Development and Characterization of Acetylated Cassava Starch-Based Biodegradable Packaging Films Reinforced with Kaolinite Filler.

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A RESEARCH PROJECT WRITTEN IN THE DEPARTMENT OF CHEMISTRY AND SUBMITTED TO COLLEGE OF POST GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE (M.Sc.) DEGREE IN INDUSTRIAL CHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

This is to certify that this research project was carried out by ABRAHAM OMO EDIALE with the matriculation number PG/PSC2215566 under the supervision of DR. MRS. I. G. OKUNZUWA in the DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF BENIN, Benin City, Edo State.

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DEDICATION

This work is dedicated first to the Almighty God, whose boundless grace sustained me throughout my Master's Degree journey at the University of Benin. It is also dedicated to my beloved family, whose steady love and support made this achievement possible, and to the Federal College of Education (Technical)-Ekiadolor, whose sponsorship and support played a vital role in helping me attain this milestone.

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I am deeply grateful to everyone who contributed to the success of this work. Above all, I thank the Almighty God for His guidance, strength, and unfailing grace throughout this journey.

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ABSTRACT

This study focuses on the development and characterization of biodegradable packaging films produced from acetylated cassava starch reinforced with kaolinite and plasticized with ethylene glycol. Cassava starch was extracted, chemically modified through acetylation, and confirmed using Fourier Transform Infrared (FTIR) spectroscopy. The acetylated starch spectrum displayed a distinct absorption peak around 1740 cm^{-1}, attributed to the carbonyl (C=O) stretching vibration of ester groups, confirming acetylation. An additional peak at 1230–1260 cm^{-1} corresponds to C–O stretching of the acetyl ester linkage. The observed decrease and narrowing of the O–H stretching band intensity around 3400 cm^{-1} reflects the replacement of hydroxyl groups by acetyl moieties, reducing intermolecular hydrogen bonding. Slight intensity variations in the 1000–1150 cm^{-1} region also indicate modifications to the starch backbone. Acetylation lowered the gelatinization temperature from 65 °C to approximately 51 °C, indicating reduced structural order and enhanced thermal processability. Bioplastic films were formulated using varying concentrations of ethylene glycol (2–5 g) and kaolinite filler (0–1 g) through a solution casting technique. The physicochemical analysis shows that the extracted starch had a high yield (62.3%), a moisture content of 12.3%, and a gelatinization temperature consistent with high-quality cassava starch. Film characterization revealed notable variations in colour, thickness, solubility, and absorption behaviour across formulations. Water, moisture, and acid absorption increased with higher plasticizer content (2g – 5g), while kaolinite reduced uptake due to its barrier-enhancing layered structure. All samples were soluble in 1 M NaOH, confirming the susceptibility of acetate ester linkages to alkaline hydrolysis, but remained insoluble in ethanol. Mechanical testing demonstrated that ethylene glycol improved flexibility while kaolinite enhanced strength and stiffness, with combined effects enabling fine-tuning of film performance. Tensile strength values ranged from 0.79MPa – 6.62MPa for ethylene glycol without filler and 1.67MPa - 10.74MPa across formulations, with the best balance of flexibility (22% elongation), durability, and toughness obtained at 5 g ethylene glycol with 0.5g kaolinite. ANOVA results showed that elongation at break (ethylene glycol level) had a significant effect on tensile strength ($p = 0.0079$), while moisture content and the interaction term showed marginal or non-significant effects. Regression modelling further indicated that although the overall model was significant ($p = 0.0245$), individual predictors had weak independent effects, suggesting that tensile performance is governed by the combined influence of plasticizer–filler interactions. TGA/DTA showed that ethylene glycol reduced thermal stability while kaolinite enhanced stability especially at higher ethylene glycol concentrations. Biodegradability studies confirmed substantial weight loss within one week of soil burial, demonstrating strong environmental compatibility. Overall, the study establishes that acetylated cassava starch reinforced with kaolinite can produce biodegradable films with tunable mechanical, thermal, and barrier properties. The optimal formulation of 5 g ethylene glycol with 0.5g kaolinite shows promising potential for sustainable packaging applications, providing a viable, locally sourced alternative to synthetic plastics.

CHAPTER ONE

1.0 INTRODUCTION

Across the world, the push for environmentally friendly materials has grown stronger in recent years. As the impact of plastic pollution becomes more visible, especially in oceans,rivers and soil. Scientists and industries are increasingly turning to biodegradable source. Plastics derived from petroleum sources are resistant to degradation, leading to persistent environmental pollution (Singh *et al.,* 2019).This challenge has encouraged researchers to explore materials that are renewable, biodegradable, and still functional (Otache *et al.,* 2021).

One promising option is starch. It is widely available, inexpensive, renewable, and biodegradable. Chemically, starch consists of two main components; amylose and amylopectin linked by α-1,4 and α-1,6glycosidic linkage (Zhao *et al.,* 2020). It can be obtained from crops like root/tuber crop and cereal. But native starch on its own is not ideal for producing plastic-like films because it is hydrophilic, mechanically weak, and thermally unstable (Oluwole *et al.,* 2021).

