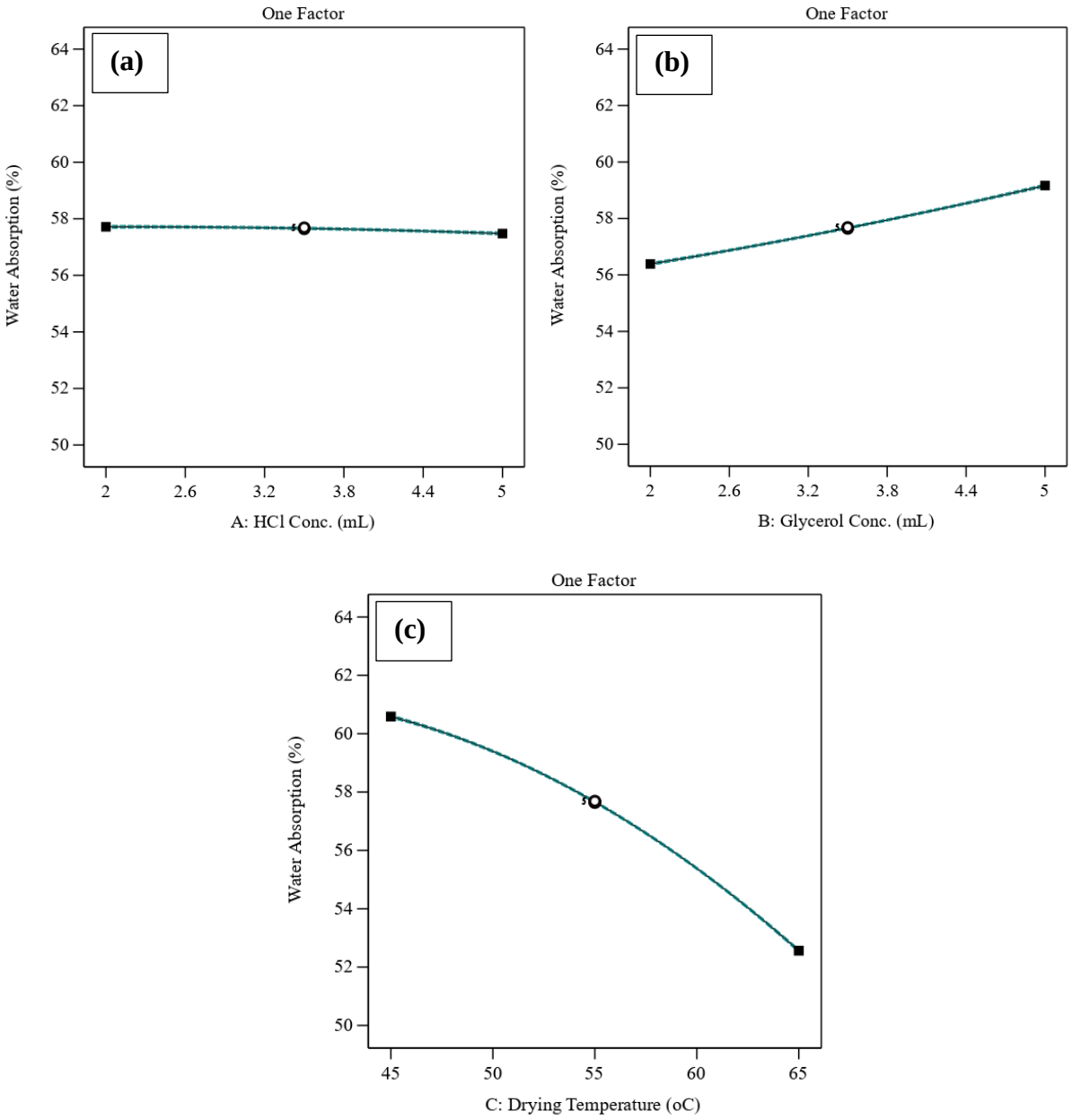


Fig. 4.10. The effect of individual factors on water absorption of the bioplastic film: (a) HCl concentration; (b) glycerol concentration; (c)drying temperature

4.9. Interaction effects of factors on the properties of PPS based bioplastic

4.9.1. Interaction effects of factors on tensile strength

The interaction effect of independent factors on tensile strength of the bioplastic film was depicted with the three-dimensional surface plot in Fig. 4.11. The dots on the surface of the plot with corresponding experimental run numbers represents the value of interacting factors showing the

result of tensile strength. The actual factor C=55°C, B=3.5mL and A=3.5mL held constant when AB, AC and BC interacts to affect tensile strength, respectively. The interaction between HCl concentration and glycerol concentration was significant ($p<0.0001$).

