

**BINDURA UNIVERSITY OF SCIENCE EDUCATION**



**FACULTY OF SCIENCE AND ENGINEERING**

**DEPARTMENT OF CHEMISTRY**

**Kidney bean (*Phaseolus Vulgaris L.*) pod husks as a potential source of cellulose for  
the synthesis of bioplastics.**

**BY**

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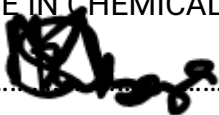
## APPROVAL FORM

The undersigned attest that they have overseen, read and endorse to Bindura University of Science Education for approbation of research project having a title:

**KIDNEY BEAN (*PHASEOLUS VULGARIS L.*) POD HUSKS AS A POTENTIAL SOURCE OF CELLULOSE FOR THE SYNTHESIS OF BIO-PLASTICS.**


Submitted by **Mitchell Shamiso Kuboya**

In partial fulfillment of the requirements for the BACHELOR OF SCIENCE HONOURS DEGREE IN CHEMICAL TECHNOLOGY

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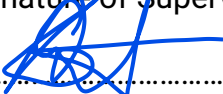
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## DECLARATION

I MITCHELL SHAMISO KUBOYA assert that this report submitted to the Department of Chemistry, Bindura University of Science Education for the conferment of Bachelor of Science Honors degree in Chemical Technology is my own authorship and that no other institution of business or higher learning has produced it. All outsourced information is referenced and a list of which is compiled at the end of the report.

Signature.....

Date 30/05/23.....

## ACKNOWLEDGEMENTS

Firstly, I sincerely thank God for nurturing, guiding and fortifying me throughout this study. I would like to express my sincere gratitude and special thanks to Bindura University for the opportunity to partake on this journey. Special thanks goes to Dr Gwatidzo, my supervisor, Mr. Chibuku, the chief laboratory technician along with Mrs. Zhou, the technical assistant for their unwavering support. I also appreciate Mr. Viriri for assisting me with relevant information whenever I needed. I also thank my colleague, Hazel Muganda for being a pillar of strength throughout this learning period. Lastly, I appreciate my parents and family for their unwavering support financially and emotionally throughout my studies.



## DEDICATION

A dedication to my family, my energy givers.



## LIST OF ABBREVIATIONS

BHT	-	Butylhydroxytoluene
DMAPP	-	Dimethylallyl Pyrophosphate
PBAT	-	Polybutylene Adipate Terephthalate
IPP	-	Isopentenyl Pyrophosphate
IUPAC	-	International Union of Pure and Applied Chemistry
PCL	-	Polycaprolactone
PBS	-	Polybutylene Succinate
PGA	-	Polyglycolic Acid
PLA	-	Polylactic Acid
PHA	-	Polyhydroxyalkanoates



## ABSTRACT

*Synthetic plastics are widely used as packaging materials in all regions of the world because of their suppleness, little weight and affordability. However, the continued use of synthetic plastics pollutes waterways and changes sites where humans or animals live, reducing the ability of ecosystems to suit accordingly to climate change. and has direct impacts on livelihoods, food production capacity and society. Agri-food operations generate billions of tonnes of agricultural waste each year. Amidst the principal parts of lignocellulosic biomass, cellulose has distinct features because it is a renewable organic material found in copious supply in the biosphere. In this study, we investigated waste from *P. vulgaris* pod husks as a potential source of cellulose for the synthesis of bioplastic. The yield of the extracted cellulose was 30%. Different concentrations of glycerol (a plasticizer) and cellulose were used to synthesize three bioplastics, 25% and 35% for sample A, 35% and 50% for sample B and 12.5% and 75% for sample C. The bioplastics were synthesized using the acid hydrolysis method and the method of casting and evaporation. The synthesized bioplastics were characterized using swelling tests and obtained weight differences in chloroform with sample A having 0.01 g, sample B and sample C with 0.02 g, in methanol with sample A having 0.05 g, sample B with 0.03 g and sample C with 0.04 g and in water with sample A having 0.09 g, sample B with 0.1 g and sample C with 0.08 g. Solubility tests where the bioplastics were insoluble in a number of organic solvents and soluble in sulfuric acid were also conducted. A biodegradability test was performed, %weight losses were noted, sample A with 22.38%, sample B with 38.97% and sample C with 41.43%. Characterization tests were also performed, peaks were observed at wavenumbers  $1019.33\text{ cm}^{-1}$ ,  $2683.41\text{ cm}^{-1}$ ,  $1600.62\text{ cm}^{-1}$ ,  $1318.99\text{ cm}^{-1}$ ,  $3329.66\text{ cm}^{-1}$  for the cellulose extract and for the bio-plastic ; $3000\text{ to }3500\text{ cm}^{-1}$  for samples A and B, sample C had a less pronounced peak at  $3000\text{ cm}^{-1}$ . Melting point was also determined and sample A had  $104^{\circ}\text{C}$ , sample B had  $102^{\circ}\text{C}$  and sample C had  $100^{\circ}\text{C}$ . This study showed that *Phaseolus vulgaris* pod husks could be a potential source of cellulose for the production a valuable product, bioplastic, thereby reducing the environmental impact of synthetic plastics.*



## TABLE OF CONTENTS

APPROVAL FORM.....	1
DECLARATION.....	ii
ACKNOWLEDGEMENTS.....	iii
DEDICATION.....	iv
LIST OF ABBREVIATIONS.....	v
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
CHAPTER 1.....	1
1.0 INTRODUCTION.....	1
1.1 BACKGROUND.....	1
1.2 PROBLEM STATEMENT.....	3
1.3 JUSTIFICATION OF THE STUDY.....	3
1.5 AIM.....	4
1.6 OBJECTIVES.....	4
CHAPTER 2.....	5
2.0 LITERATURE REVIEW.....	5
2.1 INTRODUCTION.....	5
2.2 CLASSIFICATION OF BIO-BASED PLASTICS.....	6
2.2.1 Bio-based and non-biodegradable plastics.....	6
2.2.1.1 Bio-derived polyethylene.....	7
2.2.2 Bio-based and bio-degradable.....	7
2.2.2.1 Cellulose plastics.....	7
2.2.2.2 Starch plastics.....	8
2.2.2.3 Polyhydroxyalkanoates.....	9
2.2.2.4 Polylactic Acid.....	10





2.2.2.5 Polyglycolic Acid.....	11
2.2.2.6. Polybutylene Succinate.....	11
2.2.2.7 Lignin based plastics.....	12
2.2.2.8 Cellophane.....	13
2.2.3. Fossil-based and biodegradable.....	13
2.2.3.1 Polycaprolactone.....	14
2.3 KIDNEY BEANS (PHASEOLUS VULGARIS L.).....	14
2.3.1 <i>Phaseolus vulgaris</i> botanical classification.....	15
2.3.2 Kidney bean pod husks.....	15
2.4 CHARACTERIZATION OF THE OBTAINED CELLULOSE.....	15
2.4.1 Fourier Transform Infrared Spectroscopy.....	15
2.4.2 Schultz Reagent.....	16
2.4.3 Reducing sugars tests.....	16
2.4.3.1 Benedicts' test.....	16
2.4.3.2 Fehling's' test.....	16
2.5 CHARACTERIZATION OF THE BIO-PLASTIC.....	16
2.5.1 Swelling tests.....	17
2.5.2 Determination of melting point.....	17
2.5.3 Biodegradability test.....	18
2.5.4 Solubility tests.....	18
CHAPTER 3.....	20
3.0 MATERIALS AND METHODOLOGY.....	20
3.1 INTRODUCTION.....	20
3.2 SAFETY AND CLOTHING.....	20
3.3 CHEMICALS USED.....	20
3.4 EQUIPMENT USED.....	21
3.5 GLASSWARE CLEANING AND STERILIZATION.....	21
3.6 SAMPLE COLLECTION AND PREPARATION.....	21
3.7 EXTRACTION OF CELLULOSE.....	21
3.8 OXIDATION OF CELLULOSE.....	22
3.9 CHARACTERIZATION OF CELLULOSE.....	23
3.9.1 FTIR spectroscopic analysis.....	23



3.9.2 Reducing sugars tests.....	23
3.9.2.1 Fehling’s test.....	23
3.9.2.2 Benedict’s test.....	23
3.10 SCHULTZ REAGENT.....	23
3.11 SYNTHESIS OF THE BIOPLASTIC.....	23
3.12 CHARACTERIZATION OF THE BIOPLASTIC.....	24
3.12.2 Swelling Test.....	25
3.12.3 Melting point determination.....	25
3.12.4 Biodegradability Test.....	25
3.12.5 Solubility Test.....	26
CHAPTER 4.....	27
4.0 RESULTS.....	27
4.1 INTRODUCTION.....	27
4.2 YIELD OF CELLULOSE EXTRACTION.....	27
4.2 FT-IR SPECTRUM OF THE EXTRACTED CELLULOSE.....	28
4.3 REDUCING SUGARS TEST RESULTS.....	28
4.4 SCHULTZ REAGENT TEST RESULTS.....	29
4.5 FTIR OF SYNTHESIZED BIOPLASTIC SAMPLES.....	29
4.5 SWELLING TEST RESULTS.....	30
4.6 BIODEGRADABILITY TEST RESULTS.....	30
4.7 SOLUBILITY TEST RESULTS.....	31
4.8 MELTING POINT DETERMINATION.....	33
CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS.....	34
5.1 EXTRACTION OF CELLULOSE.....	34
5.1.1 Oxidation of cellulose.....	34
5.1.2 Characterization of cellulose extract.....	35
5.2 REDUCING SUGARS TESTS.....	36
5.2.1 Schultz reagent.....	36
5.3 SYNTHESIS OF THE BIOPLASTIC.....	37
5.3.1 FTIR SPECTRA OF THE BIOPLASTIC.....	37
5.4 SWELLING TEST.....	38
5.5 SOLUBILITY TEST.....	38



5.6 BIODEGRADABILITY TEST.....	38
5.8 CONCLUSION.....	39
5.9 RECOMMENDATIONS.....	40
REFERENCES.....	41



## LIST OF TABLES

Table 3. 1: Chemicals used.....	20
Table 3. 2: Equipment used and its functions.....	21
Table 3. 3: Raw material combinations for the synthesis of the three bio-plastics.....	24
Table 4. 1: Yield of cellulose extract obtained from P.vulgaris pod husks.....	27
Table 4. 2: Reducing sugars test results.....	28
Table 4. 3: Results for the swelling test of the synthesized bioplastic.....	30
Table 4. 4: Results of biodegradability for the synthesized bioplastics.....	31
Table 4. 5: Results of solubility for synthesized bioplastics in different solvents.....	32
Table 4. 6: Melting point of the bioplastics.....	33
Table 5. 1: Characterization of cellulose.....	35

## LIST OF FIGURES

Figure 1. 1: <i>P. vulgaris</i> fruit (a), dried pod husks(b) and the plant (c)	3
Figure 2. 1: Structure of polyethylene (Xometry, 2022)	7
Figure 2. 2: Structure of cellulose (Richards et al., 2012)	8
Figure 2. 3: Structure of starch (Master Chemistry, 2021)	9
Figure 2. 4: Structure of PHA's (Doi et al., 2002)	10
Figure 2. 5: Structure of PLA (Matbase, 2012)	11
Figure 2. 6: Structure of PGA (Song et al., 2018)	11
Figure 2. 7: Structure of PBS (Polymerdatabase, 2016)	12
Figure 2. 8: Structure of lignin (Shutterstock, 2023)	13
Figure 2. 9: Structure of cellophane (Shutterstock, 2023)	13
Figure 2. 10: Structure of PCL and how it is formed (Llyas et al., 2023)	14
Figure 3. 1: Process flow diagram for the extraction of cellulose	22
Figure 4. 1: Cellulose sample extracted from <i>P. vulgaris</i> pod husks	27
Figure 4. 2: FTIR spectrum of <i>P. vulgaris</i> pod husks cellulose extract	28
Figure 4. 3: Results for Fehling's test (a) and Benedict's test (b)	28
Figure 4. 4: Positive result (purple colour) of cellulose in <i>P. vulgaris</i> pod husks	29
Figure 4. 5: Sample A synthesized bioplastic and its corresponding FTIR spectrum	29
Figure 4. 6: Sample B synthesized bioplastic and its corresponding FTIR spectrum	29
Figure 4. 7: Sample C synthesized bioplastic and its corresponding FTIR spectrum	30
Figure 5. 1: Oxidation of cellulose with hydrogen peroxide (Wen et al., 2019)	33



## CHAPTER 1

### 1.0 INTRODUCTION

#### 1.1 BACKGROUND

Synthetic plastics, as advanced materials or base materials have long played an important role in many scientific, technological, industrial and household applications. Their extensive relevancy, easy and inexpensive processability, competency ,physicochemical characteristics (e.g. ductility, sustainability, elasticity, light weight, inability to degrade and resistance to weathering) make them attractive and it has contributed to the demand and promoted swift growth of plastic industry in recent decades (Araujo, 2019).

Over 348 million tons of artificial plastics (mainly derived from fossil resources) were produced worldwide in 2017 (Statista, 2017). However, the same characteristics that make artificial plastics a widespread group of material, has a negative impact on the environment if mishandled or discarded. For example, the majority of macro plastic-related materials found in oceans and coastal areas are well consistent with principal classes of short-timed consumer goods that end up in council waste systems, primarily material for packaging uses (Ryberg *et al.*, 2018).

