Cape Peninsula University of Technology

Synthesis and characterisation of bioplastic films through valorisation of starch-rich waste from maize (*Zea mays*) wet-milling

by

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Declaration

I, Magan Ross, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

22 February 2026

Signed **Date**

Abstract

This thesis presented the synthesis and characterisation of starch-based bioplastic films developed through the valorisation of starch-rich waste derived from the maize wet-milling process. Addressing growing environmental concerns related to conventional petroleum-based plastics, this study explores sustainable alternatives by transforming agro-industrial residues into biodegradable polymer films. Starch, as a renewable and biodegradable polymer, serves as the primary matrix for bioplastic formulation. This research aimed to synthesise starch-based bioplastics for improved performance properties through the addition of filler reinforcements that are rich in lignocellulosic fibre. This research also aimed to address problems of high feedstock costs and the impact on food security through valorising starch and fillers obtained from waste rather than food crops.

A comprehensive literature review preceded the experimental work, covering starch production methods, its industrial applications, and bioplastic potential. The experimental methodology involved extracting starch from maize waste collected from a local maize wet-milling facility, followed by the formulation of bioplastic films with varied plasticiser (glycerol), filler (lignin, cellulose-lignin mixture and canola fines), and acetic acid compositions. Mechanical properties, including tensile strength and elongation at break, alongside water absorption behaviour, were assessed.

A factorial design model was employed to investigate the effects of five factors, namely starch type (spillage or extract), filler type (cellulose-only or cellulose-lignin mixture), plasticiser mass fraction, filler mass fraction, and acetic acid volume, on key bioplastic properties. Acetic acid was found to have no statistically significant influence on tensile strength, elongation at break, or water absorption and was excluded from further formulations to reduce costs and processing complexity. Spillage starch was identified as the superior raw material, yielding bioplastic films with an optimum tensile strength of 9.7 MPa, elongation at break of 24.9%, and water absorption of 54.2%, outperforming extract starch films in strength and moisture resistance.

Subsequently, response surface methodology optimised synthesis conditions for films incorporating canola fines, a lignocellulosic agro-industrial waste. The optimal formulation contained 30% glycerol and 13.5% filler (w/w dry starch), producing films with a tensile strength of 3.3 MPa, elongation at break of 52%, and water absorption of 65%. The incorporation of canola fines improved structural integrity and durability while promoting sustainability by valorising agro-industrial waste, reducing feedstock costs, and aligning with food security goals.

This research highlights the critical impact of raw material selection and plasticiser-filler optimisation in developing starch-based bioplastics with desirable mechanical and barrier properties. The elimination of acetic acid simplifies production and decreases costs without sacrificing performance. The findings provide a robust framework for developing cost-effective, eco-friendly bioplastics tailored to specific application requirements such as packaging, agricultural films, and single-use products.

Future work is recommended to explore lower acetic acid concentrations to fully assess effects influenced by concentration, alongside investigating the long-term durability and environmental biodegradability of these films to confirm commercial viability. Overall, this thesis contributes practical insights and advances the sustainable development of bioplastics derived from agro-industrial waste, supporting the growing bioeconomy and environmental sustainability efforts.

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Dedication

This thesis is dedicated to my mother, whose hard work, sacrifices, and unwavering support have profoundly inspired and shaped my academic journey.

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Nomenclature

A	Cross-sectional area	mm^2
L	Length	mm
F	Load	N
W	Weight	g

Greek letters

ε	Elongation at break	%
σ	Tensile strength	MPa

Subscripts

i	Initial specimen condition
f	Final specimen condition
o	Original specimen condition

List of Abbreviations

ASTM	American Society for Testing and Materials
ANOVA	Analysis of variance
ADLM	Association for Diagnostics and Laboratory Medicine
AOAC	Association of Official Analytical Collaboration
BDMs	Biodegradable plastic mulches
CO_2	Carbon dioxide
CHNS	Carbon, hydrogen, nitrogen, sulphur (elemental analysis)
CAF	Central Analytical Facilities
CCD	Central composite design
CSIR	Council of Scientific & Industrial Research
dH_2O	Distilled water
EtO	Ethylene oxide
FAO	Food and Agriculture Organisation
T_g	Glass transition temperature
HDPE	High-density polyethylene
HCl	Hydrochloric acid
I_2	Iodine
LDPE	Low-density polyethylene
PAA	Polyacrylic acid
PCL	Poly (ε-caprolactone)
PHAs	Polyhydroxyalkanoates
PLA	Polylactic acid
PLGA	Poly (lactic-co-glycolic acid)

PVC	Polyvinyl chloride
KI	Potassium iodide
RSM	Response surface methodology
NaCl	Sodium chloride
NaOH	Sodium hydroxide
SABS	South African Bureau of Standards
H_2SO_4	Sulphuric acid
UV	Ultraviolet
H_2O	Water
w/v	Weight by volume
w/w	Weight by weight
w/w dry starch	Weight per weight with respect to dry starch

Chapter 1: Introduction

1.1. Background

The history of plastics is a complex narrative that spans over a century, beginning with the invention of Bakelite in the early 20th century. Bakelite, developed by Leo Baekeland in 1907, marked the first synthetic plastic, which was not only durable but also heat-resistant, thus enabling the advancement of many synthetic polymers (Ekner-Grzyb et al., 2022). Following Bakelite, the mid-20th century saw the rise of other significant plastics such as polyethylene, polypropylene, and polyvinyl chloride (PVC), which became ubiquitous in consumer goods and packaging (Naeimirad, 2023). The convenience and versatility of these materials contributed to their widespread adoption, leading to a dramatic increase in plastic production, which reached approximately 8.3 billion metric tonnes by 2015 and consequently, 6.3 thousand metric tonnes of plastic waste (Singh & Chunglok, 2022; Geyer et al., 2017). Plastic packaging accounts for the largest share of global plastic consumption at approximately 30%, with 99% of these plastics currently derived from petroleum-based polymers (Zhao, et al., 2020).

Despite their functional advantages, the environmental repercussions of plastic production and disposal have become increasingly apparent over the decades. Plastic waste accumulation, especially from single-use products, has emerged as a serious environmental issue, raising concerns about their long-term impact on wildlife, marine life, and human health (Fei, 2024; Roy et al., 2022; Darni et al., 2019). Petroleum-based plastics leach toxic chemicals such as polychlorinated biphenyl and nonylphenols into water bodies, threatening water quality and aquatic organisms (McCormick et al., 2014). Plastic waste accumulating in landfills leads to contamination of groundwater, while marine debris causes entanglement, ingestion, and suffocation in animals (Al-Salem et al., 2017; Gregory, 2009). For instance, the Ocean Conservancy reported that plastic bags and fishing gear were the main causes of marine life entanglement over a 25-year period (Fox, 2011). At the Victoria and Alfred Waterfront harbour in Cape Town, 927 incidents of Cape fur seal entanglements were recorded between 2000 and 2018, largely caused by waste materials such as nylon cord, clothing fabrics, and fishing-related debris (Sadan & de Kock, 2020). Plastic particles also reach humans through seafood, drinking water and air, which leads to health problems including chronic diseases (Shafqat et al., 2021). These findings underscore the urgent need for more sustainable alternatives (Shafqat et al., 2021; Ross et al., 2017; Ismail et al., 2016).

The limitations of petroleum-based plastics have motivated the development of alternative materials that are environmentally safer and more sustainable (Elkaliny et al., 2024). Bioplastics offer the potential to reduce dependence on finite fossil fuels, minimise greenhouse gas emissions, and decrease environmental pollution at end-of-life (Mattlar & Ekholm, 2025). Additionally, by using renewable or waste-derived feedstocks, bioplastics contribute to resource efficiency and support circular economy principles, providing both economic and ecological benefits (Phillip, 2024).

A circular economy framework focuses on reducing fossil fuel reliance, maintaining material value and designing products for reintegrate into biological or technical cycles (Pal et al., 2024; Geissdoerfer et al., 2017). Within this framework, bioplastics have emerged as a promising material. Bioplastics describe a broad family of plastics that are either sourced from biomass (bio-based), designed to decompose back into the environment (biodegradable), or both (Ross et al., 2017; Philp, 2013). Importantly, bio-based origin and biodegradability are independent material characteristics: a polymer may derive from biomass yet still persist in the environment, while some fossil-based polymers are designed to biodegrade under specific conditions. Biodegradability describes the capacity of a material to undergo decomposition through microbial activity into carbon dioxide (CO_2), water, and biomass under appropriate environmental conditions, and degradation behaviour depends on factors such as temperature, oxygen availability, moisture, and microbial activity (Ross et al., 2017).

Compostable plastics are a subset of biodegradable plastics that can undergo biological decomposition under controlled composting conditions, breaking down into CO_2, water, inorganic compounds, and biomass without visible residues, and must meet recognised standards such as ASTM D6400 or EN 13432 (Ross et al., 2017). **Figure 1-1** illustrates the classifications of plastics:

1. Group I: Fossil-based, non-biodegradable plastics (conventional petroleum plastics)
2. Group II: Bio-based, non-biodegradable plastics
3. Group III: Bio-based and biodegradable plastics
4. Group IV: Fossil-based, biodegradable plastics

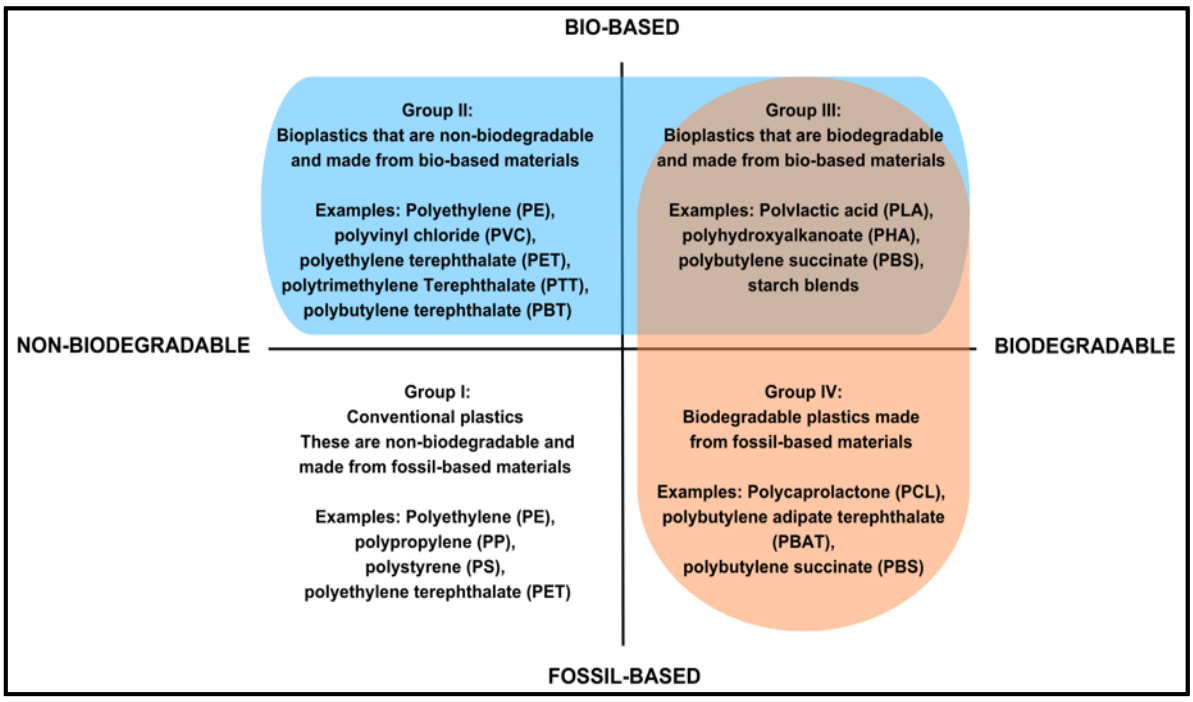

Figure 1-1: Classification of plastics (Ross, et al. (2017).

Bioplastics that are both bio-based and biodegradable are particularly aligned with circular economy goals as they combine renewable feedstocks with environmentally safe end-of-life options. However, their overall sustainability depends on feedstock sourcing, processing conditions, and waste management facilities (Molina-Besch, 2022; Keziah et al., 2018). Bio-based bioplastics are generally derived from renewable biomass, including cellulose, starch, woodchips, gluten, gelatine, animal and vegetable oils, and recycled food waste (Zoungranan et al., 2020). By replacing crude oil-based plastics to renewable feedstocks, the plastics industry can reduce reliance on fossil fuels and mitigate associated greenhouse gas emissions (Pooja et al., 2023; Ross et al., 2017; Chen, 2013).

In the agro-polymer industry, starch is valued for its low cost and complete compostability, without leaving toxic residues (Zhong et al., 2020; Zoungranan et al., 2020; Shah et al., 2008). Importantly, increased demand for starch should not compromise food security (Kringel et al., 2020). Valorising waste streams for bioplastic production not only addresses food security concerns but also reduces waste accumulation. Converting underutilised biomass to value-added polymers promotes circular supply chains, lowers disposal-related environmental costs, and enhances resource efficiency (Pal et al., 2024; Keziah et al., 2018).

1.2. Problem statement

Although starch-based bioplastics are widely recognised as promising sustainable alternatives to petroleum-derived plastics, their commercial application remains constrained by two major challenges. Firstly, starch-based films generally exhibit poor tensile strength and high water sensitivity, limiting their suitability for applications where mechanical integrity and moisture resistance are essential (Gupta, 2011). Secondly, many formulations rely on food-grade starch as feedstock, increasing production costs and raising food security concerns (Kringel et al., 2020).

Maize wet-milling generates substantial starch-rich residues; however, limited research has investigated the integration of locally available maize waste streams into bioplastic formulations (Deepak & Jayadeep, 2021). Similarly, lignocellulosic agricultural residues present potential as reinforcing agents; however, their incorporation into starch matrices has not been sufficiently optimised to effectively enhance mechanical performance while reducing moisture sensitivity (Motshabi et al., 2025).

Consequently, there is a need to develop starch-based bioplastic films derived from low-cost, starch-rich maize wet-milling by-products that exhibit improved mechanical performance and reduced water absorption through effective reinforcement strategies. Addressing these challenges is essential to advancing material performance, reduce feedstock costs, and minimise reliance on food crops. This identified gap forms the foundation of the present study.

1.3. Research questions

1. How do the mechanical and water resistance properties of bioplastic films vary based on the presence of reinforcements and the type of starch substrate used during bioplastic synthesis?
2. What is the influence of valorising locally sourced starch-rich waste and lignocellulosic fibre from canola fines on the performance and characteristics of starch-based bioplastic films?

1.4. Aim and objectives

The aim of this project was to synthesise cost-effective and sustainable starch-based bioplastic films with enhanced mechanical and water resistance properties by valorising starch-rich waste from maize wet-milling and incorporating lignocellulosic filler reinforcements derived from agricultural residues, thereby addressing feedstock cost challenges and reducing reliance on food crops.

The main objectives of this study are as follows:

1. Perform in depth characterisation of solid substrates, namely reject maize kernels (kaff-kaff) and maize starch spillage, collected from maize wet milling.
2. Identify and select the most promising substrates containing sufficient starch and synthesise bioplastic films without reinforcements and with reinforcements: (i) commercial cellulose-only, (ii) commercial cellulose and lignin mixture, and (iii) canola fines.
3. Perform product evaluation of bioplastic films through characterisation in terms of mechanical and water resistance properties.
4. Perform a characterisation and performance analysis of bioplastic films synthesised from the valorisation of locally sourced starch-rich waste and lignocellulosic fibre extracted from canola fines waste.

1.5. Significance of the study

- This project is expected to contribute new knowledge on research pertaining to starch extraction and bioplastic synthesis from waste valorisation in a South African context.
- This research will contribute to the knowledge of the feasibility of bioplastics synthesised from starch-rich waste from a maize wet-milling process as a commercially viable alternative to conventional plastics.
- Bioplastic alternatives with improved mechanical strength and reduced cost could enter the market, leading to job creation within the manufacturing stage.
- Bioplastics degrade into safer components than conventional plastics, and the production of bioplastics emits less greenhouse gases. This research will provide information that may assist in reducing plastic waste and environmental pollution.
- The valorisation of waste to produce bioplastics adds value to waste and creates a circular economy.

1.6. Delineation

The following will not be covered in this research project:

- Biodegradability analysis of bioplastics
- Antimicrobial and antifungal activity of bioplastics

1.7. Structure of the thesis

Chapter 1: Introduction

This chapter introduces the topic and provides the background information into the environmental concerns surrounding conventional plastics and why bioplastic is a better alternative. In addition, this chapter outlines the problem statement, aim and objectives, research questions, significance of the study, and delineation.

Chapter 2: Literature review

This chapter provides a comprehensive literature review of the different aspects related to the topic of this thesis.

Chapter 3: Extraction and characterisation of starch-rich waste

This chapter presents the extraction and characterisation of starch derived from maize agro-industrial waste streams. It details the methodology for starch extraction from maize wet milling waste, followed by a comprehensive characterization of both the starch-rich substrates and the extracted starch. Furthermore, the chapter evaluates the suitability of these substrates and the extracted starch, based on their physicochemical properties, for subsequent application in bioplastic film production.

Chapter 4: Screening using a factorial design

This chapter investigates the factors affecting bioplastic film properties, particularly, tensile strength, elongation at break, and water absorption. This chapter also includes the design of experiments that was generated using Design Expert software.

Chapter 5: Optimisation using response surface methodology

This chapter describes and applies the optimisation of the bioplastic films using Response Surface Methodology available in Design Expert to determine the optimal formulation to synthesise bioplastics with maximum mechanical properties and water resistance.

Chapter 6: Conclusion and recommendations

This chapter concludes all findings of the thesis and states recommendations based on these findings.

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Chapter 2: Literature review

2.1. Starch production and applications in South Africa

Starch is a biodegradable, naturally occurring polysaccharide that serves as a primary energy storage molecule in different botanical sources such as cereal grains (wheat, rice, and maize), root tubers (cassava, potatoes, tapioca), and grain legumes (chickpea, lentil, and pea) (Shoukat, et al., 2025; Surendren, et al., 2022). According to Wijesinghe & Gunathilake (2020), maize accounts for the majority of the global starch production, although significant amounts of other starch sources like cassava, sweet potato, potato and wheat starch are also produced. Starch production in South Africa is a significant agricultural activity, primarily derived from maize, which is the most widely cultivated crop in the country. Maize starch serves as a vital raw material for various industries, including food, textiles, paper, and bioplastics (Yu & Moon, 2021).

Maize is a fundamental crop in South Africa, significantly influencing food security at both household and national levels. As the leading maize producer in the Southern African Development Community (SADC), South Africa accounts for approximately 50% of the maize produced in the region, with an average annual yield of around 11 million tonnes (Meyer et al., 2019). Serving as a major dietary staple, maize accounts for nearly 30% of the caloric intake of many South Africans, a contribution that is especially crucial in rural areas where access to diverse food sources is limited (Raphela & Pillay, 2021). Beyond its nutritional importance, maize holds significant cultural value as a key ingredient in various traditional dishes (Raphela & Pillay, 2021).

In addition to its role in food security and culture, maize is the predominant source of starch in South Africa, representing approximately 83% of the national grain production (Nji et al., 2022). The country's maize farming sector is well-established and diverse, encompassing both commercial and subsistence farming systems. Commercial farms, which produce about 85% of the maize crop, utilise advanced agricultural practices and technologies, while subsistence farmers typically rely on traditional methods (Nji et al., 2022). Although other starch sources such as cassava and sweet potatoes are less prevalent, they are increasingly recognised for their adaptability to local conditions and reduced water requirements (Amelework et al., 2021).

The starch industry plays a key role in the South African economy, contributing to food security and providing raw materials for various sectors. The demand for starch is driven by its applications in food processing, where it is used as a thickener, stabiliser, and texturiser

(Tshikovhi & Wyk, 2021). Additionally, starch is increasingly utilised in the production of biodegradable plastics, aligning with global sustainability trends (Amelework et al., 2021).

2.2. Starch industrial processing

One of the starch industrial processes is the starch wet-milling process, which is a sophisticated method primarily used to extract starch from various cereal grains, most notably maize. This process involves several key steps: steeping, milling, separation, and purification, each of which plays an important role in influencing the quality and yield of the final starch product. According to Ndlovu (2013), harvested crops undergo initial screening using mechanical cleaners that employ sieving and air blowing to remove irregular grains, dust and other foreign materials, with electromagnets sometimes used to extract metal fragments.

Grains are then sent for steeping, where they are soaked in water or a dilute sulphurous acid (H_2SO_3) solution for a period typically ranging from 24 to 48 hours. This step softens the kernels, facilitating the subsequent milling process by breaking down the protein matrix that surrounds the starch granules (Zhang et al., 2005). Steeping not only enhances starch extraction but also helps in reducing the protein content of the final product, which is essential for achieving high starch purity (Uriarte-Aceves et al., 2019; Uriarte-Aceves et al., 2015). The optimal conditions for steeping can significantly influence the yield; studies have shown that varying the steeping time and temperature can lead to starch yields as high as 90.7% (Beta & Corke, 2004).

Following steeping, the grains are subjected to grinding or milling. This step can be performed using various techniques, including wet ball milling or high-impact milling, which effectively disrupt the kernel structure and release the starch granules (Liu et al., 2024). The choice of milling method and specific operational conditions affects starch quality, as excessive shear can damage granules, lowering purity and yield. Consequently, milling must be optimised to balance maximum starch extraction with minimal granule degradation to preserve functionality in food applications (Uriarte-Aceves et al., 2019; Uriarte-Aceves et al., 2015).

After milling, the mixture undergoes a series of separation and purification steps. This involves washing the milled product to remove soluble components, including proteins and fibres, which can contaminate the starch (Serna-Saldivar, 2019). The purification process aims to achieve a high starch purity, often exceeding 98%, which is a critical quality benchmark in the industry (Chew-Guevara et al., 2016; Uriarte-Aceves et al., 2015). The final product of the wet-milling process is a high-purity starch that can be utilised in various applications, ranging from food products to industrial uses. A process flow diagram of a typical maize wet-milling process is represented in **Figure 2-1** as maize starch was used in this study (Ndlovu, 2013).

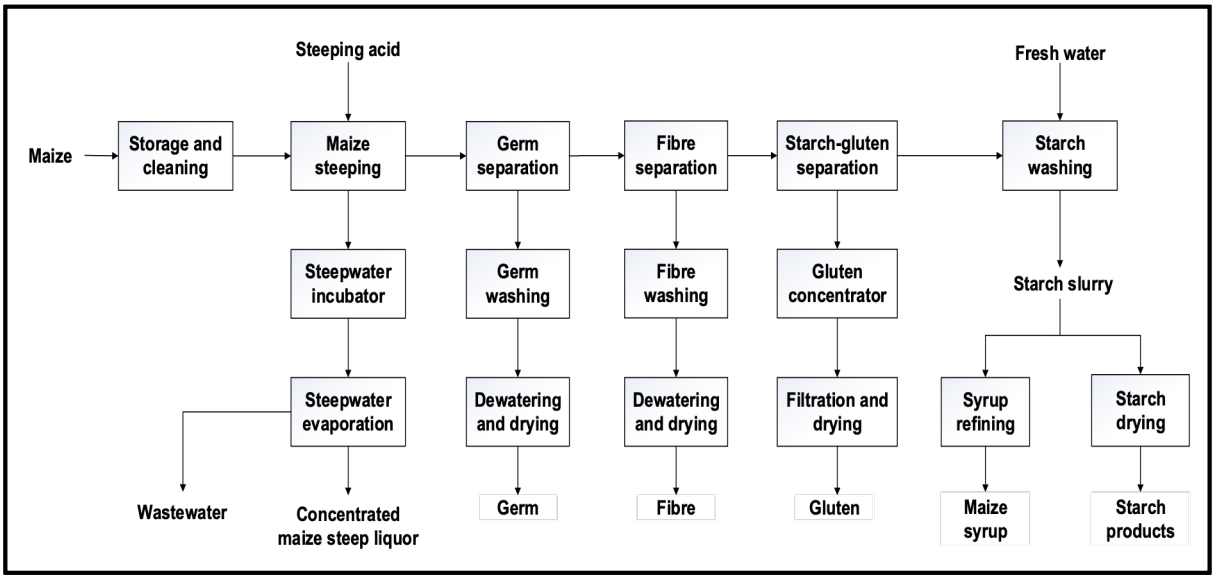

Figure 2-1: Maize wet-milling process flow diagram (Ndlovu, 2013).

2.3. Composition and structure of starch

Native starch, which is the purest form of starch after extraction processes, is a white powder that is tasteless and odourless, and is insoluble in cold water. Starch granules are semi-crystalline with a complex and highly organized arrangement consisting of two main carbohydrate polymer units: amylose and amylopectin (Surendren et al., 2022).

Amylose is a linear, unbranched polymer consisting of glucose units linked predominantly by α-D-(1,4) glycosidic bonds (**Figure 2-2a**). Linear amylose forms a single helix due to the specific angles and flexibility of the α-D-(1,4) glycosidic bonds between the glucose units, creating a natural twist in the chain. This helical structure is further stabilized by hydrogen (H) bonds between the oxygen (O_2) atoms on the outer surface, creating a more hydrophilic outer surface. Meanwhile, within the glucose rings, O_2 atoms face inward, creating a relatively hydrophobic inner surface. This means that water (H_2O) molecules are not tightly bound to the inner surface and can easily be replaced by hydrophobic molecules such as lipids and other hydrophobic molecules (Bertoft, 2017; Garcia & Franco, 2014). Amylopectin is a more crystalline, highly branched polymer which contains both α-D-(1,4) glycosidic bonds in the linear chains and α-D-(1,6) glycosidic bonds at the branch points (Ogori & Taofeek, 2022) (**Figure 2-2b**).

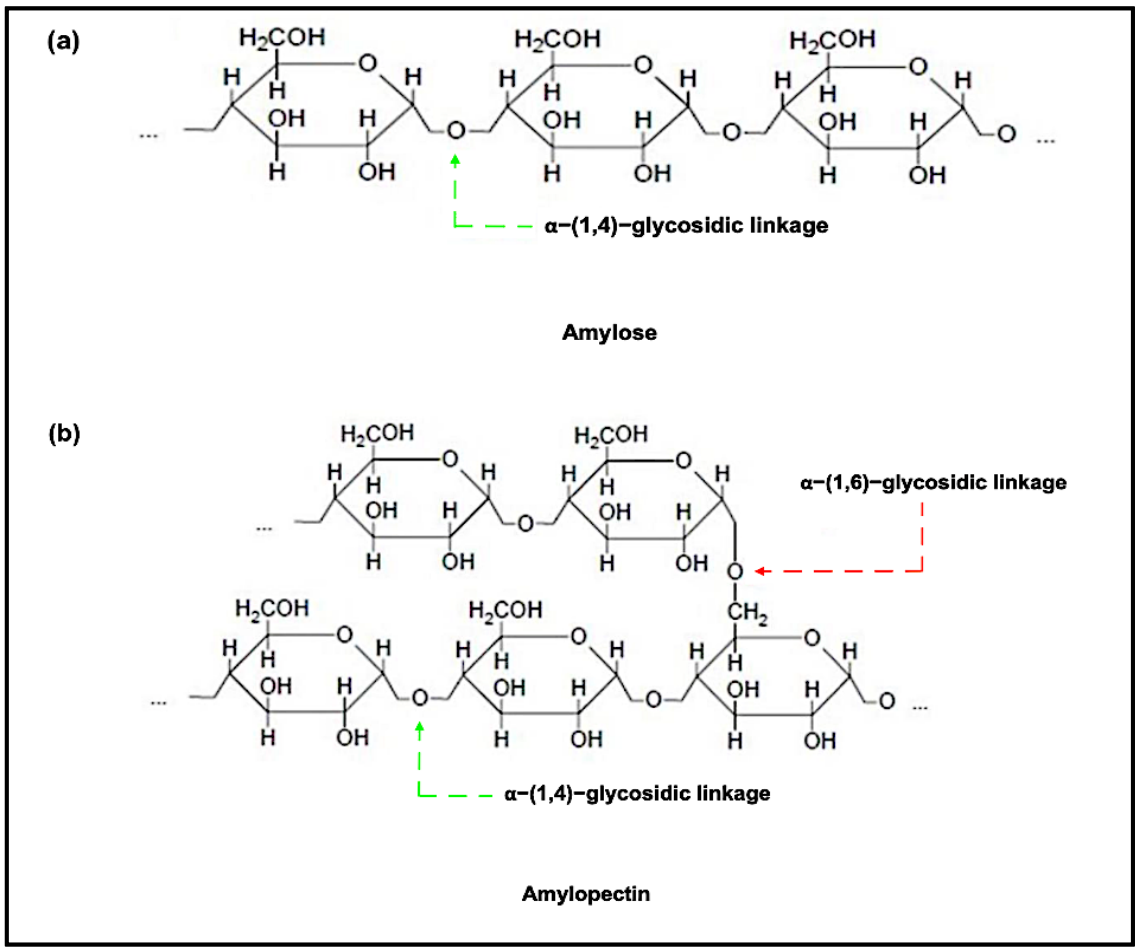

Figure 2-2: Structural composition of (a) amylose and (b) amylopectin (Zakaria et al., 2017).

Research indicates that the amylose and amylopectin contents, and the macromolecular structure of starch varies among botanical sources due to changes in geographical and climate conditions (Surendren et al., 2022; Waterschoot et al., 2015; Sharma et al., 2007). According to Avérous and Halley (2009), depending on the botanical source, granules dimensions range from 0.5–175 μm, and the weight percentage of amylose and amylopectin ranges between 15–30% and 70–85% of starch content, respectively (Mali et al., 2004). The amylose and amylopectin contents of starches from different botanical sources is given in **Table 2-1**.