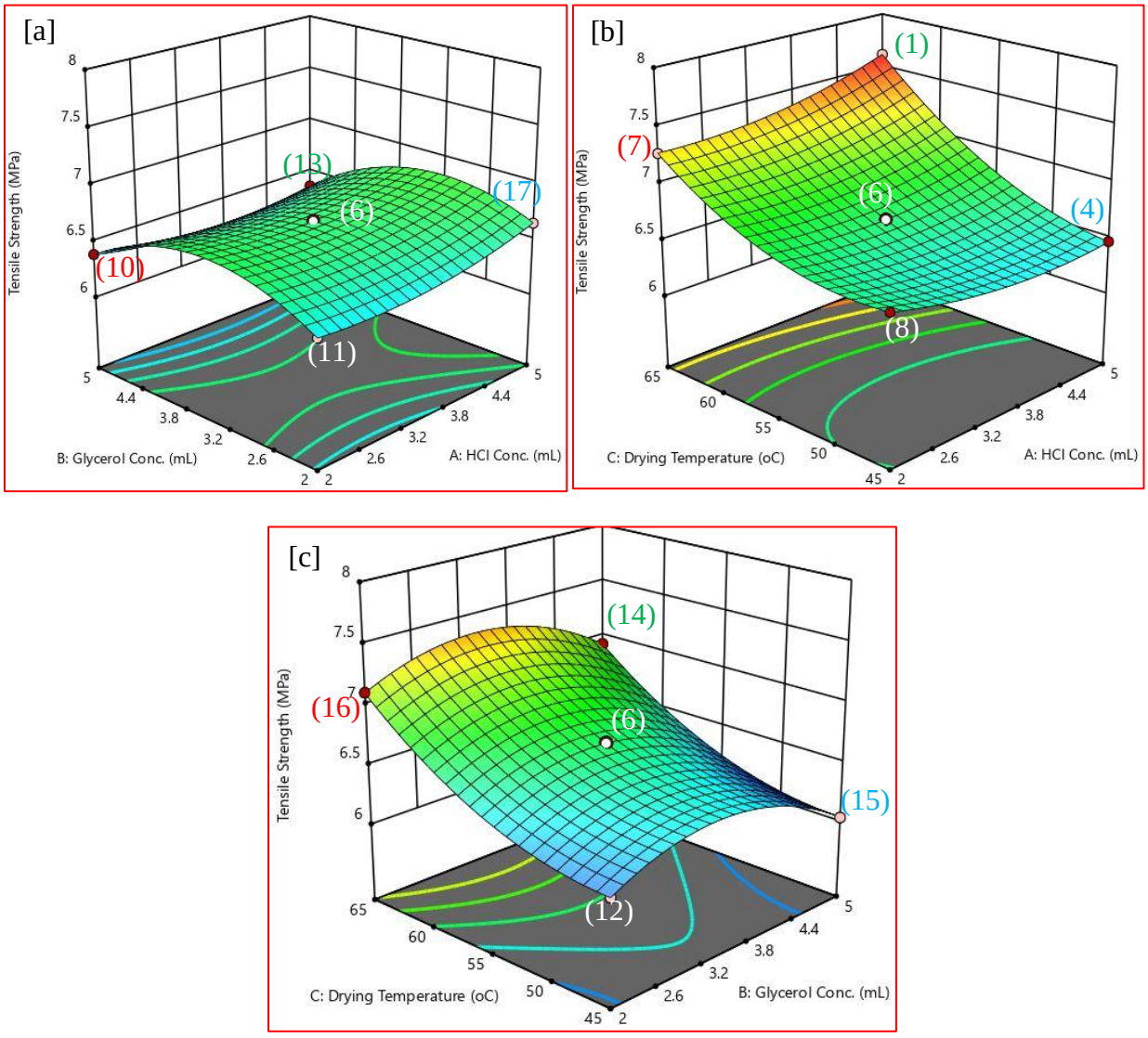


Fig. 4.11. 3D Response surface plots showing the interaction effect of factors on tensile strength of the synthesized bioplastic film: [a] HCl and glycerol concentration interaction; [b] HCl concentration and drying temperature interaction; [c] Glycerol concentration and drying temperature interaction

For interaction between HCl concentration and glycerol concentration, the concentration of glycerol shows greater interaction effect on tensile strength of the produced PPS based bioplastics. This effect can be seen clearly when we move on the 3D surface from the point 17(HCl=5mL,

Glycerol=2mL and tensile strength=6.647MPa) to point 13 (HCl=5mL, Glycerol=5mL and tensile strength=6.349MPa) and from the point 11(HCl=2mL, Glycerol=2mL and tensile strength=6.438MPa) to Point 17. On the movement over the surface for the same amount of HCl and increment of glycerol from 2mL to 5mL there is decrement in tensile strength from 6.647MPa to 6.349MPa, with a difference of 0.298, while on the contrary shift there is only 0.089 difference for the increment of HCl from 2ml to 5mL for the same amount of 2mL glycerol. For the interaction between drying temperature and HCl concentration it can be seen clearly that the interaction effect of drying temperature is dominant letting the effect of HCl concentration comparably less. Their interaction was significant with $p<0.0001$.

There was also interaction among drying temperature and glycerol concentration to affect tensile strength of the produced bioplastic film but the ANOVA result presented in Table.4.5. shows the interaction was not significant. The reason could be the unchanged chemical property of glycerol within the given range of temperature to affect the tensile strength. The dominance of drying temperature effect is vivid from Fig. 4. 11.(c) even though glycerol concentration has also affected tensile strength individually.

4.9.2. Interaction effects of factors on percent elongation at break

The interaction effect of independent factors on percent elongation at break of the bioplastic film was depicted with the three-dimensional surface plot in Fig. 4.12. The dots on the surface of the plot with corresponding experimental run numbers represents the value of interacting factors showing the result of percent elongation at break. The 3D surface plot of Design Expert® software shows that the actual factor C=55^{o}C, B=3.5mL and A=3.5mL held constant when AB, AC and BC interacts to affect percent elongation at break, respectively. The interaction between HCl concentration and glycerol concentration shows, as presented in Fig. 4.12. [a], that the concentration of glycerol had a greater interaction effect on percent elongation of the produced PPS-based bioplastics. This effect can be seen clearly when we move on the 3D surface from the point 11(HCl=2mL, Glycerol=2mL and percent elongation at break=17.02%) to point 10 (HCl=2mL, Glycerol=5mL and percent elongation at break=21.08%) and from the point 11 to Point 17(HCl=5mL, Glycerol=2mL and percent elongation at break=18.24%).

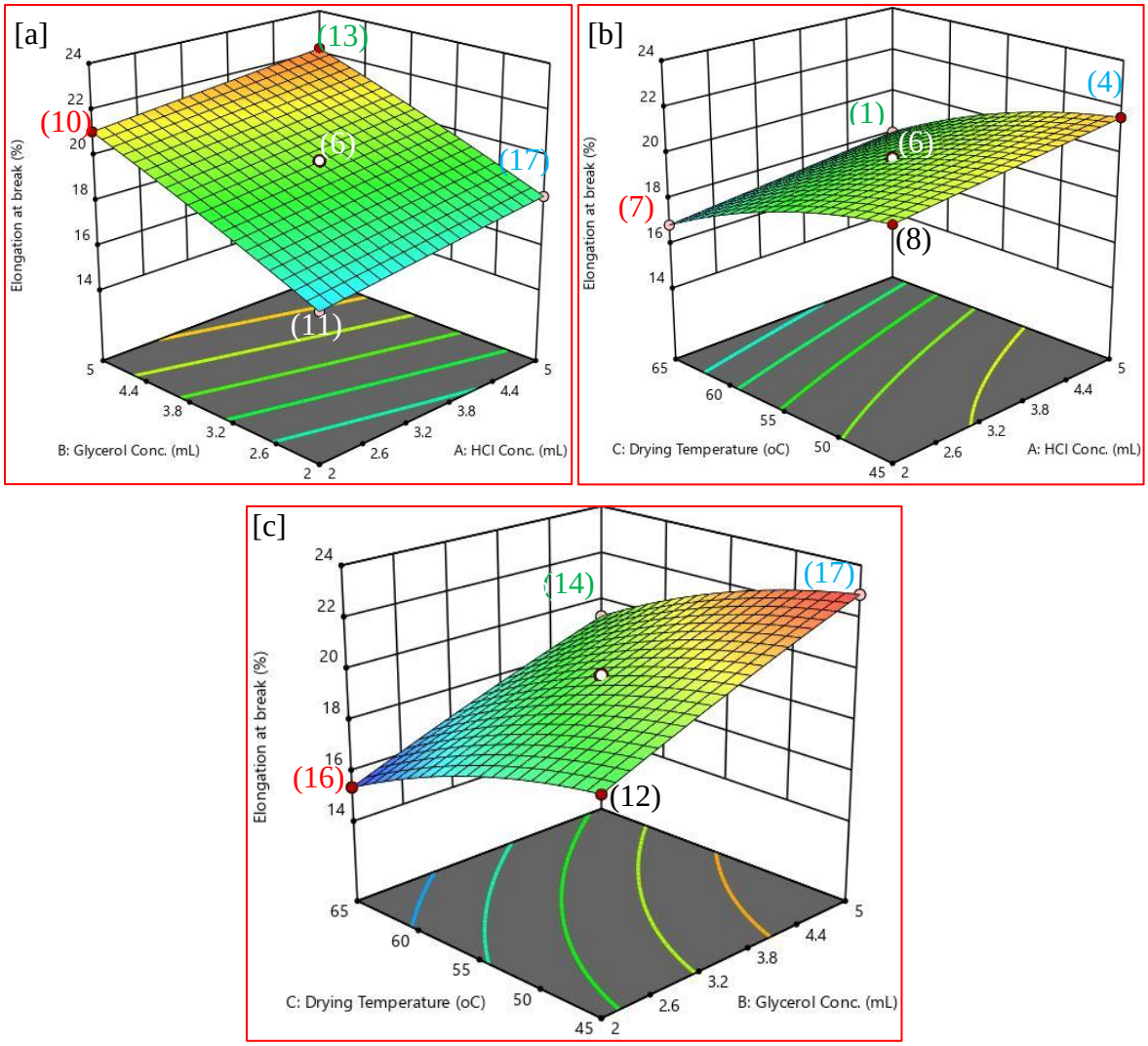


Fig. 4.12. 3D Response surface plot showing the interaction effect of factors on percent elongation at break of the synthesized bioplastic film: [a] HCl and glycerol concentration interaction; [b] HCl concentration and drying temperature interaction; [c] Glycerol concentration and drying temperature interaction