In accordance the above mentioned problems, there is a developing attention in manufacturing biodegradable plastics from renewable polymer sources that are technically, economically, environmentally sound, also unsuitable for food production. In this circumstance, lignocellulosic biomass is the most promising regeneration, as it mainly consists of polymers from nature (cellulose, hemicellulose and lignin) and is widely available in the type of agricultural, forestry and urban residues (Araujo, 2019).

Billions of tonnes of agro-industrial waste are produced each year by agro-food operations. Residues from agro-industrial products are mostly found in the form of crop remains or secondary products, most of which are used for value-added uses, but most are usually deposited on the ground for collection, incineration or decomposition, abandoned or disposed of as municipal solid waste (Araujo, 2019). Agro industrial wastes are therefore not only a low- cost, carbon-neutral alternative, but also a widely



available source of biopolymers, whose use can help prevent pollution and aim to keep resources in a closed loop.

Amidst the principal components of lignocellulosic total mass of organic matter, cellulose is the major profuse renewable organic material in the biosphere (Wang & Li, 2015). It is readily available and has various characteristics (e.g. renewability, affordability, ability to decompose over time as a result of micro-organisms, biocompatibility, chemical and heat stability, capacity to form cognates etc.) make it an enticing biopolymer (Wang & Li, 2015; Habibi *et al.*, 2010).

Several studies have been conducted on bio plastic synthesis using cellulose from various sources. Isroi *et al.*, (2017) developed a bioplastic from cellulose from empty fruit clusters of oil palms. Empty fruit bundles of oil palm contain about 40.37% of cellulose (Isroi, 2015). A study also by Rentoy *et al.*, (2015) recounts on the development of cellulosic bioplastics from cornstalks. About 50% of cornstalk is cellulose (Rentoy *et al.*, 2015). Rambabu *et al.*, (2015) noted that pine blossoms contain 44% of cellulose and Nasihin *et al.*, (2020) developed a biodegradable plastic from cellulose extracted from pine blossoms. A research by Hayatun *et al.*, (2020) described the synthesis of a bioplastic film from rice husk cellulose and Johar *et al.*, (2012) had reported 50% content in rice husks.

No research has been done on the use of cellulose from *Phaseolus vulgaris* for the synthesis of bio-plastics as there is no exact amount known of cellulose in these pod husks. The husk of *Phaseolus vulgaris* could be another source of cellulose for bioplastic production. According to Food and Agriculture Organization Statistics (FAOSTAT, 2019), the global production of common bean has risen from 16.6 million tons (Mt) in 1988 up to the record of 29.3 Mt per year in 2017. This important production of kidney beans was primarily because of their nutritional value and also as an income-generating crop.

Kidney bean is part of the many staple crops of Zimbabwe's communal farming. The crop is multifunctional because it has nutriment benefits, provides funding and also provides sufficient and safe food that meets dietary needs. Acknowledging these advantages, the government of Zimbabwe, through the Health sector, adopted

initiatives to encourage kidney bean consumption and production (Katungi *et al.*, 2017). *P.vulgaris* production in Zimbabwe has been enhanced by the Pan African Bean Research Alliance (PABRA), the Department of Research & Specialist Services (DR&SS) and the Agricultural Research & Extension Services (AGRITEX) through encouraging common bean intensification technologies. This effort has increased *P.vulgaris* by more than 30% (Katungi *et al.*, 2017). Figure 1.1 shows kidney beans and its pod husks.



**Figure 1.1:** *P. vulgaris* fruit (a) (Haileslassie, 2019), dried pod husks (Easy bean, 2023) (b) and the plant (c) (CIAT, 2015)

## 1.2 PROBLEM STATEMENT

The increase in production of common bean (*P. vulgaris*) has also encouraged the increase in production of undesirable residues, such as pod husks on farms. Furthermore, resources from petroleum continuously decline and the environmental problems caused by their products, pollution arising from their products such as synthetic plastics, continue to give headache to governments around the world. It is against this background that this study is conducted to investigate the potential of *P. vulgaris* pod husks as a source of cellulose for the production of bioplastic.

## 1.3 JUSTIFICATION OF THE STUDY

The act of regaining lignocellulosic fractions and biologically active compounds from kidney bean husks may lead to the growth of viable base products and income for farmers, promoting economic development. No studies have been conducted before using (*Phaseolus vulgaris*) pod husk as a natural source of cellulose for bio-plastic synthesis. Problems associated with improper waste disposal of *Phaseolus vulgaris*



may be greatly alleviated by the recovery and biotransformation of their cellulose to produce bio-plastic. Being biodegradable, it can substitute synthetic plastics from non-biodegradable sources. There is need to develop a set of procedures which are light to bring about, cheaper, more sustainable and environmentally friendly. This turns regular bean unwanted produce into elevated quality added product to meet existing and challenges that are yet to come.

### 1.5 AIM

- ▶ To extract cellulose from *P.vulgaris* pod husks and use it to synthesize and evaluate cellulose-based bio-plastic.

### 1.6 OBJECTIVES

1. To extract, purify and analyze cellulose from *Phaseolus vulgaris* pod husks using the acid hydrolysis method.
2. To synthesize bio-plastic from the extracted cellulose.
3. To evaluate properties of the synthesized bioplastic such as stability, sustainability, solubility swelling and biodegradability.
4. To identify the functional groups, present in the cellulose-based bioplastic using Fourier-Transform Infrared Spectroscopy (FTIR) spectrometer.

## CHAPTER 2

### 2.0 LITERATURE REVIEW

#### 2.1 INTRODUCTION

According to Bashir, (2013), plastics are a subspecies of the class of materials known as polymers. The rise of petroleum- based plastics is a prominent interest for the world plus the society at large (Jain and Twain, 2015). Petroleum-based plastics have proven useful in recent years, but their inability to decompose lead to environmental pollution. Bioplastic production is of the many ways being explored to achieve a greener planet (Shor *et al.*, 2020). According to the IUPAC, bioplastics are bio-based polymers derived from biomass or made from biomass-derived monomers, which at certain stages of their final processing can be formed into final products that can be formed by flow (Bioplastics, 2023).

More recently, the effective management of a community and environmental issues associated with the artificial plastics' value chain, particularly unpredictability marketing of oil prices, the suitability of non-renewable fuels, and growing concerns about biotic and abiotic contamination of the Earth's compartments has become a core support for new sustainable thinking of polymers (Araujo, 2019). While the tightening of legal framework for the generation, consumption and disposal of plastics is changing the position at the social and organizational level. Advances in best repetitions in this area are likely to proceed as governments and businesses scale back policies also aligning with circular economy models (Araujo, 2019).

Circular economy thinking emphasizes applying regenerative approaches through restoring and breaking down of components, reusing to maximize the degree of importance and time of products while reducing resource needs and waste generation (Korhonen *et al.*, 2018). As a component of public policy, the process of change from straight economy to a circular economy could avail economic growth and chances in agreement to social development and environmental protection.

It is a promising avenue used in countries seeking to make use of circular economy as national policy address with replacement of resources that are not renewable with biomaterials. Circular economy works in synergy with the principles of bio-economics.

The bio-economy includes any chain of value that makes use of biological materials as a point of departure, such as agricultural and forestry sources (Birner, 2018).

Bio-plastics can be bio-based, biodegradable or have both properties. Ammala *et al.*, 2011 defines plastics that are biodegradable as those that can be broken down into carbon dioxide, water and bagasse by living organisms (usually micro-organisms). The worldwide production capability of bio plastics is projected to multiply from about 2.11 million tons in 2018 to about 2.62 million tons in 2023 (Araujo, 2019). To present date ,fastest growing bio-plastic on the market are bio-based polyethylene (PE), bio-based polyethylene terephthalate (PET), polylactic acid (PLA) and polyhydroxyalkanoates (PHA) (Baltus *et al.*, 2013; Araujo, 2019). While they have an environmental branch that is better compared to petroleum -based plastics , still they face criticism for not being fully bio-based, biodegradable or obtained from preceding - generation products.

## 2.2 CLASSIFICATION OF BIO-BASED PLASTICS

Bio based plastics may or may not be biodegradable as they are derived from materials and biodegradability depends on chemical composition rather than raw material origin (Robertson, 2014).

Bioplastics are classified into three main groups related to whether they are biodegradable and the source of raw materials used in their manufacture, namely:

- Bio-based and non-biodegradable
- Bio-based and bio-degradable
- Fossil-based and biodegradable

### 2.2.1 Bio-based and non-biodegradable plastics

This group includes non-biodegradable polymers that are wholly or partially from biological origin and have the same composition as typical fossil-based plastics (Jankova, 2018). This means that plastics can fragment into minute particles, forming micro-plastics accumulating in the environment (Europa, 2020). Examples of these materials are bio-based polyethylene (bio-PE) and bio-based technical high-performance

polymers such as polyamides and bio-based polyurethanes (European Bioplastics, 2017).

### 2.2.1.1 Bio-derived polyethylene

The elementary monomer of the polymer polyethylene is ethylene. Bioethylene (also called renewable polyethylene) is made from ethanol, the removal of water resulting in ethylene (Sreejth, 2020). The structure of bio-derived polyethylene is shown in Figure 2.1.

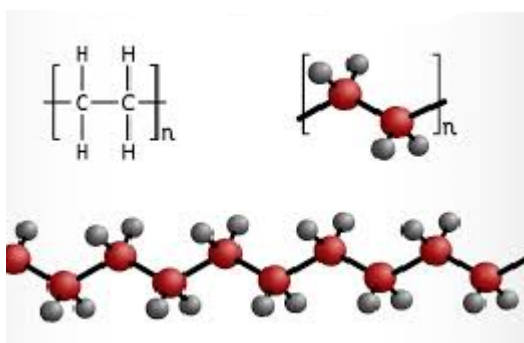


Figure 2. 1: Structure of polyethylene (Xometry, 2022)

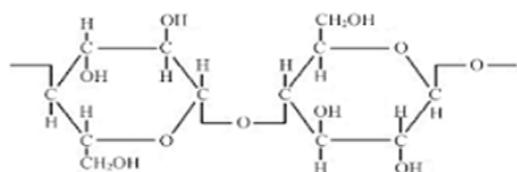
Bio-derived polyethylene can be made from an extensive range of raw materials, including sugarcane and wheat. Low density polyethylene is made by addition polymerization and bio-based polyethylene is made by the same process, the only difference being the ethanol made by fermentation of biomass feedstock instead of ethane from crude oil distillation (Sreejth, 2020). It has the advantages of being environmentally friendly, recyclable and a safe and effective alternative to traditional polyethylene packaging (Sreejth, 2020).

### 2.2.2 Bio-based and bio-degradable

This group consists of bio-based and biodegradable polymers. This means that over time, the plastics can be degraded and metabolized by the motion of micro-organisms presented naturally like bacteria and fungi (Jankova, 2018).

### 2.2.2.1 Cellulose plastics

Cellulose a compound of organic matter with the formula  $(C_6H_{10}O_5)_n$ , is a polymer made of many saccharide units linked by glycosidic bonds and composed of straight chains of hundreds to thousands of  $\beta$  linked D-glucose units (Crowford, 1981), its structure is shown in Figure 2.2 :



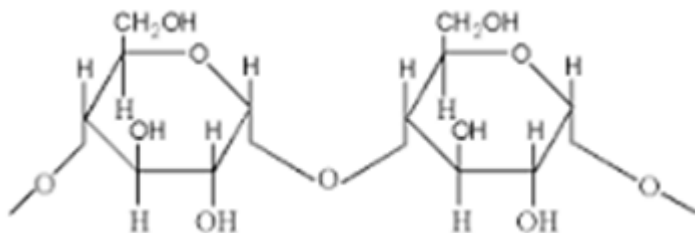
**Figure 2. 2:** Structure of cellulose (Richards et al., 2012)

Cellulose is a crucial structural self-contained part of a larger entity of cell walls and is a richly available organic polymer on earth (Klemm *et al.*, 2005). It is mainly used in the production of cardboard and paper and in this study; it will be used in the production of bioplastics. Cellulose is tasteless, has no smell, hydrophilic with contact angles between  $20^{\circ}$  and  $30^{\circ}$ , does not dissolve in water and most organic solvents, cannot be superposed on its mirror images and can be decomposed by microorganisms (Bishop and Charles, 2007). Cellulose does not undergo a melting transition, so it has no melting point. Instead, when heated a pyrolysis process occurs. Cellulose first undergoes thermal decomposition at about  $200-300^{\circ}\text{C}$  to produce volatile decomposition products. As the temperature rises further decomposition occurs, eventually forming to carbonaceous residue. Cellulose can be easily fragmented by use of chemical principles into glucose units by treatment with strong mineral acids at elevated temperatures (Wymer, 1994). Cellulose is a linear polymer and unlike starch is not coiled or branched, the molecule adopting an elongated and rather rigid rod-like arrangement, aided by the perpendicular arrangement of the glucose residues (Bidhendi *et al.*, 2020). The mechanical attributes of cellulose in early plant chambers are mutually related with increase and new addition of plant cells (Bidhendi *et al.*, 2020). In comparison to starch, cellulose is to the same degree highly crystalline and starch experiences crystalline-to-

amorphous change if made hot in water above 70°C, whereas it takes 320°C for cellulose to be amorphous in water and a pressure of 25MPa (Deguchi *et al.*, 2006). Many attributes of cellulose are dependent on the chain distance measured or extent of polymerization (Klemm *et al.*, 2005). Cellulose of plant origin is usually mixed with substances such as hemicellulose, lignin and pectin and must be separated to obtain its pure form (Klemm *et al.*, 2005).