Table 2-1: Average starch percentages in various starch sources (Waterschoot et al., 2015; Whistler et al., 1984).

Source	Amylose (%)	Amylopectin (%)
Arrowroot	20.5	79.5
Banana	16	83
Cassava	18-24	76-82
Maize (*Zea mays*)	23-28	72-77
Potato	17-27	73-83
Rice	18.5	81.5
Tapioca	16.7	83.3
Wheat	25-26	74-75

The semi-crystalline nature of starch arises from the lamellar organization within the internal structure of starch granules, where amylose and amylopectin chains are arranged in alternating crystalline and amorphous regions (Chen et al., 2016; Noosuk et al., 2003). The branch chains of amylopectin structured as double helices is responsible for the crystalline region of starch granules. Amylose is mainly situated in the amorphous regions of starch granules with individual amylose units also dispersed throughout both the crystalline and amorphous phases of amylopectin clusters, often within close proximity (Ningrum et al., 2023).

2.4. Plasticisation of native starch

When starch is heated in the presence of H_2O, it undergoes gelatinisation, a process in which starch granules absorb water, swell, and eventually lose their crystalline structure. Upon cooling, retrogradation occurs as the starch molecules reassociate, leading to changes in functional properties. These structural transitions influence characteristics such as viscosity, texture, and moisture retention, underpinning starch's versatility in diverse applications, including food processing and the formulation of biodegradable materials (Whistler et al., 1984).

2.4.1. Gelatinisation

When starch granules are heated in the presence of H_2O, the crystalline structure of the starch granules begins to break down, leading to significant swelling and the absorption of H_2O. This process is called gelatinisation, which results in the disruption of H bonds within the granules and causes the starch granules to swell to many times their original size, allowing the amylose and amylopectin molecules to leach out into the surrounding water, forming a viscous gel (Prisby et al., 2024). The process of gelatinisation is characterised by a specific temperature range and varies depending on the type of starch and its composition, with amylose generally contributing to the gel's thickening properties due to its linear structure, while amylopectin provides stability and viscosity (Han et al., 2021). The gelatinisation temperature range is thus

defined as the temperature at which the granules start to swell and end at the temperature, where nearly all granules have fully gelatinised (Chakraborty et al., 2022; Whistler et al., 1984). **Table 2-2** shows the gelatinisation temperature ranges of various starch sources.

Table 2-2: Chemical and physical properties of starches derived from different botanical sources (Whistler et al., 1984).

Starch Source	Gelatinisation Temperature Range (°C)
Wheat	58-64
Maize	62-72
Potato	50-68
Rice	68-78

2.4.2. Retrogradation

Retrogradation occurs after gelatinisation when gelatinised starch cools and the amylose and amylopectin chains begin to reassociate and recrystallise. The mechanism of retrogradation involves the reformation of H bonds between the hydroxyl (OH^-) groups of starch molecules, leading to the recrystallisation of starch (Lu et al., 2024). The rate of retrogradation can vary significantly based on the amylose-to-amylopectin ratio; Starches with a higher amylose content typically result in a faster retrogradation rate due to its tendency to form more stable crystalline structures (Gong et al., 2019). Additionally, the degree of retrogradation is influenced by various factors, including temperature, storage time, and the presence of other components such as lipids and proteins (Gao et al., 2022).

Native starch has a high tendency to retrograde, leading to undesirable outcomes such as high brittleness and rigidity, which limits their industrial applications as plastic alternatives. As the starch molecules realign and form crystalline regions, the material can become less flexible and more prone to cracking under stress. This brittleness is particularly problematic in applications where flexibility and durability are required, such as in packaging materials (Surendren et al., 2022; Agustin et al., 2014). Plasticisers such as glycerol (propane-1,2,3-triol, $C_3H_8O_3$) and sorbitol are commonly used in starch bioplastics to prevent the retrogradation of starches during the plasticisation stage as they are compatible with polysaccharides, consequently preventing brittleness and enhancing the flexibility of starch-based bioplastics (Abe et al., 2021; Amin et al., 2019).

2.5. Starch-based bioplastics

The processing of starch into starch-based bioplastics involves heating starch in the presence of H_2O and plasticisers like glycerol. Different processing techniques are used to manufacture starch-based bioplastics, including extrusion, compression moulding, and solution casting. Solution casting, used in this study, involves preparing the film solution by dissolving the starch in H_2O while adding the plasticiser. The mixture is heated to the gelatinisation temperature range with continuous stirring for approximately 20 minutes to ensure complete gelatinisation (Saberi et al., 2017) when starch granules have swollen and lost their crystalline structure, becoming more amorphous (Gabriel et al., 2021). Plasticisers intercalate between the amylose and amylopectin chains, reducing intermolecular H bonding of starch granules. This increases molecular mobility and lowers the glass transition temperature (T_g), facilitating easier processing into flexible films (Surendren et al., 2022). The type of plasticiser used in the bioplastic must be a hydrophilic polar molecule that is compatible with the given starch or efficient plasticisation will not be achieved. Furthermore, the optimal mass fraction of the plasticisers is also important for efficient plasticisation. For instance, a glycerol mass fraction of around 20% w/w of dry starch has been shown to produce flexible films with efficient plasticisation (Abdullah et al., 2019).

Researchers have conducted studies with starch-based bioplastics synthesised from various starch sources incorporating different plasticisers ranging in quantity. **Table 2-3** presents the optimum mechanical properties (tensile strength and elongation at break) and water absorption results from previous studies. These were all synthesised by the solution casting method. These results show that flexibility is achieved with the addition of a plasticiser. However, the addition of plasticisers can lead to a trade-off between flexibility and mechanical strength, complicating the formulation of effective bioplastics (Yang et al., 2022). Thus, it is important to optimise the glycerol content to produce bioplastics that have good flexibility (elongation at break) while still achieving good tensile strength. In this study, glycerol was used as a plasticiser with varied amounts similar to the investigations provided in **Table 2-4**. This was done to optimise the plasticiser content and the performance of the resultant bioplastic films.

Table 2-4: Mechanical properties and water absorption of different starch-based bioplastics films.

Starch source (%, w/v)	Tensile strength (MPa)	Elongation at break (%)	Water absorption (%)	Plasticiser (%, w/w dry starch)	Reference
Arrowroot (2)	3.9	45.3	-	Glycerol (30)	(Makishi et al., 2017)
Arrowroot (5)	2.42	46.62	190	Glycerol (30)	(Tarique et al., 2021)
Cassava (2)	7.0	132.1	-	Glycerol (32.5)	(Alves et al., 2007)
Cassava (3)	0.45	30.7	-	Glycerol (30)	(Yang et al., 2022)
Cassava (5)	20.9	266	-	Glycerol (25)	(Harunsyah et al., 2017)
Maize (5)	10	33.1	-	Glycerol (30)	Agustin, 2014
Maize (5)	6.4	3.8	-	Glycerol (25)	(Hermansyah et al., 2014)
Maize (5)	1.49	51	-	Glycerol (50)	(Żołek-Tryznowska & Kałuża, 2021)
Maize (10)	2.61	200	-	Glycerol (30)	(El Mogy et al., 2024)
Pinhão (5)	18.56 2.14	2.8 57.4	-	Glycerol (20) Glycerol (25)	(Daudt et al., 2016)
Rice (5)	10.9 1.6	2.8 59.8	-	Glycerol (20) Glycerol (30)	(Dias et al., 2010)
Sweet Potato (2.5)	7.96	77.96	-	Sorbitol (40)	(Ehivet et al., 2011)
Wheat (5)	7.0 7.6	60.7 47.5	140 187	Glycerol (35) Fructose (35)	(Mohammed et al., 2023)

2.6. Limitations of starch-based bioplastics

Starch-based bioplastics, while promising as biodegradable alternatives to conventional plastics, face several limitations that hinder their broader application. One of the primary challenges is their high water sensitivity, which significantly affects their mechanical properties and performance in humid environments. Starch-based bioplastics tend to absorb moisture, leading to a reduction in tensile strength and overall structural integrity (Azevedo et al., 2020). This hydrophilicity can result in swelling and loss of mechanical properties, making them less suitable for applications where moisture exposure is likely (Oluwasina et al., 2018). To improve these properties, several strategies have been developed to enhance the performance of starch-based bioplastics, including the chemical modification of starch, the addition of crosslinking agents to improve the compatibility of different polymers, addition of reinforcing agents, and blending with nanoparticles and other biodegradable polymers as reinforcing agents (Kadir et al., 2023; Suri & Singh, 2023; Chen et al., 2021; Awolu et al., 2020; Zakaria et al., 2017; John & Anandjiwala, 2008)

2.7. Natural fibre-reinforced bio-composites

Native starch bioplastics often exhibit poor mechanical properties and high water sensitivity, limiting their practical applications (Oluwasina et al., 2021). To address this limitation, the incorporation of natural fibres as reinforcements has garnered significant attention in recent years. The incorporation of lignocellulosic fibres into starch matrices can significantly improve these properties, enhancing the overall performance of the bioplastic (Yang et al., 2019). Furthermore, bioplastics derived from starch reinforced with lignocellulosic fibres represent a promising avenue for developing sustainable materials. The properties and applications of these bioplastics are discussed in this section.

2.7.1. Lignocellulosic biomass sources

Lignocellulosic biomass is a significant renewable resource primarily composed of three main components: cellulose, hemicellulose, and lignin (Ranjan et al., 2024). These components are derived from various sources, including agricultural residues (such as straw and husks), forestry by-products (like wood chips and sawdust), and dedicated energy crops (Sid et al. 2021). The composition and structure of lignocellulosic biomass can differ widely depending on the source, which influences its properties and potential applications in bioproducts and biofuels (Haq et al., 2021; Huang et al., 2021).

2.7.2. Structure and properties of lignocellulosic fibres

2.7.2.1. Cellulose

Cellulose is the most abundant organic polymer on Earth, constituting about 40-50% of lignocellulosic biomass (Narancic et al., 2020). It is a linear polysaccharide composed of D-

glucose monomers connected by β-(1,4)-glycosidic linkages, forming crystalline microfibrils that provide structural strength to plant cell walls (Yan et al., 2019; Simmons et al., 2016). **Figure 2-3** illustrates the molecular structure of cellulose and the inter- and intra-molecular interactions involved. The crystalline structure of cellulose contributes to its high tensile strength and resistance to microbial degradation, making it a valuable material for various industrial applications, including textiles and composites (Farhat et al., 2016).

Yousuf, A., Pirozzi, D. and Sannino, F., 2020. Fundamentals of lignocellulosic biomass. In *Lignocellulosic biomass to liquid biofuels* (pp. 1-15). Academic Press.

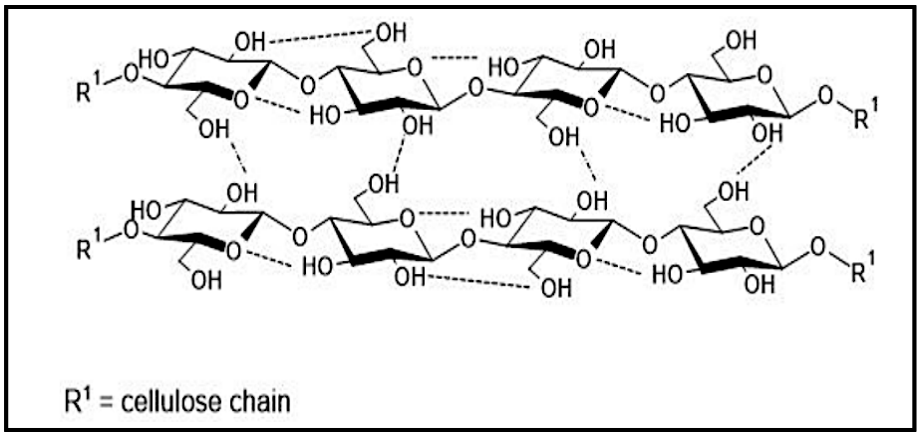

Figure 2-3: Structural composition of cellulose and chain interactions (Abe et al., 2021).

2.7.2.2. Hemicellulose

Hemicellulose is a heterogeneous group of polysaccharides that includes xylans, mannans, and galactans, and typically represents 20-35% of lignocellulosic biomass (Pan et al., 2022; Sun et al., 2021). Hemicellulose has a branched structure, which allows it to interact with cellulose and lignin, thus greatly impacting the mechanical properties of plant cell walls (Berglund et al., 2020; Martínez-Abad et al., 2017). The presence of hemicellulose can enhance the ductility and flexibility of cellulose-based materials, making it an important component in the development of biodegradable films and composites (Pereira et al., 2017; Bahcegul et al., 2014). The composition of hemicellulose varies depending on its source. For example, xylan (**Figure 2-4**) is the dominant form in agricultural materials such as straw and grasses, whereas glucomannan (**Figure 2-5**) is the main hemicellulose component in softwoods (Piazza et al., 2024).

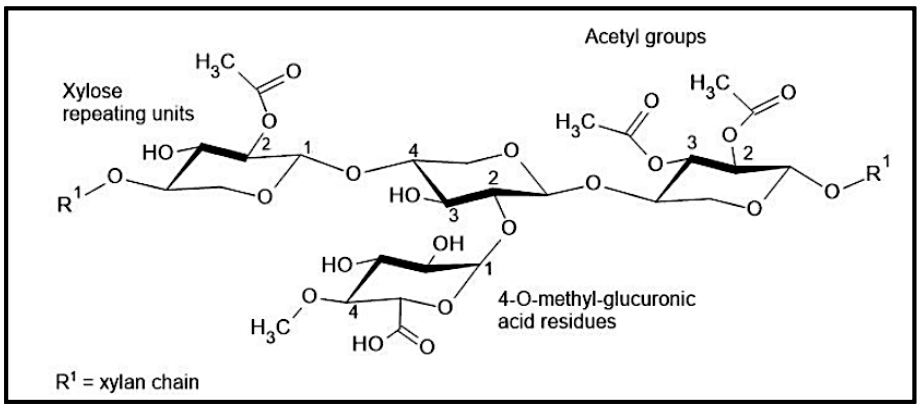

Figure 2-4: Structural composition of arabinoglucuronoxylans (Abe et al., 2021).

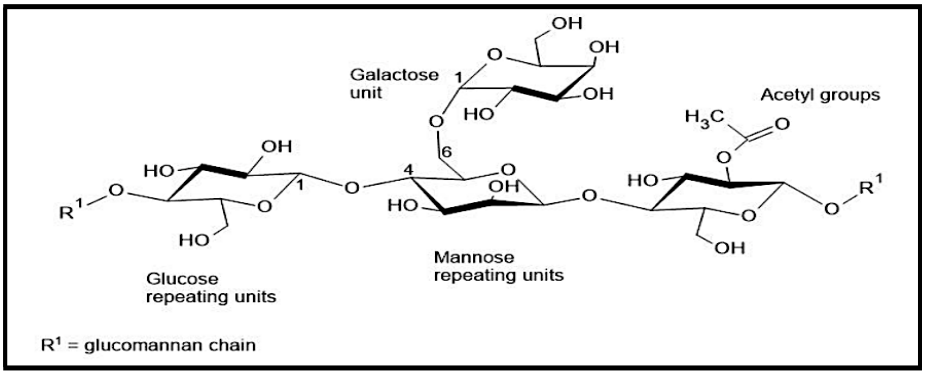

Figure 2-5: Structural composition of galactoglucomannans (Abe et al., 2021).

2.7.2.3. Lignin

Lignin, constituting about 15-30% of lignocellulosic biomass, is a complex, cross-linked phenolic polymer consisting of three phenylpropane monomeric units, including sinapyl alcohol, p-hydroxyphenyl alcohol, and coniferyl alcohol (**Figure 2-6**), that provides rigidity and hydrophobicity to plant structures (Huang et al., 2021; Yang et al., 2011). Its intricate structure contributes to the recalcitrance of lignocellulosic biomass, making it resistant to enzymatic degradation and thus complicating the conversion processes for biofuels and bioproducts (Zhang et al., 2019). Lignin's unique properties, including its ability to absorb UV light and its antioxidant capacity, make it a potential candidate for various applications, including as a natural preservative and in the production of bio-based materials (Shao et al., 2020; Yan et al., 2019).

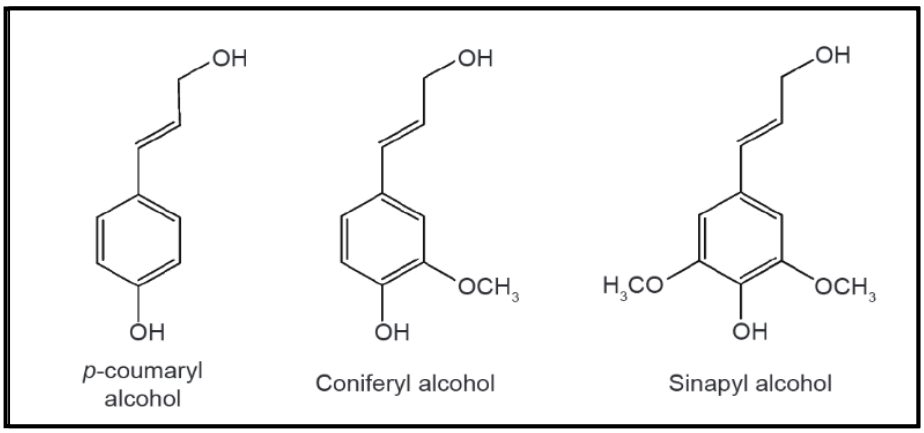

Figure 2-6: Basic structures of lignin monomers (Achinas et al., 2017).

The lignocellulosic fibre (cellulose, hemicellulose, and lignin) contents of different botanical sources are shown in **Table 2-5**, with canola fines used as a reinforcement fibre in this study.

Table 2-5: Lignocellulosic fibre contents of different botanical sources.

Botanical source	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	Reference
Bagasse	55.2	16.8	25.3	(John & Anandjiwala, 2008)
Canola fines	21.4	15.9	23.0	(Ranjan et al., 2024)
Canola straw	22.4	16.9	19.6	(Adapa et al., 2011)
Maize stalk	10.8	60.3	1.97	(Ibrahim et al., 2020)
Rice rusk	35.0-45.0	19.0-25.0	20.0	(Mohammed et al., 2015)
Wheat straw	38.0-45.0	15.0-31.0	12.0-20.0	(Mohammed et al., 2015)

The interactions among cellulose, hemicellulose, and lignin are essential for the overall properties of lignocellulosic fibres. Hemicellulose acts as a compatibiliser between the hydrophilic cellulose and the hydrophobic lignin, reducing interfacial tension and enhancing adhesion within the cell wall matrix (Busse-Wicher et al., 2014). This synergy not only contributes to the mechanical integrity of plant tissues but also influences the processing and performance of lignocellulosic materials in industrial applications (Martínez-Abad et al., 2017; Farhat et al., 2016; Peng et al., 2011). Understanding the structural and compositional nuances

of these components is essential for optimising the utilisation of lignocellulosic biomass in sustainable materials and energy production.

2.7.3. Canola production in South Africa

Canola is a crop derived from rapeseed (*Brassica napus* and *Brassica rapa*) that has been genetically modified to have low levels of erucic acid and glucosinolates, making it a healthier and more versatile oilseed (Kandel et al., 2011; Dupont et al., 1989). Canola production in South Africa has seen a notable increase in recent years, driven by a combination of favourable agronomic practices and market demand for canola oil. According to the Bureau for Food and Agricultural Policy (BFAP), canola production is projected to reach approximately 200 000 tonnes by 2027, indicating a growing interest in this oilseed crop within the South African agricultural landscape (Becker et al., 2020). The crop is particularly valued for its oil, which is often blended with other oils such as soybean and sunflower, making it a staple in the food industry (Seetseng et al., 2023).

2.7.4. Canola fines as reinforcement

Canola seed processing by-products can serve as viable raw materials for the synthesis of high-value products. Canola fines are lignocellulosic biomass residues, comprising straw-like fragments and other plant-derived debris, separated from canola seeds during the primary and secondary sorting stages of processing (Ranjan et al., 2024). The potential of canola fines waste as a reinforcement for starch-based bioplastics in South Africa is an area of significant interest, particularly in the context of advancing sustainable materials. Owing to their high lignocellulosic fibre content, canola fines are well-suited for improving the mechanical performance of bioplastics. The incorporation of such agricultural waste into bioplastic formulations not only addresses waste management issues but also contributes to the development of environmentally friendly materials.

Starch-based bioplastics are recognized for their biodegradability and renewability, which are key attributes in mitigating the environmental impact of conventional plastics (Ratnawati, 2023). The use of canola fines as a reinforcing agent can potentially improve the tensile strength and flexibility of starch bioplastics, as observed in various studies where natural fibres and agricultural by-products were utilised (Anwajler, 2023; Nasir & Othman, 2020). For instance, the addition of natural fillers has been shown to enhance the mechanical properties of bioplastics, making them more competitive with conventional petroleum-based plastics (Lackner, 2023; Bucio-Galindo et al., 2023).

Research indicates that the mechanical performance of bioplastics can be significantly improved by incorporating various natural fillers, including those derived from agricultural

waste (Bartolucci et al., 2023; Patel et al., 2022). The fibrous nature of canola fines could provide a reinforcing effect, enhancing the structural integrity of the bioplastic matrix. This is particularly relevant in applications where mechanical strength is essential, such as packaging materials (Wahyuningtiyas & Suryanto, 2017; Tan et al., 2016). Furthermore, the use of Canola fines aligns with the principles of a circular economy by valorising agricultural waste, thus reducing the overall environmental footprint of bioplastic production (Amin et al., 2019).

Moreover, the biodegradability of starch-based bioplastics can be further enhanced by the addition of canola fines, as they can facilitate microbial activity during the degradation process (Wahyuningtiyas & Suryanto, 2017; Tan et al., 2016). This characteristic is essential in addressing the growing concerns regarding plastic waste accumulation in the environment. The integration of canola fines into bioplastics could lead to materials that not only perform well but also decompose more readily, thus contributing to sustainable waste management practices (Bucio-Galindo et al., 2023; Amin et al., 2019).

Therefore, the utilisation of canola fines waste as a reinforcement for starch-based bioplastics presents a promising opportunity for developing sustainable materials in South Africa. Notably, this study represents the first known application of canola fines as a reinforcing agent in starch-based bioplastics, introducing a novel use for an underutilised agricultural by-product. By leveraging agricultural by-products, it is possible to enhance the mechanical properties and biodegradability of bioplastics, thereby addressing both environmental and economic challenges associated with plastic waste. Continued research in this area could lead to innovative solutions that benefit both the agricultural and materials science sectors.

2.7.5. Properties of starch-based bioplastics reinforced with lignocellulosic fibres

Research has shown that the interaction between starch and lignocellulosic fibres can lead to improved tensile strength, elasticity, and thermal stability of the bioplastics. For instance, the formation of H bonds between the OH^- groups of starch and the fibres contributes to a more cohesive material (Yang et al., 2019). Additionally, the presence of lignin can further enhance the mechanical properties and provide additional hydrophobicity, reducing H_2O absorption and improving the durability of the bioplastic (Rizal et al., 2022).

2.7.5.1. Preparation and composition

Starch bioplastics are typically produced through a plasticisation process that involves heating starch in the presence of H_2O, leading to gelatinisation. This process allows the starch granules to swell and dissolve, forming a viscous solution that can be moulded into films or other shapes (Yang et al., 2020). The incorporation of lignocellulosic fibres, such as those

derived from agricultural residues, enhances the mechanical properties of the resulting bioplastics. These fibres provide additional strength and stiffness due to their high tensile strength and modulus (Waluyo, 2024). The interaction between starch and lignocellulosic fibres is facilitated by H bonding, which contributes to the formation of robust composite structures (Agustin et al., 2014).

2.7.5.2. Mechanical properties

The mechanical performance of starch-based bioplastics can be significantly improved through the addition of lignocellulosic fibres. Studies have shown that the reinforcement of starch with cellulose nanocrystals or other lignocellulosic materials results in a more rigid and durable bioplastic (Agustin et al., 2014). For instance, the compatibility of cellulose with starch enhances the overall structural integrity of the bioplastic, allowing it to withstand external tensile forces better (Waluyo, 2024). However, the dispersion of fibres within the starch matrix is important; poor dispersion can lead to fibre aggregation, resulting in a heterogeneous phase that negatively impacts mechanical properties (Yang et al., 2019).

2.7.5.3. Hydrophobicity and water absorption

One of the challenges associated with starch-based bioplastics is their inherent hydrophilicity, which can lead to high water absorption and reduced durability (Xu, 2024). The incorporation of lignocellulosic fibres can improve the hydrophobicity of the bioplastic, thereby reduce water uptake and enhance its resistance to moisture (Abe et al., 2021). This is particularly important for applications in packaging and other environments where exposure to moisture is a concern. The use of surface treatments on lignocellulosic fibres can further enhance their compatibility with starch, leading to improved mechanical properties and reduced water sensitivity (Asyraf et al., 2022). **Table 2-6** summarises previous research on starch bioplastics reinforced with lignocellulosic fibres, highlighting the types of starch, the botanical sources of the fibres, the quality and quantity of the plasticisers, and their effects on mechanical properties and water absorption.

Table 2-6: Mechanical properties and water absorption results of starch-based bioplastic films reinforced with lignocellulosic fibre.

Starch Source (%, w/v)	Tensile Strength (MPa)	Elongation at Break (%)	Water Absorption (%)	Lignocellulosic fibre (%, w/w dry starch)	Plasticiser (%, w/w dry starch)	Reference
Arrowroot (5)	4.77-15.2	6.21-46.6	100-172	Arrowroot fibre (10)	Glycerol (30)	(Tarique et al., 2022)
Cassava (3)	10.6	18.9	-	Cassava fibrous filler (3)	Glycerol (25)	(Versino & García, 2014)
Maize (5)	12.8	2.5 mm (3.57%)*	96.4	Maize husk (8)	Fructose (25)	(Ibrahim et al., 2019)
Maize (5)	11.9	0.9 mm (1.30%)*	85	Maize husk (8)	Fructose (25)	(Ibrahim et al., 2020)
Maize (5)	6.56 7.29	3.5 5.2	-	Banana pseudo-stem fibre (10) Bacterial cellulose (10)	Glycerol (25)	(Hermansyah et al., 2014)
Maize (5)	19.1	1 mm (1.43%)*	90.4	Maize husk (8); sugar palm (6)	Fructose (25)	(Ibrahim et al., 2020)

**% calculated from data provided in manuscripts*

2.7.6. Challenges and optimisation strategies

2.7.6.1. Fibre dispersion

The effective dispersion of lignocellulosic fibres within the starch matrix is important for achieving optimal mechanical properties. Poor dispersion can lead to fibre aggregation, resulting in a heterogeneous phase that negatively affects the mechanical performance of the bio-composite (Agustin et al., 2014). Techniques such as surface modification of fibres, such as alkali treatment, can improve fibre-matrix adhesion and dispersion, enhancing the overall properties of the bio-composite (Edhirej et al., 2018; Sanyang et al., 2016).

2.7.6.2. Fibre content

The concentration of lignocellulosic fibre is important in bioplastic formulation. While increasing fibre content can enhance mechanical properties, excessive amounts may lead to brittleness and reduced flexibility (El Mogy et al., 2024; Sanyang et al., 2017). Therefore, it is essential to identify an optimal fibre loading that maximises strength without compromising the material's ductility.

2.7.6.3. Chemical modifications

Chemical treatments can improve the compatibility of lignocellulosic fibres with the starch matrix. For example, acetylation or silane coupling can enhance interfacial bonding, leading to improved mechanical performance (Yang et al., 2019; Sulaeman, 2023). Additionally, the use of plasticisers like glycerol can facilitate better interaction between the fibres and the starch, further enhancing the mechanical properties of the bio-composite (Jannah et al., 2021).

2.7.6.4. Blending with other polymers

Combining starch with other biodegradable polymers, such as polyvinyl alcohol (PVA) or polyhydroxyalkanoates (PHAs), alongside lignocellulosic fibres can improve the overall properties of the bio-composite. This approach can enhance water resistance and mechanical strength, making the bioplastics more versatile for various applications (Sanyang et al., 2016; Sanyang et al., 2017).

The addition of lignocellulosic fibres as reinforcements in starch-based bioplastics offers significant benefits, including improved mechanical properties, biodegradability, and cost-effectiveness. By addressing challenges related to fibre dispersion, content optimisation, and chemical modifications, the performance of starch-based bioplastics can be significantly enhanced, paving the way for their broader application in sustainable materials. In this study, the fibre loading was varied in order optimise the performance of the resultant bioplastic films in a similar manner to previous studies (**Table 2-6**).

2.8. Applications of starch-based bioplastics

2.8.1. Packaging industry

Starch-based bioplastics find significant application in the packaging sector. According to the European Association of Plastics Recycling and Recovery Organizations (EPRO), plastics comprised about 40.5% of packaging materials in the six major European nations in 2020. By 2021, bioplastics made up roughly 48% of packaging materials, equating to approximately 1.15 million tonnes of global bioplastic production. Bags, films, and wraps are common uses of bioplastics in packaging (Kong et al., 2023). According to Jariyasakoolroj et al. (2020), non-biodegradable bioplastics are utilised in beverage packaging. For example, Coca-Cola developed PlantBottle®, consisting of 30% Bio-PET derived from bio-based ethylene glycol and PepsiCo used switchgrass, corn husks, and pine bark to create 100% Bio-PET for their product containers.