On the movement over the surface for the same amount of HCl and increment of glycerol from 2mL to 5mL there is increment in percent elongation at break from 17.02% to 21.08% with a difference of 4.06%, while on the direction of HCl there is only 1.22% increment difference for the increment of HCl from 2ml to 5mL for the same amount of 2mL glycerol. The higher percent elongation at break was recorded when both HCl and glycerol concentrations were high at experimental run 13. For the interaction between drying temperature and HCl concentration it can be seen clearly from Fig.4.12(b) the interaction effect of drying temperature is dominant over the

effect of HCl concentration as in tensile strength. There was also interaction among drying temperature and glycerol concentration to affect percent elongation at break of the produced bioplastic film but not significant. The equal dominance of drying temperature and glycerol concentration effect is vivid from Fig. 4. 8.(c) even though drying temperature interacts to decrease the percent elongation at break and glycerol interact for increasing. Generally, the combined interaction effects among concentration of HCl, glycerol and drying temperature to affect percent elongation at break didn't show remarkable interaction as it can be seen from analysis of variance.

4.9.3. Interaction effects of factors on biodegradability by enzyme action

The interaction effect of independent factors on biodegradability of the bioplastic film was depicted with the three-dimensional surface plot in Fig. 4.13. The dots on the surface of the plot with corresponding experimental run numbers represents the value of interacting factors showing the result of biodegradability. The 3D surface plot of Design Expert® software shows that the actual factors C=55^{o}C, B=3.5mL and A=3.5mL held constant when AB, AC and BC interacts to biodegradability, respectively. The interaction between HCl concentration and glycerol concentration shows, as presented in Fig. 4.13. [a], that the concentration of glycerol had a greater interaction effect on biodegradability of the produced PPS-based bioplastics letting the interaction of HCl insignificant. This effect can be seen clearly when we move on the 3D surface from the point 11(HCl=2mL, Glycerol=2mL and biodegradability=82.41%) to point 10 (HCl=2mL, Glycerol=5mL and biodegradability=83.75%) and from the point 11 to Point 17(HCl=5mL, Glycerol=2mL and percent elongation at break=82%). On the movement over the surface for the same amount of HCl and increment of glycerol from 2mL to 5mL there is increment in biodegradability from 82.41% to 83.75% with a difference of 1.34%, while on the direction of HCl there is only 0.41% decrement for the increment of HCl from 2ml to 5mL for the same amount of 2mL glycerol. From this finding, when glycerol interacts to increase biodegradability HCl interacts to decrease. Drying temperature interacts greatly to decrease the biodegradability as it can be seen from the interaction with HCl concentration, which is depicted in Fig.4.9. (b).

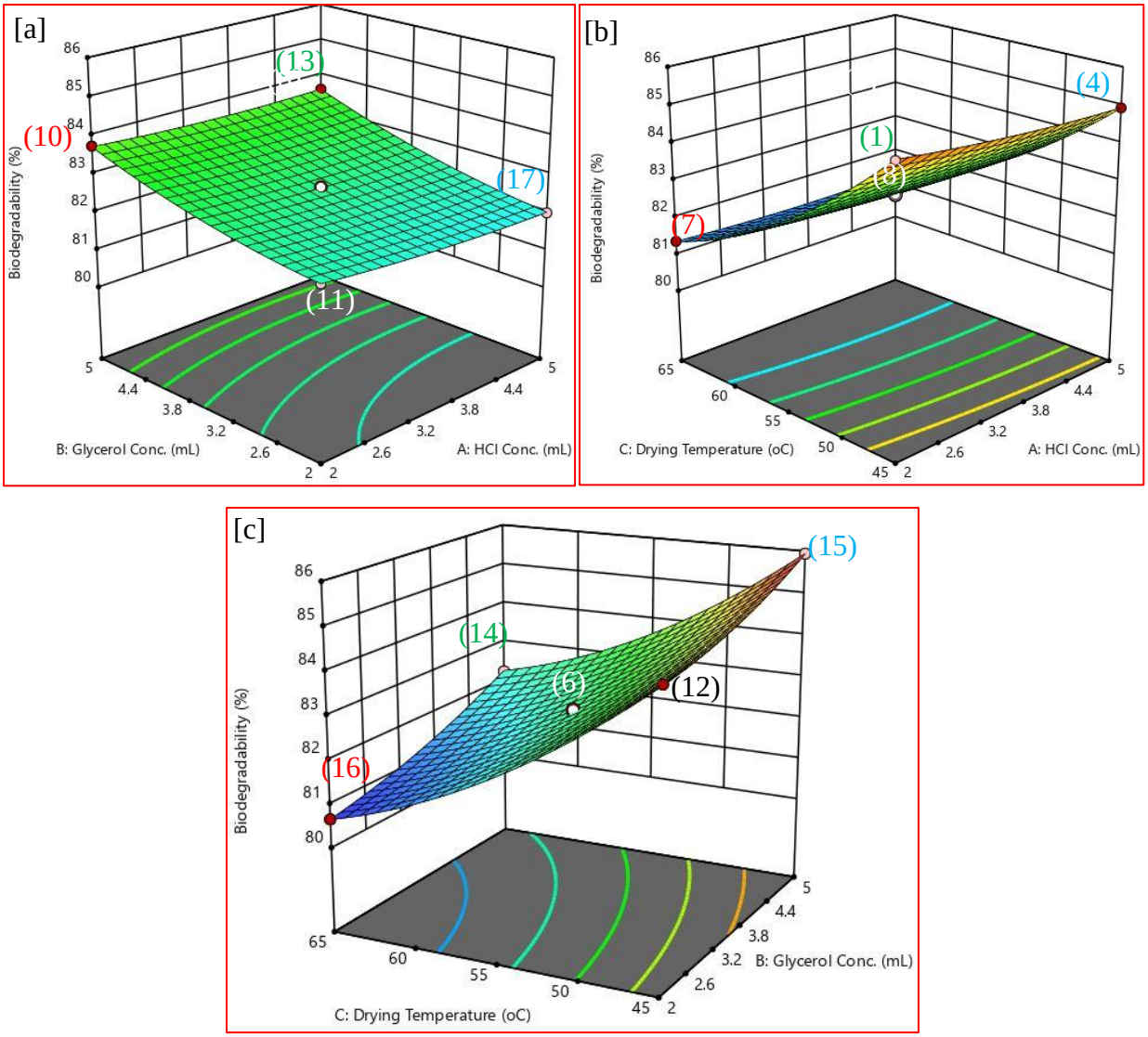


Fig. 4.13. 3D Response surface plot showing the interaction effect of factors on biodegradability of the synthesized bioplastic film: [a] HCl and glycerol concentration interaction; [b] HCl concentration and drying temperature interaction; [c] Glycerol concentration and drying temperature interaction

From drying temperature glycerol interaction 3D plot of Fig. 4.13.(c) the interaction dominance of drying temperature over glycerol concentration is vivid to affect biodegradability in a great way, even though drying temperature and glycerol interacts to increase the biodegradability. The higher interaction of glycerol to increase biodegradability in the case of interaction with HCl was swallowed when it interacts with drying temperature.