### 2.2.2.2 Starch plastics

Starch is abundant in corn, rice and wheat. However, using starch from food-grade sources is wasteful. A more economical and resourceful alternative is to use starch made from plant waste such as starch made from corn husks. It accounts for about 50% of the market (types of bioplastics, accessed 27 February 2023). Starch constitutes of anhydroglucose polymers, amylose and amylopectin. Amylose is a straight polymer and has anhydroglucose units which are linked through  $\alpha$ -D-(1, 4) -glucosidic bonds. Amylopectin is a branched polymer which contains periodic branches connected to a fundamental support by  $\alpha$ -D-(1, 6)-glucosidic bonds. Figure 2.3 shows the structure of starch.



**Figure 2. 3:** Structure of starch (Master Chemistry, 2021)

The amount of amylose and amylopectin in starch is variable and highly dependent on the starch wellspring (Wool and Sun, 2005); (Niranjana and Prashantha, 2016). Plastics from starch are intricate mixtures of starch with suitable for compost plastics like Polylactic acid. Mixtures of starch with plastics enhances water resistance, processing ownoms and mechanical properties (Gadhav *et al.*, 2018). However, the challenge for researchers and the packing industry to produce starch-based mixtures with

commercial satisfaction is to overcome issues related with mixing together proportions at elevated starch levels and reduce costs (Ochoa *et al.*, 2016). Strength outlook for the section that deals with material used to wrap products for sale continues to improve as the sustainable plastics market drives further innovation and development (Niranjana and Prashantha, 2016).

### 2.2.2.3 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA's) are straight line polyesters produced in nature by the state of agitation of bacteria in sugars or lipids. PHA's are made from bacteria to accumulate carbon and vigour (Chen, 2009). In the transformation of raw materials into finished products, polyesters are abstracted from bacteria and refined by optimizing sugar fermentation conditions. PHA's are easy to mold into new forms, less capable of being stretched than other plastics and are also decomposable (NPG Asia materials, 2016). PHA's are classified narrow-rackle length PHAs (C<sub>3</sub>-C<sub>5</sub>) which comprises of 3-5 carbon building blocks and intermediate-rackle length PHA's (C<sub>6</sub>-C<sub>14</sub>) which comprises of 6-14 carbon monomers in the 3-hydroxyalkanoate units (NPG Asia materials, 2016). PHA's have limited uses due to some undesirable physical properties. Due to the large crystallites, it is not suitable for packaging films as it has poor mechanical properties (Koller *et al.*, 2003). Also, since the liquefying temperature is alike to its thermal temperature, PHA's are rendered not congruous with traditional heat treatment techniques and prone to thermal decomposition. Regarding the biodegradability of PHA's, the slow deterioration rate and high hydrophobicity of unmodified PHA's are disfavored for many enhanced biomedical applications (Li Z, 2016). Therefore, using PHA's as direct replacements for synthetic plastics remain a major challenge. Figure 2.4 exhibits the structure of PHA's.

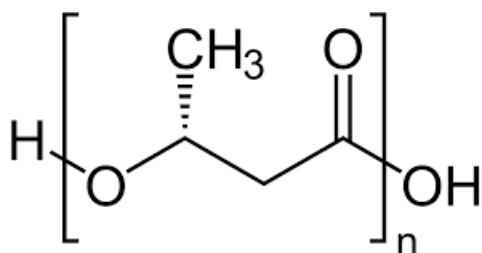


Figure 2. 4: Structure of PHA's (Doi et al., 2002)

#### 2.2.2.4 Polylactic Acid

Polylactic acid (PLA) an aliphatic polyester that softens when heated and cooled when hardened , making it easier to mould. PLA is made out of renewable raw materials for instance, corn starch or sugarcane. According to the Bioplastic Market Report Industry Analysis, 2023, in 2010, PLA was the world's top bioplastic by consumption. The advantages of PLA are that, it is obtained from plants and is readily decomposable. However, Andreas Kunkel *et al.*, 2016 point out that PLA suffers from poor impact design, thermal evincing strength and barrier attributes (obstructing air transport across the membrane). PLA is used to a limited extent in the manufacturing of pellicles , fibers, thermoplastic receptacles, mugs and feeding bottles (Lunt, 2018). The majority of regular class of plastic filament, PLA is mainly for moulding domestic enamel layers (Lunt, 2018), its structure is exhibited in Figure 2.5

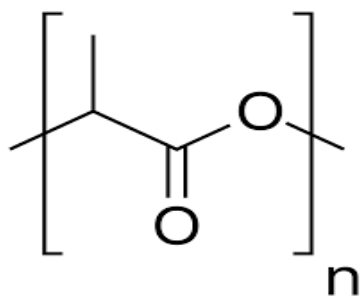


Figure 2. 5: Structure of PLA (Matbase, 2012).

#### 2.2.2.5 Polyglycolic Acid

According to Niaoumakis, 2015, Polyglycolic acid (PGA) is synthetic aliphatic polyester obtained from renewable resources. This is a new decomposable polymer with elevated mechanical properties and elevated gas barrier properties. PGA (Figure 2.6) has been shown to have excellent short-term stability and environmental conditions and has also been shown to biodegrade within about 12 months (Niaoumakis, 2015). Since the 1970's, PGA has been made use of as a biodegradable Dexon, an absorbable synthetic suture material for general surgical applications due to its material properties and an extremely elevated tensile strength (Manoukian and Kumbar, 2019). PGA is insoluble in many



organic solvents, but exhibits high susceptibility to hydrolysis. PGA can be modified through manufacture by extrusion, injection and compression molding, but the processing window between its melt and degradation temperatures is very narrow (Pascault and Furies, 2012).

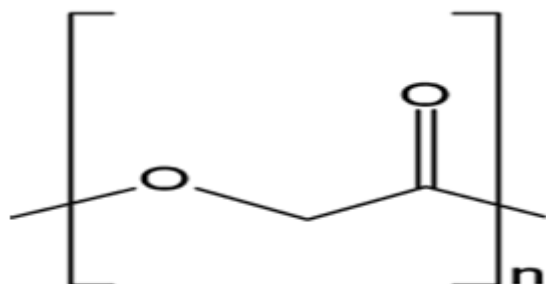
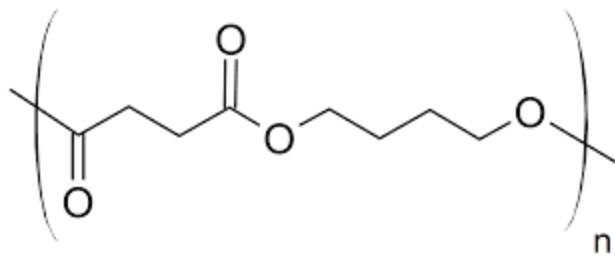


Figure 2. 6: Structure of PGA (Song et al., 2018)

#### 2.2.2.6. Polybutylene Succinate

Polybutylene succinate (PBS) (Figure 2.7) is a bio-degradable, semi-crystalline thermoplastic polyester synthesized by polycondensation of succinic acid and 1,4-butanediol (Polymerdatabase, 2023). It is a promising biopolymer because its mechanical attributes are comparable to those of the extensively used elevated-density polyethylene and isotactic polypropylene (Polymerdatabase, 2023). PBS can break down into biomass such as carbon dioxide and water and thus able to be discarded of with other organic garbage (Novamont news and media, 2016). It is flexible, has a low melting point, does not require plasticizers, is part of the many recent biopolymers, can be used as a cheaper option to other biopolymers for instance, PLA for food packaging, mulch film and fishing nets etc. (Novamont news and media, 2016). PBS has several drawbacks such as, inflexibility, resistance to heat changes and low molecular weight required for industrial applications, which limit its commercial use (Savitha *et al.*, 2022).



**Figure 2. 7:** Structure of PBS (Polymerdatabase, 2016)

### 2.2.2.7 Lignin based plastics

Lignin is a composite polymer derived from benzene with alcohols known as monolignands (Figure 2.8). Commonly obtained from wood, is an important part of the structure situated between the primary cell wall and the plasma membrane of plants and some algal microorganisms. Lignin is also a part of the many macromolecules composed of carbon atoms on earth (Bioplastics, 2023). Lignin, as a biopolymer, is out of the ordinary due to its diversity and absence of a definite primary structure (Bioplastics,2023). It is established as a secondary product of polysaccharide drawn out from plants in manufacture of bookfell, ethyl alcohol, etc. (Tharkur, 2014). According to Taniguchi *et al.*, 2019, lignin is abundant with 50 million tons reportedly produced by pulp industry each year. It is useful because of its light-weight and , eco-friendly nature compared to other options and innocuous to CO<sub>2</sub> emissions during the biodegradation process (Tharkur 2014). Lignin has chemical properties that can be compared to current plastic chemistries such as functional groups that react, film forming ability, high carbon content and versatility related to different chemical mixtures used in plastics (Bioplastics, 2023). It is also inherently antibacterial, manufactured in very high volumes and readily available as a new eco-friendly polymer that could improve current plastic standards (Tharkur, 2014).

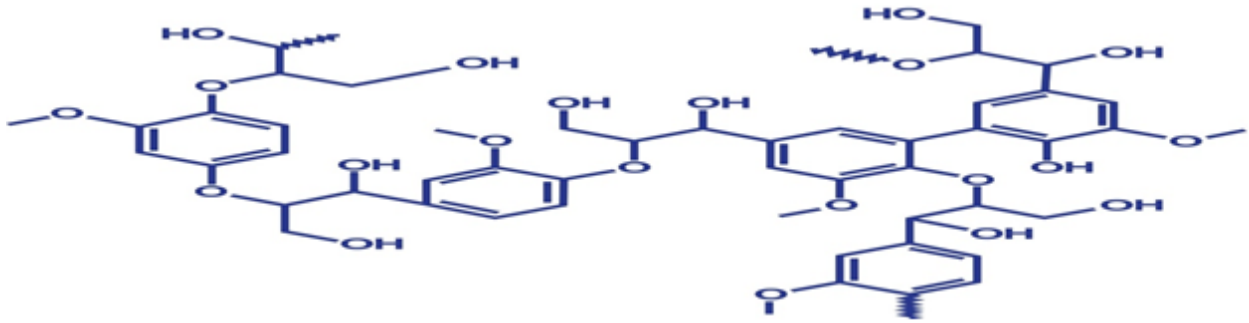


Figure 2. 8: Structure of lignin (Shutterstock, 2023)

### 2.2.2.8 Cellophane

It is transparent and thin film made from reconstructed cellulose. Its low absorbance and allowed movement of air, oil, fat, bacteria and liquid water allows it to be beneficial in food packaging (Morris and Barry, 2017). Cellophane (Figure 2.9) is compostable, biodegradable and derived from biomass. There are two types of cellophane: real cellophane and man-made cellophane. Real cellophane is made from a combination of vegetable cellulose or wood mass of chemically processed fibers and of different materials that cannot be recycled at the same time. In the degree of this difference, synthetic cellophane is from polypropylene (Conserve energy, 2023). Cellophane is biodegradable and therefore more sustainable than plastic.

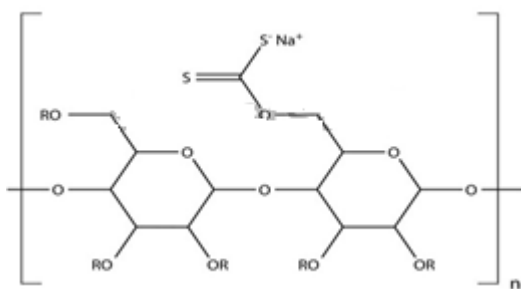


Figure 2. 9: Structure of cellophane (Shutterstock, 2023)

### 2.2.3. Fossil-based and biodegradable

This is the lone class in which polymers are partly or wholly based on fossil fuels (European Bioplastics, 2017). These polymers are durable, long lasting polymers made from petrochemicals (Biodegradability testing, 2020). Examples are Polybutylene adipate terephthalate (PBAT) and Polycaprolactone (Biodegradability testing, 2020).

#### 2.2.3.1 Polycaprolactone

Amidst diverse biodegradable polymers, Polycaprolactone (PCL) is a water aversion, semi-crystalline straight aliphatic polyester with a low melting point and a glass transition temperature of about 60°C making it easy to process (Woodruff and Hutmacher, 2010). Sarasm *et al.*, (2006) found that despite the advantages of PCL, its low melting point makes it easy to process but limits its end uses and its low thermal stability makes it one of the biodegradable materials for food packaging. This indicates that it is being used in departmental research. PCL has little solubility in acetonitrile, insoluble in petroleum ether, diethyl ether and alcohol (De Gryter, 2021). It features tailored degradation rates and mechanical properties as well as ease of manufacture. The structure of PCL allows for good cellular responses due to the inclusion of OH groups that enhance the biocompatibility of the polymer (Gapitira *et al.*, 2011). Figure 2.10 is an equation to show how PCL is formed from caprolactone.