Bioplastics typically exhibit poor mechanical and permeability properties that limit their applicability in packaging, but these limitations can be mitigated by incorporating reinforcing materials (Kakadellis & Harris, 2020). For instance, the synthesis of nanocomposites using materials such as carbon fibre microelectrodes, titanium dioxide (TiO_2), and nanoclay has further enhanced the mechanical and barrier properties of starch-based bioplastics, making them suitable for packaging (Marichelvam et al., 2019).

In food packaging, safety and environmental considerations lead to the increased use of bio-based materials for reinforcing bioplastics as opposed to fossil-based materials. Food packaging necessitates low permeability to block moisture and gas, which could otherwise lead to food spoilage (Hong et al., 2021). Incorporating antibacterial and antioxidant additives to starch-based food packaging offers additional benefits, such as extending food product shelf life (Falua et al., 2022). Reinforced bioplastics are used in food packaging for items like dairy product bottles, containers, films, dishes, and takeaway bags (Hong et al., 2021). These films can extend the shelf life of food goods by acting as moisture and gas barriers (Usha, 2023; Azevedo et al., 2020).

2.8.2. Agriculture

In agriculture, starch bioplastics are utilised for biodegradable mulch films and seed coatings. These applications help reduce plastic waste in farming practices while improving soil quality. The biodegradable nature of these films allows them to decompose in the soil, enriching it without leaving harmful residues (Hidayat et al., 2023). Biodegradable plastic mulches (BDMs) are a notable application, made from materials like PLA, PHA, starch, and cellulose. After use, these BDMs are incorporated into the soil through ploughing, where microorganisms break them down, improving soil quality by enhancing microbial activity (Bandopadhyay et al., 2018). PHA has also been utilised as carriers for insecticides, as components of crop-protection films, as controlled-release systems for fertilisers, and as encapsulating materials for seeds (George et al., 2021).

Research investigating bioplastics reinforced with empty fruit bunch fibres have shown that incorporating these fibres produces high-quality BDMs with enhanced mechanical strength, while also supporting plant adaptation to climate conditions (Ayu et al., 2020; Iriany et al., 2018). Additionally, lignocellulosic fibre-reinforced bioplastics have been found to accelerate the degradation rate of the polymeric matrix in soil, making them more suitable for plant nursery bags or pots in agriculture (Cinelli et al., 2019; Díez-Pascual, 2019). Kong et al. (2023) states that using natural resources to reinforce bioplastics for plant nurseries could provide similar benefits to BDMs, increasing soil quality as the materials decompose but further

research is needed to fully understand the elements and gases emitted during decomposition to avoid potential negative impacts on agricultural crops.

2.8.3. Medical and pharmaceutical industry

Starch-based bioplastics are being explored for drug delivery systems due to their biocompatibility and biodegradability. These materials can be engineered to release drugs in a controlled manner, minimising side effects and improving therapeutic efficacy (Abral et al., 2019). The ability to modify the mechanical properties of starch bioplastics through the incorporation of various additives allows for tailored drug delivery applications (Usha, 2023).

Plastics are widely used in the healthcare sector, particularly for medical devices such as intravenous (IV) tubes, surgical gloves, blood bags, syringes, and for packaging medical instruments to maintain hygiene. This is due to their superior properties (Kong et al., 2023). Recently, biodegradable bioplastics have found applications in healthcare, including controlled drug delivery systems and implantable therapeutic devices (Narancic et al., 2020).

Medical instruments must undergo sterilisation processes including high-temperature steam, ethylene oxide (EtO), and gamma irradiation and may encounter various chemicals or bodily fluids. These conditions often accelerate biodegradation rates and reduce polymer molecular weight (Pérez Davila et al., 2021). Consequently, materials used in such applications should resist different chemicals and sterilisation methods while maintaining instrument functionality and safety (Sastri, 2014).

Biodegradable bioplastics like poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), and poly (ε-caprolactone) (PCL) are widely used in medical applications as they can be broken down by fungi and bacteria within the human body (Bano & Pandey, 2018). Additionally, research by Yang et. al. (2019), indicates that bioplastics have antimicrobial qualities when reinforced with materials like lignocellulosic fibre and lignin.

2.8.4. Textile industry

The textile industry is beginning to adopt starch-based bioplastics for biodegradable fibres and non-woven fabrics. These materials align with the increasing need for environmentally friendly and sustainable textiles (Gökçe, 2018). Blending starch with other biodegradable polymers can enhance the mechanical properties of the resulting fabrics, making them suitable for a range of textile applications (Jayarathna et al., 2022).

2.9. References

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Chapter 3: Extraction and characterisation of starch from maize (*Zea mays*) agro-industrial wastes

3.1. Introduction

The rising global demand for bioplastics as alternatives to conventional plastics, driven by environmental concerns and the need to reduce reliance on fossil fuels, has heightened the need for sustainable, non-food raw materials to meet both environmental and social needs. Traditionally, starch is considered an essential biopolymer in bioplastic production and is sourced from staple crops such as maize (*Zea mays*). However, this reliance creates competition with food supplies, exacerbating food insecurity and driving up prices in vulnerable regions (Chakraborty et al., 2022). Moreover, diverting agricultural land for bioplastic feedstock production contributes to deforestation, biodiversity loss, and unsustainable land use changes, further aggravating environmental challenges (Amin et al., 2019; Marichelvam et al., 2019). In response to these concerns, agro-industrial wastes present a viable alternative source of starch, offering opportunities to reduce environmental footprints by utilising by-products that do not require additional land, water, or fertilisers (Zhang et al., 2022; Wahyuningtiyas & Suryanto, 2018).

The valorisation of starch-rich agro-industrial residues aligns closely with global sustainability and circular economy goals by addressing plastic pollution, reducing reliance on fossil fuels, and promoting efficient waste management (Navasingh et al., 2023; Sidek et al., 2019). By decoupling bioplastic production from food supply chains through the use of agricultural by-products, food processing waste, or non-food crops, this approach not only safeguards food security but also minimises waste generation while contributing to resource reuse and repurposing (Oluwasina et al., 2021; de Azevedo et al., 2020). Several studies have demonstrated the potential of various agro-industrial wastes, such as tapioca, rice, lotus rhizomes, maize, mango, and jackfruit residues, for starch extraction and subsequent bioplastic synthesis, highlighting the broad applicability of this strategy (Rendón-Villalobos et al., 2022; Ibrahim et al., 2019; Krishnamurthy & Amritkumar, 2019; Sukhija et al., 2019).

A critical determinant of starch suitability for bioplastics is its amylose-to-amylopectin ratio, which governs film properties such as tensile strength and flexibility. Higher amylose content contributes to improved film thickness, tensile strength and water resistance through enhanced intermolecular hydrogen bonding facilitated by its linear molecular structure, whereas higher amylopectin content contributes to improved flexibility (Maraveas, 2020; Patkar et al., 2020; Silva et al., 2023; Wang et al., 2017; Rindlav-Westling et al., 1998).

However, the starch yield and quality from agro-industrial wastes can be compromised by factors such as kernel composition variability, mechanical damage, and the presence of impurities including proteins, lipids, and fibre (Rausch & Eckhoff, 2015). These impurities inhibit starch granule swelling and gelatinisation, which impacts viscosity, solubility, and functional properties critical to bioplastic formation (Xiang et al., 2023; Malki et al., 2023). Consequently, optimising extraction techniques to efficiently remove such impurities while maximising starch recovery is essential for producing high-quality starch suited to industrial applications (Rashwan et al., 2024).

Globally, food loss and waste at farming and post-harvest stages account for approximately 20 to 30% of agricultural production, largely due to inadequate storage and transport infrastructures (Maraveas, 2020). In South Africa, maize production approaches 8 million tonnes annually, but post-harvest losses are estimated at nearly 14.9%, equivalent to approximately 350 215 tonnes per year (Adisa et al., 2019; APHILIS, 2014).

Maize itself exhibits genotypic variations in starch composition; normal dent maize, waxy maize, and high-amylose maize differ considerably in amylose and amylopectin content, which directly influences bioplastic properties (Serna-Saldivar, 2019). For instance, high-amylose maize contains 37 to 65% amylose, while waxy maize has approximately 5% amylose and 95% amylopectin. The wet milling facility supplying samples for this study processes normal dent maize, with amylose content ranging between 23 and 28% and amylopectin between 72 and 77% (Serna-Saldivar, 2019). Additionally, extraction parameters such as temperature and pH strongly affect amylose solubility and gelatinisation behaviour, altering both yield and starch quality (Palupi et al., 2024; Ogori & Taofeek, 2022; Shoukat et al., 2025; Alcázar-Alay & Meireles, 2015). Lower pH tends promote a more orderly granular structure within the starch, thereby reducing its solubility and resulting in higher yield and amylose content, whereas higher pH leads to enhanced protein solubility and increased starch content (Palupi et al., 2024; Ogori & Taofeek, 2022). Furthermore, higher temperatures facilitate better amylose leaching during extraction by promoting gelatinisation (Shoukat et al., 2025; Alcázar-Alay & Meireles, 2015).

Extraction methods encompassing mechanical, chemical, enzymatic, and physical techniques each present trade-offs between yield, purity, and functional properties of the recovered starch (Rashwan et al., 2024). The aim of this study was to investigate the potential of various maize waste streams from the wet milling process as alternative starch sources for bioplastic production. This involved detailed extraction and characterisation of their physicochemical and compositional properties, including starch yield, moisture content, pH, amylose and

amylopectin contents, total carbohydrate, protein and lipid contents, as well as swelling power and water solubility, to evaluate their suitability in bioplastic applications.

3.2. Materials and Methods

3.2.1. Raw materials

In the context of maize processing, substantial quantities of starch-rich by-products are generated at various stages of the wet-milling process (**Figure 3-1**), offering a valuable source of raw materials for bioplastic production. The main raw materials used in this study were starch-rich substrates namely, reject maize kernels (kaff-kaff) and maize starch spillage (**Figure 3-1**), collected from a local starch mill operating industry within the Western Cape to investigate their starch quality for bioplastic synthesis. The samples were stored in a cool, dry cupboard at 25^{o}C and used for experimentation. Spillage refers to the starch-rich waste material collected during the final stage of the wet-milling process, typically comprising residual starch that escapes recovery and is often discarded. In contrast, kaff-kaff is the by-product obtained at the initial stage of maize processing, where viable kernels are separated from irregular kernel cuttings and other debris. This fraction contains a mixture of broken kernels, and other debris. The starch extracted from kaff-kaff, referred to as the extract, is isolated through a series of purification steps to obtain a substrate suitable for bioplastic film formation. In this study, the physicochemical properties of these starch-rich substrates namely, spillage, kaff-kaff, and extract, will be discussed to evaluate the potential of these underutilised waste streams as sustainable feedstocks for bioplastic applications.

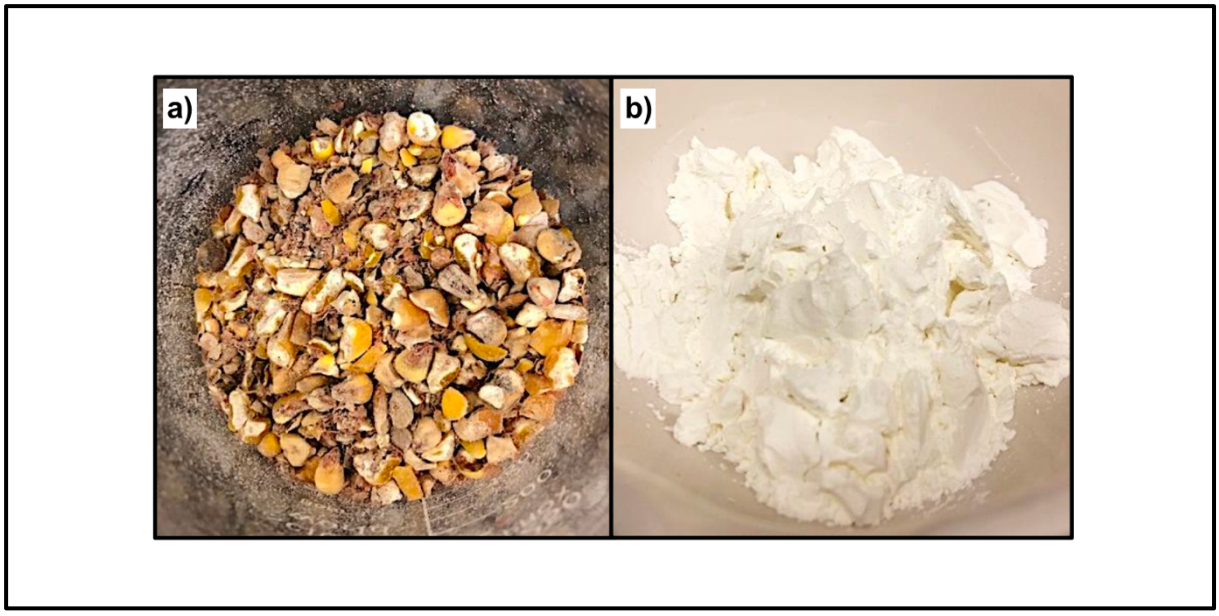

Figure 3-1: Samples of (a) reject maize kernels and (b) maize starch spillage.

3.2.2. Sample preparation

The extraction of starch from reject maize kernels (kaff-kaff) was carried out according to the extraction method described by Zoungranan et al. (2020) with some modifications. An outline of the extraction process is presented in **Figure 3-2**. A mass of 80 g of the substrate was placed in the top of sieve trays and left on a shaker for 30 minutes at 300 rpm. Following that, the dust and other debris was easily removed, and the maize grains hand-picked. The maize grains were then placed in a 1 000 mℓ beaker with distilled water (dH_2O) (100 g grains to 400 mℓ water) to steep for 48 hours at 20ºC. After steeping, the sedimented maize grains were removed and processed in a blender. The slurry formed was filtered through a 75 μm sieve placed above a 1 000 mℓ beaker, followed with continuous rinsing with dH_2O. The resultant sediment formed in the beaker was the raw starch. To remove fats and proteins, the starch was washed 3 times in 4% w/v sodium chloride (NaCl) (cat no: 71382, Merck, Darmstadt, Germany) decanting between each wash. A total volume of 300 mℓ solution was used. The starch was further purified by washing 4 times with 100 mℓ dH_2O. The purified starch was then dried in a drying oven until a constant weight was achieved. Finally, the dried starch was ground with a mortar and pestle and passed through a 75 μm sieve. The resulting starch extracted from the kaff-kaff substrate is hereafter referred to as the extract. Similarly, the spillage substrate was placed in an oven to dry, then ground with a mortar and pestle, and finally sifted through a 75 μm sieve.

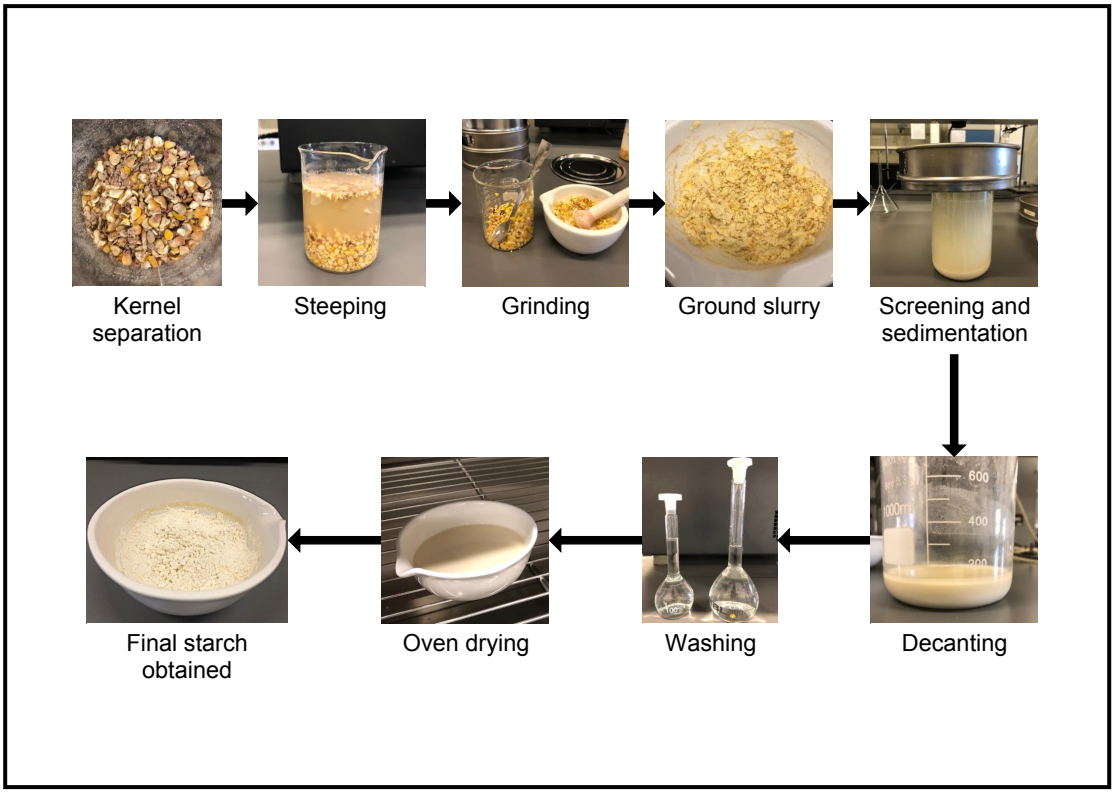

Figure 3-2: Starch extraction process.

3.2.3. Substrate characterisation

This section presents the starch characterisation and calculation of yield of the kaff-kaff, spillage, and extract. The evaluated properties provide insight into the extraction efficiency and assess their suitability for bioplastic film formulation and performance.

3.2.3.1. Starch yield

Starch yield (%) represents the amount of starch extracted from the kaff-kaff substrate relative to the initial dry weight of the sample. It was calculated according to the method described by Tessema and Admassu (2021) and Ji et al. (2004).

$$\textbf{Starch yield}\ (\%) = \frac{\textbf{Dry weight of extracted starch(g)}}{\textbf{Dry weight of sample(g)}} \times \mathbf{100} \qquad \text{(Equation 3-1)}$$

3.2.3.2. Total starch

Total starch analysis was performed at the Food and Allergy Consulting and Testing Services (FACTS) SA laboratories (Stellenbosch, South Africa) using a combination of the Association of Official Analytical Collaboration (AOAC) method 996.11 and the Association for Diagnostics and Laboratory Medicine (ADLM) method 76.13.

3.2.3.3. Carbohydrates

The carbohydrate content was determined using the anthrone method as described by (Hedge & Hofreiter, 1962) with slight modifications. To enable quantitative analysis of the unknown starch samples, a standard curve was first established. The blank consisted of dH_2O. The standard solutions were made from anhydrous D(+) glucose (cat no: G8270, Merck, Darmstadt, Germany) from a 1:1 working solution made from a stock solution of 100 mg of glucose made up to 100 mℓ with dH_2O in a volumetric flask. The preparation of the glucose standard solutions used for generating the standard curve is summarised in **Table 3-1.**

Table 3-1: Preparation of the glucose standard

Tube	Concentration (ug/mℓ)	Volume of working solution (mℓ)	Volume of distilled water (mℓ)
Blank	0	0.0	1
1	50	0.1	0.9
2	100	0.2	0.8
3	150	0.3	0.7
4	200	0.4	0.6
5	250	0.5	0.5
6	300	0.6	0.4
7	350	0.7	0.3
8	400	0.8	0.2
9	450	0.9	0.1
10	500	1	0.0

The anthrone reagent consisted of 200 mg of anthrone (cat no: 319899, Merck, Darmstadt, Germany) dissolved in 100 mℓ of ice-cold 95% sulphuric acid (H_2SO_4) (cat no: 258105, Merck, Darmstadt, Germany). Thereafter, 4 mℓ of the anthrone reagent was added to each test tube and placed in a boiling water bath for 8 minutes. The test tubes were cooled rapidly after heating, and the absorbance was determined at 630 nm using a Rayleigh (Beijing, China) UV-900 spectrophotometer. Subsequently, the absorbance results were plotted against the glucose concentrations to create the glucose standard curve.

The samples were prepared using acid hydrolysis to break down complex carbohydrates into simple sugars: 100 mg of each sample was added to 5 mℓ of 2.5 N hydrochloric acid (HCl) in test tubes and hydrolysed in a boiling water bath for 3 hours. Once cooled to room temperature, the samples were centrifuged at 6 000 rpm for 10 minutes. Thereafter 100 μL of the supernatant of each sample was pipetted into new test tubes and made up to 1 mℓ with dH_2O. This dilution was repeated once more to achieve a dilution factor of 100. Thereafter,

4 mℓ of anthrone reagent was added to each test tube which were then placed in a boiling water bath for 8 minutes. After cooling, the absorbance was read at 630 nm using a using a Rayleigh (Beijing, China) UV-900 spectrophotometer. The carbohydrate content was determined from the straight-line equation generated from the glucose standard curve and adjusted by the dilution factor.

3.2.3.4. Amylose/amylopectin

The amylose contents of all substrates and extracts were determined by a colorimetric iodine method similar to that described by Tessema and Admassu (2021). This process began with the construction of a standard curve using known concentrations of an amylose standard (cat no: A0512, Merck, Darmstadt, Germany). The resulting standard graph was then used to determine the percentage of amylose in the substrate samples. Subsequently, the percentage of amylopectin was estimated by difference, subtracting the amylose content from 100%. The procedure is described as follows:

(i) Preparation of the standard amylose curve

A standard stock solution of amylose was prepared by adding 40 mg of a potato amylose standard (cat no: A0512, Merck, Darmstadt, Germany) to 1 mℓ of 95% ethanol and 9 mℓ of 1 N sodium hydroxide (NaOH) in a 100 mℓ volumetric flask. The flask was covered with foil, the contents mixed thoroughly and placed in a boiling water bath for 10 minutes to allow the starch to gelatinise. After cooling to room temperature, the solution was made up to 100 mℓ with dH_2O and mixed thoroughly. Different volumes of the stock standard solution were pipetted into five separate 100 mℓ volumetric flasks according to **Table 3-2** to prepare standard amylose solutions of 4%, 8%, 12%, 16% and 20%. To each of the five volumetric flasks (as outlined in **Table 3-2**), 1 N acetic acid and an iodine solution were added. The iodine solution was prepared by dissolving 0.2 g iodine (I_2) and 2.0 g potassium iodide (KI) in 100 mℓ of dH_2O. Each solution was then diluted to the 100 mℓ mark with distilled water and mixed thoroughly. A blank solution was prepared simultaneously with 1 mℓ of 95% ethanol and 9 mℓ of 1 N NaOH in a 100 mℓ volumetric flask, placed in a boiling water bath for 10 minutes, and made up to the 10 mℓ mark with distilled water. Finally, 5 mℓ of the blank solution was pipetted into a new 100 mℓ volumetric flask with 1 mℓ of 1 N acetic acid and 2 mℓ of 0.2% iodine solution, diluted to the 100 mℓ mark with dH_2O, and mixed thoroughly. The absorbance of the blank and amylose standard solutions were read at 620 nm using a Rayleigh (Beijing, China) UV-900 spectrophotometer. The blank was used to standardize the spectrophotometer by deducting its absorbance from the absorbance of the amylose standard solutions. Consequently, a standard amylose curve was constructed based on the absorbance results to give a straight line equation of the relationship between the absorbance and concentrations of the amylose standard solutions.

Table 3-2: Reaction mixtures for standard amylose curve

Acetic acid (mℓ)	Iodine solution (mℓ)	Standard solution (mℓ)	Water to adjust volume (mℓ)	Amylose Equivalent (%)
0.2	2	1	100	8
0.4	2	2	100	16
0.6	2	3	100	24
0.8	2	4	100	32
1	2	5	100	40

(ii) Determination of amylose and amylopectin content in starch substrates

Subsequently, 100 mg of each substrate sample was added to a 100 mℓ volumetric flask with 1 mℓ of 95% ethanol and 9 mℓ of 1 M NaOH solution. The contents were mixed thoroughly and placed in a boiling water bath for 10 minutes to allow the starch to gelatinise. After cooling to room temperature, the solutions were made up to 100 mℓ with distilled water and mixed thoroughly. In a new set of 100 mℓ volumetric flasks, 5 mℓ of each cooked starch solution, 1 mℓ of 1 M acetic acid, and 2 mℓ of 0.2% iodine solution were added, then made up to 100 mℓ mark with dH_2O. The final solutions were mixed thoroughly, and the absorbance of each substrate sample was read at 620 nm using a JASCO (Tokyo, Japan) spectrophotometer.

The percentage of amylose content in each starch substrate was determined using a standard amylose calibration curve. The amylopectin content (%) was subsequently estimated by difference, based on the following equation:

$$\textbf{Amylopectin content}\ (\%) = \mathbf{100} - \textbf{Amylose content}\ (\%) \qquad \text{(Equation 3-2)}$$

This analysis was performed in triplicates, and the mean values were taken.

3.2.3.5. Protein

The carbon, hydrogen, nitrogen and sulphur (CHNS) contents in the substrates were quantified at the Central Analytical Facilities (CAF) of Stellenbosch University using an Elementar (Langenselbold, Germany) Vario EL Cube Elemental Analyser. The protein content was determined by multiplying the measured N content by the appropriate correction factor which is 6.25 for maize (Iheanacho et al., 2024; Krotz et al., 2019; Müller, 2017; VELP, 2017).

3.2.3.6. Oil and grease (lipids) and pH

Oil and grease (lipids) and pH analyses were performed at A.L. Abbott & Associates (Cape Town, South Africa) using the South African Bureau of Standards (SABS) 1051 (Nov.1982) and SABS 11 methods, respectively.

3.2.3.7. Moisture content

The determination of moisture content was determined by weighing a sample of each substrate and extract, placing into crucibles and drying in an oven at 50°C for 24 hours or until constant weights were achieved. The moisture content (%) of the substrates and extracts was determined according to the method described by Shafqat et al. (2021) using the following equation:

$$\textbf{Moisture content}\ (\%) = \frac{\mathbf{W_{initial} - W_{final}}}{\mathbf{W_{initial}}} \times \mathbf{100} \qquad \text{(Equation 3-3)}$$

Where $W_{initial}$ represents the initial weight of the sample prior to drying, and W_{final} represents the weight of the sample following the drying process. Moisture content of each substrate and extract was performed in triplicate and average values were taken.

3.2.3.8. Swelling power and water solubility

The methodology employed to measure the swelling power and water solubility of the substrates as a function of temperature was adapted from Silva et al. (2020). In centrifuge tubes, aqueous starch suspensions (2% w/w) of each substrate were homogenized with a vortex mixer and heated at different temperatures of 50°C, 60°C, 70°C, 80°C, and 90°C for 30 minutes using a water bath. Following heating, the suspensions were cooled and centrifuged at 6000 rpm for 15 minutes. The supernatant was decanted into previously weighed crucibles and dried in an oven at 100°C until a constant mass was achieved. This mass of the dried supernatant was used to calculate the water solubility (%). The sedimented swollen granules remaining in the decanted centrifuge tubes were weighed and used to determine the swelling power (g water/g dry sample). Water solubility and swelling power were calculated following the method described by Tessema and Admassu (2021) using the equations below:

$$\textbf{Water solubility}\ (\%) = \frac{\textbf{Weight of dried supernatant}}{\textbf{Dry weight of original starch}} \times \mathbf{100} \qquad \text{(Equation 3-4)}$$

$$\textbf{Swelling power}\ (\mathbf{g/g}) = \frac{\textbf{Weight of swollen granules}}{\textbf{Dry weight of original starch}} \qquad \text{(Equation 3-5)}$$

3.3. Results and discussion

A summary of the physicochemical and compositional properties of the substrates (spillage and kaff-kaff) and extract examined in this study is presented in **Table 3-3.**

3.3.1. Starch yield

The average starch yield achieved from the kaff-kaff waste in this study was 45.4% (**Table 3-3**), which aligns with laboratory-scale extractions reported by Paraginski et al. (2014), ranging from 46.0% to 66.9% for freshly harvested maize starches, and Ji et al. (2004), who reported yields between 45.0% and 63.8% for maize starch extractions. These results indicate that the extraction yield of the reject kernels achieved in this study falls within a reasonable range for laboratory-scale extraction, especially given the inferior quality of the waste feedstock. In comparison, typical starch yields in the maize wet-milling industry for normal dent maize range between 60% and 72% (Serna-Saldivar, 2019).

Several factors may account for the lower starch yield observed in this study. Experimental losses, such as starch loss during the steeping stage of extraction, and the poor quality of the waste material likely contributed. Rausch and Eckhoff (2015) note that changes in the kernel compositional characteristics and mechanical damage are some of the factors that can affect starch yield. Fox et al. (1992) explains that higher protein content in maize kernels makes it difficult to achieve clean separation of protein and starch, leading to greater starch retention within the protein matrix and reduced starch yield. Additionally, Rausch and Eckhoff (2015) report that physical damage to kernels increases exposed internal surfaces, which accelerates both water uptake and the leaching of soluble solids, including starch and protein, into the steepwater. Supporting this mechanism, Wang & Eckhoff (2000) found a linear relationship between the percentage of broken kernels and increased solids and protein content in the steepwater ($r^2 = 0.98$ and $r^2 = 0.96$, respectively), further reducing final starch yield. Unlike whole kernels, which have predictable ratios of starch, protein, fibre, and fat, industrial waste material is highly variable in composition, contributing further to the reduced yield observed in this study.