4.9.4. Interaction effects of factors on water absorption

The interaction effect of multiple independent factors on water absorption of the bioplastic film was depicted with the three-dimensional surface plot in Fig. 4.14. The dots on the surface of the plot with corresponding experimental run numbers represents the value of interacting factors showing the result of water absorption in percent. The 3D surface plot of Design Expert® software shows that the actual factor C=55°C, B=3.5mL and A=3.5mL held constant when AB, AC and BC interacts to affect water absorption of the bioplastic film, respectively.

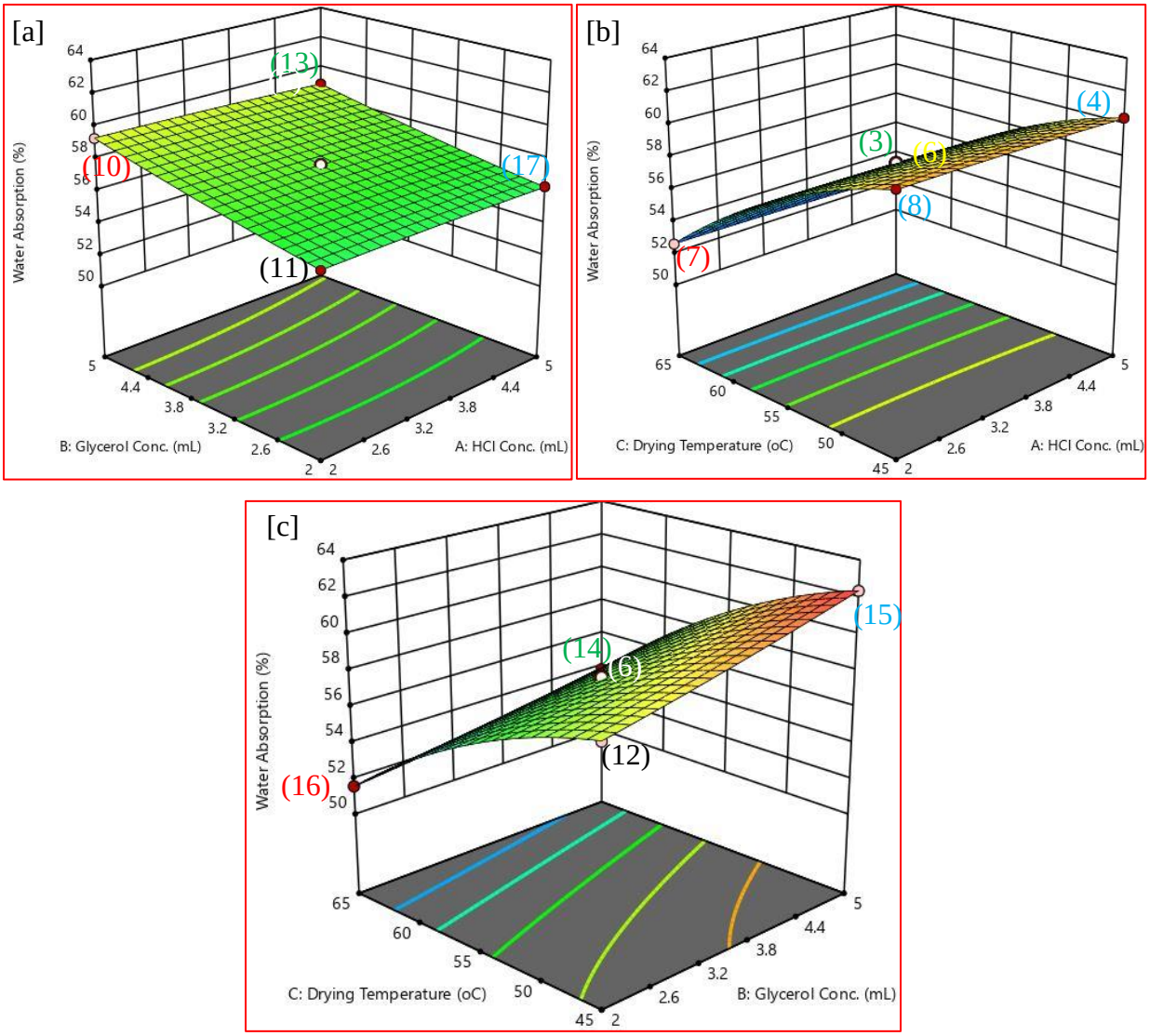


Fig. 4.14. 3D surface plot showing the interaction effect of factors on water absorption of the synthesized bioplastic film: [a] HCl and glycerol concentration interaction; [b] HCl concentration and drying temperature interaction; [c] Glycerol concentration and drying temperature interaction.

The interaction between HCl concentration and glycerol concentration shows that the concentration of glycerol had a greater dominant interaction effect on water absorption of the produced PPS-based bioplastics, as presented in the 3D surface plot of Fig.4.14. (a). The interaction effect of glycerol concentration can be significantly seen when moving on the surface of the plot from point 11 to point 10 and from point 17 to point 13 showing the increase of water absorption from 56.35% to 59.29% and from 56.32% to 58.89%, respectively. On contrary, the interaction effect of HCl concentration is very insignificant which is very plainly seen when moving on the surface of the plot from point 11 to point 17 and from point 10 to point 13 showing very small decreasing changes of the value of water absorption from 56.35% to 56.32% and from 59.29% to 58.89%, respectively.

Drying temperature interacted to decrease the water absorption as it can be seen from the interaction with HCl concentration, which is depicted in Fig.4.14. (b). Water absorption was decreased from 60.67% to 52.61% as drying temperature increased from 45oC to 65oC, respectively, for 2mL HCl and 3.5mL glycerol usage in both cases even though the interaction among them was not significant. The insignificant interaction among drying temperature and HCl concentration would be because of the neutralization of gelatin with NaOH solution. This means drying temperature had no interaction with the concentration of HCl since drying of the casted gelatin was performed after the neutralization. Water absorption result was changed from 60.67% to 60.42 with the change of HCl amount from 2mL to 5mL correspondingly. The interaction between drying temperature and glycerol resulted to decrease and increase water absorption correspondingly. The decreasing interaction of drying temperature had got more significant effect than the increasing effect of glycerol to change the result of water absorption of the bioplastic film.

4.10. Optimization of the process parameters

An optimum setting for the formulation was generated by the numerical optimization technique following desirability approach for multiple responses (Chakraborty et al. 2013). Multi-objective optimization was aimed at to achieve better quality coupled with higher productivity (Aziz and Aziz 2018). The process was optimized for the four response variables namely tensile strength, percent elongation at break, biodegradability and water absorption according to the optimization criteria set in Table 4.10. for the constraints.