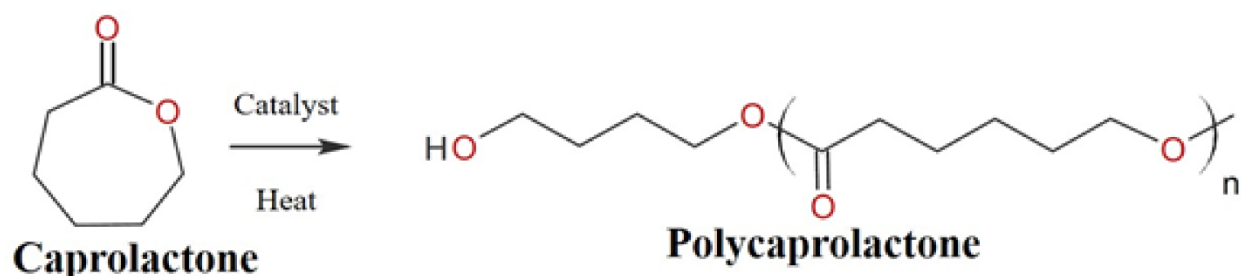


Figure 2. 10: Structure of PCL and how it is formed (Llyas et al., 2023)

### 2.3 KIDNEY BEANS (*PHASEOLUS VULGARIS L.*)

*Phaseolus vulgaris L.* is also known as kidney bean (FAO, 1999). It is an annual botanical plant grown globally for its edible dried seeds or immature fruit (both

commonly called beans) (Sharasia *et al.*, 2017). It is a part of the ancient crops in the New World and has been a major staple food in the low to Middle Americans for thousands of years (Sharasia *et al.*, 2017). Global production is high, over 27 million tonnes of which 23 million tonnes are produced in Latin America and Africa (Broughton *et al.*, 2003). Its botanical classification in addition to other kidney bean species is a part of the legume class, Fabaceae (FAO, 1999). Kidney beans are a worthy source of amino acids such as lysine and tryptophan, minerals such as iron, zinc and copper as well as flavonoids, phytochemicals and antioxidants (FAO, 1999).

### **2.3.1 *Phaseolus vulgaris* botanical classification**

Kingdom : Plantae.

Phylum : Tracheophyte.

Class : Magnoliopsida.

Order : Fabaces.

Family : Fabaceae.

Genus : Phaseolus L.

Species : P. vulgaris.

Botanical identity: Phaseolus vulgaris L.

### **2.3.2 Kidney bean pod husks**

Bean pods are part of the unwanted products produced during bean processing and are frequently inappropriately discarded on agricultural land (Elaigwu *et al.*, 2017). Bean pod husks are obtained after removing the outer layer of the bean leaving only the seeds. The fruit are separated from the hulls and remaining shells are used for various purposes, including feeding ruminants (Elaigwu *et al.*, 2017). In this research, husks of beans are used as a source of cellulose to synthesize bioplastics.

## **2.4 CHARACTERIZATION OF THE OBTAINED CELLULOSE**

### **2.4.1 Fourier Transform Infrared Spectroscopy**

Fourier Transform Infrared Spectroscopy (FT-IR) is a tool for determining the structural knowledge of definite kind. This practical aspect is used to determine the quantitative



intensity of infra-red radiative waves or particles in terms of wavelength (Sachdeva *et al.*, 2008). Infra-red rays are transmitted through the sample and determine the incident radiation absorbed at specific energies. The energies at which the peaks occur in the absorption spectrum correspond to the vibrational frequencies of some of the sample (Munajad *et al.*, 2018). Furthermore, chemical bonds absorb different intensities and frequencies in different environments. In infra-red spectroscopy, absorption data are collected and analyzed in spectra kind. Frequencies of infrared inclusion bands of radiation ('peaks' or 'signals') are likely to be undeviatingly related to binding in scope of the compound in consideration. Between atoms, bond vibrations in various progressions of lengthening and curving single bonds can be absorbed at multiple infra-red frequencies. Stretching absorption usually produces stronger peaks than bending absorption. However, weaker bending absorptions helps distinguish similar bond types.

#### **2.4.2 Schultz Reagent**

Schultz's' reagent is also known as Chlorine-Zinc-Iodine solution. It is an oxidizing reagent comprising of an aqueous homogeneous mixture, a solution of potassium chlorate and fluctuating quantities of concentrated nitric acid (Ruppersburg *et al.*, 2022). It was invented and used by Max Schultz to determine if a substance contains cellulose. Depending on cellulose concentration or fiber structure, cellulose presence is signified by a blue to brown- purple dye (Ruppersburg *et al.*, 2022). Although the mechanism underlying this color change remains unclear, zinc and chloride ions promote swelling of the cellulose fibers interactions between iodine and cellulose molecules with zinc ions and cellulose involved (Ruppersburg *et al.*, 2022).

#### **2.4.3 Reducing sugars tests**

##### ***2.4.3.1 Benedicts' test***

Cellulose is a polysaccharide that is an integral part of the plant cell walls. Like other polysaccharides, cellulose has no free hemiacetal or aldehyde groups therefore non-reductant sugars (Vedantu, 2023). Positive test of Benedict's test yields a brick red or bright orange color, while non-reducing sugars such as cellulose produce a faint blue color or no color change.



#### **2.4.3.2 Fehling's' test**

This reagent is also used for estimation and identification of reducing and non-reducing sugars (Vedantu, 2023). The positivity of reducing sugars is signified by the apparition of red-brown precipitate. A negative result is signified by the lack of reducing sugars.

### **2.5 CHARACTERIZATION OF THE BIO-PLASTIC**

#### **2.5.1 Swelling tests**

A swelling test is performed to assess whether the developed bioplastic materials retain their original properties when molded during manufacturing (Kalia., *et al* 2009). It is a process in which the degree of swelling of a bio-plastic film is measured when it comes into contact with a liquid. In the context of bio-plastic synthesis, swelling tests are commonly used to assess the ability of bio-plastic films to absorb water and other liquids (Yin *et al.*, 2019). This is an important property to measure as the liquid absorption capacity of the bio-plastic can affect its mechanical/ barrier properties. The extent of proturberance of the film can affect strength as well as the flexibility (Kalia *et al.*, 2009). The greater the swelling, the more flexible and the more likely it is to tear or puncture. The degree of swelling can also affect the film's ability to act as an impedement to gases as well as liquids (Yin *et al.*, 2019).As the bio-plastic film swells, the gaps between the polymer chains increase, allowing more molecules to pass through the film, making the bio-plastic less effective as a barrier. Swelling can also affect the clarity and transparency of bio-plastics. A severely swollen bio-plastic can become cloudy or opaque, which can make it less attractive and unsuitable for other applications (Yin *et al.*, 2019).

#### **2.5.2 Determination of melting point**

Melting point determination is an important test for evaluating the thermal properties of bioplastics. The melting point of a polymer is the temperature at which the polymer changes from a solid to a liquid state. Melting point is a crucial property to determine since as it can affect the processing and end-use properties of bio-plastics (Kijchavengkul *et al.*, 2008).The higher the melting point, the stronger and stiffer the



material can be and the lower the melting point, the more flexible and elastic the material can be (Ojijo and Ray, 2015). Melting point can also be an indicator of the thermal stability of bio-plastics (Kijchavengkul *et al.*, 2008). A high melting point may indicate that the material is more heat resistant and less likely to decompose at high temperatures. Various methods can be used to determine the melting point of bio-plastics. The melting point of bio-plastics can be evaluated using differential scanning calorimetry, in this method; a small sample of bio-plastic is heated at a constant rate its and heat flow is measured (Kijchavengkul *et al.*, 2008). Thermo-mechanical analysis is another method that can be used to determine the melting point of bio-plastics. In this method, a small sample of bioplastic is heated or cooled while its dimensional changes are monitored (Ojijo and Ray, 2015). Rheological measurements can also be performed to determine the melting point of bio-plastics. Bio-plastics undergo shear stress when heated. Melting point can be determined by observing the changes in viscosity or flow behavior of bio-plastics as they transition from solid to liquid state (Ojijo and Ray, 2015).

### **2.5.3 Biodegradability test**

The biodegradability of bio-plastics can have a significant implication on their environmental influence and terminal disposal alternatives. Biodegradability is the ability of a material to break down and degrade in the presence of inherently existing microbes, such as bacteria, fungi and algae (Bikiaris and Panayioutou, 2013). Biodegradable plastics can be broken down by micro-organisms into natural compounds that do not persist in the environment, potentially reducing the environmental influence of plastic garbage, and reduces micro-plastic pollution (ISO Standards, 2019). Biodegradability can also affect the material properties of bioplastics. Biodegradable bioplastics can have different mechanical, thermal and barrier properties compared to non-biodegradable plastics affecting how they degrade when exposed to the environment. The chemical configuration of bio-plastics has an important influence on its biodegradability (ISO Standards, 2019). Chemical bonding between monomers within the polymer chain can affect its biodegradability (Bikiaris and Panayioutou, 2013). The ester bonds of Polylactic acid and Polyhydroxyalkanoates are more susceptible to microbial hydrolysis compared to the stronger carbon-carbon bonds (ISO Standards,





2019). In this study, the biodegradability of cellulose harvested from *P. vulgaris* husks was tested. Cellulose-based bioplastics typically consists of cellulose fibers or particles that are embedded in a polymer matrix (Kalia *et al.*, 2011). The biodegradability of these materials can depend on the degree of alkalinity and the size of the cellulose fibers of particles (Kalia *et al.*, 2011). Highly crystalline cellulose fiber has a larger surface area and may be more resistant to biodegradation than amorphous cellulose particles, which are more accessible to micro-organisms (Chiellini *et al.*, 2003).

#### **2.5.4 Solubility tests**

Solubility testing is a method of determining the chemical properties of bio-plastics and identifying their polymer type. Solubility testing involves dissolving a small amount of bioplastic in a solvent and observing the solubility (ISO 1183, 2019). Solubility testing can be used to assess the purity of bio-plastics and identify any potential contaminants (Kalia *et al.*, 2011). Solubility testing also provides information on the suitability of bio-plastics for specific applications. For example bio-plastics that are soluble in water or certain organic solvents may be suitable for applications such as coatings and adhesives, whereas bio-plastics that are insoluble in water or certain organic solvents may be suitable for applications such as packaging or durable goods (Kalia *et al.*, 2011). The choice of solvent for bio-plastic solubility testing depends on the chemical configuration of the polymer and the planned aims of the test. Various solvents such as, water, acetone, chloroform, ethanol and hexane are used. These solvents are used because they have different polarities and can solubilize different types of bioplastics (Kalia *et al.*, 2011). Solvent selection can affect solubility test results of , so it is important to use the appropriate solvent for the particular bioplastic being tested.



## CHAPTER 3

### 3.0 MATERIALS AND METHODOLOGY

#### 3.1 INTRODUCTION

This section reflects on the experimental procedures that were carried out to achieve the objectives and aims of this research. Procedures described include the sampling method, preparation and extraction of cellulose and characterization techniques.

#### 3.2 SAFETY AND CLOTHING

Lab coat

Safety shoes

Latex gloves

Safety gloves

#### 3.3 CHEMICALS USED

The chemicals that were utilized during the course of this research were analytical reagents and a few standard reagents grade. The table below shows the chemicals:

**Table 3.1:** Chemicals used.

Entry	Items	The Company	Grade
1	Starch soluble	ACE	
2	Sodium hydroxide	ACE	Analytical Reagent
3	Sodium metabisulphite	ACE	Analytical Reagent
4	Glycerol	Glassworld	Analytical Reagent
5	Acetic acid	Glassworld	Analytical Reagent
	Chloroform	Skylabs	Analytical Reagent
7	Butyl hydroxyl toluene (BHT)	ACE	Standard
8	Hydrochloric acid	Glassworld	Analytical Reagent
9	Sodium hypochlorite	Minema	Analytical Reagent
10	Hydrogen peroxide	Skylabs	Analytical Reagent
11	Benedicts solution	Skylabs	Analytical Reagent
12	Fehling's solution	Skylabs	Analytical Reagent
13	Methanol	Minema	Analytical Reagent
14	Acetone	Glassworld	Analytical Reagent
15	Sulphuric acid	Glassworld	Analytical Reagent
16	Ammonia	Glassworld	Analytical Reagent
17	Ethanol	Skylabs	Analytical Reagent
18	Glucose	ACE	Standard

### 3.4 EQUIPMENT USED

**Table 3. 2:** Equipment used and its functions.

Equipment/Instrument	Purpose
Analytical balance (Sartorius-BP3105)	Used to weigh samples and reagents
Blender	Crushing dried pod husks
Oven (Labcon R.T.D hot air oven)	Drying samples
Melting point apparatus	Measuring the melting point of bioplastic
Thermometer	Measuring temperature
FTIR Spectroscopy(FT-IR Nicole Atr diamond accessory)	Characterization of cellulose extract and bioplastic film
Water bath	Heating samples
pH meter	Measuring pH of solutions
Hot plate	Heating samples
Electric shaker	Continuous agitation of samples

### 3.5 GLASSWARE CLEANING AND STERILIZATION

Prior to the start of the study, all glassware that was required was thoroughly cleaned and sterilized for the prevention of contamination from previous studies. Dilute hydrochloric acid was used for intermediate cleaning followed by rinsing with distilled water.

### 3.6 SAMPLE COLLECTION AND PREPARATION

Kidney pod husks were obtained from local farmers in Mashonaland East Province of Zimbabwe. Pod husks were washed with distilled water and immersed in sodium metabisulphite preservative for 2 hours. After this, they were dried and ground with a blender in preparation for the next step.