3.3.2. Total starch

Spillage exhibited the highest starch content at 88.8%, a value comparable to that of commercial maize starch (Maizena), which typically contains around 89% total starch (**Table 3-3**). This was followed by the extract with 78.2%, while kaff-kaff showed a lower content at 57.7% (**Table 3-3**). The extract exhibited a higher starch content compared to the original kaff-kaff substrate, primarily due to the extraction and purification processes employed. While kaff-kaff, as a raw material, contains a mixture of starch, broken kernels, fibres, and other impurities, the extraction procedure selectively isolates starch granules, effectively removing

non-starch components such as proteins and fibres. This selective separation enhances the quality of the starch in the extract. The efficiency of the extraction method, including steps like washing, sedimentation, and drying, plays a crucial role in maximising starch recovery and quality. Therefore, despite the lower initial starch content of the starting material, the targeted extraction process yields an extract with enhanced starch quality. These results directly impact their suitability for starch-based bioplastic film synthesis, as starch content is the main constituent and correlates with film integrity and mechanical properties (Maraveas, 2020).

The high starch purity of the spillage, closely aligning with commercial standards, suggests that less extensive purification steps are required, thereby reducing processing costs and environmental footprint. This makes spillage a highly promising substrate for bioplastic production, as it can yield a large quantity of primary polymer needed for film formation.

3.3.3. Carbohydrates

The average carbohydrate content of these substrates varied, with spillage (92.5%) demonstrating the highest carbohydrate content, followed by extract (79.6%) and kaff-kaff (68.7%) (**Table 3-3**). These values represent all organic compounds present in each substrate including sugars, starch, and fibres (Ogori & Taofeek, 2022; Hedge & Hofreiter, 1962).

The small difference between the carbohydrate content (92.5%) and starch content (88.8%) in the spillage substrate suggests a high degree of starch purity within its organic matter. Since the total carbohydrates measure all organic polysaccharides, comprising starch, sugars, and fibres, a minimal gap between these values indicates that starch constitutes the overwhelming majority of the carbohydrate fraction. In other words, non-starch polysaccharides such as soluble sugars and dietary fibres are present only in minor amounts relative to starch. Thus, the small discrepancy between total carbohydrates and starch content further supports the potential of the spillage substrate as a high-quality starch source suitable for valorisation in bioplastic synthesis.

Conversely, the larger discrepancy between the carbohydrate content (68.7%) (**Table 3-3**) and starch content (57.7%) (**Table 3-3**) observed in the kaff-kaff substrate indicates a significant proportion of non-starch carbohydrates such as dietary fibres, hemicelluloses, and complex polysaccharides (Sangwan et al., 2014). Unlike starch, which is a glucose polymer primarily used as a bioplastic precursor, these other carbohydrates, common in fibrous maize residues and related agro-industrial wastes, do not contribute effectively to film formation.

The extract sample, with a carbohydrate content of 79.6% (**Table 3-3**) and a starch content of 78.2% (**Table 3-3**), shows a relatively small difference (1.4%) between its total carbohydrate and starch content. This indicates that, similar to spillage, most of the carbohydrates present

in the extract is starch. The non-starch carbohydrate fraction in the extract is minimal, suggesting a high level of starch purity within this specific stream of the maize wet milling process. This high purity suggests that the extract could also be efficiently utilised as a high-quality starch suitable as a raw material for bioplastic production.

3.3.4. Amylose and amylopectin

The amylose content was found to be positively correlated with the starch content, with the spillage exhibiting the highest amylose content of 44.9%, followed by the extract with 38.4%, and the kaff-kaff with 25.3% (**Table 3-3**). A Pearson's correlation coefficient of approximately 0.99 was calculated between the starch content and amylose content across these substrates, indicating an exceptionally strong positive linear relationship. This suggests that as the total starch content increases, the amylose content increases almost proportionally, with near-perfect predictability. Such a strong correlation is expected given the molecular composition of starch, which primarily comprises two polysaccharides namely, amylose and amylopectin. Since starch content reflects the combined presence of these two components, variations in starch quality are closely mirrored by changes in amylose concentration.

In contrast, the amylopectin content, being complementary to amylose as the other major starch component, has important implications for the functional properties of starch-based films derived from these substrates. Higher amylopectin levels, such as those found in the extract sample with lower amylose content, typically lead to bioplastic films with reduced tensile strength but increased elongation capacity, making the films more flexible and less brittle, allowing for better moldability and adaptability during processing. This is because amylopectin's highly branched molecular structure disrupts tight packing and crystallinity, allowing greater polymer chain mobility (Maraveas, 2020; Patkar et al., 2020; Silva et al., 2023; Wang et al., 2017; Rindlav-Westling et al., 1998).

However, films with higher amylopectin content also tend to exhibit increased water absorption and reduced barrier properties due to their amorphous regions that readily absorb moisture. This results in greater sensitivity to humidity and potentially less suitable where water resistance is critical (Rindlav-Westling et al., 1998). Consequently, the variations in amylose and amylopectin content among the substrates in this study suggest that the kaff-kaff, with its higher amylopectin proportion of 74.7% (**Table 3-3**), may produce more flexible but weaker films with higher water uptake, whereas the spillage and extract, with higher amylose content, are likely to yield films with greater tensile strength, lower elongation, and improved water resistance. Additionally, given the relatively higher amylopectin content in the extract (61.6%) compared to the spillage (55.1%), it is reasonable to expect that films from the extract will be more flexible but reduced tensile strength and exhibit higher water absorption than those from

the spillage (**Table 3-3**).The subsequent increase in amylose content observed during the starch extraction process, from the whole maize dent kernel (23 to 28%) to the spillage (44.9%), and from the kaff-kaff substrate to the extract, can be attributed to several interrelated factors.

The 25.3% amylose content in the kaff-kaff substrate falls within the typical range for normal dent maize (23 to 28%) as reported by Serna-Saldivar (2019). The subsequent increase in amylose content observed during the starch extraction process, from the whole maize dent kernel (23 to 28%) to the spillage (44.9%), and from the kaff-kaff substrate to the extract, can be attributed to several factors. Unlike the use of intact kernels, damage to the kernel structure during the cleaning stage of the wet-milling process, facilitated by mechanical treatments, enhances the leaching of amylose during steeping (Alcázar-Alay & Meireles, 2015). This likely accounts for the substantial increase in amylose content obtained in this study from the kaff-kaff substrate to the extract (only 6.5% lower than spillage), despite the absence of extensive extraction techniques such as alkali or acid steeping employed by Palacios-Fonseca et al. (2013).

Chemical treatments, particularly alkaline treatments, can disrupt the crystalline structure of the granules by allowing hydroxyl ions to penetrate amorphous regions (Palupi et al., 2024). In contrast, acid treatments hydrolyse starch molecules, primarily targeting amorphous regions. This process leads to depolymerization and increased solubility, though excessive treatment can result in amylose degradation (Wang & Copeland, 2015). According to Serna-Saldivar (2019), maize wet-milling typically involves acid steeping, which may explain the high amylose content of the spillage substrate (44.9%) collected from the final starch product of the wet-milling facility that provided samples for this study. This highlights the critical role of extraction methods, as procedural variations can significantly influence amylose yield.

3.3.5. Protein and lipids content

Serna-Saldivar (2019) reported that starch products from the maize wet-milling process typically have small amounts of protein (0.35%) and lipids (0.02%) which are considered acceptable levels. In this study, the spillage presented low protein and lipid contents of <0.03% and <1.0%, respectively (**Table 3-3**). Furthermore, the extract also presented low protein and lipid contents of <0.03% and <1.0%, respectively (**Table 3-3**). This low content indicates that the extraction process employed was efficient in isolating starch, minimising the co-extraction of proteins and lipids that could complicate the material properties of the starch. Serna-Saldivar (2019) further mentions the issue of amylose-lipid complexes where lipids, in the presence of amylose, increase the gelatinisation temperature required and restricts swelling of the starch granules. Lipids can influence the gelatinisation behaviour of starch; when

amylose interacts with lipids, it can increase the gelatinisation temperature required for starch granules, thereby affecting their swelling capacity. High lipid content typically results in restricted swelling of starch granules, which can limit their usability in applications such as bioplastics. In this study, the low levels of lipids present in both the spillage and extracts suggest that the starch would have a more favourable swelling behaviour and enhanced gelatinisation properties, making it more suitable for bioplastic production.

3.3.5.1. Moisture and pH

The moisture content of the spillage (5.8%) (**Table 3-3**) and extract (6.9%) (**Table 3-3**) were lower than the moisture content of maize starch previously reported ranging from 10% to 12% by Amin et al. (2019); Ibrahim et al. (2019); and Marichelvam et al. (2019). The pH of the spillage (5.23) (**Table 3-3**) and the extract (4.11) (**Table 3-3**) were lower than previously reported for maize starch with pH levels between 6.47 and 6.72. This could be ascribed to starch processing during the maize wet-milling process and the extraction process used in this study. Interestingly, Sakkara et al. (2020) investigated the effects of pH on properties of maize starch films and reported improved mechanical properties of films made from starch at pH of 3 to 5 than those made from starch at pH of 7 to 11. Moreover, water absorption was also lower at pH 3 to 5 than at pH 7 to 9. However, despite the lower pH, the films demonstrated enhanced mechanical strength, indicating inverse proportionality between pH and mechanical strength. The pH levels investigated in this study fall within the optimal range for tensile strength reported by Sakkara et al. (2020). This suggests that the spillage and extract in this study have substantial potential for the development of bioplastic films with improved mechanical properties and water absorption.

Table 3-3: Physicochemical properties of substrates and extract (*n*=3)

Properties	**Spillage**	**Kaff-Kaff (ground)**	**Extract**	**Commercial maize starch (Maizena)**
Starch extraction yield (%)	**-**	**-**	45.4	
Carbohydrates (%)	92.5±0.009	68.7±0.004	79.6±0.043	89
Starch content (%)	88.8±0.14	57.7±0.85	78.2±0.42	89
Amylose apparent content (%)	44.9±0.14	25.3±0.001	38.4±0.12	23-28
Amylopectin content* (%)	55.1±0.14	74.7±0.001	61.6±0.12	72-77
Protein content (%)	<0.03	1.94	<0.03	0
Lipids (%)	<1.0	0.23±0.1	<1.0	0
Moisture content (%)	5.8	-	6.9	
pH	5.23±0.01	5.63±0.02	4.11±0.01	

**Obtained by difference*

3.3.6. Swelling power and water solubility

The swelling power of the spillage, kaff-kaff, and extract all increased with temperature, as shown in **Figure 3-3a**. At 90°C, the swelling power was maximised across all samples, with the extract exhibiting a swelling power of 14.5 g/g, while maize spillage and kaff-kaff demonstrated swelling powers of 10.6 g/g and 10.5 g/g, respectively. High swelling power at elevated temperatures suggests that the starch granules can absorb more water, swell, and expand, which is critical for achieving the desired texture and mechanical properties in bioplastics. Generally, a higher swelling power coupled with lower water solubility is indicative of improved gelatinisation and retrogradation properties, which are essential for producing durable and effective bioplastics (Kadir et al., 2023).

The water solubility demonstrated a more variable relationship with temperature, as shown in **Figure 3-3b**. The lowest water solubility observed for the spillage and extract was at 80°C whereas the highest water solubility for both were observed at 90°C. The lowest water solubility for both spillage and extract occurred at 80°C, whereas the highest solubility was recorded at 90°C. This suggests that as the temperature increases, some granules break down and dissolve, which can facilitate the leaching of amylose. This effect is important since amylose contributes to the cohesive properties of the resulting bioplastic matrix.

According to Wang and Copeland (2013), the relationship between swelling power and water solubility is important when evaluating the suitability of starch sources for bioplastic applications. Ma et al. (2015) adds that the ability of starch granules to expand and form a viscous gel while minimising solubility helps maintain integrity in bioplastic formulations. The key finding is that gelatinisation at 80°C strikes the right balance for these starch sources, promoting effective swelling while moderating excessive solubility that could compromise the mechanical strength of the final product.

This suggests that heating spillage and extracts to 80°C represents an ideal condition for maximising their gelatinisation, thereby enhancing their functionality in bioplastic synthesis. By maintaining optimal gelatinisation at this temperature, the resultant blends are likely to exhibit improved structural integrity and performance in bioplastic applications. The swelling power behaviour and water solubility characteristics observed in spillage and extracts signal their potential as ideal substrates for bioplastic production. The favourable interaction of these properties likely results in enhanced mechanical performance of the generated bioplastics, making those starch types particularly valuable for sustainable material development.

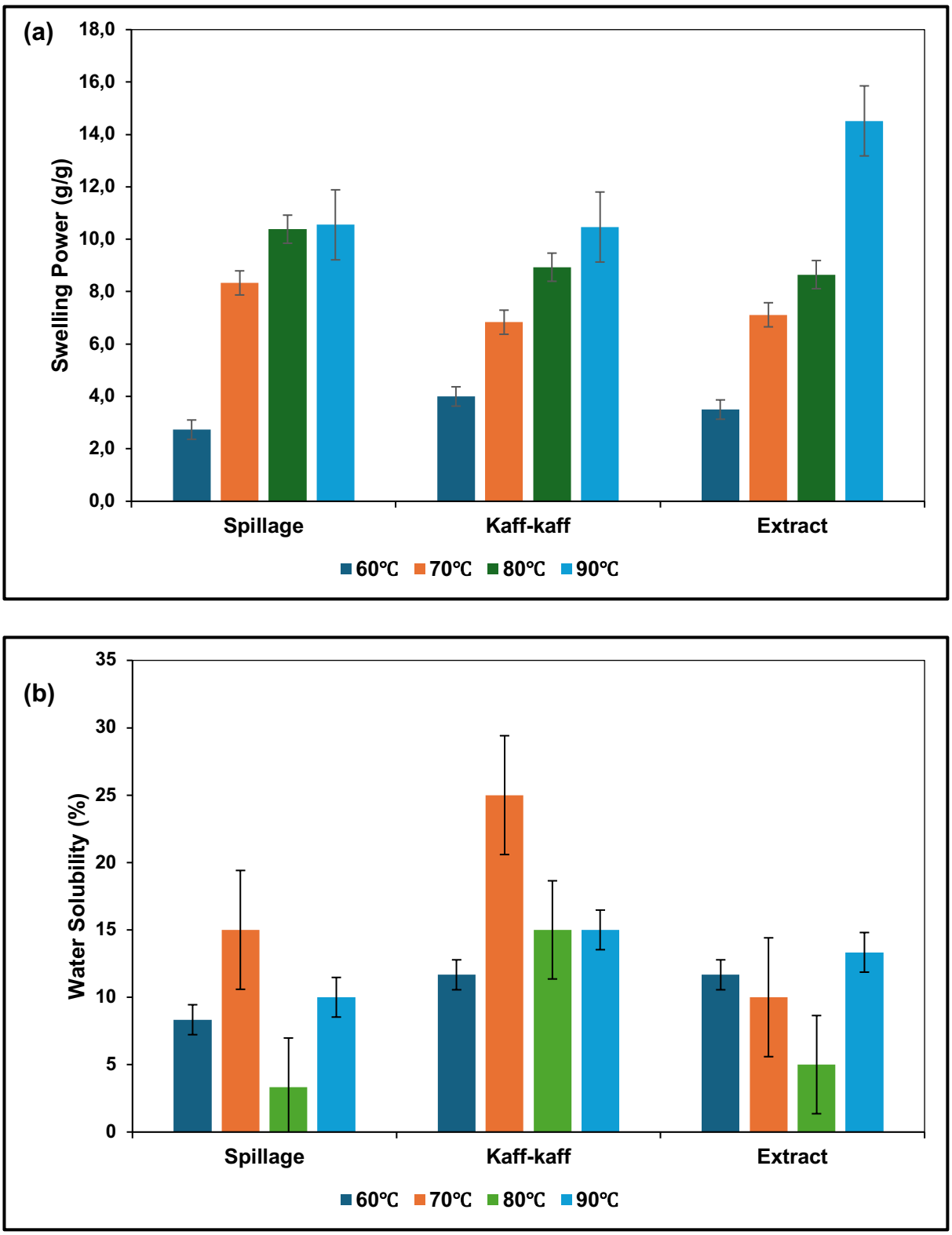

Figure 3-3: Swelling power (a) and Water absorption (b) of all substrates as a function of temperature (*n*=3), error bars represent standard deviation from the mean.

3.4. Summary

The findings demonstrate that kaff-kaff can be effectively used to extract quality starch suitable for bioplastic synthesis. The extraction yield achieved from the kaff-kaff substrate was 45.4%, which is comparable to laboratory-scale extractions reported in previous studies. The chemical composition and physicochemical properties of the substrates and extract were analysed. Spillage exhibited the highest starch content (88.8%), followed by the extract (78.2%). The spillage and extract showed increased amylose content (44.9 % and 38.4%, respectively) compared to the original kaff-kaff substrate, indicating potential improvements in mechanical properties for bioplastic applications.

Swelling power and water solubility analyses revealed that the spillage and extract demonstrated ideal gelatinisation conditions at 80°C. These properties are important for the formation of a viscous gel during bioplastic synthesis. Additionally, the measured pH levels of the spillage (5.2) and extract (4.1) suggest potential for enhancing the mechanical properties and water absorption behaviour of the resulting bioplastic films.

This study demonstrated that maize wet milling waste materials, particularly kaff-kaff and spillage, show promising potential as alternative starch sources for bioplastic production. The spillage and extract exhibited superior starch properties and was used for further bioplastic synthesis in this study. Utilising these waste materials could contribute to a more sustainable and circular economy approach in bioplastic manufacturing while addressing concerns related to food security and environmental impact.

Considering the comprehensive physicochemical and compositional analyses conducted in this study, encompassing parameters such as starch yield, moisture content, pH, amylose and amylopectin ratios, total carbohydrate, protein and lipid content, as well as functional attributes like swelling power and water solubility, both spillage and extract substrates emerged as the most promising candidates for bioplastic production. These two waste streams demonstrated superior starch and amylose contents and purity, alongside physicochemical properties conducive to enhanced mechanical performance in the resulting bioplastic films. Specifically, their favourable amylose-to-amylopectin ratios suggest optimal film-forming capability, contributing to improved tensile strength and flexibility critical for packaging applications. Additionally, the relatively low levels of impurities such as proteins and lipids in these substrates imply reduced interference with starch gelatinisation and polymer network formation, thereby facilitating optimised bioplastic synthesis. Consequently, spillage and extract were selected as the preferred raw materials for bioplastic synthesis in this study due

to their balanced composition and favourable functional properties, aligning with the aim of valorising maize agro-industrial waste for sustainable material development.

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Chapter 4: Factorial screening of starch type, plasticiser mass fraction, and reinforcement type and mass fraction on the mechanical properties of bioplastic films

4.1. Introduction

The synthesis of starch-based bioplastic composites is influenced by multiple variables, including the type of starch used, the mass fraction of plasticisers, the type and mass fraction of reinforcements (fillers), and additives such as acetic acid. Previous studies have demonstrated that starch type significantly affects mechanical, thermal, and barrier properties of bioplastics. For example, cereal starches like corn and wheat have been extensively investigated due to their well-characterised amylose-to-amylopectin ratios, which influence film flexibility and strength (Edhirej et al., 2017; Zhang & Han, 2010). In contrast, tuber starches such as cassava offer advantages in clarity and gelatinisation temperature but have been less frequently studied. Moreover, much research has focused on commercially purified or laboratory-grade starches, often overlooking starches derived from agro-industrial waste such as potato peels, discarded cassava, or maize wet-milling waste streams. These waste-derived starches represent a more sustainable and cost-effective resource (Aulia et al., 2022; Tedeschi et al., 2020). Addressing this, this study incorporates waste-derived starches to expand the applicability of bioplastic films and contribute to circular economy initiatives.

Plasticisation strategies are key in overcoming the brittleness of native starch films. Common plasticisers such as glycerol, sorbitol, and fructose have been widely reported for their ability to increase elongation at break and improve processability (Edhirej et al., 2017; Zhang & Han, 2010). As shown in **Table 2-6**, the plasticiser mass fraction in previous studies was typically between 25 to 30% (w/w dry starch), with combinations such as 30% glycerol for arrowroot starch (Tarique et al., 2022), and 25% glycerol or fructose for cassava and maize starch-based films (Versino & García, 2014; Ibrahim et al., 2019; Hermansyah et al., 2014). Ibrahim et al. (2019) further explored a wider range of plasticiser contents (0%, 25%, 40%, 55%), demonstrating the influence of increasing plasticiser concentration on film flexibility.

In addition to plasticisers, acetic acid has been incorporated in several studies to enhance the film-forming ability and tensile strength of starch-based bioplastics. For instance, Pradeep et al. (2022) used 2 mℓ of acetic acid, while Ansanay et al. (2024) investigated effects at 2%, 4% and 6% v/v loadings. Abdel Hamid et al. (2025) explored even higher ranges, applying 5 to 50% w/w relative to dry starch.

Lignocellulosic fibres have garnered significant attention recently as natural reinforcement materials. These fibres consist mainly of cellulose, hemicellulose, lignin, pectin, waxes, and various water-soluble constituents, with their composition and proportions varying depending on the biomass source. This variability leads to a wide range of mechanical properties among lignocellulosic fibres (Boey et al. 2022). Cellulose-based fillers such as arrowroot fibre, cassava fibrous filler, maize husk, banana pseudo-stem fibre, and bacterial cellulose effectively enhance the tensile strength of starch matrices. For instance, arrowroot fibre loadings between 0 and 10% increased tensile strength from 2.42 to 15.2 MPa (Tarique et al., 2022), while sugar palm fillers added at 0 to 8% yielded tensile strengths ranging from 12.84 to 19.1 MPa in maize starch films (Ibrahim et al., 2020) (**Table 2-6**). The aromatic structure of lignin contributes to improved water resistance by reducing water uptake in bioplastics (Yang et al. 2019). Additionally, Yang et al. (2019) also reported that starch-lignin composites exhibit enhanced tensile strength compared to composites without lignin, although this improvement is accompanied by a reduction in elongation.

This chapter investigates the effects of cellulose-based fillers alone and in combination with lignin, aiming to understand their individual and synergistic effects on the mechanical properties and water absorption behaviour of starch-based films. Cellulose primarily enhances tensile strength, while lignin imparts hydrophobicity and durability (Yang et al. 2019). This focused approach allows for the clear distinction between cellulose-only and cellulose-lignin fillers to be distinguished, providing foundational insights before exploring more complex lignocellulosic blends in **Chapter 5**. Despite progress, there remains a clear gap in systematically evaluating the combined effects of various factors, particularly the starch type, filler type and mass fraction, plasticiser mass fraction, and acetic acid, within a single experimental framework. Most existing research focuses on limited subset of variables, often optimising one or two parameters at a time, potentially overlooking complex interactions among variables. Moreover, many studies continue to rely on commercial or laboratory-grade starches, rarely considering waste-derived starches, which offer sustainability and cost benefits.

This study addresses these gaps by selecting a comprehensive range of influential factors, informed by literature, to systematically evaluate their effects on tensile strength, elongation at break, and water absorption of starch-based bioplastics. The findings will inform the optimisation study presented in Chapter 5, guiding the formulation of canola fines-reinforced starch-based bioplastics. Ultimately, this work aims to advance the development of bioplastic films with enhanced mechanical properties and reduced water absorption, thereby promoting their viability for practical applications.

4.2. Materials and methods

This research employed a quantitative experimental approach to systematically investigate the variables and interactions involved in the development of starch-based bioplastics. The bioplastic films were synthesised using (i) a cellulose-only filler, and (ii) a mixture filler composed of 21% cellulose and 23% lignin to assess their potential in enhancing bioplastic performance. It is important to clarify that this study focused on bioplastics synthesised from the spillage and extract substrates, while kaff-kaff was excluded from further consideration due to its unfavourable properties. The performance of this mixture filler was later compared with that of canola fines, a lignocellulosic waste, used as a reinforcing material in **Chapter 5**.

4.2.1. Raw Materials

The main raw materials used in this study were two starch-based substrates namely maize spillage and extract, as selected based on results described in Chapter 3. Analytical-grade reagents including glycerol (cat no: G5516), acetic acid (cat no: A6283), cellulose (cat no: 435236), and lignin (cat no: 471003) were procured from Merck (Darmstadt, Germany). The cellulose and lignin were employed as control additives to evaluate the performance of bioplastic films reinforced with: (i) analytical-grade cellulose alone, and (ii) a mixture of analytical-grade cellulose and lignin, incorporated at mass fractions equivalent to those reported in canola fines (23% lignin and 21% cellulose) as characterised by Ranjan et al. (2024).

4.2.2. Experimental design

A quantitative experimental approach was employed to screen factors influencing the properties of synthesised bioplastic films, using Design Expert software version 10.0 (Stat-Ease Inc, Minneapolis, USA). A five-factor, two-level fractional factorial design was formulated to evaluate the effects of (i) plasticiser mass fraction (15 to 30% w/w dry starch), (ii) filler mass fraction (0 to 12% w/w dry starch), (iii) volume of 5% w/v acetic acid solution (0 to 2.5 mℓ), (iv) starch type (spillage or extract), and (v) filler type (cellulose or mixture) on three responses, namely tensile strength (MPa), elongation at break (%), and water absorption (%). The selected factor ranges were informed by previous studies summarised in **Table 2-6.** Each factor was tested at a low (−1) and high (+ 1) levels detailed in **Table 4-1**, with additional centre point runs conducted at the medium (0) level. Experimental runs were performed in duplicate, and the average was recorded. The conditions for each experimental run are presented in **Table 4-1**. The Analysis of Variance (ANOVA) was used to assess the significance and magnitude of each factor's effect on the responses.

4.2.3. Synthesis of bioplastic films

A series of starch-based bioplastic films were synthesised in beakers based on the traditional casting method described by Ibrahim et al. (2020) with a few modifications. Initially, 5g of starch was added to each beaker followed by reinforcements, glycerol, and 5% w/v acetic acid corresponding to the amounts for each run given in the experimental design (**Table 4-1)**. The volume in each beaker was made up to 100 mℓ with dH_2O and the mixtures were maintained at 85°C in a water bath for 20 minutes with constant stirring. The casted solutions were then poured evenly into a 150 mm diameter petri dishes and dried in an oven dryer at 27°C for 36 hours. The experimental setup consisted of a water bath equipped with four overhead stirrers, each with a submerged beaker clamped to it, enabling simultaneous execution of four experimental runs. To ensure uniform temperature distribution throughout the water bath, a circulating pump was installed. The bioplastic synthesis of starch bioplastic films is illustrated in **Figure 4-1.**

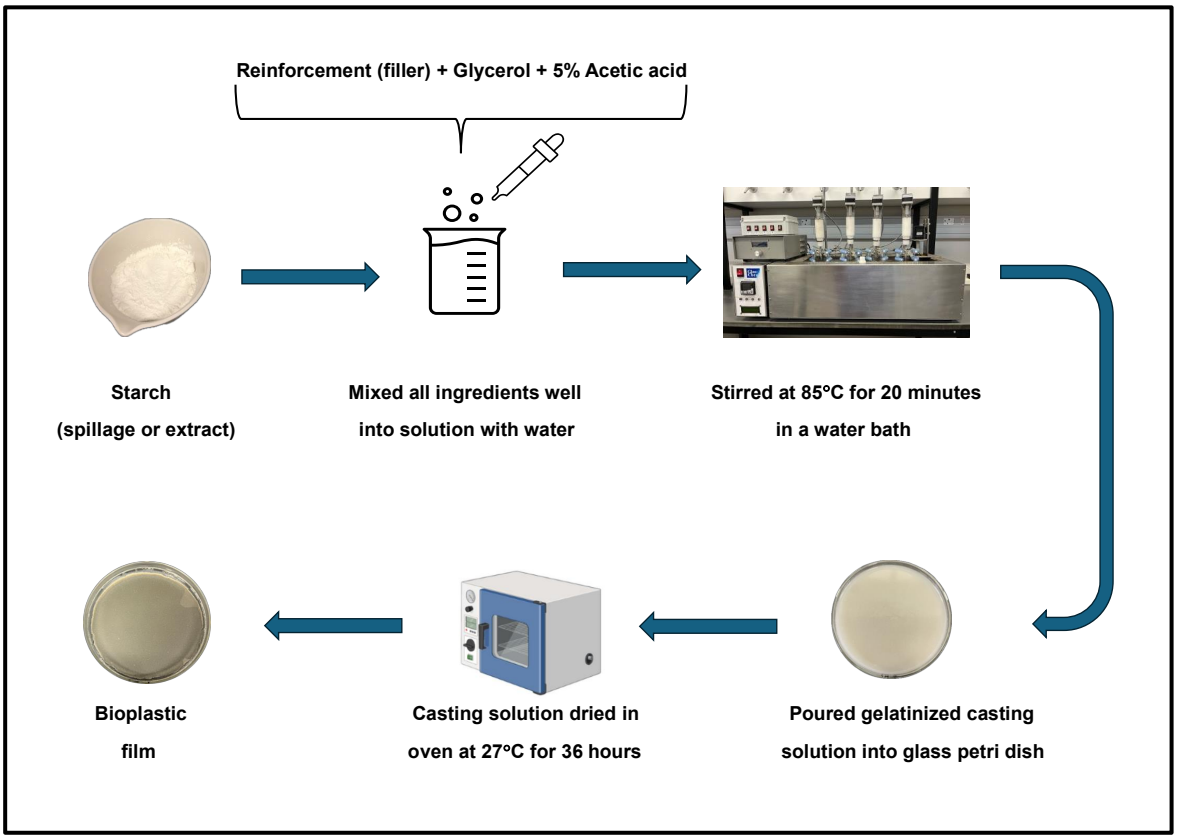

Figure 4-1: Schematic diagram of the bioplastic synthesis process.