Table 4.10. Constraints of the numerical optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: HCl Conc.	is target = 3.5	2	5	1	1	3
B: Glycerol Conc.	minimize	3	5	1	1	3
C: Drying Temp.	minimize	48	60	1	1	5
Tensile Strength	maximize	6.029	7.619	1	1	5
Elongation at break	maximize	15.37	22.9	1	1	5
Biodegradability	maximize	80.66	85.94	1	1	4
Water Absorption	minimize	51.58	62.36	1	1	3

Expert design® software was used to get the optimal solution by numerical optimization method with 103 starting points and had found 13 Solutions. The solution with best minimum of glycerol concentration and water absorption with corresponding best maximum drying temperature, tensile strength, percent elongation at break and biodegradability with desirability of 0.676 was selected as shown below in Table 4.11.

The optimal values found by numerical optimization for the independent variables (factors) were 3.5 mL, 3mL and 48oC for HCl concentration, glycerol concentration and drying temperature, respectively, to have the optimized result of 6.462 MPa, 20.05%, 84.05% and 59.423% for tensile strength, percent elongation at break, biodegradability and water absorption correspondingly. After optimization, experiment was conducted to produce a bioplastic film from PPS at the optimal process variables to confirm these optimized results of responses. The PPS based biodegradable bioplastic synthesized at optimal process variables had results of 6.449MPa, 19.87%, 83.92% and 59.94% for tensile strength, percent elongation at break, biodegradability and water absorption, respectively. The results were not significantly different from those values numerically optimized by Design Expert® software.

Table 4.11. The numerical optimization solutions found

No	HCl Conc.	Glycerol Conc.	Drying Temp	TS	EB	BioD	WA	Desirability	
1	**3.500**	**3.000**	**48.000**	**6.462**	**20.050**	**84.050**	**59.423**	**0.676**	**Selected**
2	3.500	3.020	48.000	6.464	20.077	84.057	59.442	0.676	
3	3.512	3.000	48.000	6.462	20.054	84.049	59.422	0.675	
4	3.488	3.000	48.028	6.463	20.042	84.045	59.417	0.675	
5	3.500	3.000	48.140	6.464	20.036	84.013	59.389	0.675	
6	3.462	3.000	48.000	6.463	20.034	84.056	59.425	0.673	
7	3.500	3.000	48.388	6.468	20.010	83.948	59.328	0.673	
8	3.553	3.002	48.000	6.462	20.075	84.043	59.422	0.672	
9	3.570	3.001	48.000	6.461	20.080	84.040	59.420	0.671	
10	3.500	3.154	48.002	6.472	20.265	84.108	59.578	0.669	
11	3.500	3.000	48.819	6.475	19.964	83.835	59.219	0.669	
12	3.357	3.000	48.000	6.466	19.993	84.073	59.430	0.664	
13	3.500	3.000	49.382	6.486	19.902	83.691	59.071	0.663	

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this study proximate composition of potato peel was performed in the very beginning of laboratory work and PPS was extracted from the fresh potato peels. After examining the proximate composition of PPS, starch-based biodegradable bioplastic was produced using the PPS extracted from waste potato peel by the method of water extraction. The process parameters influencing the properties of synthesized bioplastic film were concentration of HCl, glycerol and drying temperature. A three-variable, three-level Box–Behnken design using Response Surface Methodology was used for optimization and to see the effect of factors on the responses. At the optimum values of factors, pure bioplastic and False banana (*Ensete ventricosum*) fiber reinforced bioplastic films were produced.

The produced bioplastic with the variation of concentration of HCl, concentration of glycerol and drying temperature has shown statistically significant quadratic models for responses of tensile strength, percent elongation at break, biodegradability and water absorption with $p<0.0001$ and all determination coefficients, R^2, almost equal to one. The optimum bioplastic film production conditions predicted for maximum tensile strength, elongation at break, biodegradability and minimum water absorption were 23.33 % HCl concentration, 20 % glycerol concentration and 48^{o}C. Under these optimal values, a bioplastic film with tensile strength of 6.449MPa, percent elongation at break of 19.87%, biodegradability of 83.92% and water absorption of 59.94 % was produced. The incorporation of False banana (*Ensete ventricosum*) fiber has shown the increase of tensile strength resulting maximum of 8.878MPa. The FTIR analysis of pure bioplastic and fiber reinforced bioplastic film exposed the incorporation of fiber didn't change chemical structure of the film.

In general, this research confirms the utilization of potato peel waste as a source of starch for the production of starch-based biodegradable bioplastic film. The produced bioplastic films showed good enough tensile strength coupled with possessing a character of extensibility when reinforced with False banana fiber which can fit the bioplastic film to be used in broad application areas like shopping bags and packaging films substituting petroleum based synthetic plastics.

5.2. Recommendations

The long journey to accomplish this thesis work has exposed the researcher to many observable complications beginning from the extraction of potato peel starch to the development of bioplastic film with some of the problems solved and others left for another researchers. One of the complications left unsolved was the formation of foam when gelation process had been going. Researchers who are interested to conduct the same area of research in the future are recommended to do extensive research to combat this problem.

The effect of varying the amount of starch used in relation to the changing parameters was not covered in this study. Hence, researchers in the coming years can focus on this to disclose if it has any significant effect on the properties of PPS based bioplastic film. In line with this, to examine the effect of using different plasticizer rather than glycerol was left as duty for researchers interested on this part.

The enhancement of tensile strength with the reinforcement of False banana (Ensete ventricosum) fiber at different loading ratio has shown remarkable result with increasing effect to a defined point. The effect of using different sized fiber and fiber orientation is recommended for further studies.

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APPENDICES

Appendix A: Functional groups and its quantified frequencies.

Functional groups	Wavenumbers (cm^{-1})
1. Saturated Aliphatic (alkene/alkyl)	
a) Methyl (−CH3)	
➢ Methyl C-H asym./sym. Stretch	2970–2950/2880–2860
➢ Methyl C-H asym./sym. Bend	1470–1430/1380–1370
b) Methylene (>CH2)	
➢ Methylene C-H asym./sym. Stretch	2935–2915/2865–2845
➢ Methylene C-H bend	1485–1445
2. Olefinic (alkene)	
➢ Alkenyl C=C stretch	1680–1620
➢ Aryl-substituted C=C	1625
➢ Vinyl C-H in-plane bend	1420–1410
➢ Conjugated C=C	1600
3. Aromatic ring (aryl)	
➢ C=C-C Aromatic ring stretch	1615–1580, 1510–1450,
➢ Aromatic C-H stretch	3130–3070
➢ Aromatic C-H in-plane bend	1225–950 (several)
4. Acetylenic(alkyne)	
➢ C≡C Terminal alkyne (monosubstituted)	2140–2100
➢ C≡C Medial alkyne (disubstituted)	2260–2190
➢ Alkyne C-H stretch	3320–3310
5. Alcohol and hydroxy compound	
➢ Hydroxy group, H-bonded OH stretch	3570–3200 (broad)
➢ Normal ‘‘polymeric’’ OH stretch	3400–3200
➢ Phenols, OH stretch	3620–3540
➢ Primary or secondary, OH in-plane bend	1350–1260

Appendix B: Additional data of Experimental Design

Table B1. Model Summary Statistics for Tensile Strength

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.2385	0.6945	0.6241	0.3606	1.55	
2FI	0.2562	0.7287	0.5659	-0.3937	3.37	
Quadratic	**0.0040**	**1.0000**	**0.9999**	**0.9996**	**0.0011**	**Suggested**
Cubic	0.0035	1.0000	0.9999		*	Aliased