### 3.7 EXTRACTION OF CELLULOSE

80 g of the powdered sample of *P. vulgaris* pod husks was weighed on a weighing balance in a 250 ml glass beaker. The sample was boiled in distilled water for 10 minutes and vacuum filtered thereafter. This step was aimed at removing any phenolic

compounds and soluble polysaccharides. A solution of 0.5 M hydrochloric acid was prepared and was added to the obtained residue and incited for 30 minutes at 85 °C using a magnetic stirrer on a hot plate. The residue was filtered and then stirred with 1.0 M sodium hydroxide on an electric shaker for 2 hours. This process was repeated until the solution turned to almost colorless when filtered. Residue bleaching was performed with 1% of sodium hypochlorite solution for 60 minutes at 95°C and the process was repeated thrice. The resulting precipitate was cellulose which was cleaned separte times with hot de-ionized water up to the time a neutral pH of the filtrate was obtained. The extracted cellulose was weighed on an electronic balance to determine it yield. A simplified flow chat for the extraction of cellulose is shown in Figure3.1.

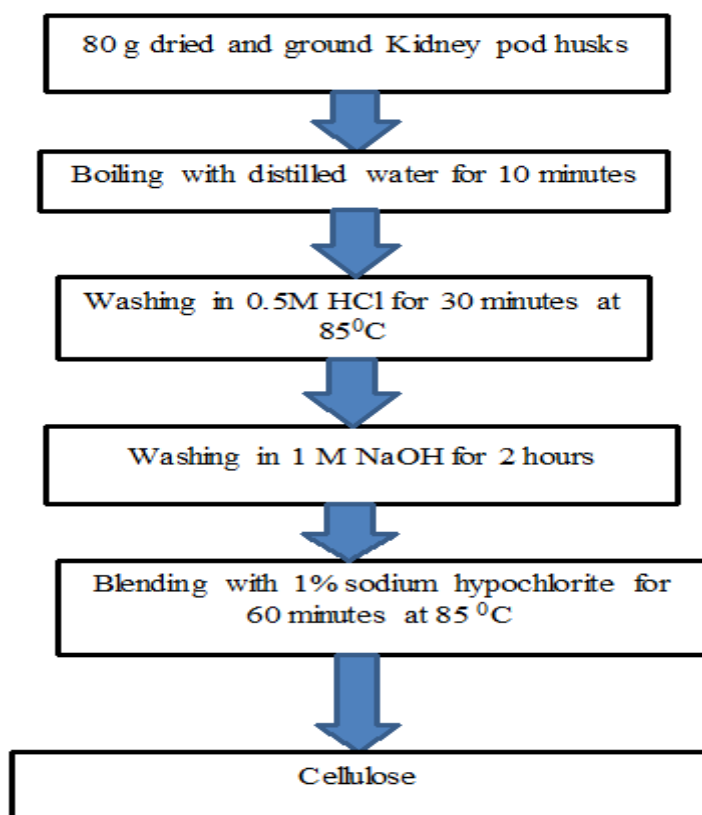


Figure 3. 1: Process flow diagram for the extraction of cellulose

### 3.8 OXIDATION OF CELLULOSE

3 replicates of 5 g of cellulose were added to a 100 ml Erlenmeyer flask. 12%, 9% and 6% hydrogen peroxide were prepared and used to impregnate three samples of

cellulose respectively. 0.1 M NaOH and 0.1 M HCl were used to maintain the pH at 11. The cellulose dormant was constantly shook on an electric shaker for a period of 24 hours. The pulp was filtered and washed with de-ionized water three times and air dried preliminary to upcoming treatment analysis.

### **3.9 CHARACTERIZATION OF CELLULOSE**

#### **3.9.1 FTIR spectroscopic analysis**

FTIR was made use of to determine the specific group of elements characteristic of samples. FTIR spectroscopic analysis of extracted cellulose was done using a Nicolet thermoscientific FTIR spectrometer with an ATR diamond accessory. Sample introduction was done by putting the powdered sample of cellulose on ATR diamond plate and was made to make contact with the crystal on top. The spectra of cellulose were recorded in order to confirm and compare the structure of the extracted cellulose with the one in literature.

#### **3.9.2 Reducing sugars tests**

##### ***3.9.2.1 Fehling's test***

1 ml of both Fehling's (A, B) reagent was added to 1 g of the sample and the glucose in a glass tube then heated in a water bath. Results were obtained and recorded.

##### ***3.9.2.2 Benedict's test***

1 g of the sample and the glucose standard were placed into clean separate test tubes 2 ml's of Benedict's reagent were placed in each of the test tubes and the solution was subjected to heating in a water bath for 5 minutes. Color transformations were obtained and recorded.

### **3.10 SCHULTZ REAGENT**

20 g of anhydrous zinc chloride was placed in a beaker and dissolved in 8.5 ml of water and the mixture was cooled. In another beaker, 1 g of potassium iodide and 0.5 g iodine was dissolved in 20 ml of water. The resulting homogeneous mixture was added in small amounts to the zinc chloride solution upto the time iodine precipitate persisted



on perturbation. Schultz reagent was then added to 2 g of the sample and results were obtained.

### 3.11 SYNTHESIS OF THE BIOPLASTIC

All raw materials were weighed and used in concentrations according to table 3.3 below:

**Table 3. 3:** Raw material combinations for the synthesis of the three bio-plastics

SAMPLE	INGREDIENTS	WEIGHT (%)
A	Cellulose	35
	Glycerol	25
	Starch	3
	BHT	5
	Acetic acid	5
B	Cellulose	50
	Glycerol	35
	Starch	3
	BHT	5
	Acetic acid	5
C	Cellulose	75
	Glycerol	12.5
	Starch	3
	BHT	5
	Acetic acid	5

The bioplastic was prepared using the method of casting and evaporation. Starch was used as a copolymer; 15 g of soluble starch was adjoined in 500 ml of de-ionized water and was heated at 60 °C for 15 minutes on a hot plate to allow gelatinization to occur. Cellulose homogeneous mixture was proliferated gradually to the gelatinized starch and stirred using a stirring rod. Cellulose concentrations which were used were in the concentrations 35%, 50% and 75% for sample A, B and C respectively, glycerol addition was 25%, 35% and 12.5% respectively. The mixture was cooled on watch glasses and peeled off after cooling and stored in a desiccator.



## **3.12 CHARACTERIZATION OF THE BIOPLASTIC**

### **3.12.1 FTIR analysis**

All synthesized samples were analyzed on a thermo-scientific Nicolet FTIR spectrometer with an ATR diamond accessory to identify the functional groups present in the synthesized bioplastics.

### **3.12.2 Swelling Test**

The protuberant capability of a polymer is ascertained by the amount of liquid corporeal that can be absorbed by it (Wikipedia, 2011). The swelling properties of the synthesized bioplastic were analyzed by immersing pre-weighed samples of the bioplastic in mediums containing different solvents such as water, methanol and chloroform for up to 2 hours at normal temperature (25 °C) and weighing final weight of the specimen on a balance and results were recorded. Swelling tests were done to check pro-turbulence and to determine morphological structure.

### **3.12.3 Melting point determination**

It is the thermal determination most occasionally used to characterize solid crystalline materials and to assess their purity, (Mettler Toledo, 2023). Undefined substances melt at precise, elevated defined temperatures, in contrast, contaminated substances basically demonstrate a large melting intermittent (Mettler Toledo, 2023). Melting point of the bioplastic was determined by cutting bioplastic films into 2cm by 2cm and placed into corresponding test tubes. 100ml of water was poured into a beaker and test tubes with the bioplastic were placed into the beaker and placed on a hot plate. A thermometer was placed in the beaker and temperature was observed as the structure of the bioplastic changed. Once a liquid was observed in the test tube, temperature was recorded and also when the whole bioplastic was melted the temperature was recorded. The differences in temperature were recorded

### **3.12.4 Biodegradability Test**

Biodegradability tests are used to determine the biodegradability of product in a given or interval use in the environment. They measure the intricate biochemical process that betide when microbes devour a given class of material. In this research, bioplastic films



were cut into 2 cm by 2 cm and were pre-weighed prior to the burial test. Pre-weighed bioplastic samples were placed in the beaker enclosing soil at 5 cm bottom from the surface. The soil was covered with water to allow bacterial enzymatic undertakings to be enriched. Specimens were preserved in the beaker for 5 days of interval, a reduction in weight of the bioplastic was observed. Results were obtained and each sample was analyzed in triplicates to improve the accuracy of results.

### **3.12.5 Solubility Test**

Bioplastics should be able to absorb or repel water depending on their use (Lui, 2013). The sample was incised into small chips and was placed into various test tubes inholding different solvents namely ammonia, acetic acid, acetone, chloroform, methanol, sulphuric acid and ethyl alcohol. The solvents where selected in a way that enabled the determination of traits such as activity in high acidic , polar and non polar solvents also in weak acids.



## CHAPTER 4

### 4.0 RESULTS

#### 4.1 INTRODUCTION

This section presents results of the research conducted.

#### 4.2 YIELD OF CELLULOSE EXTRACTION

**Table 4. 1:** Yield of cellulose extract obtained from *P. vulgaris* pod husks

Mass of powdered sample (g)	80
Mass of cellulose (g)	24
Yield (%)	30



**Figure 4. 1:** Cellulose sample extracted from *P. vulgaris* pod husks

## 4.2 FT-IR SPECTRUM OF THE EXTRACTED CELLULOSE

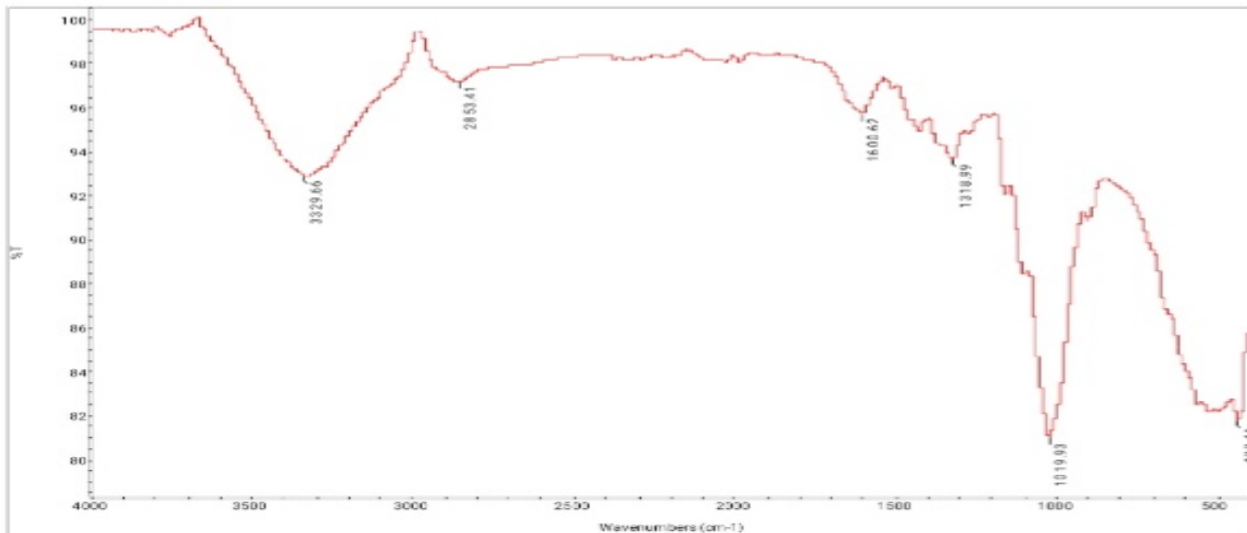


Figure 4. 2: FTIR spectrum of *P. vulgaris* pod husks cellulose extract

## 4.3 REDUCING SUGARS TEST RESULTS

The obtained results of the Fehling's test and Benedicts test are presented in the table below

Table 4. 2: Reducing sugars test results

TEST	RESULTS FOR CONTROL	RESULTS FOR SAMPLE
Fehling's test	Reddish brown precipitate	No precipitate Blue colour observed
Benedicts test	Orange precipitate	Blue precipitate

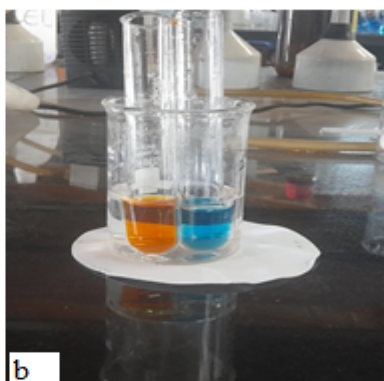
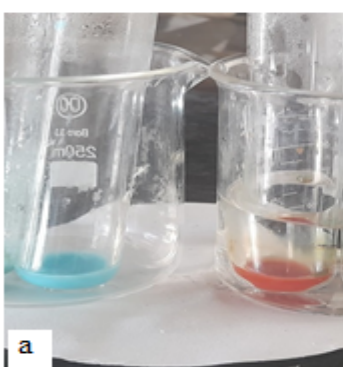


Figure 4. 3: Results for Fehling’s test (a) and Benedict’s test (b)

#### 4.4 SCHULTZ REAGENT TEST RESULTS



Figure 4. 4: Positive result (purple colour) of cellulose in *P. vulgaris* pod husks