4.2.4. Characterisation of bioplastic films

The synthesised bioplastic films were characterised for tensile strength, elongation at break, and water absorption. The methodology for these is provided in **Sections 4.2.4.1** and **4.2.4.2**.

4.2.4.1. Tensile strength and elongation at break

The mechanical properties of samples were tested using a 5 kN 3344 universal testing machine (Instron, Norwood, MA, USA) with Bluehill 3 software. The film samples and settings of the tensile machine were conditioned based on the standard method described by ASTM D638-14 (2014) at room temperature. The films were cut using a manual Arbor press and a specimen cutting die, the specimen width of the narrow section was 13 mm, the length of narrow section was 57 mm, the overall length was 165 mm, and the gauge length was 50 mm. The thickness of each specimen was measured using a 0 to 25 mm (±0.01 mm) micrometre (Adendorff, Brackenfell, South Africa). Specimens were fixed properly between the tensile machine grips and stretched at a strain rate of 5 mm/min until breaking, generating load-extension data for each film.

The tensile strength is expressed in units of force per unit area (megapascals, MPa), as described by Abdel Hamid et al. (2025), and calculated using the following equation:

$$\boldsymbol{\sigma}\ (\mathbf{MPa}) = \frac{\mathbf{F}}{\mathbf{A}} \qquad \text{(Equation 4-1)}$$

where σ denotes the tensile strength (MPa), F is the load (N), and A represents the cross-sectional area of the specimen (mm^2).

The elongation at break was calculated using the following equation, as described by Abdel Hamid et al. (2025) and Pradeep et al. (2022):

$$\boldsymbol{\varepsilon}\ (\%) = \frac{\mathbf{L_o - L_f}}{\mathbf{L_o}} \qquad \text{(Equation 4-2)}$$

where ε denotes the elongation at break (%), L_o is the original gauge length of specimen (mm), and L_f represents the length of the specimen after it is stretched (mm). Two replicates were tested for each film, resulting in average results for tensile strength and elongation at break.

4.2.4.2. Water absorption

The water absorption of the bioplastic films was determined in accordance with the ASTM D570-98 (2010) standard, in which homogeneous plastic test specimens were immersed in

water for a period of 24 hours. Water absorption (%) was calculated using the following equation:

$$\textbf{Water Absorption}\ (\%) = \frac{\mathbf{W_f - W_i}}{\mathbf{W_i}} \times \mathbf{100} \qquad \text{(Equation 4-3)}$$

where W_i is the initial weight of a specimen before being submerged in distilled water and W_f is the final weight of a specimen after being submerged in distilled water.

4.3. Results and discussion

Figure 4-2 illustrates experimental runs with intact bioplastic films that did not exhibit any crack formation. Runs 5 and 11 correspond to spillage-based films reinforced with cellulose and mixture fillers, respectively, while Runs 12 and 18 represent extract-based films reinforced with cellulose and mixture fillers, respectively. As presented in **Table 4-1**, the mechanical properties of certain starch-based bioplastic films could not be determined due to their brittleness or cracking during the casting process, resulting in incomplete data. Crack formation in these films was influenced by several interrelated factors. Uneven drying rates across the film thickness led to uneven shrinkage, as surface layers contract faster than inner regions, generating internal stresses that result in cracking (Jayarathna et al., 2022; Yang et al., 2019). Additionally, the quality of fibre-matrix interactions plays an important role. Poor adhesion or incompatibility between starch and reinforcing fibres can cause interfacial debonding, creating localised stress concentrations that promote cracking (Jayalath et al., 2025; Abe et al., 2021). Plasticisers can also affect water retention and vapor permeability, leading to swelling and shrinkage cycles that weaken the film and encourage cracking. These factors collectively contribute to the mechanical fragility observed during the casting process. Furthermore, the specific drying conditions used in this study (drying oven at 27°C for 36 hours) influenced the extent and nature of crack formation.

Table 4-1 shows that incomplete mechanical property data mainly correspond to films with low glycerol mass fractions (15% w/w dry starch) and those combining 22.5% glycerol with 9% filler (w/w dry starch). This pattern suggests that cracking primarily occurs at lower glycerol levels or when moderate glycerol content is paired with high filler loading, resulting in brittle, rigid films. It is well documented that insufficient plasticiser limits polymer chain mobility, causing films to be brittle and prone to cracking, consistent with findings in this study (Mohamed et al., 2023; Tarique et al., 2021). Similarly, Ibrahim et al. (2019) reported microcracks in starch films lacking glycerol. Moreover, Adel Hamid et al. (2025) found that cellulose fibre contents above 5% caused filler agglomeration and uneven stress distribution, degrading mechanical properties. Together, these results indicate that both glycerol concentration and filler loading significantly influence the mechanical integrity and susceptibility to cracking in starch-based bioplastics. The interplay between plasticiser concentration and filler content is critical: plasticisers improve flexibility, but excessive filler reduces matrix continuity, leading to easy crack initiation. Gadhave et al. (2018) highlighted that optimal mechanical integrity occurs at intermediate plasticiser and filler levels, which aligns with our observations that very low plasticiser or high filler loadings result in mechanical failure.

From a production perspective, minimising the use of additives is crucial to reduce raw material costs and simplify manufacturing operations. Both plasticisers and reinforcing materials contribute significantly to the overall production expense of bioplastic films. However, excessively reducing glycerol content compromises polymer chain mobility, resulting in decreased film flexibility and mechanical performance. Conversely, high filler concentrations increase viscosity, promote agglomeration, and create dispersion challenges, thereby adversely affecting processability and final product quality.

Therefore, optimisation of bioplastic formulation requires a careful balance, including identifying the minimum effective plasticiser and filler loadings that meet tensile strength, elongation, and water resistance property targets while ensuring consistent and efficient production operations. Literature indicates that glycerol concentrations of approximately 20 to 30% (w/w of dry starch) and filler contents below 10% achieve a practical balance between bioplastic performance and production cost (Gadhave et al., 2018; Ibrahim et al., 2019). These ranges also help avoid problems such as phase separation or filler agglomeration that compromise product uniformity and scalability.

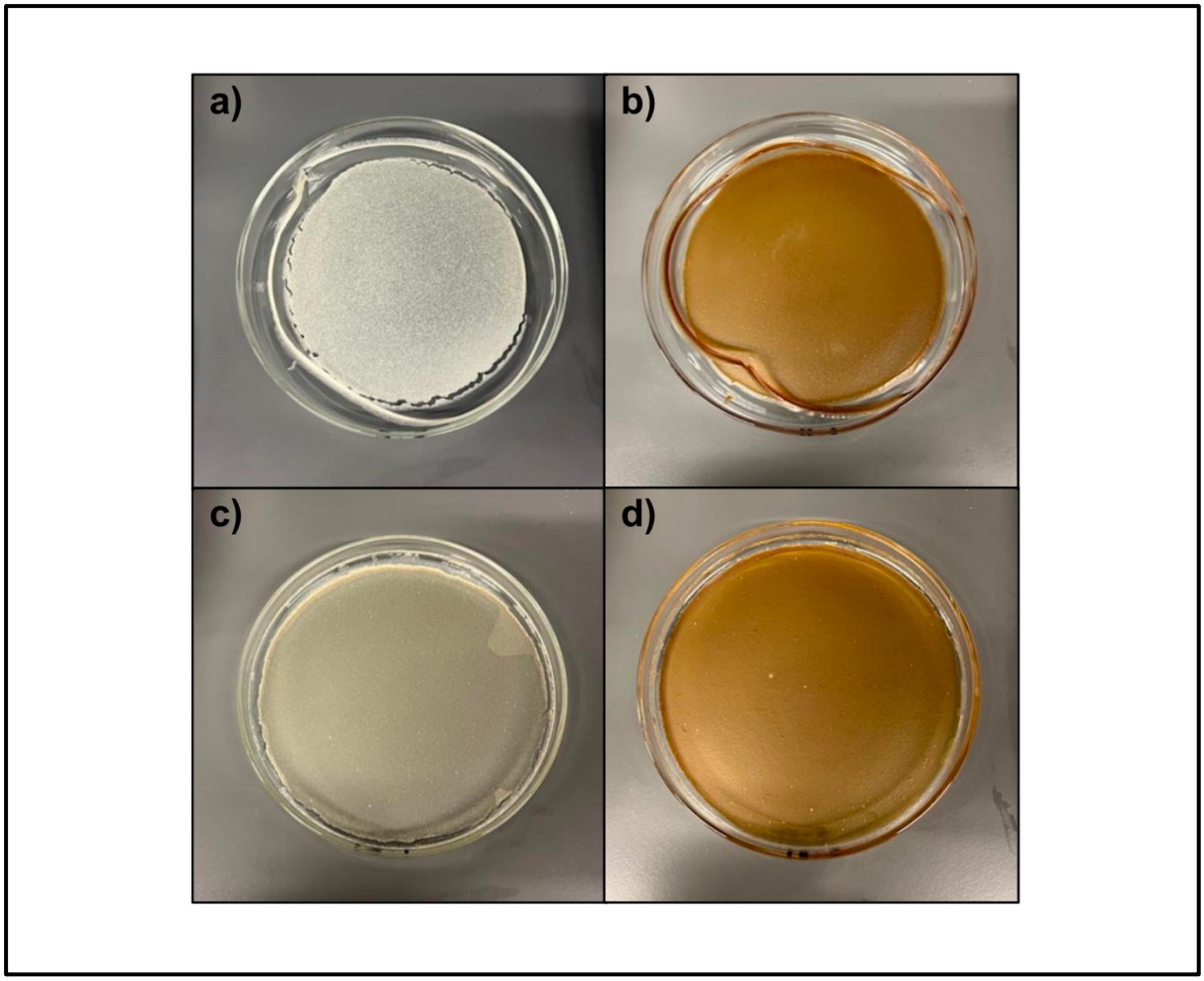

Figure 4-2: Pictures of bioplastic films synthesised according to (a) run 5, (b) run 11, (c) run 12, and (d) run 18.

Table 4-1: Experimental design matrix and experimental results for bioplastic synthesis.

Run	Factors					Responses		
	A	B	C	D	E	R1	R2	R3
1	15	6	2.5	Extract	Cellulose	-	-	184
2	22.5	9	1.25	Spillage	Cellulose	15.73	5.29	92
3	22.5	9	1.25	Spillage	Mixture	-	-	101
4	22.5	9	1.25	Spillage	Mixture	-	-	95
5	15	12	2.5	Spillage	Cellulose	11.64	1.66	109
6	22.5	9	1.25	Spillage	Mixture	-	-	108
7	30	12	0	Extract	Mixture	5.58	45.07	76
8	15	12	0	Spillage	Mixture	-	-	137
9	22.5	9	1.25	Spillage	Cellulose	14.93	3.97	69
10	22.5	9	1.25	Extract	Cellulose	12.41	5.11	85
11	30	6	0	Spillage	Mixture	7.40	32.47	52
12	22.5	9	1.25	Extract	Cellulose	10.18	10.14	112
13	30	12	2.5	Extract	Cellulose	7.32	22.71	35
14	22.5	9	1.25	Spillage	Mixture	16.25	5.33	90
15	15	6	0	Extract	Mixture	-	-	165
16	22.5	9	1.25	Extract	Mixture	-	-	109
17	22.5	9	1.25	Extract	Mixture	-	-	134
18	30	6	2.5	Extract	Mixture	6.34	46.47	64
19	22.5	9	1.25	Extract	Cellulose	11.29	4.23	122
20	22.5	9	1.25	Spillage	Cellulose	-	-	110
21	22.5	9	1.25	Extract	Mixture	7.59	32.30	144
22	30	12	2.5	Spillage	Mixture	7.61	41.00	65
23	15	12	2.5	Extract	Mixture	-	-	160
24	22.5	9	1.25	Extract	Cellulose	-	-	123
25	15	6	2.5	Spillage	Mixture	-	-	113
26	30	6	0	Extract	Cellulose	3.85	60.69	69
27	30	12	0	Spillage	Cellulose	10.38	16.89	67
28	22.5	9	1.25	Extract	Mixture	-	-	101
29	15	12	0	Extract	Cellulose	-	-	128
30	15	6	0	Spillage	Cellulose	14.44	3.94	98
31	22.5	9	1.25	Spillage	Cellulose	9.42	3.57	94
32	30	6	2.5	Spillage	Cellulose	8.58	28.09	40
33	22.5	0	0	Spillage	Mixture	5.51	116	144
34	37.5	0	0	Spillage	Mixture	2.59	108	63
35	22.5	0	0	Extract	Mixture	2.79	148	156
36	30	0	0	Extract	Mixture	2.77	104	57
37	37.5	0	0	Extract	Mixture	1.68	117	47

[a]*Factors: A, plasticiser mass fraction (% w/w dry starch); B, filler mass fraction (% w/w dry starch); C, volume of 5% w/v acetic acid solution (mℓ); D, starch type; E, filler type.*

[b]*Responses: R1, tensile strength (MPa); R2, elongation at break %), R3, water absorption (%)*

4.3.1. Effect of factors on the properties of synthesised bioplastic films

4.3.1.1. Tensile strength

The predicted R^2 and the adjusted R^2 were in reasonable agreement with values 0.8508 and 0.8245, respectively, indicating a strong model fit and suggesting that the model successfully explains a substantial portion of the variability in the tensile strength of the synthesised bioplastic films. The close alignment between these two values typically signifies that the model is robust and reliable for making predictions. The adequate precision ratio of 14.460 indicates that the statistical model used in the analysis has a good signal-to-noise ratio, which suggests that the model can predict the responses with a high degree of confidence. In general, a ratio greater than 4 is considered acceptable, implying that the variation explained by the model is adequate relative to the unexplained variation. This high precision ratio reinforces the reliability of the model in predicting the tensile strength of the bioplastic films based on the factors analysed, ensuring that the conclusions drawn from the experimental data are statistically valid and meaningful.

The ANOVA results between the five factors toward tensile strength are presented in **Table 4-2**. The model demonstrated high predictive capability, as indicated by a significant model p-value (<0.0001) and a non-significant lack of fit p-value (0.6628), allowing for accurate predictions in further analyses. The model terms plasticiser mass fraction (A), filler mass fraction (B), and starch type (D) had p-values (<0.05) indicating that they had significant impacts on the tensile strength of the synthesised bioplastics.

Table 4-2: ANOVA results for tensile strength

Tensile strength					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	11.02	3	3.67	32.32	< 0.0001
A-Plasticiser mass fraction	1.19	1	1.19	10.43	0.0049
B-Filler mass fraction	2.08	1	2.08	18.26	0.0005
D-Starch type	1.49	1	1.49	13.12	0.0021
Curvature	1.12	2	0.5601	4.93	0.0205
Residual	1.93	17	0.1137		
Lack of Fit	1.39	13	0.1073	0.7980	0.6628
Pure Error	0.5377	4	0.1344		
Corrected Total	14.07	22			

(i) Effect of filler type and acetic acid

The results show that filler type did not have significant effects ($p>0.05$) on the tensile strength of the synthesised bioplastic films (**Table 4-2**). Yang et al. (2019) reported that while lignin can enhance hydrophobicity, it may negatively affect tensile strength if poorly dispersed. However, in this study, the addition of lignin to cellulose did not result in a significant reduction in tensile strength, as indicated by the insignificant effect of filler type ($p>0.05$), suggesting that lignin was uniformly dispersed within the bioplastic matrix (**Table 4-2**). This uniform dispersion implies that the casting solution was homogeneous, allowing for consistent distribution of components and optimal interaction between the filler and starch matrix, thereby preserving the mechanical integrity of the final bioplastic films.

Furthermore, the addition of acetic acid did not result in a statistically significant effect ($p>0.05$) on the tensile strength of the synthesised bioplastic films, as shown in **Table 4-2**. This was observed across the testing range of 0 mℓ, 1.25 mℓ, and 25 mℓ (equivalent to 0%, 26%, and 52% w/w of dry starch), which aligns with those reported in previous studies. For instance, Pradeep et al. (2022) synthesised bioplastic using 2 mℓ of acetic acid, while Abdel Hamid et al. (2025) evaluated concentrations of 5%, 27.5%, and 50% (w/w of dry starch). Additionally, Ansanay et al. (2024) explored lower concentrations of 2%, 4%, and 6% (v/v). All three of these studies reported statistically significant effects of acetic acid on the mechanical properties of starch-based bioplastics. It should be noted that the present study did not investigate acetic acid addition within this lower range explored by Ansanay et al. (2024), which may partially explain the absence of a significant effect.

Testing considerably higher acetic acid concentrations (up to 52% w/w dry starch) may have induced excessive hydrolysis or chain scission, negated potential benefits and resulted in unchanged mechanical strength. The lack of a significant effect of acetic acid may be attributed to formulation and processing differences, such as starch source, plasticiser-filler interactions, or drying conditions, which could have altered the chemical environment and polymer network, limiting acetic acid's capacity to modify the bioplastic matrix effectively. It is possible that the formulation already achieved near-optimal chain mobility and mechanical properties, reducing sensitivity to further chemical modification. This unexpected result suggests that, under the specific formulation and processing conditions used in this study, acetic acid may not exert the mechanical-enhancing effects commonly reported in literature.

(ii) Effect of plasticiser: glycerol

In this study, glycerol was used as a plasticiser at concentrations ranging from 15 to 30% (w/w dry starch) in both spillage-based and extract-based bioplastic films. As illustrated in **Figure**

4-3a, increasing the glycerol content in spillage-based films resulted in a decrease in tensile strength from 14 MPa (15% glycerol) to 9.5 MPa (30% glycerol). Similarly, **Figure 4-3b** shows a decrease in tensile strength from 10.5 MPa (15% glycerol) to 7 MPa (30% glycerol) in extract-based films. These tensile strength measurements were obtained at a constant filler mass fraction of 12% (w/w dry starch).

This effect of glycerol as a plasticiser is consistent with research conducted by Tarique et al. (2021) who tested glycerol concentrations from 15% to 45% (w/w dry starch) in arrowroot starch films, resulting in a drop of tensile strength from 9.34 MPa (15% glycerol) to 1.95 MPa (45% glycerol). Similarly, Nasir & Othman (2021) who synthesised maize starch-based bioplastic films with varied glycerol concentrations of 20%, 30%, and 40%, reported a decrease in tensile strength of 0.43 MPa, 0.38 MPa, and 0.15 MPa, respectively. These findings highlight the typical plasticising effect of glycerol that, while it enhances flexibility, it weakens intermolecular interactions within the polymer matrix, leading to reduced tensile strength. Consequently, minimising the glycerol mass fraction may be beneficial for improving the tensile strength of starch-based bioplastics, particularly those derived from spillage.

(iii) Effect of starch type

Moreover, spillage-based bioplastics demonstrated superior tensile strength compared to extract-based counterparts, achieving a peak of 14 MPa (**Figure 4-3a**) versus 10.5 MPa (**Figure 4-3b**), respectively. This difference is likely due to variations in amylose content. Higher amylose concentrations are known to contribute to greater tensile strength due to increased intermolecular H bonding among linear amylose chains. This observation is consistent with the findings of Alves et al. (2007) who reported that cassava starch-based bioplastic films enriched with amylose exhibited increased tensile strength due to stronger intermolecular hydrogen bonding among the linear amylose chains.

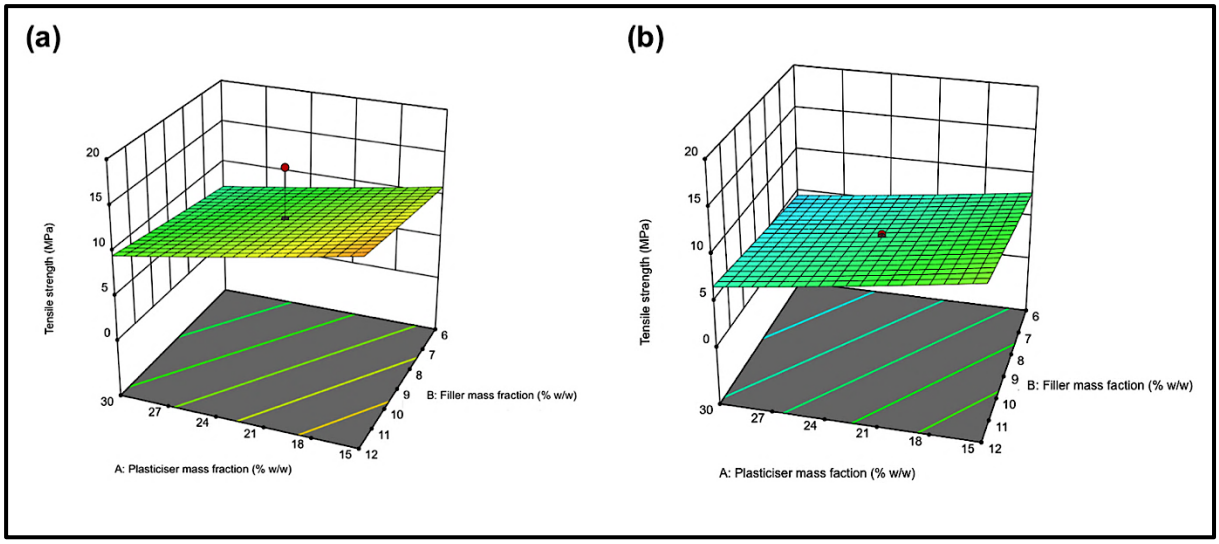

Figure 4-3: Response surface plots of the effects of plasticiser mass fraction and filler mass fraction on tensile strength using (a) spillage and (b) extract.

4.3.1.2. Elongation at break

The relationship of the five factors on elongation at break was assessed using ANOVA and presented in **Table 4-3**. The model p-value was <0.0001, indicating a robust predictive model. The non-significant lack of fit p-value of 0.3686 further supports the model's validity for further analysis. The factors identified as having a significant effect on the elongation at break, with p-values <0.05, were plasticiser mass fraction (A), filler mass fraction (B), starch type (D), and filler type (E), with corresponding p-values of 0.0007, 0.0005, 0.0131, and less than 0.0001, respectively. This highlights the significant influence of these factors on the flexibility and ductility of these bioplastics.

The predicted R^2 and adjusted R^2 were in reasonable agreement with values of 0.8504 and 0.9121, respectively, indicating a well-fitting model, with the adjusted R^2 suggesting strong predictive capability in the context of elongation at break. The adequate precision ratio of 18.679 further affirms the reliability of the model, suggesting a good signal-to-noise ratio, for predicting the elongation at break performance of the bioplastics studied.

Table 4-3: ANOVA results for elongation at break

Elongation at break					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	36.88	5	7.38	44.60	< 0.0001
A-Plasticiser mass fraction	2.88	1	2.88	17.41	0.0007
B-Filler mass fraction	3.19	1	3.19	19.31	0.0005
D-Starch type	1.29	1	1.29	7.79	0.0131
E-Filler type	4.92	1	4.92	29.77	< 0.0001
AE	2.75	1	2.75	16.62	0.0009
Curvature	2.55	1	2.55	15.40	0.0012
Residual	2.65	16	0.1654		
Lack of Fit	2.17	12	0.1807	1.52	0.3686
Pure Error	0.4771	4	0.1193		
Corrected Total	42.07	22			

(i) Effect of acetic acid

Acetic acid did not show a significant impact on elongation, paralleling its negligible influence on tensile strength (**Table 4-3**). Abdel Hamid et al. (2025) reported that the addition of acetic acid had no statistically significant effect on the elongation of bioplastic films derived from maize, a finding that is consistent with the results of this study. In both cases, variations in the quantity of acetic acid added within the tested range did not produce meaningful changes in the flexibility of the bioplastic films. However, Hamid et al. (2025) observed a statistically significant interaction effect between the mass ratio of glycerol to starch and acetic acid addition on elongation at break. These findings imply that the impact of acetic acid on elongation becomes evident only when combined with varying glycerol levels, highlighting a potential synergistic relationship that influences the flexibility of the bioplastic network. In contrast, this study did not find a significant interaction between acetic acid and any other factor (**Table 4-3**), indicating that under the formulation and processing conditions applied, acetic acid did not modify elongation behaviour even when combined with varying glycerol content.

(ii) Effect of starch type

Figure 4-4 illustrates compelling results that bioplastic films made from extract outperformed those made from spillage in terms of elongation at break, achieving a maximum elongation at break of approximately 70%, while spillage-based films peaked at 40%. Tafa et al. (2023) attributes this to the amylopectin proportions of starch sources. By varying amylose/amylopectin ratios, it was discovered that high-amylose bioplastic films exhibited with higher tensile strength but lower flexibility whereas, high-amylopectin bioplastic films acted as natural plasticisers, increasing elongation but reducing strength. This indicates that extract-

based bioplastics may be more suitable for applications where flexibility and stretchability are vital, such as in packaging or envelopes that require bending without breaking.

(iii) Effect of plasticiser and filler type

The significant interaction term (AE) with a p-value of 0.0009 means that the effect of plasticiser mass fraction on elongation at break is influenced by the type of filler used. Analysis of the results shown in **Figure 4-4a** and **Figure 4-4b** suggests that utilising the mixture as a filler improved the elongation at break, reaching a maximum of roughly 30%. In contrast, cellulose alone (**Figure 4-4c** and **Figure 4-4d**) achieved a maximum elongation at break of about 20%. These findings emphasise the potential benefits of selecting appropriate starch and filler sources to optimise bioplastic performance characteristics, contributing to their applicability in various uses where flexibility and ductility are essential, such as in packaging or envelopes that require bending without breaking. The enhanced elongation at break seen with mixture fillers over cellulose-only fillers highlights the potential of canola fines (lignocellulosic waste), not just to improve tensile strength, but also as an integral element that could improve flexibility characteristics.

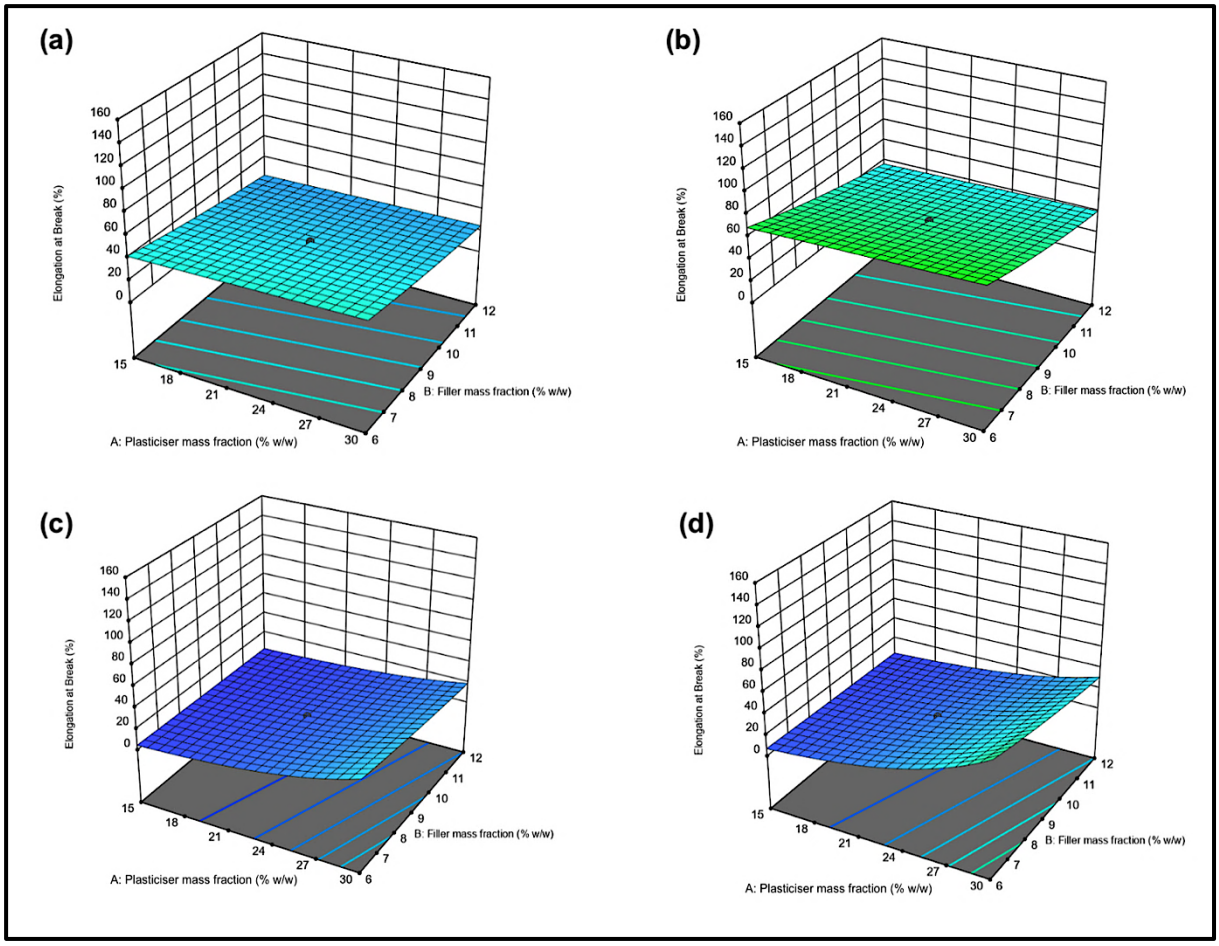

Figure 4-4: Response surface plots of the effects of plasticiser mass fraction and filler mass fraction on elongation at break using (a) spillage reinforced with mixture, (b) extract reinforced with mixture, (c) spillage reinforced with cellulose, and (d) extract reinforced with cellulose.