Table B2. Model Summary Statistics for Percent Elongation at break

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.3220	0.9780	0.9729	0.9619	2.34	
2FI	0.3665	0.9781	0.9649	0.9203	4.88	
Quadratic	**0.0331**	**0.9998**	**0.9997**	**0.9994**	**0.0145**	**Suggested**
Cubic	0.0432	0.9999	0.9995		*	Aliased

Table B3. Model Summary Statistics for Biodegradability

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.3411	0.9571	0.9471	0.9298	2.47	
2FI	0.3778	0.9595	0.9352	0.8736	4.45	
Quadratic	**0.0225**	**0.9989**	**0.9985**	**0.9981**	**0.0348**	**Suggested**
Cubic	0.0195	1.0000	0.9998		*	Aliased

Table B4. Model Summary Statistics for Water Absorption

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.6449	0.9639	0.9556	0.9321	10.17	
2FI	0.7113	0.9662	0.9459	0.8583	21.21	
Quadratic	**0.0329**	**0.9999**	**0.9999**	**0.9993**	**0.0995**	**Suggested**
Cubic	0.0192	1.0000	1.0000		*	Aliased

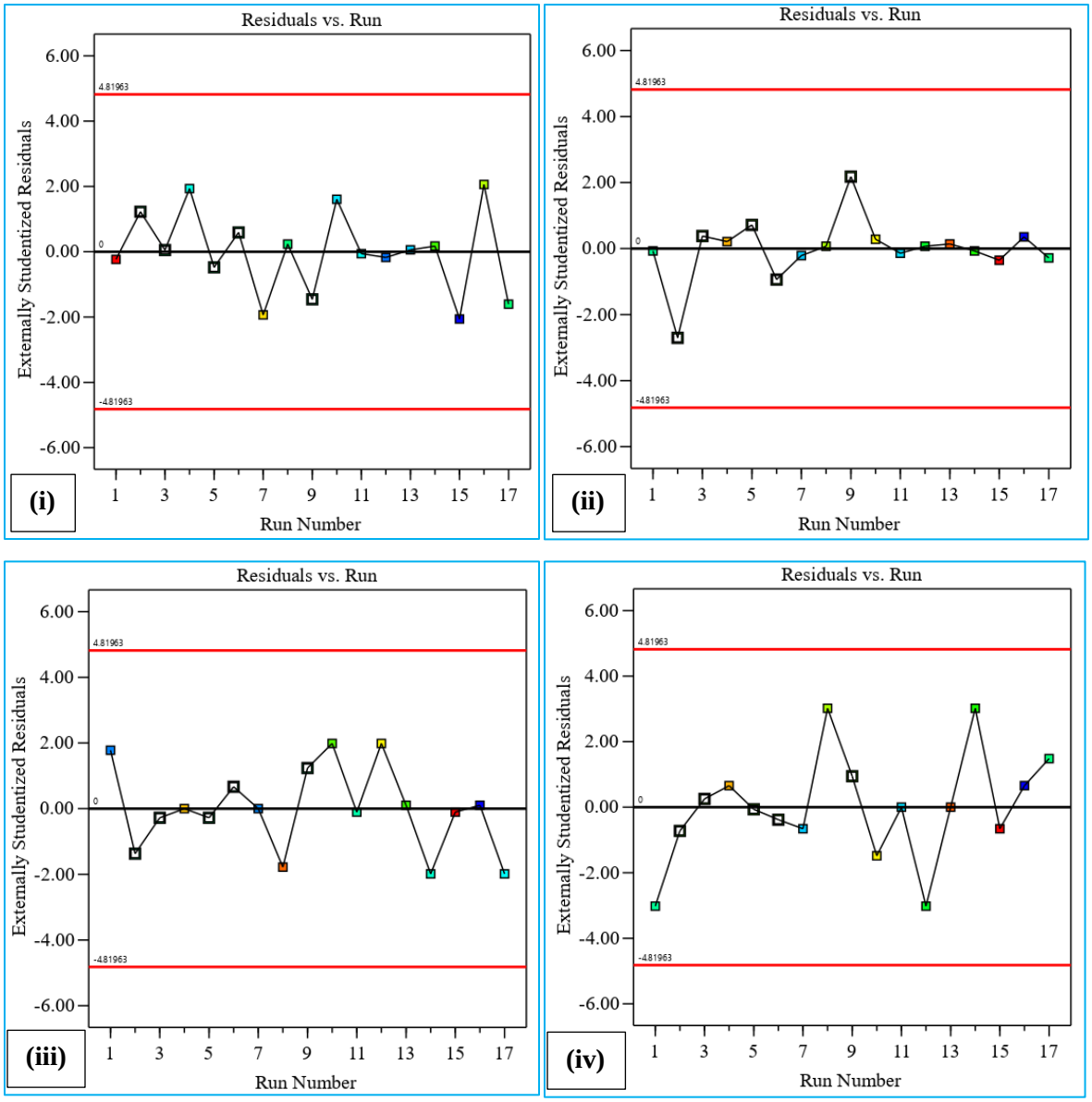

Fig. B1. Plot of Externally Studentized Residuals versus Run Number for: (i) Tensile Strength; (ii) Percent Elongation at break; (iii) Biodegradability; (iv) Water absorption

Appendix C: Additional data used when analyzing tensile strength

Table C1: The data of produced bioplastic film thickness (measured by analog thickness gauge with 20kPa dead weight), maximum force and extension at rapture for different experimental runs

Run	Thickness (mm)	Maximum Force (N)	Extension at rapture (mm)
1	0.18	13.7142	9.022
2	0.19	12.6939	9.855
3	0.18	12.0168	9.893
4	0.2	12.9360	10.792
5	0.18	12.0132	9.895
6	0.18	12.0204	9.872
7	0.19	13.8111	8.434
8	0.2	13.2740	10.195
9	0.17	11.3407	9.912
10	0.22	14.0536	10.541
11	0.17	10.9446	8.514
12	0.18	11.1744	9.423
13	0.21	13.3329	11.113
14	0.19	13.1613	9.662
15	0.21	12.6609	11.451
16	0.17	12.0836	7.685
17	0.17	11.2999	9.124

Table C2: The data of False banana fiber reinforced bioplastic film thickness, maximum force and extension at rapture

Fiber Loading	0%	2%	4%	6%	8%	10%
Thickness (mm)	0.19	0.21	0.21	0.23	0.238	0.245
Maximum Force (N)	12.2531	17.3187	18.0999	20.4194	17.0741	16.7507
Extension at rapture (mm)	9.935	9.232	8.873	8.855	8.472	8.483

Appendix D: Photos Taken During Laboratory Work

Fig. D1. Pictures showing potato peel starch extraction: (a) Waste potato peel; (b) Potato peel disintegration with cutting mixer; (c) Filtration by doubled muslin cloth; (d) Settled starch with unclear supernatant; (e) Starch suspended in clear distilled water; (f) Settled starch with clear supernatant; (g) Extracted wet potato peel starch; (h) Grinding of dried starch by mortar and pistil; (i) Packed potato peel starch (PPS).