#### 4.5 FTIR OF SYNTHESIZED BIOPLASTIC SAMPLES

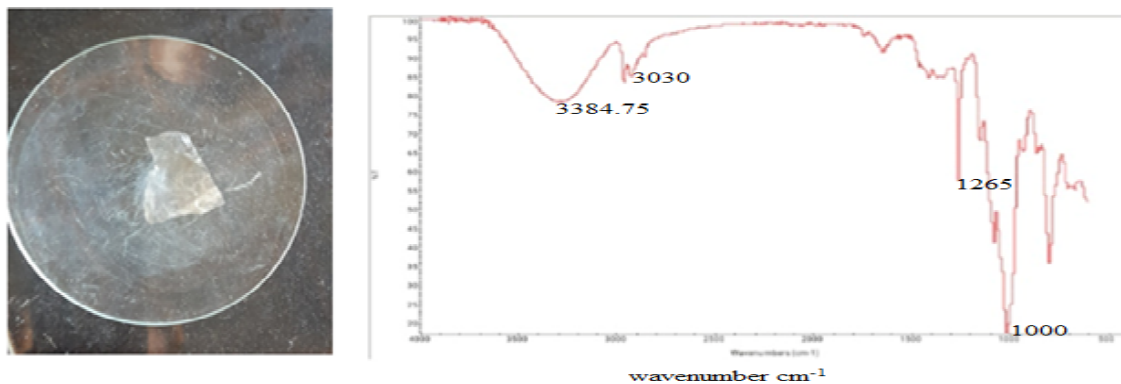


Figure4.5: Sample A synthesized bioplastic and its corresponding FTIR spectrum

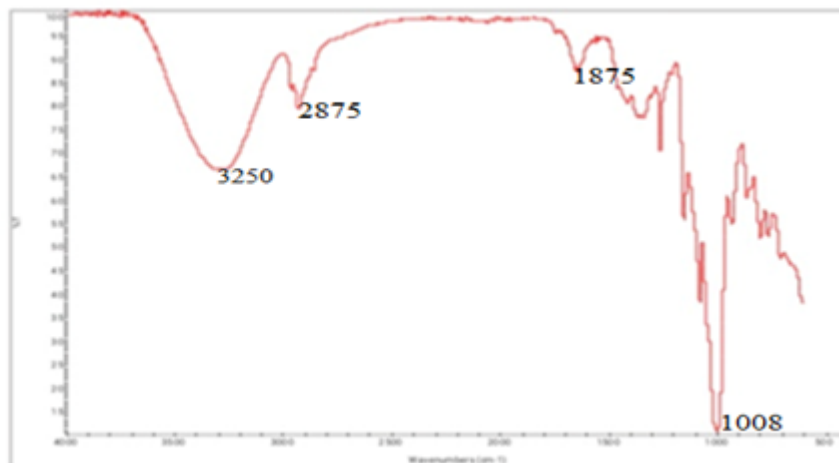


Figure4.6: Sample B synthesized bioplastic and its corresponding FTIR spectrum

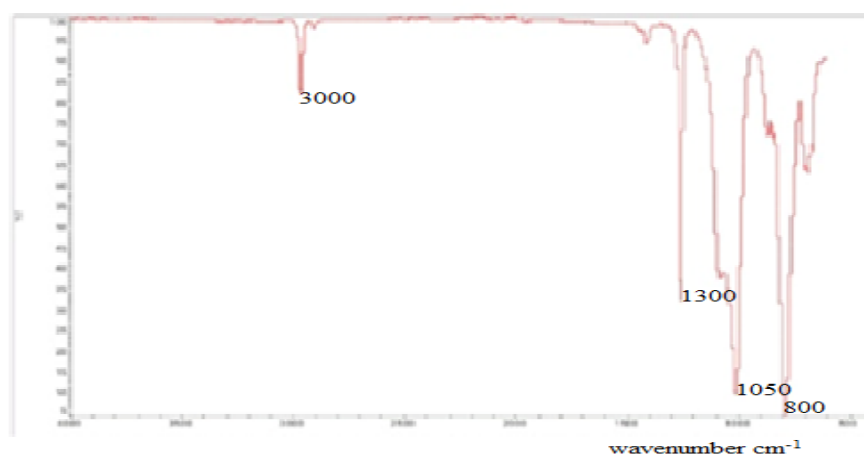


Figure4. 7: Sample C synthesized bioplastic and its corresponding FTIR spectrum

#### 4.5 SWELLING TEST RESULTS

Table4. 3: Results for the swelling test of the synthesized bioplastic

Sample	Solvent medium	Quantity (ml)	Initial weight of sample (g)	Final weight of sample (g)	Difference in weight (g)
A	Water	20	1.00	1.09	0.09
	Chloroform	5	1.00	1.01	0.01

	Methanol	5	1.00	1.05	0.05
<b>B</b>	Water	20	1.00	1.10	0.10
	Chloroform	5	1.00	1.02	0.02
	Methanol	5	1.00	1.03	0.03
<b>C</b>	Water	20	1.00	1.08	0.08
	Chloroform	5	1.00	1.02	0.02
	Methanol	5	1.00	1.04	0.04

#### 4.6 BIODEGRADABILITY TEST RESULTS

The percentage weight loss of the bioplastic was calculated using the formula:

$$\% \text{Weight loss} = \frac{[W_1 - W_0]}{W_0} \times 100\%$$

**Table 4. 4:** Results of biodegradability for the synthesized bioplastics

<i>Test No</i>	Mass of sample A			Mass of sample B			Mass of sample C		
	Initial mass (g)	Final mass (g)	Weight loss (%)	Initial mass (g)	Final mass (g)	Weight loss (%)	Initial mass (g)	Final mass (g)	Weight loss (%)
1	0.52	0.37	<b>40.05</b>	0.53	0.34	<b>55.58</b>	0.56	0.39	<b>43.59</b>
2	0.56	0.46	<b>21.74</b>	0.51	0.38	<b>34.21</b>	0.51	0.37	<b>37.84</b>
3	0.59	0.56	<b>5.36</b>	0.52	0.41	<b>26.83</b>	0.52	0.35	<b>42.86</b>
Average	0.55	0.46	<b>22.38</b>	0.52	0.38	<b>38.97</b>	0.53	0.37	<b>41.43</b>

#### 4.7 SOLUBILITY TEST RESULTS

**Table 4. 5:** Results of solubility for synthesized bioplastics in different solvents

Solvent used	Samples	Solubility test		
		Insoluble	Partially soluble	Completely soluble
Acetone	A	+	-	-
	B	+	-	-
	C	+	-	-
Acetic acid	A	+	-	-
	B	+	-	-
	C	+	-	-
Ammonia	A	-	+	-
	B	-	+	-
	C	-	+	-
Chloroform	A	+	-	-
	B	+	-	-
	C	+	-	-
Ethyl alcohol	A	+	-	-
	B	+	-	-
	C	+	-	-
Methanol	A	-	+	-
	B	-	+	-
	C	-	+	-
Sulfuric Acid	A	-	-	+
	B	-	-	+
	C	-	-	+
Water	A	+	-	-
	B	+	-	-
	C	+	-	-

**Key:** + positive result; - negative result

#### 4.8 MELTING POINT DETERMINATION

Table 4. 6: Melting point of the bioplastics

Sample	Rapid melting point (°C)	Final melting point (°C)
A	100	104
B	98	102
C	96	100

## CHAPTER 5:

### DISCUSSION, CONCLUSION AND RECOMMENDATIONS

#### 5.1 EXTRACTION OF CELLULOSE

The method for extracting cellulose used in this study was adopted from that reported by Rentoy *et al.*, 2015 and Rehman *et al.*, 2018. Cellulose extraction was performed on *P.vulgaris* husks and the process was applied in an environmentally friendly manner. Pod husks were delignified, hydrolyzed and bleached as shown in the flow chat in Figure 3.1 Alkaline treatment was utilized in the delignification process and a dark brown colour was noted, indicating the presence of lignin still entrapped in the cellulose (Hayatun *et al.*, 2020).Lignin is insoluble in simple solvents but soluble in dilute alkali, so 1 M NaOH was used to separate lignin from cellulose. This is because the hydroxide ion of NaOH breaks bonds in the lignin base structure, while Na<sup>+</sup> binds to lignin to form lignin sodium which is readily soluble in water due to its polarity (Safaria *et al.*, 2013). The yield of extracted cellulose was found to be 30% and the cellulose obtained is shown in Figure 4.1. The cellulose content obtained from kidney pod husks was lower than cotton hemp which is 97% (Wakelyn *et al.*, 2007), rice husk which is 42% (Kumar *et al.*, 2009) and in sugarcane bagasse which is 48% (Jacobsen *et al.*, 2002). Differences in cellulose extraction yields are likely due to the differences in plant fibers, concentrations of extraction chemicals and pretreatment processes.

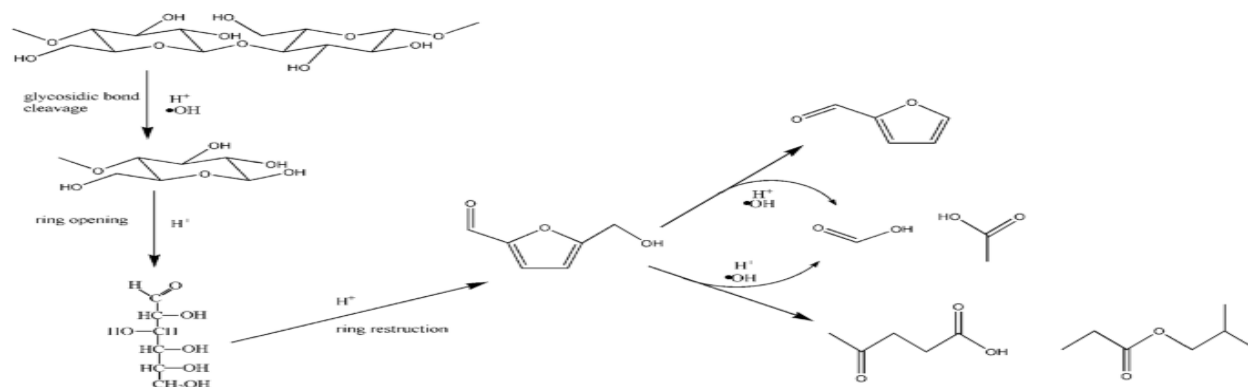
##### 5.1.1 Oxidation of cellulose

Oxidation of cellulose can be defined as the treatment of elementary C<sub>6</sub> with next in line (C<sub>2</sub> and C<sub>3</sub>) hydroxyl collections with any substance that receives electrons from another ,thereby oxidizing cellulose to aldehyde/ carboxyl ones (Tashikj *et al.*, 2019). In this study, hydrogen peroxide was used as the oxidizing agent. Peroxides can decolorize and remove all stains on cellulose (Zeronian and Inglesby, 1995). Both free radicals and peroxyanions have been proposed as intermediates in the reaction between cellulose and hydrogen peroxide. The oxidative activity of hydrogen peroxide arises from the presence of the two oxygen atoms compared to the structure of water (Woodard and Amp, 2006). The mechanism by Figure 5.1 demonstrates the reaction between cellulose





and hydrogen peroxide.



**Figure 5. 2:** Oxidation of cellulose with hydrogen peroxide (Wen *et al.*, 2019)

Oxidation of cellulose reduces the crystallinity as the amorphous regions of the cellulose chains become more susceptible to attack (Isroi *et al.*, 2017). Cellulose chain scission in amorphous regions and damage in crystalline regions are believed to be responsible for the decrease in cellulose crystallinity (Isroi *et al.*, 2017). Decreased crystallinity is beneficial for bioplastics production as it facilitates the transformation of the dense structure of cellulose into a flexible polymer. The apparent lack of purity of the extracted cellulose even after oxidation with hydrogen peroxide compares with other studies using different bleaching steps, such as sodium hypochlorite, extraction with NaOH and hydrogen peroxide, hydrogen peroxide and sodium hypochlorite (Thiab, 2015). The lack of purity of extracted cellulose could be attributed to the 1% that was used instead of a higher concentration.

### 5.1.2 Characterization of cellulose extract

**Table 5.1:** Characterization of cellulose.

Observed wavelength( $\text{cm}^{-1}$ )	Functional groups present	Wavelength in literature ( $\text{cm}^{-1}$ )
3329.66	OH stretching	3600-3300( MaO et al.,2019)
2683.41	Asymmetric C-H stretching	2800-3000(Adullah et al.,2018)
1600.62	C=C stretching	1600-1650(Nakamoto, 2009)
1318.99	C-O in carboxylic acid	1100-1350(Larkin, 2011)
1019.33	C-O stretch of secondary and tertiary alcohol	1000-1200(Marilyn, 2003)

Cellulose obtained in this study has an absorption area of  $3329.66\text{cm}^{-1}$  representing OH groups, enriched by an area of  $1318.99\text{cm}^{-1}$  representing C-O group and an asymmetric elongation of C-H groups at  $1600.62\text{cm}^{-1}$  (Figure 4.2). This is also corroborated by presence of certain cellulose collections apart from the hydroxyl group for instance the methylene group ( $\text{CH}_2$ ) at absorption band,  $2683.41\text{cm}^{-1}$  this is a C-H stretch. Also, the -O- groups that build up cellulose emerged in the absorption bands  $1318.99\text{cm}^{-1}$  and  $1019.33\text{cm}^{-1}$ . OH groups appearing in the uptake area of  $3329.66\text{cm}^{-1}$  demonstrate that bioplastic films can be decomposed by microbial activity in the soil (Sato *et al.*, 2017). The resulting Fourier Transform Infra-Red (FTIR) characterization is similar to research by Fajrin *et al.*, 2017, with OH emerging at a wavelength of  $3080\text{cm}^{-1}$  and C=C stretch at  $1640\text{cm}^{-1}$ .