4.3.1.3. Water absorption

The ANOVA results pertaining to water absorption, as presented in **Table 4-4**, demonstrate a statistically significant model with a p-value of <0.0001, which is indicative of the model's robustness. The lack of fit p-value of 0.2275 suggests that the model is adequate for further analysis since it is greater than the threshold of 0.05. The analysis identified three significant factors influencing water absorption in the bioplastic films: plasticiser mass fraction (A), filler mass fraction (B), and starch type (D). The p-values for these factors were <0.0001, 0.0076, and 0.0018, respectively (**Table 4-4**), confirming their considerable impact on the water absorption properties. Consistent with the results for tensile strength and elongation at break, acetic acid did not exhibit a significant effect on water absorption.

Table 4-4: ANOVA results for water absorption.

Water absorption					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	38444.10	4	9611.02	27.36	< 0.0001
A-Plasticiser mass fraction	31903.27	1	31903.27	90.82	< 0.0001
B-Filler mass fraction	2873.17	1	2873.17	8.18	0.0076
D-Starch type	4111.97	1	4111.97	11.71	0.0018
AD	2846.11	1	2846.11	8.10	0.0079
Curvature	595.91	2	297.96	0.8482	0.4382
Residual	10538.54	30	351.28		
Lack of Fit	7343.92	18	408.00	1.53	0.2275
Pure Error	3194.62	12	266.22		
Corrected Total	49578.55	36			

(i) Effect of starch type

The clear delineation between the performance characteristics of spillage and extract-based bioplastics based on their water absorption percentages depicted in **Figure 4-5** indicates that choice of starch directly affects both functionality and potential applications.

(ii) Effect of plasticiser: glycerol

Glycerol mass fraction plays a critical role in modulating the water absorption capacity of starch-based bioplastics, with higher glycerol levels contributing to improved water resistance, characterised by lower water absorption percentages. This insight is valuable for optimising the formulation of bioplastic films to achieve desired water absorption characteristics, which is essential for their functionality and application.

(iii) Plasticiser and starch type

Additionally, the interaction term between plasticiser mass fraction and starch type (AD) was significant, with a p-value of 0.0079, suggesting that the effect of varying levels of plasticiser on water absorption is influenced by the type of starch used. **Figure 4-5** show that high water absorption percentages (above 100%) at lower glycerol mass fractions. However, as glycerol mass fractions increased, there was a significant improvement in the water resistance (decreased water absorption), for both spillage and extract-based bioplastic films. The findings presented in **Figure 4-5a** show that spillage-based bioplastic films exhibited water absorption ranging from 50% to 120%, whereas the extract-based bioplastic films shown in **Figure 4-5b** demonstrated a wider absorption range of 50% to 160%. The enhanced water resistance in spillage-based films implies their applicability in situations requiring durability against water exposure, such as in agricultural films or moisture-sensitive packaging. In contrast, extract-

based films, while exhibiting potentially beneficial flexibility with greater elongation at break of up to 70% versus 40% for spillage-based bioplastic films, may face challenges in applications where moisture control is vital.

This suggests that spillage-based bioplastic films are inherently more water-resistant, aligning with the findings Yang et al. (2023). Their study on thermoplastic starch films derived from different botanical sources reported that pea starch bioplastic films exhibited the highest tensile strength (6.28 MPa) and superior water resistance, characterised by lower water solubility (15.70%) and reduced water absorption (42.35%). The enhanced tensile strength and reduced water absorption properties were attributed to high amylose content and specific chain structures.

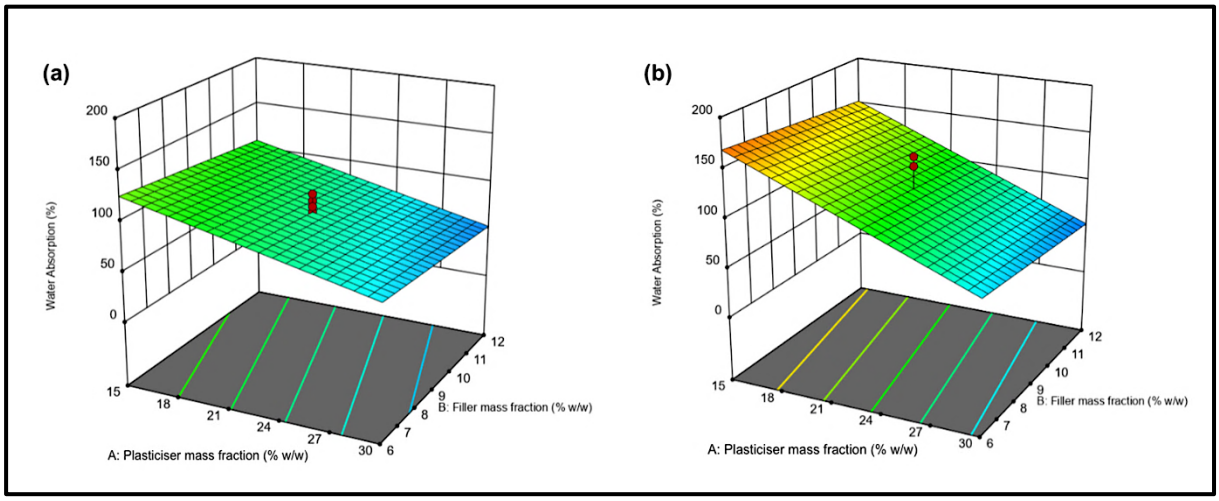

Figure 4-5: Response surface plots of the effects of plasticiser mass fraction and filler mass fraction on water absorption using (a) spillage and (b) extract.

4.4. Formulation factors on bioplastic film properties

The study showed that plasticiser mass fraction, filler mass fraction, and starch type significantly influenced the bioplastic film properties. Plasticiser and filler mass fractions had a significant impact on tensile strength and elongation at break. A five-factor, two-level fractional factorial experimental design was employed to investigate the influence of starch type, filler type and mass fraction, plasticiser mass fraction, and the presence of acetic acid. Among the factors explored, starch type was critical. Films made from spillage starch demonstrated superior tensile strength, reaching up to 14 MPa, compared to 10.5 MPa for extract-based films. This makes spillage starch particularly advantageous for applications

requiring high durability and mechanical resistance, such as packaging materials and construction components.

However, while spillage-based films excelled in tensile strength, extract-based films exhibited greater elongation at break up to 70% versus 40% for spillage films under optimal filler conditions. This highlights a trade-off where spillage starch offers strength, whereas extract starch provides enhanced flexibility and ductility. This difference allows for strategic formulation depending on the intended application. For example, extract-based films are better suited to uses where extensibility and toughness are prioritised over maximum strength, such as in flexible packaging or deformable films.

Water absorption characteristics also favoured spillage starch, particularly at 30% glycerol content, where both starch types showed similar absorption rates around 50%. However, extract-based films exhibited a much broader absorption range up to 160% which could cause swelling and mechanical degradation in moisture sensitive environments. Thus, spillage-based films may be better suited to applications demanding water resistance, including food packaging coatings and construction materials.

Interestingly, acetic acid was found to have no significant effect on any measured properties, suggesting its exclusion from further formulation studies. This omission is expected to simplify production and reduce raw material costs without compromising film performance. From a production viewpoint, spillage starch offers an advantage as it can be used in its native form without requiring extraction or purification, reducing processing time, energy consumption, and overall costs. In contrast, extract starch requires a time-consuming extraction process and increased costs. Additionally, extract-based films often demand higher modifier usage to match performance, further elevating costs. Their higher moisture absorption also complicates storage and handling, indirectly affecting cost-effectiveness.

The differences between extract and spillage-based bioplastics confirm that raw material selection is critical to final film performance. The optimum mechanical properties for the bioplastic synthesis were identified through analysis using Design-Expert software. As shown in **Table 4-5**, parameter constraints were systematically adjusted to maximise tensile strength and elongation at break while minimising water absorption. For the choice of spillage starch reinforced by the cellulose-lignin mixture, the optimal formulation for determined to consist of 29.8% glycerol, 12% mixture filler (w/w of dry starch), and no acetic acid to achieve a bioplastic film exhibiting a tensile strength of 9.7 MPa, elongation at break of 24.9%, and water absorption of 54.2%, as detailed in **Table 7-1** in the **Appendix**.

Table 4-5: Constraints parameters for synthesis of bioplastic films.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Plasticiser mass fraction	is in range	15	30	1	1	3
B: Filler mass fraction	is in range	0	12	1	1	3
C: Acetic acid	is in range	0	2.5	1	1	3
D: Starch type	is equal to Spillage	Extract	Spillage	1	1	3
E: Filler type	is equal to Mixture	Cellulose	Mixture	1	1	3
Tensile Strength	maximise	1.6821	16.2522	1	1	5
Elongation at Break	maximise	1.66248	147.832	1	1	5
Water Absorption	minimise	35.123	184.254	1	1	4

The tensile strength of the formulated bioplastic falls within the range of typical LDPE (8 to 10 MPa) and exceeds that of thermoplastic starch-based films (3 to 10 MPa) and other bioplastic formulations tested in this study, which ranged from 1.4 MPa to 7.7 MPa. Although the elongation at break (24.9%) is significantly lower than that of LDPE (150 to 600%) and PCL (600 to 1000%), it surpasses the flexibility reported for thermoplastic starch (3%) and the more rigid formulations in this study, which exhibited elongation values as low as 6.1%. The water absorption of the optimal formulation (54.2%) is considerably higher than that of conventional plastics such as HDPE and LDPE (<0.01%) and PCL (~1%), but it demonstrates improved water resistance relative to several starch-based formulations in this study, which exhibited absorption values exceeding 100%. Overall, the optimal bioplastic film demonstrates a favourable balance between tensile strength and flexibility, with moderate water sensitivity. These properties suggest potential applications in dry or semi-dry environments, particularly for non-load-bearing rigid packaging, compostable containers, or agricultural films intended for use in low-humidity conditions.

4.5. Summary

This chapter explored the effects of starch type, filler type and mass fraction, plasticiser mass fraction, and acetic acid on the mechanical properties and water absorption of starch-based bioplastic films using a fractional factorial experimental design. The study revealed that spillage starch significantly enhanced tensile strength, reaching up to 14 MPa, compared to 10.5 MPa for extract-based starch, while extract-based films demonstrated greater elongation at break, up to 70%, compared to 40% for spillage, indicating a trade-off between strength and flexibility. Both filler and plasticiser mass fractions were found to significantly influence tensile strength and elongation. Water absorption was lower and more consistent in spillage-based films, with extract-based films exhibiting higher moisture uptake that could compromise their performance in humid environments. Acetic acid showed no significant effect on any properties and will therefore be excluded from further formulation optimisation in **Chapter 5**. The optimal formulation consisting of 29.8% glycerol, 12% cellulose-lignin filler by dry starch weight, and no acetic acid yielded a bioplastic film with a tensile strength of 9.7 MPa, elongation at break of 24.9%, and water absorption of 54.2%. This formulation demonstrated mechanical properties superior to many starch-based bioplastics and comparable tensile strength to conventional plastics such as LDPE.

Additionally, the use of spillage starch reduces processing complexity and costs by eliminating extraction steps, supporting more sustainable and economically viable bioplastic production. Overall, the findings highlight spillage starch-based bioplastics as promising materials for applications requiring a balance of strength and flexibility in low-moisture environments, such as packaging and agricultural films, and lay a foundation for the final phase of study focusing on further optimisation of glycerol and filler content.

4.6. References

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Chapter 5: Optimisation of plasticiser and canola fines (reinforcement) mass fractions for enhanced mechanical properties of starch-based bioplastic films

5.1. Introduction

The current study aimed to explore the potential of canola fines as a reinforcing material to improve mechanical properties and water absorption of starch-based bioplastic films. Canola fines, a fibrous by-product rich in lignocellulosic, consists mainly of straw-like fragments and other plant-derived debris removed during the primary and secondary stages of canola seed sorting. Canola fines like any lignocellulose-based materials, consist of a rigid structure made up of cellulose, hemicellulose, and lignin (lignin = 23.0%, hemicellulose = 15.9%, cellulose = 21.4%) (Ranjan et al., 2024). They represent an ideal agro-industrial substrate for beneficiation in South Africa due to the year-round storage and processing of oilseeds in silos, ensuring a consistent and non-seasonal supply of this lignocellulosic by-product (Ranjan et al., 2024). The relative amounts of cellulose, hemicellulose, and lignin vary depending on the biomass source, influencing their reinforcing potential (Haq et al., 2021; Huang et al., 2021). Lignocellulosic fibres are good as reinforcing materials due to their availability and biodegradability (Abe et al. 2021). Fitch-Vargas et al. (2019) studied acetylated corn starch bioplastics reinforced with sugarcane bagasse fibres and found that the composite exhibited improved mechanical strength and enhanced water resistance. Another study by Wu et al. (2018) reported the use of corn straw as a reinforcement for starch bioplastics, resulting in enhanced mechanical properties.

The study in **Chapter 4** demonstrated that spillage-based bioplastic films demonstrated superior tensile strength and water absorption to extract-based bioplastic films, while glycerol was shown to improve its elongation. Furthermore, the mixture filler demonstrated positive effects of elongation as opposed to cellulose filler alone. Therefore, this chapter focused on developing spillage-based bioplastic films with glycerol as a plasticiser to improve the mechanical properties and water absorption of starch-based bioplastic films. Canola fines contain the same cellulose (%) and lignin (%) contents as the mixture filler used in **Chapter 4**. By systematically investigating the effects of glycerol as a plasticiser and canola fines as a reinforcing material on spillage-based bioplastic films, this research aimed to optimise the mechanical properties and water absorption characteristics of starch-based bioplastics.

The study employed response surface methodology (RSM) to model and optimise the interactions between these variables, with the goal of developing bioplastics that can meet

specific performance requirements for various industrial applications. This approach aimed to address the key challenges in achieving materials that can match the performance of conventional plastics while offering environmental benefits. By focusing on the valorisation of agricultural by-products and optimising the balance between plasticiser and reinforcement contents, this research contributes to the broader effort of developing sustainable alternatives to conventional plastics that are both environmentally friendly and commercially viable.

Previous studies have demonstrated that plasticisers such as glycerol, sorbitol, and fructose, coupled with reinforcing materials (fillers) from natural plant fibres like cassava, arrowroot, and maize residues can significantly improve the mechanical properties (tensile strength and elongation at break) and water resistance of starch-based bioplastics, thereby enhancing their functionality for many applications (Rendón-Villalobos et al., 2022; de Azevedo et al., 2020). Specifically, de Azevedo et al. (2020) noted that the inclusion of glycerol enhances flexibility and reduce brittleness, thereby rendering the bioplastic films more applicable across various industries. Furthermore, exploring the role of agricultural by-products offers an innovative pathway to augment both the mechanical properties and water absorption of starch-based bioplastic films (Safitri et al., 2022).

Although significant progress has been made, finding the optimal balance between the plasticiser and reinforcement contents remains a key challenge in achieving materials that match the performance of conventional plastics (Shafqat et al., 2021; Versino & García, 2014). Therefore, the aim of this study was to model and optimise the effects of glycerol and canola fines on the mechanical (tensile strength and elongation at break) and water absorption properties of spillage-based bioplastic films.

5.2. Materials and methods

5.2.1. Raw materials

Collection of maize spillage and canola fines are described in **Section 3.2.1** and Ranjan et al. (2024), respectively. Canola fines were selected due to its lignocellulosic fibre content to evaluate its effectiveness as a potential reinforcing material for bioplastic films, comparative to the mixture filler (23% lignin and 21% cellulose) as outlined in **Chapter 4**. Analytical-grade glycerol (cat no: G5516) was procured from Merck (Darmstadt, Germany).

5.2.2. Canola fines preparation

The size of the canola fines was initially reduced to shorter sticks of approximately 5 to 9 mm, followed by grinding them in a Bennett Reed (Tevo, South Africa) 1 200 W food processor to

increase the surface area of the sample. The canola fines were then sieved through a set of sieve trays to ensure that the particles were reduced to 200 μm to 1 mm. **Figure 5-1** illustrates the sample preparation of the canola fines.

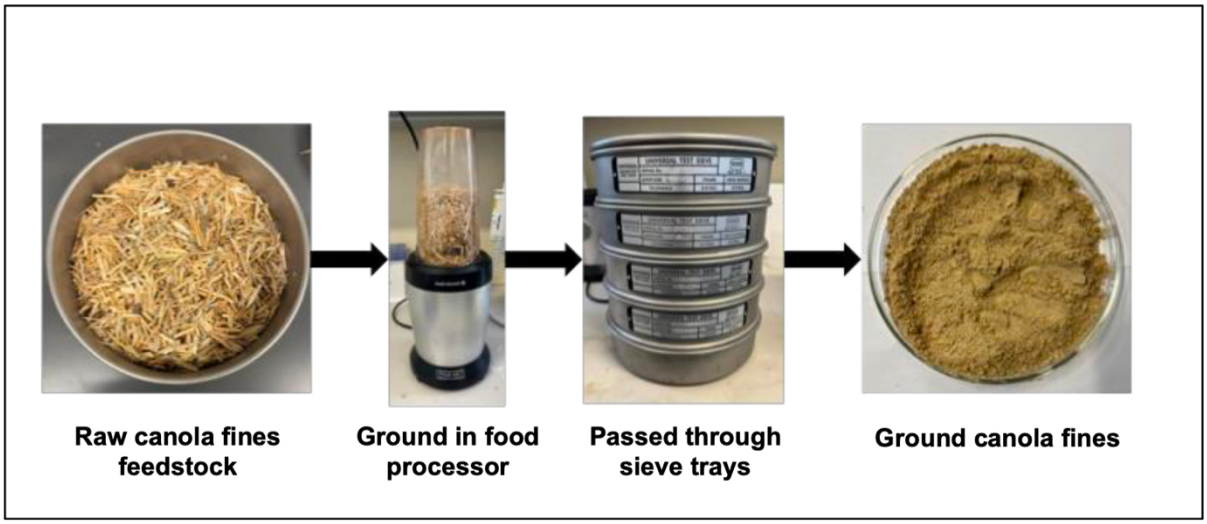

Figure 5-1: Canola fines sample preparation.

5.2.3. Experimental design

Response surface methodology (RSM) was used to evaluate the effects of 2 numeric factors, namely plasticiser mass fraction (19.5 to 50 % w/w dry starch) and filler mass fraction (0 to 20 % w/w dry starch) on 3 responses, namely tensile strength (MPa), elongation at break (%), and water absorption (%). The RSM was based on a central composite design (CCD) with 13 runs. Additional runs were added to strengthen the predictability of the model and increase the robustness of the response surface model, resulting in a total of 20 experimental runs. This allowed for the effective optimisation of these formulations for the desired properties. The experimental design generated using Design Expert Software version 10.0 (Stat-Ease Inc, Minneapolis, USA) is illustrated in **Table 5-1**. The Analysis of Variance (ANOVA) was used to assess the effect of each factor on the 3 responses.

5.2.4. Synthesis of bioplastic films

A series of spillage-based bioplastic films were synthesised based on the traditional casting method described in **Section 4.2.6**. Varying mass fractions of the plasticiser (glycerol) and the filler (canola fines), expressed as percentage of dry starch (w/w), were incorporated into the casting solution according to the CCD outlined in **Table 5-1.**

5.2.5. Characterisation of bioplastic films

The bioplastic films were characterised by tensile strength, elongation at break, and water absorption. The methodologies for these are as follows:

5.2.5.1. Tensile strength and elongation at break

The mechanical properties of the samples, specifically tensile strength and elongation at break, were measured according to the methodology outlined in **Section 4.2.7.1**.

5.2.5.2. Water absorption

The water absorption of the bioplastic films was measured according to the methodology outlined in **Section 4.2.7.2**.

5.3. Results and discussion

The following **Table 5-1** shows the experimental design and outcome of the experiments. Due to its insignificant effect in **Chapter 4**, acetic acid was not investigated further, and filler type was maintained as canola fines.

Table 5-1: Experimental design matrix showing results for bioplastic film synthesis.

Run	Factors		Responses		
	Plasticiser (% w/w dry starch)	Filler (% w/w dry starch)	Tensile strength (MPa)	Elongation at break (%)	Water absorption (%)
1	22.5	15	5.70	12.40	65.37
2	30	10	3.00	41.81	78.24
3	30	10	3.22	39.18	78.04
4	30	10	3.26	41.76	79.80
5	50	10	1.37	45.98	39.50
6	19.5	10	7.67	6.12	113.48
7	30	20	3.12	7.87	81.07
8	37.5	5	2.29	50.96	60.72
9	30	10	3.08	49.85	77.95
10	37.5	15	2.37	31.72	61.03
11	30	0	2.57	146.05	109.58
12	22.5	5	5.41	11.92	142.76
13	30	10	3.17	42.51	80.85
14	37.5	15	2.34	32.58	64.95
15	37.5	15	2.41	30.86	57.12
16	22.5	0	5.51	115.52	143.50
17	37.5	0	2.59	108.39	62.36
18	22.5	15	6.86	13.74	66.64
19	22.5	15	4.45	11.07	64.11
20	22.5	0	4.79	125.52	143.04

5.3.1. Bioplastic synthesis

The synthesis of spillage-based bioplastic films reinforced with canola fines rich in lignocellulosic fibre was carried out, resulting in thin bioplastic films, as shown in **Figure 5-2**. The composition of materials in bioplastics has an impact on its morphology and bioplastic performance characteristics. The effect of varying glycerol and canola fines on the mechanical

properties and water absorption of the bioplastic films is provided in **Table 5-1**. Bioplastic films with various levels of rigidity and flexibility were obtained.

The shrinkage behaviour of starch-based bioplastic films reinforced with lignocellulosic fibres during drying, as evident in the selected films presented in **Figure 5-2**, arises from multifaceted physicochemical interactions. As water evaporates from the film, capillary forces develop within the porous starch-fibre network, pulling polymer chains closer together and causing shrinkage. This effect stems from the dual-pore structure in starch-based films, where both inter-granular and intra-granular pores undergo dehydration, generating significant internal stress (Goehring, 2009). Shrinkage can also be influenced by binder/fibre migration which occurs as starch redistributes toward evaporating surfaces, creating uneven reinforcement gradients and differential shrinkage across the film thickness, particularly under accelerated drying conditions (Jayarathna et al., 2022; Yang et al., 2019). 25 28

As with the previous experiment (**Chapter 4**), the drying temperature was also maintained at ambient conditions of 27°C within an oven dryer. Although rapid heating was avoided, the presence of a fan likely influenced the drying process by enhancing air circulation during the drying stage. Collier et al. (2022) investigated the effects of different drying methods on potato starch-based bioplastic films and found that using a waxed paper lid and petri dish lid, combined with hybrid natural and forced convection drying, significantly reduced shrinkage and warping of the films. These interconnected mechanisms highlight the delicate balance between processing parameters and material interactions in controlling dimensional stability.

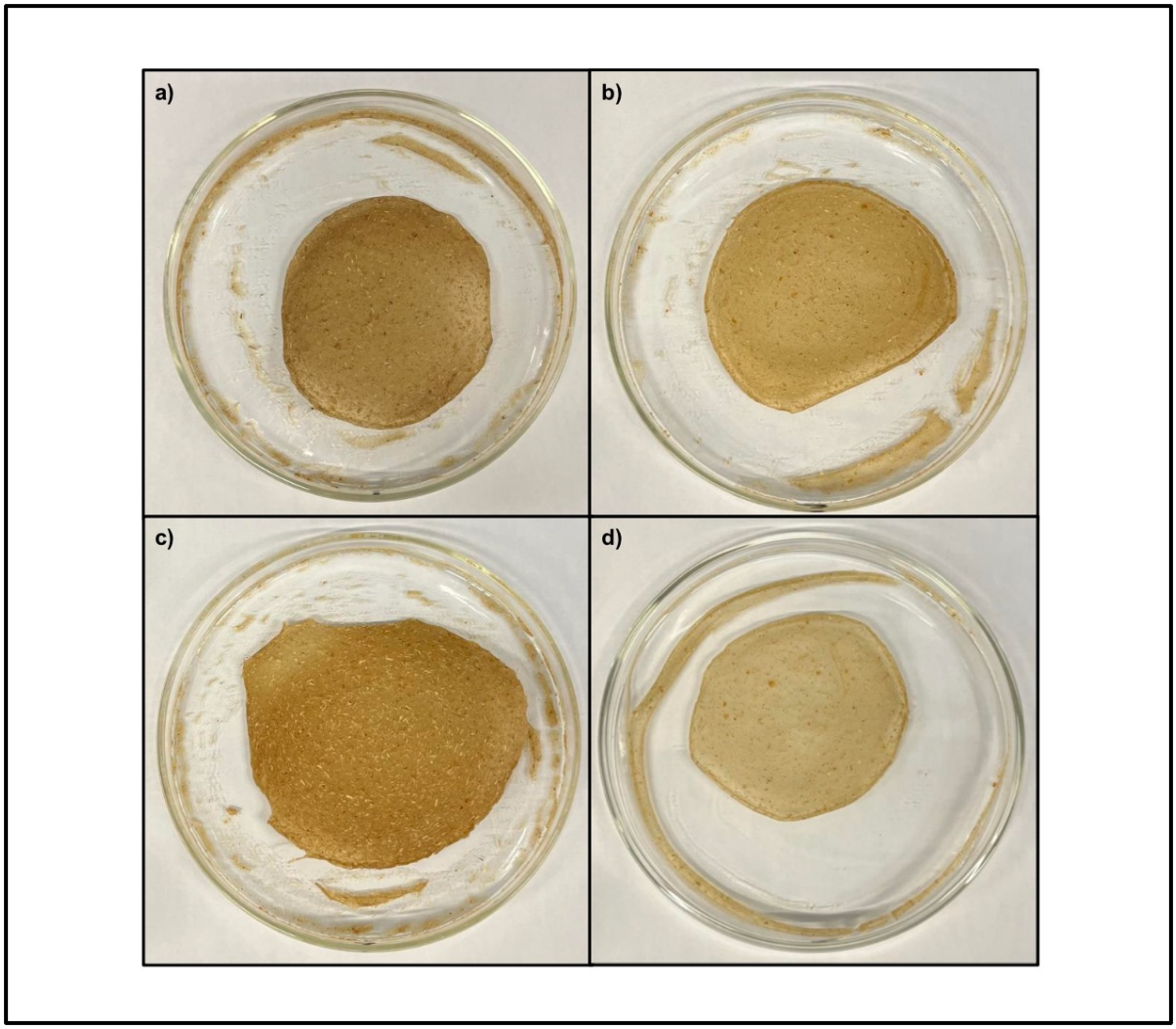

Figure 5-2: Examples of spillage-based bioplastic films reinforced with canola fines according to (a) run 1, (b) run 3, (c) run 7, and (d) run 8.

5.3.2. Effect of plasticiser and reinforcement mass fractions on the properties of synthesised bioplastic films

5.3.2.1. Tensile strength

The ANOVA results presented in **Table 5-2** indicate that the plasticiser mass fraction has a statistically significant effect on the tensile strength of starch-based bioplastic films ($p<0.0001$). As shown in **Figure 5-3a**, tensile strength decreases with increasing plasticiser content. This result is attributed to the action of glycerol, which disrupts intermolecular forces within the starch matrix, thereby enhancing flexibility but reducing the overall mechanical strength (Tarique et al., 2021; Darni et al., 2019; Harunsyah et al., 2017; Dias et al., 2010). Meanwhile, variations in filler content have negligible impact within the tested range. This effect may be due to the accumulation of the canola fines in the starch matrix. According to the response surface model presented in **Figure 5-3a**, the predicted maximum tensile strength in this study is approximately 5 MPa. This value is lower than the 14 MPa reported for the bioplastic reinforced with a mixture of cellulose and lignin in (**Section 4.3.1.1**). The relatively low tensile strength observed with canola fines reinforcement may be attributed to inherent poor interfacial adhesion between the fibres and the starch matrix, as well as fibre agglomeration, which can compromise stress transfer efficiency (Kong et al., 2023; Yang et al., 2019). This limitation could be addressed by treating the canola fines fibres before incorporating them into the starch matrix. Researchers have reported that treatments such as alkalisation or acetylation improve interfacial bonding between the fibre components and the starch matrix, thereby enhancing the tensile strength of bioplastics (Siddiqui et al., 2024; Thamarai Selvi et al., 2023; Diah et al., 2024).

In the broader context of existing polymeric materials (**Table 6-1**), predicted maximum tensile strength of 5 MPa observed in this study is notably inferior to that of typical commercial plastics. For example, high-density polyethylene (HDPE) typically exhibits tensile strengths between 21 and 52 MPa, while low-density polyethylene (LDPE) ranges from 8 to 10 MPa. Similarly, polycaprolactone (PCL), a biodegradable polymer known for its enhanced mechanical properties, demonstrates tensile strengths in the range of 15 to 41 MPa. This disparity underscores the inherent challenge in matching the robust mechanical strength of conventional petroleum-based plastics with starch-based bioplastics. However, the tensile strength range between 1.4 to 7.7 MPa achieved in this study (**Table 6-1**) is comparable to values reported for some thermoplastic starch materials (3 to 10 MPa) (Zhao et al., 2020), indicating that the developed bioplastic formulations perform competitively within this specific category. This suggests the potential applicability of these formulations in certain low-strength or non-structural applications where LDPE is commonly employed.