Fig. D2. Pictures taken at laboratory during primary stage for bioplastic film development: (A) Chemicals used for bioplastic film development; (B) Mixing of chemicals and ingredients in a beaker; (C) Gelatinization process; (D) Gelatin formed

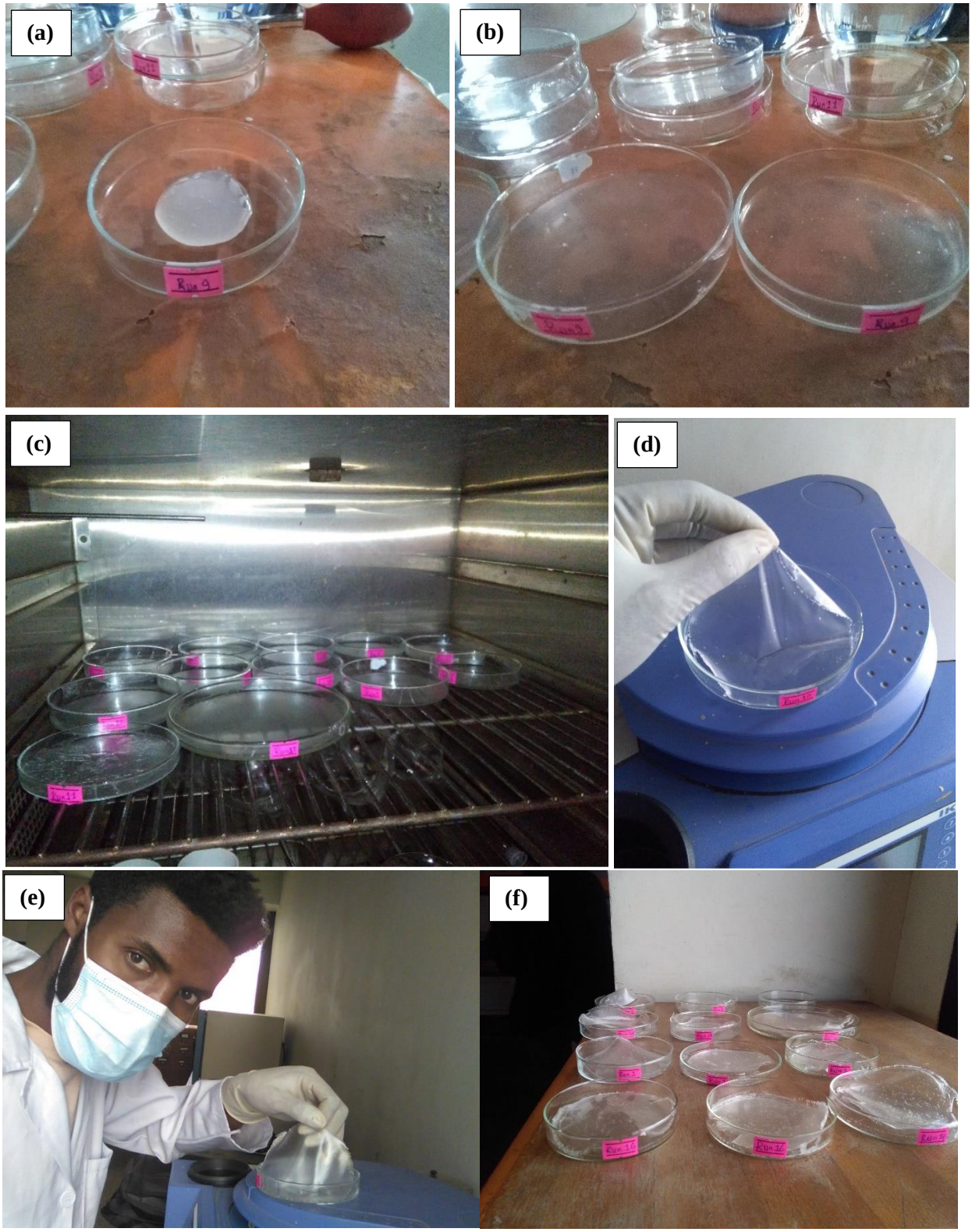

Fig. D3. Pictures taken at laboratory during secondary stage for bioplastic film development: (a) Gelatin ready for casting; (b) Casted gelatin on glass petri dishes; (c) Casted gelatin in drying oven; (d & e) Peeling off the bioplastic film; (f) Peeled off bioplastic films of different experimental run

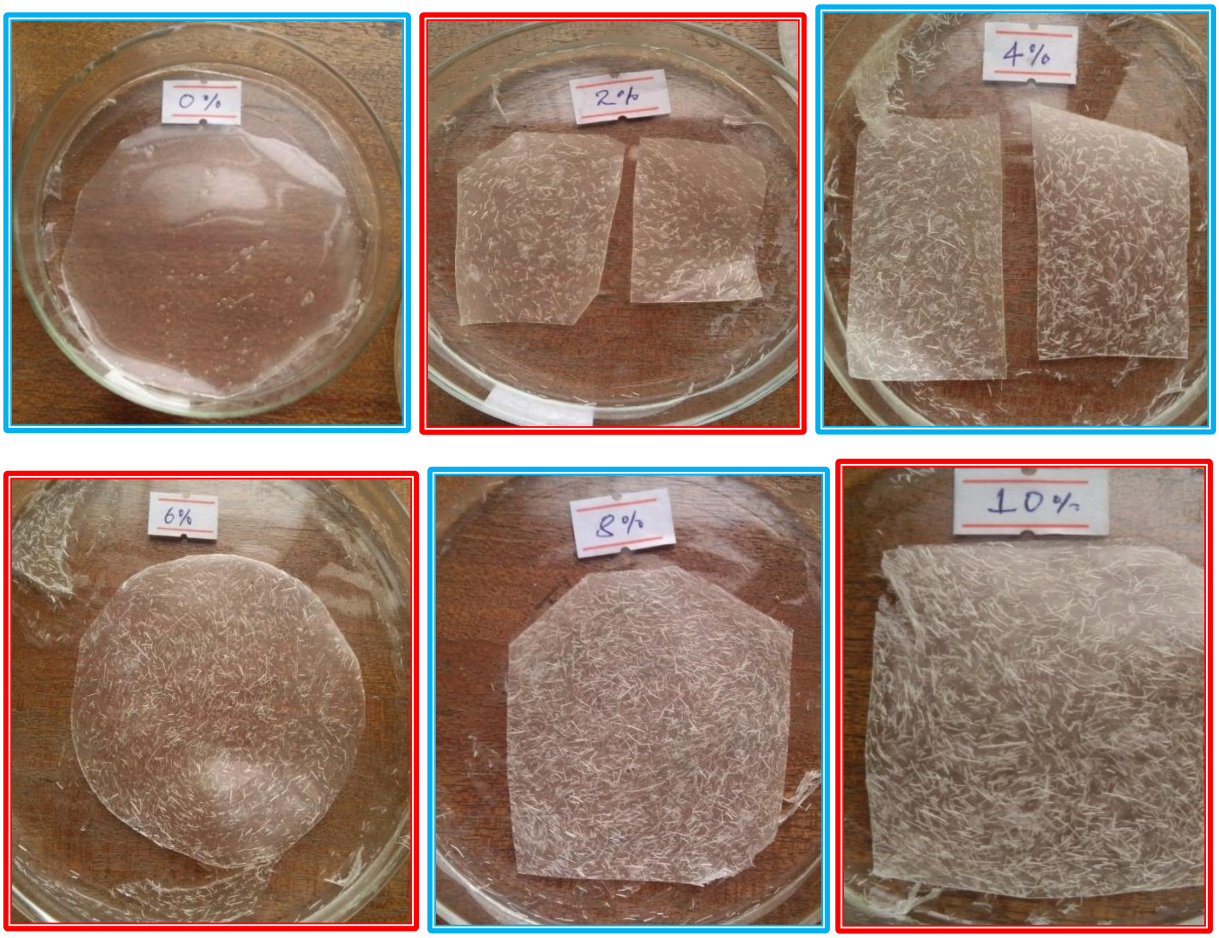

Fig. D4. Bioplastic film produced at optimum values of the variables with different ratios of False banana fiber loading as reinforcement