## 5.2 REDUCING SUGARS TESTS

Figure 4.3 exhibits the findings of the Fehling's test where no precipitate is observed and a blue colour is observed and Benedict test results when a blue precipitate is obtained. Results obtained serve as a confirmatory test for cellulose extracted from

*P.vulgaris* pod husks. A negative result of the reducing sugars test indicates that the obtained cellulose is not hydrolyzed to glucose and is therefore stable in bioplastic synthesis.

### 5.2.1 Schultz reagent

Since the exact amount of cellulose has not been reported previously in literature on *P.vulgaris* pod husks, it was important to estimate the amount of cellulose that was present in *P.vulgaris* pod husks. The Schultz reagent was used. The test was run and results were obtained as depicted in Figure 4.4. The purple color obtained is a positive test presence of cellulose.

## 5.3 SYNTHESIS OF THE BIOPLASTIC

The synthesis of the bioplastic was done basing on a method by Rentoy *et al.*, 2015 and Isroi *et al.*, 2017. Three bioplastic samples were synthesized from cellulose as shown in sample A (Figure 4.5), sample B (Figure 4.6) and sample C (Figure 4.7), respectively. The use of glycerol in the synthesis of bioplastics aims to reduce the molecular bond strength of cellulose and increase the flexibility of bioplastics. It also prevents the samples from drying out during the experiment. This is because glycerol is more viscous than water due to strong attractive forces between particles. Differences in synthetic sample quality may be due to different concentrations of cellulose and glycerol, as shown in Table 3.3. The opacity of bioplastics decreases with increasing cellulose concentration (Isroi *et al.*, 2017). This observation is consistent with the results of this study, where sample A is highly opaque at 35% cellulose concentration, then sample B at 50% cellulose concentration and finally sample C at 75% cellulose concentration. Further tests to analyze physical properties of bioplastics such as tensile strength, contact angle, elongation rupture and elastic modulus related to fiber concentration could not be performed due to lack of equipment.

### 5.3.1 FOURIER TRANSFORM INFRA-RED SPECTRA OF THE BIOPLASTIC

The Fourier Transform Infra-Red (FTIR) spectra of the synthesized bioplastics are shown in Figures 4.5, 4.6 and 4.7. The Figures show the presence of broad OH peaks in the range of 3000 to 3500  $\text{cm}^{-1}$  for samples A and B. Sample C has a less pronounced



peak at  $3000\text{ cm}^{-1}$ . The presence of the O-H is attributed to the presence of glycerol used as a plasticizer. The lack of the C-H stretch between  $2800$  and  $3000\text{ cm}^{-1}$  and C-O stretch between  $1100$  and  $1350\text{ cm}^{-1}$  compared to cellulose confirms the great progress made in this study in converting cellulose to bioplastics. The results obtained differ from those obtained by Beevi *et al.*, (2020) which had peaks at  $716\text{ cm}^{-1}$  for N-H stretches and a stretch at  $857\text{ cm}^{-1}$  results in the C-H bond. These differences are caused by the different materials used.

#### 5.4 SWELLING TEST

A swelling test study was conducted using a method by Jayachandra *et al.*, (2016) to assess the sustainability of the synthesized bioplastics. According to the results in Table 4.3, when samples A, B and C were immersed in chloroform, there was little change in volume and the total volume difference was  $0.01\text{ mL}$ ,  $0.02\text{ mL}$  and  $0.02\text{ mL}$ . Methanol also contributed to a slight increase in samples with volumes of  $0.05\text{ mL}$ ,  $0.03\text{ mL}$  and  $0.04\text{ mL}$  for samples A, B and C, respectively. However, using water as the solvent, a significant increase in volume is observed with volumes of  $0.09\text{ mL}$ ,  $0.10\text{ mL}$  and  $0.08\text{ mL}$  for samples A, B and C. This is because the hydroxyl groups of cellulose that are not involved in crosslinking with plasticizer may be exposed, which can react with water and cause swelling of the film (Parida *et al.*, 2022). Similarity of swelling tests results was also seen in Noorjahan *et al.*, (2022) with small volumes when samples were immersed in chloroform and methanol but increased slightly when samples were soaked in water.

#### 5.5 BIODEGRADABILITY TEST

Mssardier-Nageotte *et al.*, (2006) points out that at the right requirements, decomposable plastics can be decomposed completely into water and decayed remains of organic matter by microbes. In this study, biodegradability test method of Jayachandra *et al.*, (2016) was adopted, and the results shown in Table 4.4 were obtained. All samples A, B and C showed weight loss, indicating that the samples were degraded by soil bacteria. The large difference in weight loss could also be due to different concentrations of cellulose and plasticizer. The results obtained in the biodegradation test are similar to previous studies by Noorjahan *et al.*, (2022), which



also showed weight losses when samples were exposed to soil bacteria with weight loss of 21%.

## 5.6 SOLUBILITY TEST

Bioplastic material was also subjected to solubility tests with an aim to assess its durability and sustainability. The analytical method was adopted from Jayachandra *et al.*, (2016). The solubility test results are shown in Table 4.5 where it was noted that samples A, B and C were insoluble in water and hence suitable to be bioplastic material. Starch was added to cellulose to create a bioplastic that has enhanced mechanical properties. Bioplastics are synthesized without pores because their properties weaken when they absorb water. The sample could not dissolve in acetone (polar solvent), ethyl alcohol (non-polar solvent) and acetic acid (polar solvent). However, prepared samples were incompletely dissolved in ammonia (polar solvent) and utterly dissolved in sulphuric acid (strong acid solvent). Solubility test results demonstrate how efficient the bioplastic from *P.vulgaris* pod husks is, as it is insoluble in water and other organic solvents making it profitable to a greater extent to manufacture at affordable prices. The results obtained in this study are similar to those of Jayachandra *et al.*, (2016), as the prepared samples did not dissolve in water and other organic fluids that dissolve solids and liquids but completely dissolved in strong acid solvents. The presence of glycerol in the bioplastic samples also affects solubility as reported by Sanyang *et al.*, (2016). Glycerol is hydrophilic in nature and this causes water to diffuse into the polymer matrix increasing the solubility of plasticized bioplastic samples. Due to its low molecular weight, glycerol has a high affinity for water penetrates into polymer chains.

## 5.7 MELTING POINT DETERMINATION

The melting point temperature of bioplastics has a sturdy ability on enzymatic degradation of the polymer. An elevated melting temperature has less decomposable effects on the polymer (Takiwa and Calabia, 2015). The method for determining the melting point in this study was adapted from ECOTEK, (2010). In this study, all samples A, B and C had melting temperatures slightly above 100 °C, as shown in Table 4.6, indicating that the bioplastic is highly biodegradable. Results obtained are largely similar to those by ECOTEK, (2010) where the melting point of bioplastics was

estimated at 110<sup>0</sup> C. Differences could be attributed to the difference in materials used for bioplastic synthesis.

## 5.8 CONCLUSION

*P. vulgaris* pod husks have proved to be a potential source of cellulose for the synthesis of bioplastic. The bioplastic synthesized had the ability to biodegrade, was insoluble in polar solvents and soluble in strong acidic solvents like sulfuric acid. Swelling tests conducted were to ascertain durability and sustainability of the bioplastic and the tests proved that the bioplastic is stable due to less ability to fill with water. The use of *P. vulgaris* pod husks as an alternative source of cellulose for the synthesis of bioplastics is a promising endeavor to help reduce the environmental pollution caused by the inability of microorganisms to degrade synthetic plastics.

## 5.9 RECOMMENDATIONS

The researcher recommends the use of an instrument that can identify the chemical composition of *P.vulgaris* before and after being treated with hydrogen peroxide. The bio-plastics obtained have to be characterized using Scanning electron microscopy, Transmission electron microscopy and X-Ray diffraction for the identification of the chemical composition and morphology. Further tests to analyze physical properties of bioplastics such as tensile strength, contact angle, elongation rupture and elastic modulus related to fiber concentration should also be looked into by future studies.

## REFERENCES

1. Araujo D. J. C. (2019). *Production of cellulose-based bioplastics from agroindustrial residues*.
2. Araujo, J.R. (2019). *Polymers: Properties and Applications*. In: Encyclopedia of Polymer Science and Technology, 4th Edition. John Wiley & Sons, Inc. <https://doi.org/10.1002/0471440264.pst676>.
3. Baltus W., Beckmann J., Carrez D., Carus M. (2013). Market study on Bio-based Polymers in the World Capacities, Production and Applications: *Status Quo and Trends towards 2020*.
4. Bashir, M.J.K. (2013). *Plastics and Their Applications*. In: Materials Science and Engineering. IntechOpen. <https://doi.org/10.5772/56909>.
5. Bidhendi, A.J., Kumar, R., and Dhiman, S.S. (2020). *Cellulose and Its Derivatives: Synthesis, Modification, and Applications*. In: Handbook of Sustainable Polymers. Springer. [https://doi.org/10.1007/978-3-319-97148-3\\_6](https://doi.org/10.1007/978-3-319-97148-3_6).
6. Biodegradability Testing (2020). *What are Fossil-Based Polymers?* <https://www.biodegradabilitytesting.com/what-are-fossil-based-polymers/>.
7. Bioplastics (2023). *Cellulose* <https://www.bioplasticsmagazine.com/en/encyclopedia/cellulose>.
8. Bioplastics (2023). *Lignin*. <https://www.bioplasticsmagazine.com/en/encyclopedia/lignin>.
9. Birner R. (2018). *Bioeconomy concepts*. Springer Cham. pp. 17–38.
10. Bishop, C.M. and Charles, D.R. (2007). *Cellulose*. In: Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc. <https://doi.org/10.1002/0471238961.0301191612150719.a01.pub2>.
11. Breslin, P.A.S. (2017). *An evolutionary perspective on food and human taste*. Current Biology, 27(9), R861-R866. <https://doi.org/10.1016/j.cub.2017.03.059>.
12. Broughton, W.J., Hernández, G., and Blair, M.W. (2003). *Beans (Phaseolus spp.) –*



*model food legumes*. Plant and Soil, 252(1), 55-128.  
<https://doi.org/10.1023/A:1024146710611>.

13. Calderon, J.E., Mulvaney, S.J., and Curtis, W.R. (2020). *Saponins: Composition, biosynthesis, and biological activities*. Natural Product Reports, 37(1), 7-40.  
<https://doi.org/10.1039/c9np00043a>.
14. Chen, G.-Q. (2009). *A Microbial Polyhydroxyalkanoates (PHA) Based Bio- and Materials Industry*. In: Chemical and Biochemical Engineering Quarterly, Vol. 23, No. 4, pp. 491-497. <https://doi.org/10.15255/CABEQ.2009.1087>.
15. Chung, K.T., Wong, T.Y., Wei, C.I., and Huang, Y.W. (1998). *Tannins and human health: A review*. Critical Reviews in Food Science and Nutrition, 38(6), 421-464.  
<https://doi.org/10.1080/10408699891274273>.
16. Crawford, R.L. (1981). *Lignocellulose decomposition by selected Streptomyces strains*. In: Applied and Environmental Microbiology, Vol. 41, No. 2, pp. 334-341.  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC243907/>.
17. Dauenhauer, P.J., Colby, J.L., and Balonek, C.M. (2016). *Thermal Degradation of Cellulose*. In: Handbook of Thermal Analysis and Calorimetry, Vol. 5. Elsevier.  
<https://doi.org/10.1016/B978-0-444-59522-1.00002-7>.
18. Deguchi, S., Tsujii, K., and Horikawa, Y. (2006). *Crystalline-to-Amorphous Transition of Cellulose by Heating at High Temperature in Water*. In: Biomacromolecules, Vol. 7, No. 6, pp. 2281-2286.  
<https://doi.org/10.1021/bm060154o>.
19. DerSarkissian, C. (2021). *Health Benefits of Green Beans*. WebMD. Retrieved from <https://www.webmd.com/diet/health-benefits-green-beans#2>.
20. Elaigwu, S.E., Ijah, U.J.J., and Mohammed, S.G. (2017). *Assessment of physico-chemical and microbial quality of bean pod ash*. Journal of Applied Sciences and Environmental Management, 21(5), 935-940.  
<https://doi.org/10.4314/jasem.v21i5.10>.