Table 5-2: ANOVA results for tensile strength

Tensile strength					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Block	0.0094	1	0.0094		
Model	0.2485	1	0.2485	297.73	< 0.0001
A-Plasticiser mass fraction	0.2485	1	0.2485	297.73	< 0.0001
Residual	0.0142	17	0.0008		
Lack of Fit	0.0090	10	0.0009	1.23	0.4023
Pure Error	0.0051	7	0.0007		
Corrected Total	0.2721	19			

5.3.2.2. Elongation at break

Both plasticiser and filler mass fractions significantly influenced elongation at break, indicated by p-values of < 0.0001 and 0.0043, respectively (**Table 5-3**). Furthermore, the interaction term between these variables also showed statistical significance (p-value < 0.05), indicating that the effect of glycerol mass fraction on elongation at break is influenced by the mass fraction of canola fines used, highlighting the synergistic effects between glycerol and canola fines in modifying film flexibility (**Table 5-3)**. These results are further supported by the response surface plot (**Figure 5-3b**) which demonstrates that increasing glycerol mass fraction enhances elongation at break significantly, particularly at lower mass fractions of canola fines. Glycerol acts as a plasticiser, reducing intermolecular forces within the starch matrix and increasing chain mobility, which results in improved flexibility.

However, excessive glycerol levels may lead to phase separation or over-softening, diminishing structural integrity (Mohammed et al., 2023; Marichelvam et al., 2019; Versino & García, 2014). At low glycerol levels, increasing canola fines slightly reduces elongation at break (**Figure 5-3b**). This is likely due to the reinforcing effect of canola fines, which introduces rigidity to the matrix and limits chain mobility. At higher glycerol mass fractions, the negative impact of canola fines on the elongation at break diminishes, evident by the increase in elongation at break between 30 to 37.5% glycerol in **Figure 5-3b**, suggesting a synergistic effect where glycerol compensates for the rigidity introduced by the canola fines. This behaviour is consistent with findings by Darni et al. (2019) who found that lower glycerol levels resulted in a slight decrease in elongation at break when systematically increasing sorghum stalk powder as the reinforcing material (0.25 to 1g) while higher glycerol levels of 15 to 20% resulted in increased elongation at break.

The ANOVA results (**Table 5-3**) reveal nonlinear effects, with significant squared and cubed terms for both factors (p-values<0.05). This indicates complex behaviour where optimal

elongation is achieved at moderate plasticiser and filler levels, particularly between 30 to 37% glycerol and between 10 to 13% canola fines. The response surface plot (**Figure 5-3b**) highlights this region of optimised glycerol and canola fines mass fractions at moderate levels to achieve maximum elongation at break. This region suggests the balanced combination of plasticiser and filler necessary to enhance flexibility without compromising on the structural integrity of these starch-based bioplastic films. The lack-of-fit p-value of 0.9643 (**Table 5-3**) suggests that the model fits the observed data well, providing reliable predictions for elongation performance under varying formulations.

Compared to the materials listed in **Table 6-1**, the bioplastic films produced in this study especially those from Runs 11, 16, and 20 exhibit notably high elongation at break values, ranging from 115.5% to 146%, indicating high flexibility. While these values are generally lower than the broad range of typical LDPE (150 to 600%) and substantially less than the highly ductile PCL (600 to 1000%) (Gupta, 2011), they represent a notable improvement over other reported starch-based materials such as thermoplastic starch (3%) (Zhao et al., 2020) and avocado seed starch and yarn (3.1%) (Tesfaye et al., 2018). This indicates that the plasticiser and filler optimisation effectively enhance the flexibility of starch-based bioplastic films for certain applications. Although the developed films do not yet match the extreme stretchability of conventional HDPE and LDPE, their performance approaches the lower limit of commercial LDPE, demonstrating promising ductility. The synergistic effect identified between glycerol and canola fines is crucial for developing films with balanced mechanical properties, advancing them towards the flexibility required for certain applications currently dominated by LDPE.

Table 5-3: ANOVA results for elongation at break

Elongation at break					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Block	0.0045	1	0.0045		
Model	0.1574	9	0.0175	231.46	< 0.0001
A-Plasticiser mass fraction	0.0079	1	0.0079	104.77	< 0.0001
B-Filler mass fraction	0.0011	1	0.0011	14.29	0.0043
AB	0.0060	1	0.0060	79.32	< 0.0001
A^2	0.0066	1	0.0066	87.83	< 0.0001
B^2	0.0032	1	0.0032	42.93	0.0001
A^2B	0.0010	1	0.0010	13.81	0.0048
AB^2	0.0062	1	0.0062	82.54	< 0.0001
A^3	0.0011	1	0.0011	14.20	0.0044
B^3	0.0082	1	0.0082	108.95	< 0.0001
Residual	0.0007	9	0.0001		
Lack of Fit	7.027E-06	2	3.513E-06	0.0366	0.9643
Pure Error	0.0007	7	0.0001		

5.3.2.3. Water absorption

The ANOVA results (**Table 5-4**) demonstrated that both plasticiser and filler mass fractions significantly impact water absorption, with p-values < 0.0001 for both factors. Higher plasticiser levels increased water absorption due to the hydrophilic nature of glycerol, which facilitates moisture uptake (Versino & García, 2014). In contrast, increasing the filler mass fraction reduced water absorption as the lignocellulosic fibre from canola fines act as barriers to water penetration. Nonlinear effects were observed, with significant squared and cubed terms for both factors (p values < 0.05), indicating that water absorption behaviour is influenced by complex interactions between plasticiser and filler mass fraction. The response surface plot (**Figure 5-3c**) illustrates this relationship, showing reduced water absorption at higher filler levels combined with moderate plasticiser levels. This outcome is particularly relevant for enhancing bioplastic durability in humid environments, which is a critical consideration in their industrial application. The ability to vary water absorption through chemical composition has been previously mentioned by Gadhave et al. (2018), who emphasised that optimising filler mass fraction can successfully reduce hydrophilicity, thereby enhancing the overall usability of bioplastics.

Despite these advancements in varying water absorption, a critical comparison with conventional plastics (**Table 6-1**) reveals a persistent challenge. Typical HDPE and LDPE exhibit extremely low water absorption, generally less than 0.01%. In contrast, the starch-

based bioplastic films developed in this study demonstrated significantly higher water absorption rates, ranging from 39.5% to 143.5%. This distinct hydrophilicity, largely resulting from the glycerol content and inherent nature of starch, remains a major limitation for applications requiring sustained moisture resistance (Gupta, 2011). While increasing filler mass fraction evidently reduced water absorption and run 5 shows a comparatively lower absorption of 39.5% (**Table 6-1)**, these values still far exceed those of conventional polyethylenes and even other bioplastics like PCL (~1%) (Gupta, 2011) and thermoplastic starch (4 to 8%) (Zhao et al., 2020). This necessitates careful consideration of the intended application environment and may restrict the use of these films to dry or short-term exposure scenarios.

Table 5-4: ANOVA results for water absorption

	Water absorption				
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Block	64.81	1	64.81		
Model	17658.50	8	2207.31	353.01	< 0.0001
A-Plasticiser mass fraction	1694.45	1	1694.45	270.99	< 0.0001
B-Filler mass fraction	2074.46	1	2074.46	331.76	< 0.0001
AB	2753.20	1	2753.20	440.31	< 0.0001
A^2	4.15	1	4.15	0.6644	0.4340
B^2	46.42	1	46.42	7.42	0.0214
AB^2	318.52	1	318.52	50.94	< 0.0001
A^3	94.72	1	94.72	15.15	0.0030
B^3	393.06	1	393.06	62.86	< 0.0001
Residual	62.53	10	6.25		
Lack of Fit	21.92	3	7.31	1.26	0.3594
Pure Error	40.61	7	5.80		
Corrected Total	17785.83	19			

5.3.3. Bioplastic synthesis optimisation

The high predicted R^2 values of 0.9261, 0.8872, and 0.9265 (**Table 5-5**) for tensile strength, elongation at break and water absorption, respectively, demonstrate that the developed models possess strong predictive capability when forecasting bioplastic properties under new experimental conditions (**Table 5-5**). This predictive power is particularly valuable when optimising bioplastic formulations or scaling up production by enabling reliable material property estimation without exhaustive experimentation. The adjusted R^2 values of 0.9428, 0.9914, and 0.9936 (**Table 5-5**), respectively, indicate that the models explain over 94 to 99 %

of the observed variability in the data while accounting for the number of predictors used. These statistics reflect the models' ability to capture the complex relationships between critical factors influencing bioplastic synthesis, particularly the glycerol plasticiser and canola fines filler mass fractions.

Additionally, the models yield high adequate precision values of 40.71, 49.74, and 59.19 (**Table 5-5**) for tensile strength, elongation at break, and water absorption, respectively. Such values signify excellent signal-to-noise ratios, affirming that the models can reliably differentiate between diverse bioplastic formulations within the experimental design space. Such precision is vital in bioplastics research and production, where minor variations in composition can substantially impact mechanical properties and water resistance. Consequently, this level of precision provides a robust foundation for fine-tuning formulation parameters, enabling the optimisation of processes to enhance bioplastic performance and meet specific application requirements.

Table 5-5: Summary of the statistical results of the fitted models on bioplastic film synthesis

Models	Standard deviation	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
Tensile strength linear	0.0289	0.9460	0.9428	0.9261	40.7119
Elongation at break cubic	0.0087	0.9957	0.9914	0.8872	49.7390
Water absorption cubic	2.50	0.9965	0.9936	0.9265	59.1862

The optimum mechanical properties for the spillage-based bioplastic synthesis were identified through analysis using Design-Expert software. As shown in **Table 5-6**, parameter constraints were systematically adjusted to maximise tensile strength and elongation at break while minimising water absorption, thereby achieving a balanced performance profile. The optimal formulation was determined to consist of 30.39% glycerol and 13.67% canola fines filler (w/w relative to dry starch) to achieve a bioplastic film exhibiting a tensile strength of 3.29 MPa, elongation at break of 52.11%, and water absorption of 65.19%, as detailed in **Table 5-7**. This formulation highlights the intrinsic balance between tensile strength, elongation at break, and water absorption characteristic of spillage-based bioplastics. These results demonstrate the efficacy of the optimised glycerol and filler mass fractions in enhancing the mechanical performance of starch-based films while controlling water absorption, thereby providing a foundation for further development and potential practical application. For additional visualisation of these optimal conditions, the contour plots presented in **Table 6-1.** Furthermore, **Figure 7-9** in the **Appendix** offer a detailed graphical representation.

Table 5-6: Constraints parameters for synthesis of spillage-based bioplastic films.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Plasticiser mass fraction	in range	22.5	37.5	1	1	3
B: Filler mass fraction	in range	0	15	1	1	3
Tensile Strength	minimise	1.4	7.7	1	1	3
Elongation at Break	minimise	6.1	146.1	1	1	3
Water Absorption	minimise	39.5	143.5	1	1	3

Table 5-7: Results of optimum solution for synthesis of spillage-based bioplastic films

Number	Plasticiser mass fraction	Filler mass fraction	Tensile strength	Elongation at break	Water absorption	Desirability	
1	**30.389**	**13.673**	**3.289**	**52.109**	**65.189**	**0.723**	**Selected**
2	30.410	13.661	3.285	52.283	65.174	0.723	
3	35.916	0.000	2.486	115.922	72.891	0.662	
4	36.000	0.000	2.476	115.545	72.449	0.662	
5	35.833	0.000	2.496	116.290	73.322	0.662	

The tensile strength value of 3.3 MPa at this optimum aligns with ranges reported for other native starch-plasticiser matrices, such as thermoplastic starch films exhibiting strengths between 3 and 10 MPa (Zhao et al., 2020). However, this value remains substantially lower than that of reinforced starch films, including those incorporating tea polyphenol additives, which have reported an optimum tensile strength of 17.8 (Feng et al., 2018), or cassava-based films with increased starch content achieving up to 20.9 MPa (Harunsyah et al., 2017).

Regarding elongation at break, the optimised value of 52% represents a moderate flexibility relative to broader literature values. It falls within optimum elongation percentages reported for simpler matrices, which typically range between 3.8% and 137% (Agustin, 2014; Żołek-Tryznowska & Kałuża, 2021), specifically exceeding the low elongation values (3.1%) observed for starch films derived from avocado seed and yarn (**Table 6-1)** (Tesfaye et al., 2018). However, it remains inferior to more ductile materials such as cassava starch films exhibiting elongations as high as 122.8% at a glycerol level of 30% and filler loading of 6% (Harunsyah et al., 2017).

Consequently, the optimum conditions reported for tensile strength and elongation at break in those works were determined without accounting for the limiting effects of water absorption.

In contrast, this study considers water absorption as a critical factor, which impacts the optimisation of mechanical properties and provides a more comprehensive evaluation of bioplastic performance.

Water absorption at the optimised formulation was measured at 65%, indicating the persistent challenge of hydrophilicity inherent to starch-based bioplastics. This value is considerably lower than those reported for highly hydrophilic materials, such as arrowroot starch films reinforced with arrowroot fibre, which exhibit water absorption values ranging from 89 to 190% (Tarique et al., 2021). However, it remains distinctly higher than conventional synthetic polymers, such as HDPE and LDPE, which typically exhibit water absorption below 0.01%. The reduced water absorption at moderate plasticiser and filler levels highlights the barrier effect of canola fines, consistent with findings by Gadhave et al. (2018), who demonstrated that optimising filler content can effectively mitigate hydrophilicity in bioplastics.

When benchmarked against conventional synthetic polymers summarised in **Table 6-1**, the optimised bioplastic exhibits a tensile strength of 3.3 MPa, which is considerably lower than that of common plastics such as HDPE (21 to 52 MPa), LDPE (8 to 10 MPa), and specialty PCL packaging materials (15 to 41 MPa). This substantial difference reflects the well-known trade-off in starch-based bioplastics between enhanced biodegradability and reduced mechanical robustness. Despite this limitation, starch-based bioplastics offer significant environmental sustainability benefits that synthetic polymers cannot match. In terms of elongation at break, the optimised bioplastic demonstrates moderate flexibility, but it is notably less ductile than typical synthetic plastics. HDPE shows a very broad elongation range of 10% to 500%, while LDPE exhibits even greater flexibility with elongation values from 150% to 600% (**Table 6-1**). PCL films, although high in tensile strength, show exceptional ductility with elongation ranging from 600% to 1000% (**Table 6-1**). These comparisons highlight the ongoing challenge in bioplastic development: achieving mechanical flexibility comparable to conventional polymers while preserving biodegradability.

The desirability index of 0.723 (**Table 5-7**) reflects a favourable but not perfect optimisation, highlighting the natural trade-offs that occur when simultaneously balancing tensile strength, elongation at break, and water resistance. Such trade-offs are commonly observed in starch-based bioplastic research, where improved mechanical and water resistance properties are often obtained at intermediate levels of plasticiser and filler rather than their extremes (Harunsyah et al., 2017; Zhao et al., 2020). For instance, Harunsyah et al. (2017) reported substantial increases in film flexibility at higher glycerol contents, though accompanied by reduced tensile strength and increased water absorption.

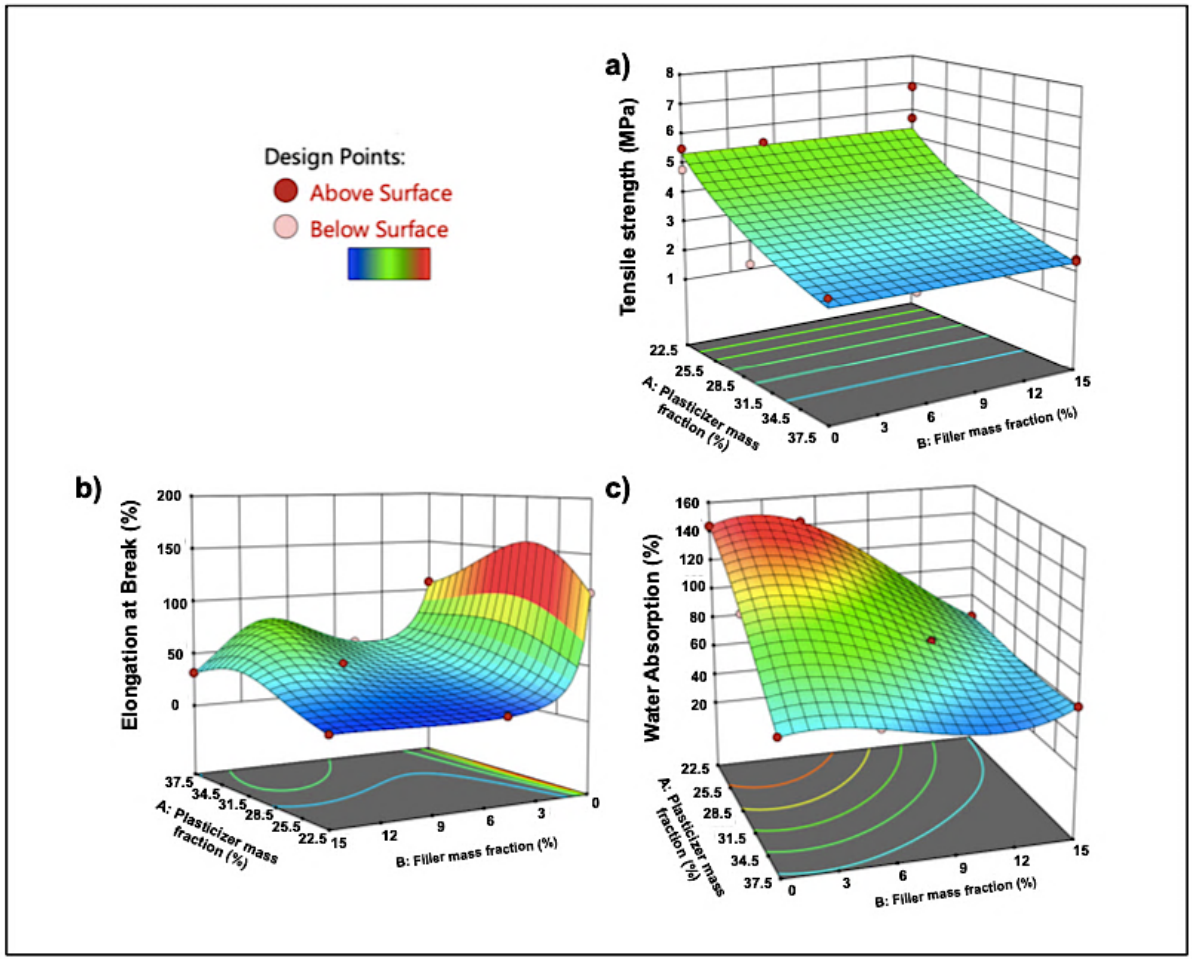

Figure 5-3: Response surface plot of the effect of plasticiser mass fraction and filler mass fraction on the three responses.

5.4. Summary

This chapter explored the synthesis and characterisation of starch-based bioplastics, focusing on the effects of varying plasticiser and filler mass fractions on their mechanical properties and water absorption behaviour. Using response surface methodology (RSM), a comprehensive statistical analysis was conducted to evaluate how these two factors influence tensile strength, elongation at break, and water absorption. The analysis of variance (ANOVA) revealed that both plasticiser and filler contents, as well as their interaction, had statistically significant effects on the target responses. The presence of significant nonlinear (squared and cubed) terms indicated complex relationships, suggesting that optimising these parameters is essential for achieving enhanced material performance.

The synthesis and characterisation of bioplastics was explored, examining how varying mass fractions of plasticisers and fillers influence their mechanical properties and water absorption characteristics. Utilising response surface methodology (RSM), a comprehensive statistical analysis was conducted to evaluate the effects of the two factors on tensile strength, elongation at break, and water absorption. In particular, the ANOVA results revealed strong statistical significance for both factors and their interaction effects, highlighting the complex interplay between these variables. Nonlinear effects, as evidenced by significant squared and cubed terms in the analysis, further emphasised that optimal mass fractions could lead to enhanced performance characteristics, particularly in terms of elongation and resistance to water absorption.

Furthermore, the response surface plots, and contour plots provided valuable insights into the relationships between the mass fractions of plasticiser and filler, identifying the optimal formulation for maximising desired responses. The optimum tensile strength, elongation at break, and water absorption was achieved at a glycerol mass fraction of 30% (w/w dry starch) and a canola fines filler mass fraction of 13.5% (w/w dry starch). At this optimum point, tensile strength, elongation at break, and water absorption are 3.3 MPa, 52%, and 65%, respectively.

The results demonstrate that canola fines can serve effectively as a natural reinforcement in starch-based bioplastics, while glycerol contributes to flexibility and processability. Together, these components enable the development of biodegradable films with a favourable balance of mechanical performance and moisture resistance. However, further optimisation may be needed to improve tensile strength without compromising elongation or water resistance. The ability to manipulate bioplastic formulations through strategic adjustments of plasticiser and filler content presents significant opportunities for advancing sustainable material solutions. It is concluded that further optimisation of the canola fines reinforcement mass fraction particularly at lower concentrations along with plasticiser, is necessary to enhance the tensile

strength and scalability of the bioplastic formulation. Additionally, to improve fibre-starch matrix interactions and enhance the overall performance of starch/canola fines bioplastics, it will be necessary to consider targeted processing of canola fines. This includes strategies such as alkaline treatment to improve interfacial adhesion, as well as optimisation of fibre size to promote better dispersion and compatibility within the starch matrix.

Importantly, the non-seasonal availability of canola fines across South Africa presents a reliable and sustainable raw material source as a reinforcement material for bioplastics. Furthermore, starch is directly obtained from maize agro-industrial spillage waste, eliminating the need for extraction and thereby significantly reducing raw material costs. These factors support the development of an economically viable and environmentally sustainable bioplastic system.

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Chapter 6: Conclusion and recommendations

6.1. Conclusion

This research successfully demonstrates the synthesis and characterisation of bioplastic films using starch-rich waste derived from maize wet-milling. The study highlights the potential of valorising agricultural waste not only as a means of waste reduction but also as a sustainable alternative to conventional plastic materials. By emphasising the environmental benefits of bioplastics, this thesis contributes to the ongoing discussion surrounding the necessity for more sustainable production practices in the bioplastic field. The experiments conducted utilised varying starch types, plasticisers, and reinforcement materials, revealing significant insights into the relationships between these components and the mechanical and water resistance properties of the bioplastics produced.

Through the application of a factorial design model, the effects of five factors, including starch type, filler type, plasticiser mass fraction, filler mass fraction, and acetic acid volume, were systematically investigated with respect to tensile strength, elongation at break, and water absorption of starch-based bioplastic films. Acetic acid was found to have no statistically significant effect on any of the measured properties. Considering both performance characteristics and operational costs, spillage starch was identified as the superior raw material, offering enhanced tensile strength and reduced moisture uptake compared to extract starch. The optimal formulation was determined to consist of 29.8% glycerol and 12% mixture filler (w/w of dry starch), without the addition of acetic acid.

This formulation yielded a bioplastic film with a tensile strength of 9.7 MPa, elongation at break of 24.9%, and water absorption of 54.2%, demonstrating a desirable balance of mechanical robustness, moderate flexibility, and controlled moisture uptake. These findings underscore the importance of starch source and plasticiser-filler optimisation in tailoring bioplastic performance. Furthermore, eliminating acetic acid from the formulation simplifies the production process and reduces raw material costs without compromising key material properties. This study provides a valuable framework for efficient and cost-effective development of starch-based bioplastics with the specific performance requirements of intended applications.

The application of response surface methodology (RSM) facilitated the optimisation of synthesis conditions, leading to enhanced mechanical performance, specifically in terms of tensile strength and elongation at break, and water absorption. The findings indicate that the incorporation of canola fines, rich in lignocellulosic fibre, improves not only the structural

integrity but also the durability of the bioplastic films, making them suitable for a variety of applications. The optimum synthesis conditions were established at a glycerol mass fraction of 30% (w/w dry starch) and a canola fines filler mass fraction of 13.5% (w/w dry starch), resulting in a tensile strength of 3.3 MPa, elongation at break of 52%, and water absorption of 65%. Moreover, the use of local agricultural waste helps mitigate the challenges associated with high feedstock costs and food security, aligning with broader sustainability goals.

Building on the findings of this study, future research should explore the effects of lower acetic acid concentrations, particularly in the range reported by Ansanay et al. (2024), to thoroughly investigate how different concentrations of acetic acid might affect mechanical and water resistance properties. Additionally, investigations into the long-term durability, biodegradation behaviour, and environmental performance of optimised spillage starch-based films would further validate their suitability for commercial applications. From a practical standpoint, the demonstrated elimination of acetic acid simplifies formulation and reduces production costs, making these bioplastics a more viable option for sustainable packaging, agricultural films, and other industrial uses that demand a balance of mechanical strength and water resistance.

Table 6-1: Comparison of bioplastic properties and potential applications from this study with literature findings

Material	Tensile strength (MPa)	Elongation at break (%)	Water absorption (%)	Potential applications
Typical HDPE (Darni et al. 2019; (Alabi et al., 2019; Rosato et al., 2004)	21 - 52	10 - 500	< 0.01	Grocery bags, detergent bottles, milk and juice bottles, agricultural piping, and crates
Typical LDPE (Alabi et al., 2019; Gupta, 2011; Rosato et al., 2004)	8 - 10	150 - 600	< 0.01	Cling film, sandwich bags, garbage bags, mulch films, squeeze bottles
PCL (Gupta, 2011)	15 - 41	600 - 1000	~1%	Biodegradable specialty packaging, medical devices
Thermoplastic starch (Zhao et al., 2020)	3 - 10	3	4 - 8	Films used for fresh produce and meat, packaging in form of film, edible film, bubble wrap, sheet, foam
Starch film incorporated with tea polyphenol (Feng et al., 2018)	16 - 20	64 - 74	-	Active food packaging
Avocado seed starch and yarn (Tesfaye et al., 2018)	2.6	3.1	-	Textiles
Corn starch/elephant grass (Debnath et al., 2022)	6.04 - 22.3	33.39 - 3.49	10.66 - 16.17	Packaging material
This study (Runs 2, 3, 4, 9, 13)	3.0 - 3.3	39.2 - 49.9	78 - 80.9	Moderate strength and flexibility - suitable for non-load-bearing flexible films.
This study (Runs 1, 6, 18)	5.7 - 7.7	6.1 - 13.7	65.4 - 113.5	Best for rigid dry-use (higher tensile strength but low flexibility) - containers, trays
This study (Runs 11, 16, 20)	2.6 - 5.5	115.5 - 146	109.6 - 143.5	Highly flexible but water sensitive High elongation, poor water resistance) - compostable bags, agricultural films if kept dry
This study (Run 5)	1.4	46	39.5	Although weak, its low water absorption could be useful for labels, barrier wraps

HDPE = High-density polyethylene; LDPE = Low-density polyethylene; PCL = Polycaprolactone
Runs refer to those presented in **Table 5-1.*

6.2. Recommendations

Based on the outcomes of this study, several recommendations for future research and development in the field of bioplastics are proposed (**Sections 6.2.1 – 6.2.3**).

6.2.1. Enhanced formulation techniques:

Further investigation into the pre-treatment and surface modification of canola fines, such as alkali treatment or acetylation, is recommended to improve filler dispersion and promote stronger interfacial adhesion. These modifications could yield bioplastics with superior properties tailored for specific applications. Additionally, chemical modification of starch molecules, such as cross-linking or grafting, may yield bioplastics with improved water resistance and structural integrity, tailored for application-specific requirements.

6.2.2. Long-term biodegradability studies:

It is necessary to conduct long-term biodegradability assessments under varying environmental conditions to determine the lifecycle impacts of the developed bioplastics. Understanding their biodegradation behaviour will provide insights into their environmental sustainability.

6.2.3. Cost-effectiveness analysis:

A detailed economic analysis of the production processes of these bioplastics should be undertaken. This will help in identifying the financial viability of large-scale production and commercialisation.