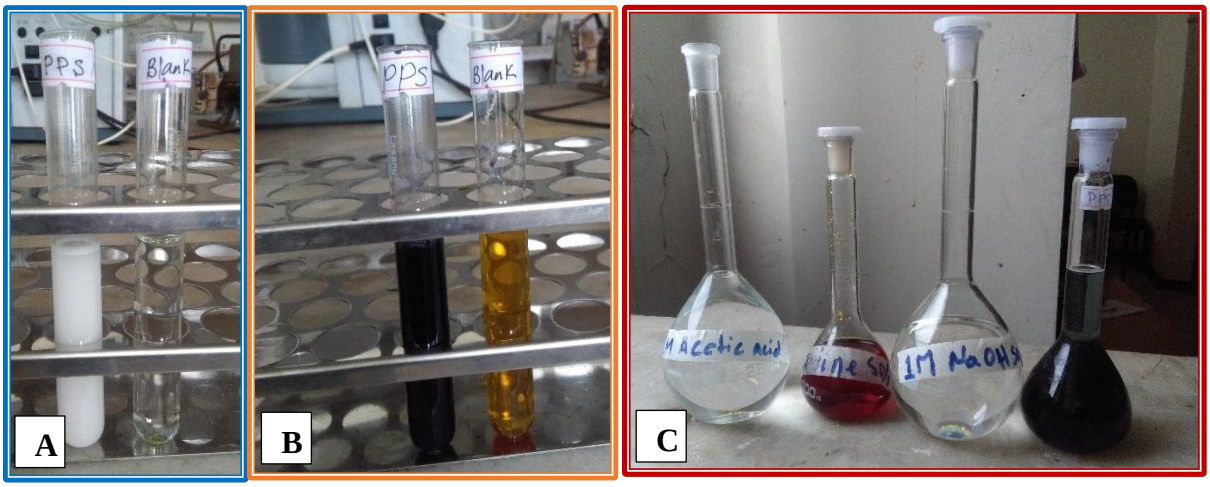

Fig. D5. Iodine test (A) before addition of iodine; (B) after addition of iodine and (C) Solutions prepared for Iodine test and amylose content analysis

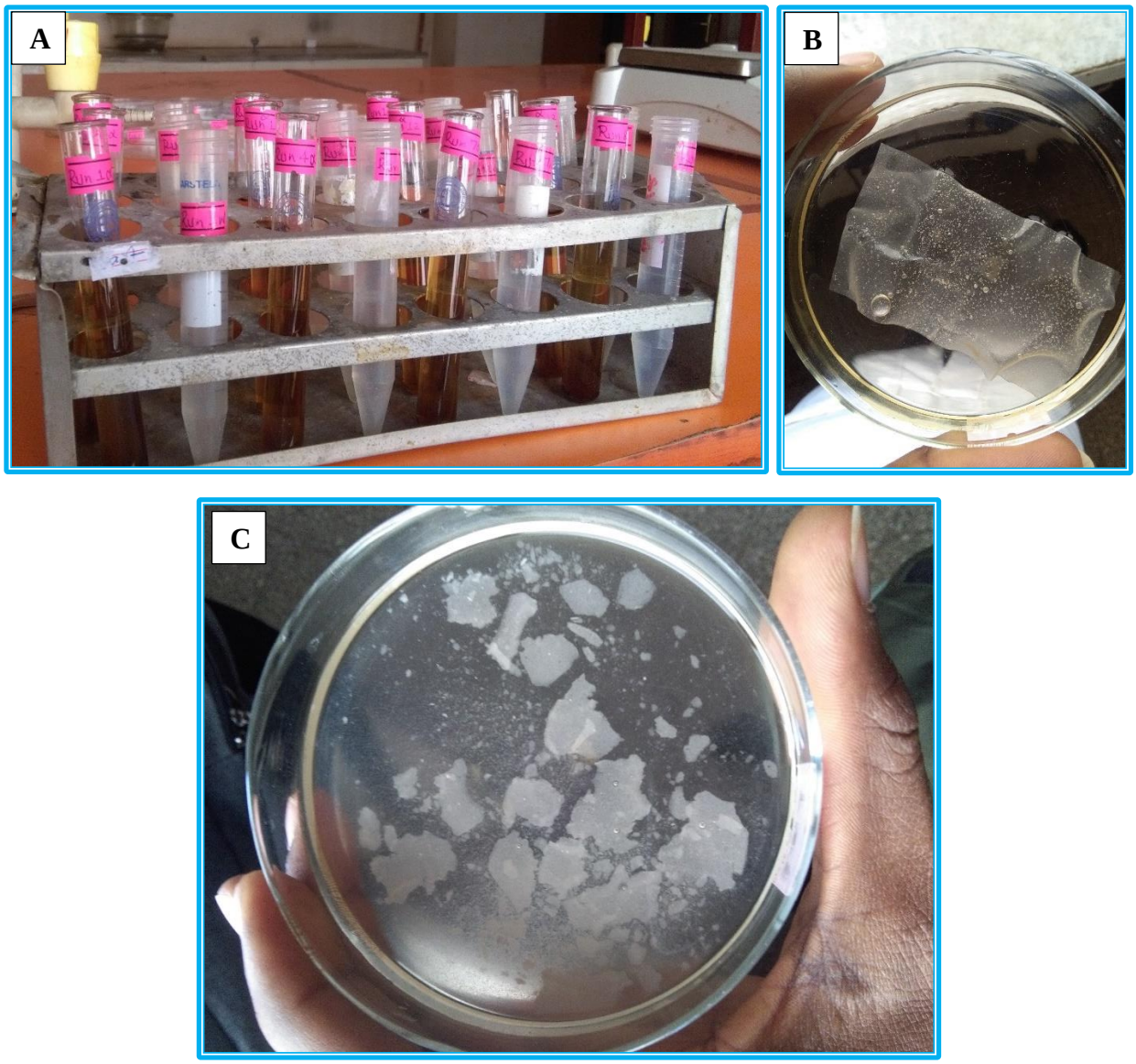

Fig. D6. Biodegradation test of bioplastic by α-amylase enzyme action: (A) bioplastic films soaked in solution containing α-amylase enzyme and in distilled water (as control); (B) Bioplastic film soaked in a solution containing one drop of α-amylase enzyme and (C) Bioplastic film degraded within 24 hrs. by the action of α-amylase enzyme.

“Wastes as a solution for wastes is the unsurpassed imagination that makes the world neat and green. In this approach there is a double victory.”

Tarekegn Yohannes Chaffa