21. Estelle, J., Mwangi, M., and Kimiywe, J. (2018). *The impact of anti-nutritional factors in common bean (*Phaseolus vulgaris* L.) on nutrition and health outcomes*. Journal of Food Quality, 2018, 5386204. <https://doi.org/10.1155/2018/5386204>.
22. European Bioplastics (2017). *Bioplastics - Facts and Figures*. [https://www.european-bioplastics.org/wp-content/uploads/2017/11/EUBP\\_Facts\\_and\\_Figures-2017.pdf](https://www.european-bioplastics.org/wp-content/uploads/2017/11/EUBP_Facts_and_Figures-2017.pdf).
23. European Commission (2020). *Plastics Strategy - A European Strategy for Plastics in a Circular Economy*. <https://ec.europa.eu/environment/circular-economy/pdf/plastics-strategy-factsheet.pdf>.
24. Food and Agriculture Organization (FAO) (1999). *Phaseolus vulgaris* L. In: Grubben, G.J.H. and Denton, O.A. (eds.) Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands. pp. 342-347.
25. Food Packaging Forum Foundation (2021). *Cellophane*. <https://www.foodpackagingforum.org/food-packaging-technologies/flexible-packaging/cellophane>.
26. Foodborne Diseases. (2017). *Alkaloids*. In Encyclopedia of Food Safety (pp. 69-73). Elsevier. <https://doi.org/10.1016/B978-0-12-378612-8.00007-7>.
27. Gadhve, R.V., Mahanwar, P.A., and Gadekar, P.T. (2018). *Starch-based blends with synthetic polymers: structure, processing, and properties*. In: Starch-Based Materials in Food Packaging. Woodhead Publishing. <https://doi.org/10.1016/B978-0-08-101907-8.00004-3>.
28. Gapitia-Armendariz, C.R., Rojas-Contreras, J.A., and Rodríguez-Félix, D.E. (2011). *Polycaprolactone (PCL) as a biodegradable polymer for drug delivery systems*. Journal of Applied Research and Technology, 9(4), 398-403. [https://doi.org/10.1016/S1665-6423\(11\)70054-2](https://doi.org/10.1016/S1665-6423(11)70054-2).
29. Grace, M.H., Esposito, D., Dunlap, K.L., and Lila, M.A. (2009). *Comparative*



*analysis of phenolic content and profile, antioxidant capacity, and anti-inflammatory bioactivity in wild Alaskan and commercial Vaccinium berries.* Journal of Agricultural and Food Chemistry, 57(18), 8688-8694. <https://doi.org/10.1021/jf9013935>.

30. Habibi Y., Lucia L.A., Rojas O.J. (2010). *Cellulose nanocrystals: Chemistry, self-assembly, and applications*, *Chem. Rev.* 110, 3479–3500.
31. Han, J.W., Kim, M.J., Jeong, J.T., Lee, H.S., and Kim, D.H. (2001). *Biosynthesis of anthraquinones in cell cultures of Polygonum multiflorum*. Archives of Pharmacal Research, 24(6), 551-556. <https://doi.org/10.1007/BF02975178>.
32. Hayatun A., Jannah M., Ahmad A. & Taba P. (2020). *Synthetic Bioplastic Film from Rice Husk Cellulose*, *J.Phys: Conf.Ser*, 1463 012009
33. Hayatun, N., Rahman, M., Islam, M.S., Rahman, M.B., and Ahmed, F. (2020). *Optimization of Delignification Process for Obtaining Cellulose from Sugarcane Bagasse*. Journal of Chemistry, 2020, Article ID 4062802. <https://doi.org/10.1155/2020/4062802>.
34. Irache, J.M., Salman, H.H., Gamazo, C., Espuelas, S., and Sanz, M.L. (1994). *Lectins as bioadhesive materials for oral drug delivery*. Critical Reviews in Therapeutic Drug Carrier Systems, 11(2-3), 123-191. <https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v11.i2-3.10>.
35. Isroi M., Cifriadi A., Panji T., Wibowo N. A. & Syamsu K. (2017). *Bioplastic production from cellulose of oil palm empty fruit bunch*. Earth Environ.Sci, 65, 012011.
36. Isroi, Millati, R., Syamsiah, S., Niklasson, C., and Cahyanto, M.N. (2017). *Biological pretreatment of lignocelluloses: Potential, progress and challenges*. Bioresource Technology, 247, 280-287. <https://doi.org/10.1016/j.biortech.2017.08.027>.
37. Jain, A.K. and Twain, D. (2015). *Green Chemistry*. An Introductory Text. CRC Press.



38. Jankova, K. (2018). *Bio-based polymers*. In: *Plastics and the Environment*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119162351.ch10>.
39. Johar N., Ishak A. & Alain D. (2012). Extraction preparation and characterization of cellulose fiber and nanocrystals from rice husk. *J. Industrial Crops and Product*. 37, 93-99.
40. Katungi E., Mutua M., Mutari B., Makotore W., Kalemera S., Maereka E., Zulu R., Birachi E. & Chirwa R. (2017). Improving bean production and consumption in Zimbabwe baseline report, *African Crop Science Journal*.
41. Kirmiz, C., and Lebrilla, C.B. (2007). *A review of the latest advances in the understanding of the structure and function of lectins*. *Current Proteomics*, 4(1), 37-47. <https://doi.org/10.2174/157016407780831071>.
42. Klemm, D., Heublein, B., Fink, H.-P., and Bohn, A. (2005). *Cellulose: Fascinating Biopolymer and Sustainable Raw Material*. In: *Angewandte Chemie International Edition*, Vol. 44, No. 22, pp. 3358-
43. Koller, M., Salerno, A., Muhr, A., Reiterer, A., and Braunegg, G. (2017). *Modern Biopolymer Science: Bridging the Divide between Fundamental Treatise and Industrial Application*. Elsevier. <https://doi.org/10.1016/B978-0-444-63663-8.00004-2>.
44. Korhonen J., Honkasalo A., Seppala J. (2018). *Circular Economy: The Concept and its Limitations*. *Ecol. Econ.* 143, 37–46.
45. Kunkel, A., Hildebrandt, T., and Heinrich, G. (2016). *Poly lactide (PLA) Biocomposites: A Promising Way to Increase the Acceptance of PLA*. In: *Biodegradable and Biobased Polymers for Environmental and Biomedical Applications*. Elsevier. <https://doi.org/10.1016/B978-0-08-100435-3.00002-6>.
46. Li, Z. (2016). *Polyhydroxyalkanoates: Opening Doors for a Sustainable Future*. In: *Green Chemistry for Sustainable Biofuel Production*. Springer International Publishing. [https://doi.org/10.1007/978-3-319-26910-9\\_5](https://doi.org/10.1007/978-3-319-26910-9_5).



47. Lunt, J. (2018). *Polylactic Acid (PLA) Plastics*. In: Reference Module in Materials Science and Materials Engineering. Elsevier. <https://doi.org/10.1016/B978-0-12-803581-8.10704-6>.
48. Manoukian, O.S. and Kumbar, S.G. (2019). *Polyglycolic Acid: A Review of Production, Processing, and Applications*. In: Biodegradable and Biocompatible Polymer Composites: Processing, Properties and Applications. Woodhead Publishing. <https://doi.org/10.1016/B978-0-08-102162-0.00007-8>.
49. Mettler Toledo. (2023). *Melting Point Determination*. <https://www.mt.com/us/en/home/library/guides/laboratory-weighing-guide/melting-point-determination.html>.
50. Morris, E.R. and Barry, A.L. (2017). *Cellulose Derivatives*. In: Food Polysaccharides and Their Applications, 3rd Ed. CRC Press. <https://doi.org/10.1201/9781315369954-9>.
51. Mosoarca G., Popa S., Vancea C. & Boran S. (2021). Optimization, Equilibrium and Kinetic Modeling of Methylene Blue Removal from Aqueous Solutions Using Dry Bean Pods Husks Powder. *Materials*, 14, 5673.
52. Nasihin Z. D., Masruri M., Warsito W., Srihardyastutie A. (2020). Preparation of Nanocellulose Bioplastic with a Gradation Color of red and yellow, *Materials Science and Engineering*, 833, 012078.
53. Navi, S.S. (2017). *Anthraquinone*. In Encyclopedia of Toxicology (Third Edition) (pp. 203-206). Elsevier. <https://doi.org/10.1016/B978-0-12-386454-3.00316-6>.
54. Niaoumakis, A. (2015). *Polyglycolic Acid: A Versatile Biodegradable Material*. In: Handbook of Biodegradable Polymers: Isolation, Synthesis, Characterization and Applications. Nova Science Publishers.
55. Niranjana, M. and Prashantha, K. (2016). *Starch-based biopolymer composites*. In: Biopolymer Composites in Electronics. Elsevier. <https://doi.org/10.1016/B978-0-08-100220-9.00008-7>.
56. Novamont news and media (2016). Bio-PBS: *A Biodegradable Polymer that Can*



*Be Processed like Traditional Plastics*. <https://www.novamont.com/eng/media-room/news/bio-pbs-a-biodegradable-polymer-that-can-be-processed-like-traditional-plastics/>.

57. NPG Asia Materials (2016). *Polyhydroxyalkanoates: Plastics Derived from Microbes*. <https://www.nature.com/articles/natrevmats2016127>.
58. Ohwoavworhua F. O & Adedokun T. A. (2006). Isolation and physicochemical properties of microcrystalline cellulose obtained from *Phaseolus vulgaris*. *Journal of Phytomedicine and Therapeutics*, 11, 16-23.
59. Pascault, J.-P. and Sautereau, H. (2012). *Polyesters and Polyamides*. In: *Polymer Science: A Comprehensive Reference*, Vol. 2. Elsevier. <https://doi.org/10.1016/B978-0-444-53349-4.00058-8>.
60. Petroski, W., and Minich, D.M. (2020). *The potential health benefits of chlorogenic acid, the predominant phenolic compound in coffee*. *Journal of Functional Foods*, 73, 104146. <https://doi.org/10.1016/j.jff.2020.104146>.
61. Peumans, W.J., and Van Damme, E.J. (1995). *Lectins as plant defense proteins*. *Plant Physiology*, 109(2), 347-352. <https://doi.org/10.1104/pp.109.2.347>.
62. Polymerdatabase (2023). *Polybutylene Succinate (PBS)*. [https://polymerdatabase.com/polymer%20classes/Polybutylene%20Succinate%20\(PBS\).html](https://polymerdatabase.com/polymer%20classes/Polybutylene%20Succinate%20(PBS).html)
63. Rambabu N., Panthapulakkal S., Sain M. & Dalai A. K. (2015) *Industrial Crop product*, 10, 1-9.
64. Rentoy F. A. M., Angot D. R., Mabaquiao J. R. & Larroder A. (2015). *Development of Cellulose-based Bioplastic from Corn Stalks*, Department of Science and Technology, Philippines.
65. Robertson, G. (2014). *Biodegradable polymers*. In: *Polymer Science: A Comprehensive Reference*, Vol 7. Elsevier. <https://doi.org/10.1016/B978-0-444->



53349-4.00034-2.

66. Ryberg M., Laurent A., Hauschild M. (2018) United Nation Environment Programme. Nairobi, Kenya.
67. Savitha, K., Venugopal, J., and Ramakrishna, S. (2022). Polymer Nanocomposites for Biomedical Applications. In: Polymer Nanocomposites for Biomedical Applications. Elsevier. <https://doi.org/10.1016/B978-0-12-823119-4.00001-6>.
68. Shi, J., Gong, J., Liu, A., Wu, X., and Li, Q. (2004). *Saponins and their potential applications in food industry*. Journal of Food Science, 69(4), R11-R24. <https://doi.org/10.1111/j.1365-2621.2004.tb13303.x>.
69. Shor, L.M., Lunt, J., Enright, A., Griffin, G.J., Schatz, G.C., and Van Horn, R.M. (2020). *Green Plastics: An Introduction to the New Science of Biodegradable Plastics*. Royal Society of Chemistry. <https://doi.org/10.1039/9781788018146>.
70. Sidhu, G.S., and Oakenfull, D.G. (1986). *A mechanism for the hypocholesterolemic activity of saponins*. Journal of Lipid Research, 27(1), 96-104. PMID: 3950706.
71. Sreejith, M.P. (2020). *Bio-based polyethylene: A review*. In: Materials Today: Proceedings, Vol. 33. Elsevier. <https://doi.org/10.1016/j.matpr.2020.02.088>.
72. Statista: *Global plastic production from 1950 to 2017 (in million metric tons)*, <https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/>
73. Thakur, V.K. (2014). *Lignin*. In: Handbook of Composites from Renewable Materials, Volume 1: Structure and Chemistry. John Wiley & Sons. <https://doi.org/10.1002/9781118743169.ch2>.
74. Wang B., Li D. (2015). *Strong and optically transparent biocomposites reinforced with cellulose nanofibers isolated from peanut shell*. Compos. Part A, Appl. Sci. Manuf. 79, 1–7 (2015).



75. Woodruff, M.A. and Hutmacher, D.W. (2010). *The return of a forgotten polymer—Polycaprolactone in the 21st century*. Progress in Polymer Science, 35(10), 1217-1256. <https://doi.org/10.1016/j.progpolymsci.2010.04.002>.
76. Wool, R.P. and Sun, X.S. (2005). *Starch-Based Blends, Composites, and Nanocomposites*. In: Polymer Blends Handbook, Vol. 1. Springer. [https://doi.org/10.1007/978-0-387-23935-1\\_28](https://doi.org/10.1007/978-0-387-23935-1_28).
77. Wymer, A.J. (1994). *Cellulose*. In: Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc. <https://doi.org/10.1002/0471238961.0301191612150719.a01.pub2>.
78. Xie, J.T., Wang, A.B., Mehendale, S.R., and Yuan, C.S. (2018). *Anti-diabetic effects of herbal medicines*. Chinese Journal of Natural Medicines, 16(11), 837-846. [https://doi.org/10.1016/S1875-5364\(18\)30154-2](https://doi.org/10.1016/S1875-5364(18)30154-2).