6.3. References

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Chapter 7: Appendices

Appendix A: Screening model supplementary information

Tensile testing results of selected screening model runs

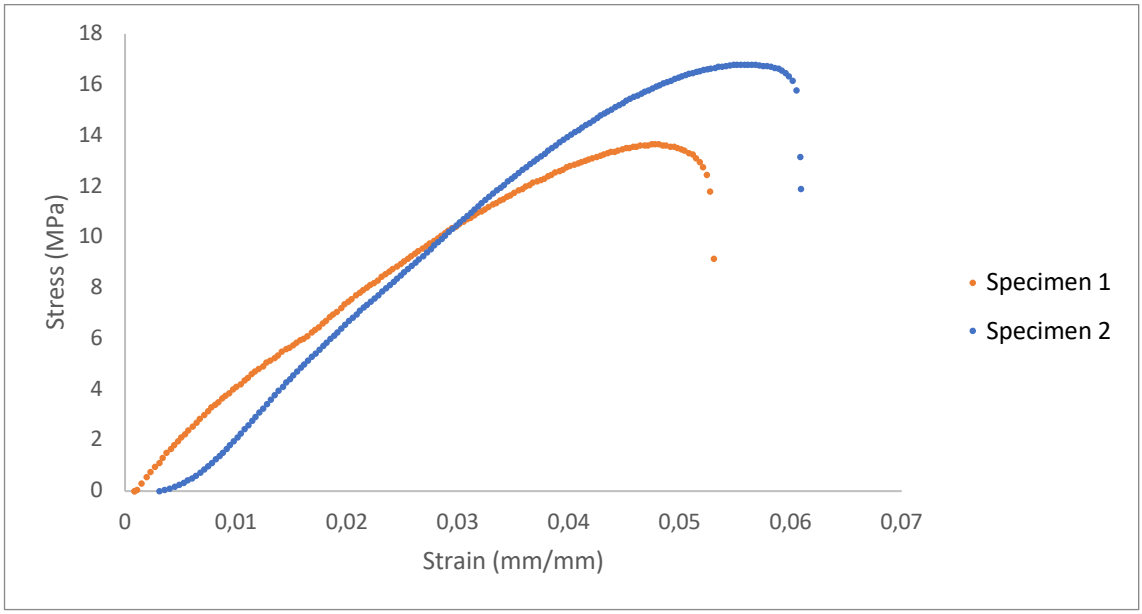

Figure 7-1: Stress vs. strain curve of screening model run 2

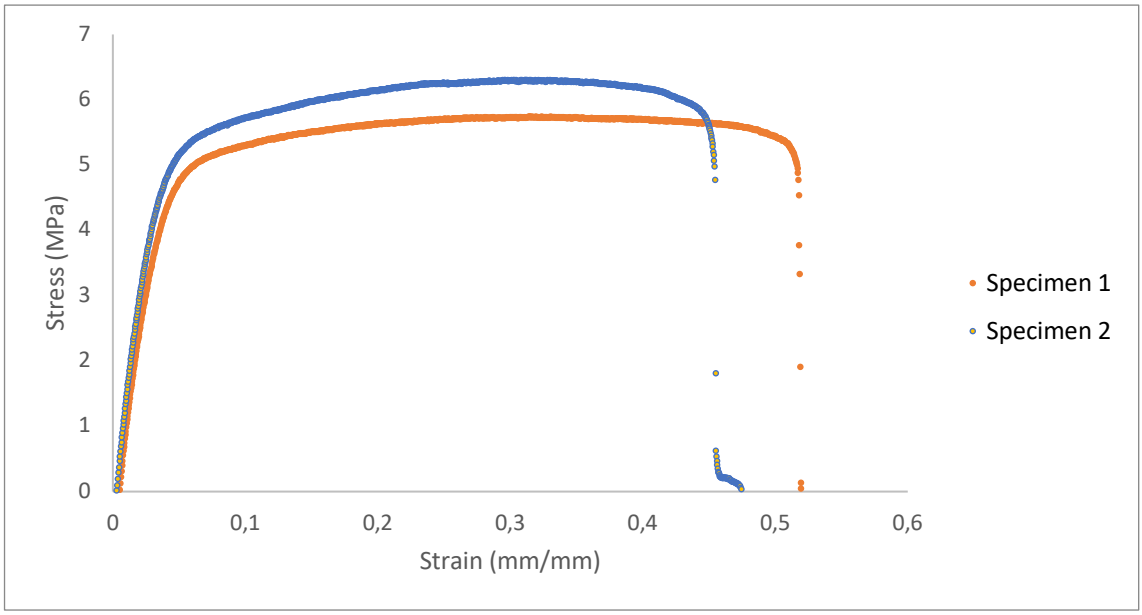

Figure 7-2: Stress vs. strain curve of screening model run 7

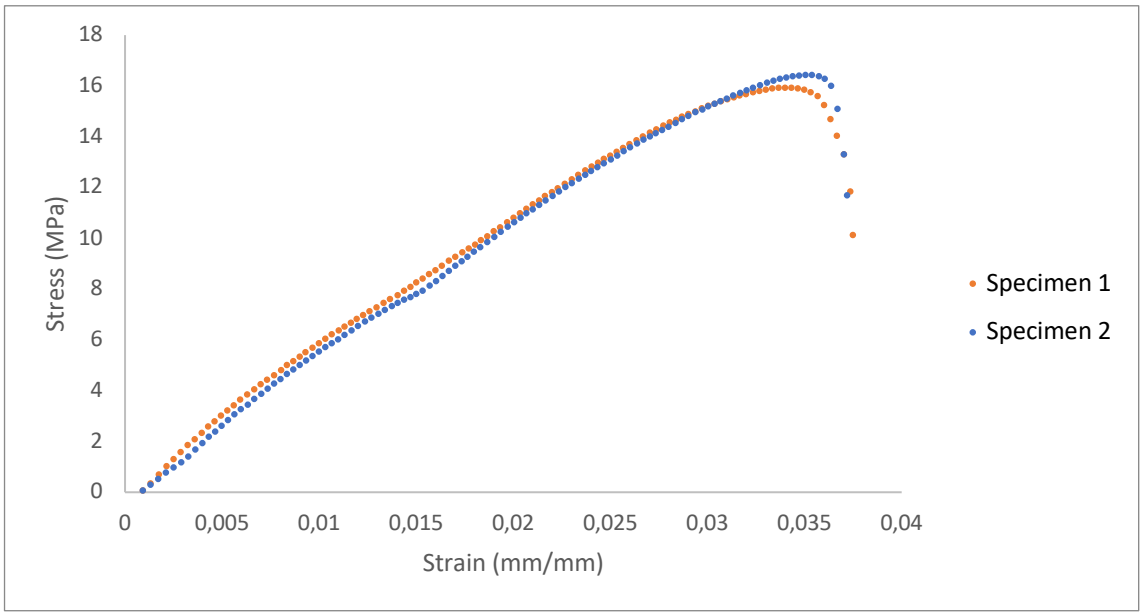

Figure 7-3: Stress vs. strain curve of screening model run 9

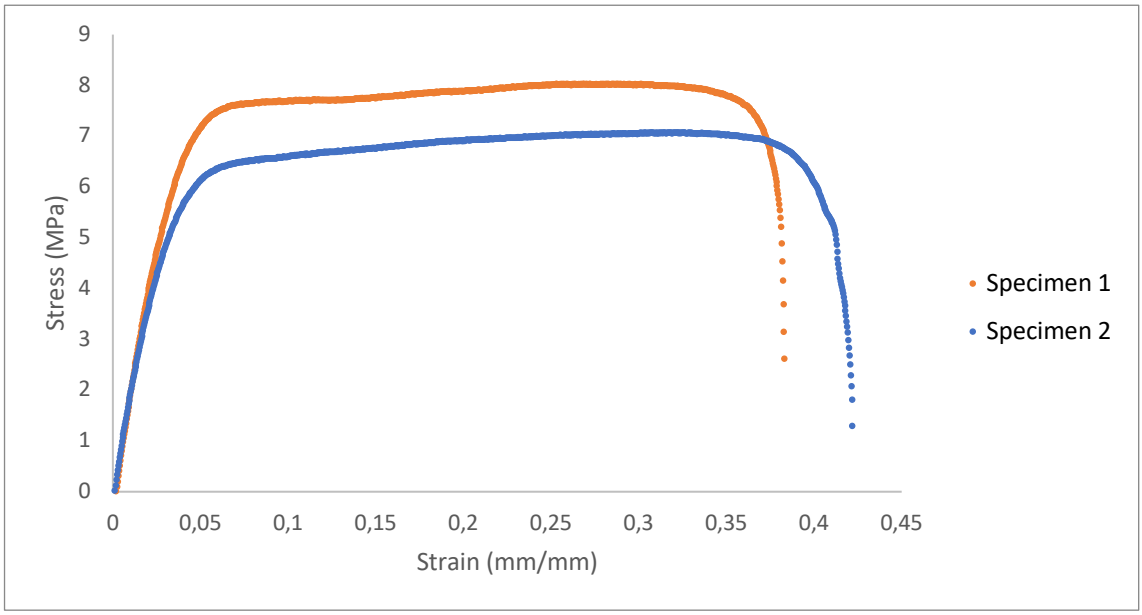

Figure 7-4: Stress vs. strain curve of screening model run 11

Table of optimum solutions of screening model bioplastic films

Table 7-1: Results of optimum solution for synthesis of bioplastic films of the screening model.

No.	Plasticiser mass fraction	Filler mass fraction	Acetic acid	Starch type	Filler type	Tensile strength	Elongation at break	Water absorption	Desirability
1	29.78	12.0	1.14	Spillage	Mixture	9.70	24.87	54.33	0.683
2	29.79	12.0	1.61	Spillage	Mixture	9.69	24.87	54.30	0.683
3	29.78	12.0	1.17	Spillage	Mixture	9.70	24.87	54.33	0.683
4	29.81	12.0	0.68	Spillage	Mixture	9.70	24.87	54.20	0.683
5	29.80	12.0	1.66	Spillage	Mixture	9.69	24.87	54.26	0.683
6	29.80	12.0	2.13	Spillage	Mixture	9.69	24.87	54.27	0.683
7	29.78	12.0	0.35	Spillage	Mixture	9.70	24.87	54.33	0.683
8	29.79	12.0	1.51	Spillage	Mixture	9.69	24.87	54.28	0.683
9	29.81	12.0	2.22	Spillage	Mixture	9.69	24.87	54.21	0.683
10	29.82	12.0	0.45	Spillage	Mixture	9.69	24.88	54.18	0.683
11	29.79	12.0	1.98	Spillage	Mixture	9.69	24.87	54.29	0.683
12	29.77	12.0	0.57	Spillage	Mixture	9.70	24.87	54.39	0.683
13	29.78	12.0	0.71	Spillage	Mixture	9.70	24.87	54.33	0.683
14	29.79	12.0	1.62	Spillage	Mixture	9.69	24.87	54.30	0.683
15	29.81	12.0	0.30	Spillage	Mixture	9.69	24.87	54.23	0.683
16	29.82	12.0	0.03	Spillage	Mixture	9.69	24.88	54.19	0.683
17	29.82	12.0	0.80	Spillage	Mixture	9.68	24.88	54.17	0.683
18	29.80	12.0	1.30	Spillage	Mixture	9.69	24.87	54.27	0.683
19	29.83	12.0	1.08	Spillage	Mixture	9.68	24.88	54.16	0.683
20	29.81	12.0	1.79	Spillage	Mixture	9.69	24.87	54.22	0.683
21	29.81	12.0	1.24	Spillage	Mixture	9.69	24.87	54.22	0.683
22	29.75	12.0	2.22	Spillage	Mixture	9.70	24.86	54.43	0.683
23	29.75	12.0	2.45	Spillage	Mixture	9.70	24.86	54.44	0.683
24	29.81	12.0	2.35	Spillage	Mixture	9.69	24.87	54.21	0.683
25	29.83	12.0	1.40	Spillage	Mixture	9.68	24.88	54.16	0.683
26	29.76	12.0	1.87	Spillage	Mixture	9.70	24.87	54.41	0.683
27	**29.82**	**12.0**	**0.00**	**Spillage**	**Mixture**	**9.69**	**24.88**	**54.20**	**0.683 Selected**
28	29.74	12.0	1.39	Spillage	Mixture	9.70	24.86	54.48	0.683
29	29.77	12.0	0.43	Spillage	Mixture	9.70	24.87	54.37	0.683
30	29.80	12.0	2.28	Spillage	Mixture	9.69	24.87	54.25	0.683
31	29.85	12.0	0.59	Spillage	Mixture	9.68	24.88	54.06	0.683
32	29.77	12.0	1.57	Spillage	Mixture	9.70	24.87	54.38	0.683
33	29.81	12.0	2.07	Spillage	Mixture	9.69	24.87	54.24	0.683
34	29.74	12.0	0.41	Spillage	Mixture	9.71	24.86	54.50	0.683
35	29.83	12.0	1.47	Spillage	Mixture	9.68	24.88	54.16	0.683
36	29.76	12.0	1.59	Spillage	Mixture	9.70	24.87	54.40	0.683
37	29.78	12.0	2.21	Spillage	Mixture	9.70	24.87	54.33	0.683

38	29.80	12.0	0.52	Spillage	Mixture	9.69	24.87	54.27	0.683
39	29.84	12.0	2.50	Spillage	Mixture	9.68	24.88	54.10	0.683
40	29.73	12.0	1.43	Spillage	Mixture	9.71	24.86	54.51	0.683
41	29.84	12.0	1.63	Spillage	Mixture	9.68	24.88	54.09	0.683
42	29.81	12.0	2.42	Spillage	Mixture	9.69	24.87	54.24	0.683
43	29.79	12.0	2.49	Spillage	Mixture	9.69	24.87	54.29	0.683
44	29.77	12.0	0.88	Spillage	Mixture	9.70	24.87	54.37	0.683
45	29.83	12.0	2.33	Spillage	Mixture	9.68	24.88	54.14	0.683
46	29.80	12.0	2.47	Spillage	Mixture	9.69	24.87	54.26	0.683
47	29.78	12.0	0.85	Spillage	Mixture	9.70	24.87	54.34	0.683
48	29.77	12.0	0.02	Spillage	Mixture	9.70	24.87	54.37	0.683
49	29.81	12.0	1.96	Spillage	Mixture	9.69	24.88	54.21	0.683
50	29.79	12.0	0.31	Spillage	Mixture	9.69	24.87	54.28	0.683
51	29.77	12.0	1.70	Spillage	Mixture	9.70	24.87	54.35	0.683
52	29.78	12.0	0.96	Spillage	Mixture	9.70	24.87	54.32	0.683
53	29.85	12.0	1.57	Spillage	Mixture	9.68	24.88	54.07	0.683
54	29.73	12.0	2.47	Spillage	Mixture	9.71	24.86	54.53	0.683
55	29.84	12.0	1.95	Spillage	Mixture	9.68	24.88	54.11	0.683
56	29.78	12.0	1.20	Spillage	Mixture	9.70	24.87	54.33	0.683
57	29.74	12.0	1.36	Spillage	Mixture	9.71	24.86	54.50	0.683
58	29.77	12.0	1.33	Spillage	Mixture	9.70	24.87	54.36	0.683
59	29.86	12.0	0.56	Spillage	Mixture	9.67	24.88	54.04	0.683
60	29.85	12.0	1.65	Spillage	Mixture	9.68	24.88	54.07	0.683
61	29.75	12.0	2.10	Spillage	Mixture	9.71	24.86	54.46	0.683
62	29.84	12.0	0.21	Spillage	Mixture	9.68	24.88	54.13	0.683
63	29.80	12.0	0.91	Spillage	Mixture	9.69	24.87	54.27	0.683
64	29.74	12.0	0.15	Spillage	Mixture	9.71	24.86	54.48	0.683
65	29.82	12.0	0.76	Spillage	Mixture	9.68	24.88	54.18	0.683
66	29.80	12.0	1.89	Spillage	Mixture	9.69	24.87	54.24	0.683
67	29.88	12.0	0.68	Spillage	Mixture	9.70	24.88	53.98	0.683
68	29.76	12.0	1.00	Spillage	Mixture	9.70	24.87	54.40	0.683
69	29.71	12.0	1.76	Spillage	Mixture	9.72	24.86	54.60	0.683
70	29.82	12.0	0.26	Spillage	Mixture	9.68	24.88	54.18	0.683
71	29.87	12.0	1.84	Spillage	Mixture	9.67	24.88	54.00	0.683
72	29.71	12.0	0.00	Spillage	Mixture	9.72	24.86	54.60	0.683
73	29.87	12.0	2.00	Spillage	Mixture	9.67	24.88	54.01	0.683
74	29.87	12.0	1.54	Spillage	Mixture	9.67	24.88	53.99	0.683
75	29.67	12.0	1.17	Spillage	Mixture	9.73	24.85	54.74	0.683
76	29.78	12.0	2.20	Spillage	Mixture	9.70	24.87	54.33	0.683
77	29.65	12.0	0.07	Spillage	Mixture	9.73	24.85	54.80	0.683
78	29.84	12.0	0.04	Spillage	Mixture	9.68	24.88	54.10	0.683
79	29.96	12.0	0.03	Spillage	Mixture	9.65	24.90	53.67	0.683
80	30.00	12.0	2.50	Spillage	Mixture	9.63	24.91	53.51	0.683

81	30.00	12.0	2.32	Spillage	Mixture	9.63	24.91	53.52	0.683
82	29.74	12.0	2.44	Spillage	Mixture	9.71	24.87	54.50	0.683
83	29.53	12.0	2.46	Spillage	Mixture	9.77	24.83	55.27	0.683
84	29.50	12.0	0.00	Spillage	Mixture	9.78	24.82	55.37	0.683
85	29.45	12.0	0.00	Spillage	Mixture	9.79	24.81	55.58	0.683
86	29.69	12.0	2.12	Spillage	Mixture	9.72	24.88	54.70	0.683
87	28.77	12.0	0.00	Spillage	Mixture	9.99	24.70	58.11	0.683
88	29.14	12.0	0.07	Spillage	Mixture	9.81	25.15	57.12	0.683
89	27.43	12.0	2.50	Spillage	Mixture	10.37	24.48	63.08	0.682
90	27.01	12.0	0.00	Spillage	Mixture	10.50	24.41	64.65	0.682
91	30.00	12.0	2.50	Spillage	Mixture	9.46	25.88	54.49	0.681
92	25.80	12.0	0.00	Spillage	Mixture	10.86	24.22	69.18	0.681

Appendix B: RSM supplementary information

Tensile testing results of selected RSM runs

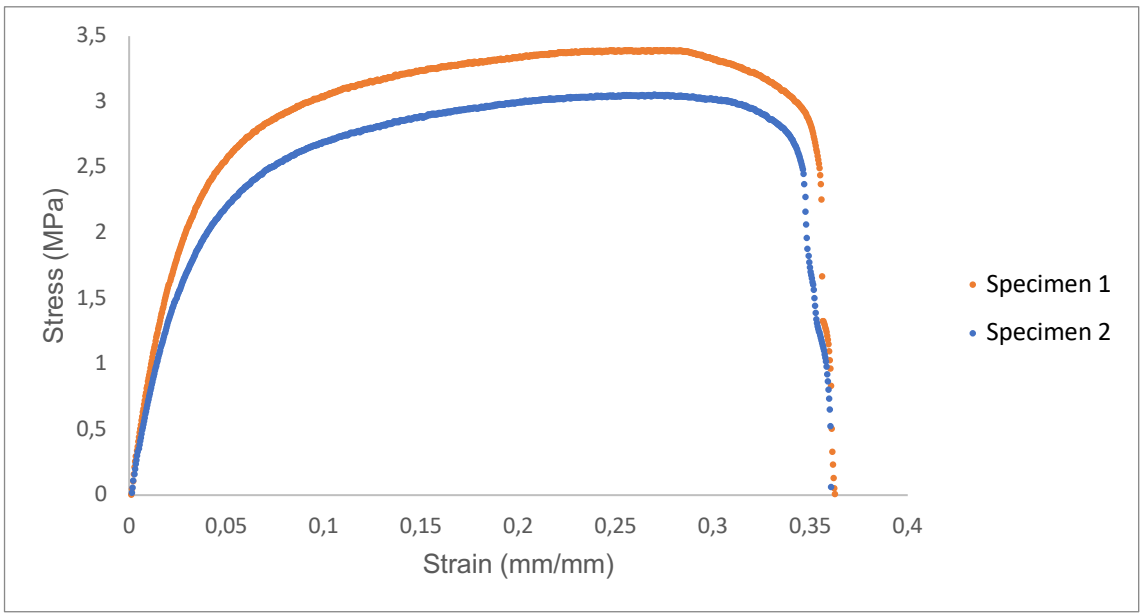

Figure 7-5: Stress vs. strain curve of RSM run 3

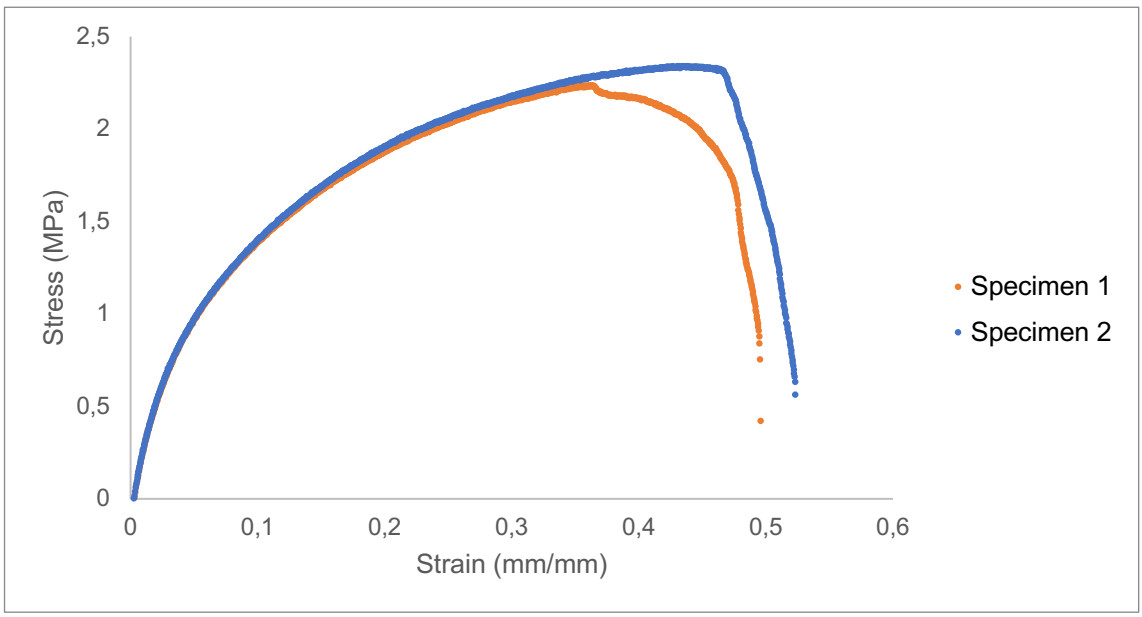

Figure 7-6: Stress vs. strain curve of RSM run 8

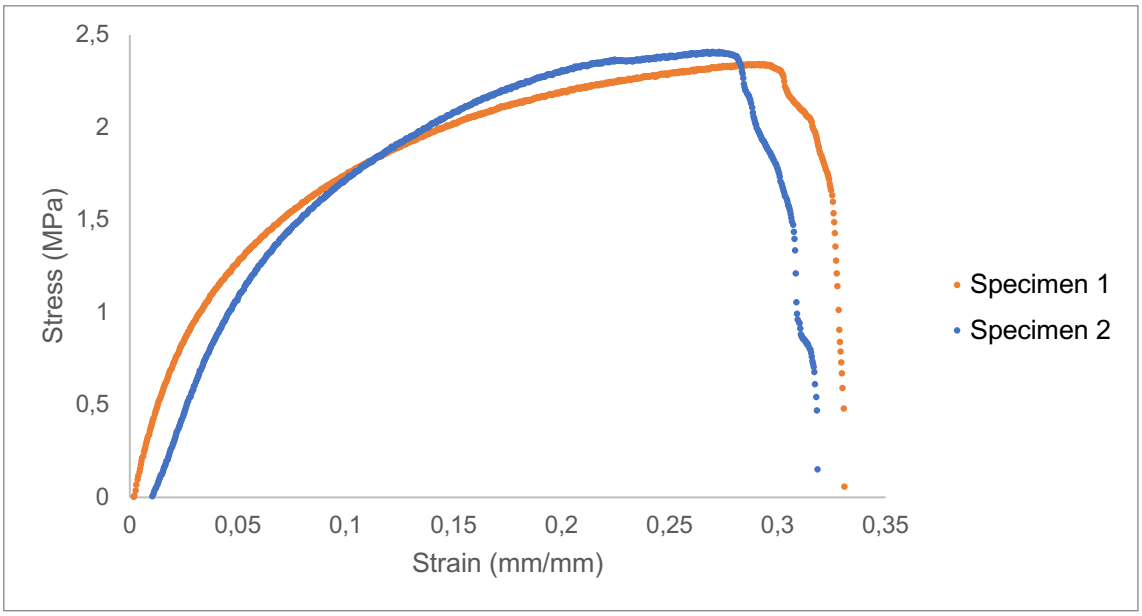

Figure 7-7: Stress vs. strain curve of RSM run 10

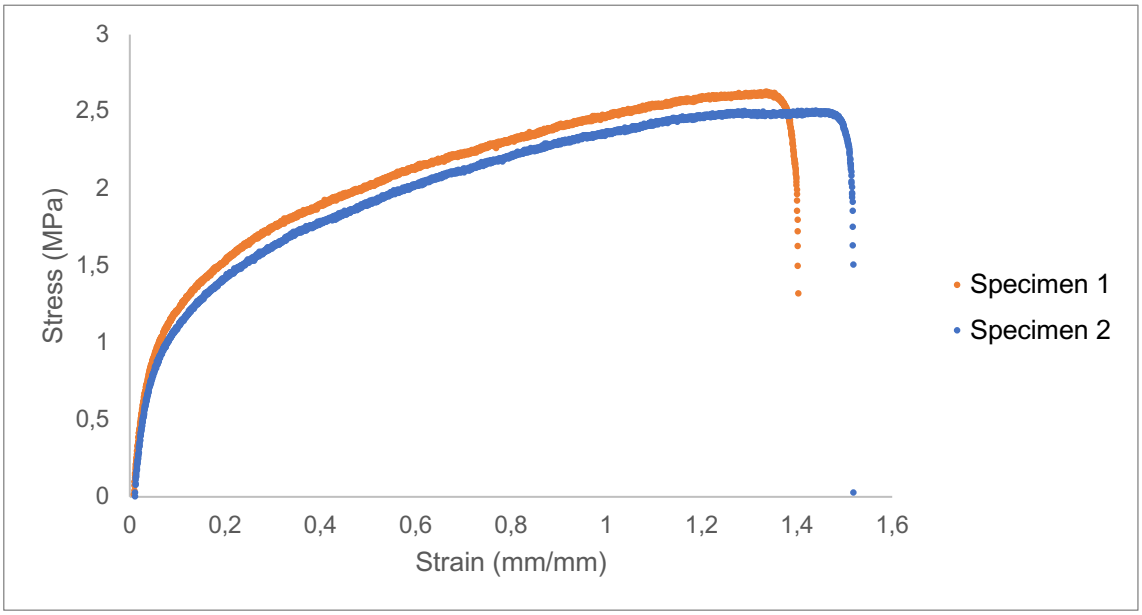

Figure 7-8: Stress vs. strain curve of RSM run 11

Contour plots of RSM optimisation

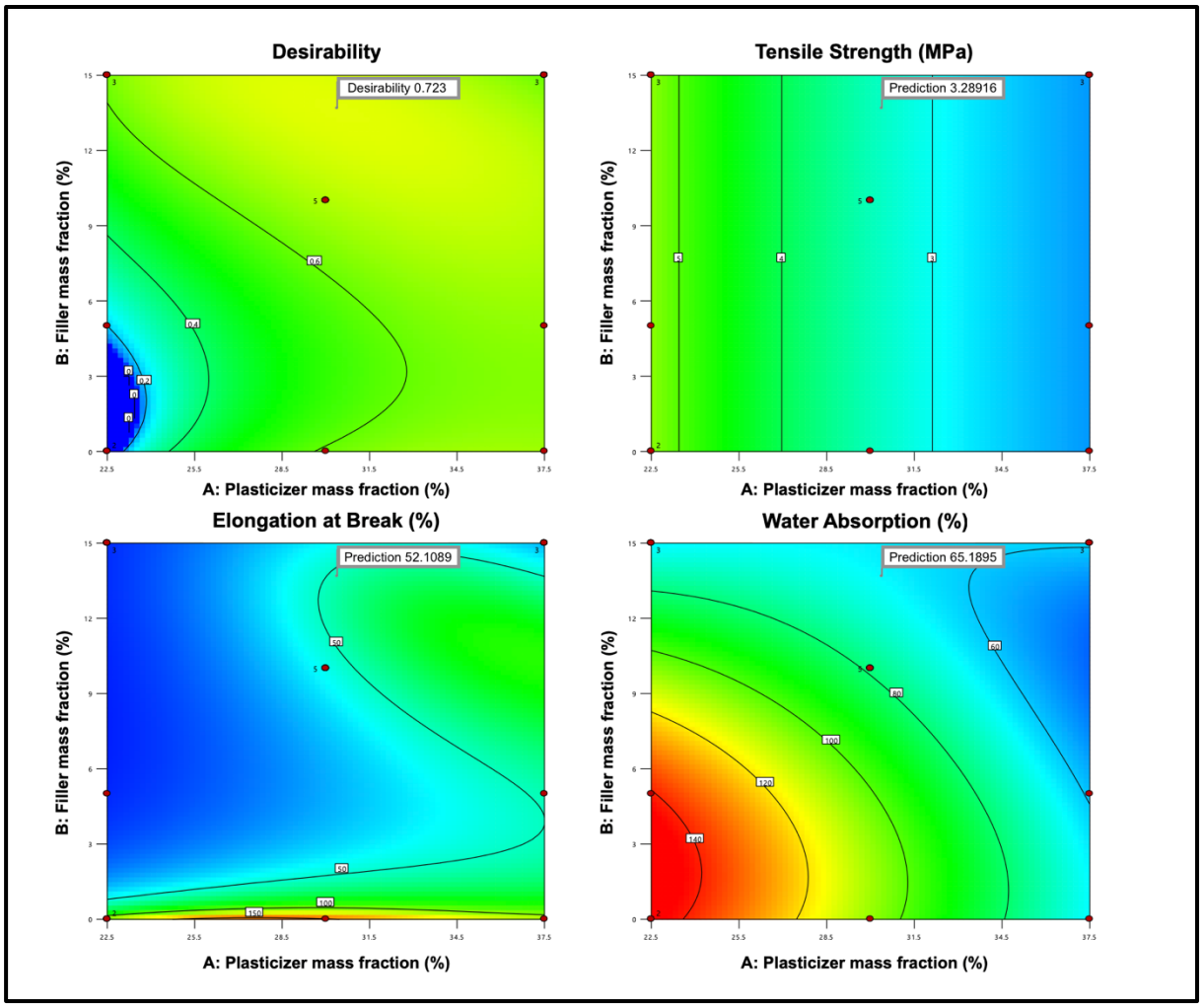

Figure 7-9: Contour plots of the optimum region of plasticiser mass fraction and filler mass fraction to achieved optimum mechanical properties and water absorption of bioplastic films.