To address these limitations, researchers modify starch chemically. One possible method is acetylation, in which some of the hydroxyl groups in starch are replaced with acetyl groups using chemicals like acetic anhydride (Awokoya *et al.,* 2020). This modification reduces the material's affinity for water, improves its flexibility and thermal behavior, and generally helps it mimic the performance of synthetic plastics more closely (Shogren and Willett, 2021).

Bioplastic films made from starch acetate are produced by dissolving or dispersing the modified starch in a plasticizer and casting it into thin films. These films can be further strengthened by adding fillers such as kaolinite, a naturally available clay mineral

($Al_2Si_2O_5(OH)_4$). Kaolinite improves the film's strength and thermal resistance because of its layered structure, which interacts effectively with polymer chains (Ibrahim *et al.* 2019; Daramola *et al.,*2022).

The global search for greener materials to replace petroleum based plastic has made biodegradable starch-based bioplastics increasingly attractive, especially for applications like packaging and disposable items. Still, it remains challenging to achieve a bioplastic that balances biodegradability with desirable mechanical and thermal properties (Okoro *et al.,* 2020).

Nigeria, being the world's largest producer of cassava, has a unique advantage. Cassava starch is abundant and affordable, making it a strong local raw material for producing biodegradable plastics. Using cassava starch for this purpose supports agricultural value addition, local industry, and environmental sustainability (Ojo *et al.,* 2022). When combined with locally sourced kaolinite, it creates a fully indigenous material system for eco-friendly plastic alternatives.

A full understanding of the performance of these starch-based films requires several forms of analysis. FTIR helps confirm that acetylation has taken place. TGA and DSC give insight into the thermal stability of the films (Yusuf *et al.,* 2021), while mechanical tests evaluate strength, flexibility, and durability (Idris *et al.,* 2023).

This study focuses on developing and analyzing bioplastic films based on chemically modified cassava starch acetate reinforced with kaolinite. It aims to explore how acetylation and filler addition affect the mechanical, structural, and thermal properties of the final material.

1.1 BACKGROUND OF THE STUDY

Starch has emerged as one of the most promising renewable materials because it is biodegradable, inexpensive, and widely available. Cassava starch, in particular, is especially important for countries like Nigeria, which leads the world in cassava production with over 59 million tonnes annually (FAO, 2021). This makes cassava starch a strategic resource for producing biodegradable polymers locally (Ojo *et al.,* 2022).

However, native starch comes with mechanical and thermal limitations. It is brittle, easily absorbs moisture, and lacks the flexibility needed for durable packaging materials (Oluwole *et al.,* 2021). These issues arise mainly from its abundance of hydroxyl groups, which encourage strong hydrogen bonding and moisture sensitivity (Zhao *et al.,* 2020).

Acetylation helps address these problems by replacing hydroxyl groups with acetyl groups, resulting in a more hydrophobic and flexible material (Shogren and Willett, 2021). The degree of substitution (DS) plays a major role in determining its final properties and its balance between performance and biodegradability (Ibrahim *et al.,* 2019; Daramola *et al.,* 2022).

To strengthen acetylated starch films, fillers like kaolinite can be added. Kaolinite's layered structure and surface hydroxyl groups allow it to interact well with modified starch, improving strength, thermal resistance, and barrier properties (Ibrahim *et al.,* 2019; Daramola *et al.,* 2022). When properly dispersed, kaolinite significantly enhances film performance. Producing starch acetate–kaolinite films generally involves extracting starch, chemically modifying it, forming films through solution casting, drying, and conditioning, followed by thermal, structural, and mechanical characterization.

Relative lack of data on these kinds of composite films, especially using cassava starch acetate, ethylene glycol, and kaolinite, makes this study important for filling a critical research gap (Ojo *et al.,* 2022).

1.2 STATEMENT OF THE PROBLEM

Plastic pollution has become a global crisis, affecting ecosystems, wildlife, and human health. Conventional plastics do not degrade easily and remain in the environment for decades, releasing harmful chemicals and micro-plastics that contaminate soil, water, and food chains (Singh *et al.,* 2019; Otache *et al.,* 2021). The need for biodegradable alternatives is therefore urgent.

While bioplastics exist, they are often expensive and depend on imported raw materials. For a country like Nigeria, this makes widespread adoption difficult (Ojo *et al.,* 2022). Nigeria also faces increasing amounts of plastic waste without adequate waste management systems.

Although starch-based bioplastics are promising, native starch films are weak, brittle, and highly water-sensitive (Oluwole *et al.,* 2021; Zhao *et al.,* 2020). Chemical modification such as acetylation improves these properties, but the films still require reinforcement to match the performance of conventional packaging materials (Shogren and Willett, 2021).

Kaolinite fillers have shown potential to enhance strength, barrier properties, and thermal stability (Daramola *et al.,* 2022; Idris *et al.,* 2023), but optimal dispersion and compatibility remain challenges.

Additionally, achieving the right degree of substitution during acetylation is crucial: too low degree of substitution, results in poor hydrophobicity, while too high reduces biodegradability (Okoro *et al.,* 2020; Yusuf *et al.,* 2021).

Despite global research efforts, little work has been done locally on cassava starch acetate films reinforced with kaolinite and plasticized with ethylene glycol. This study addresses these gaps to produce a practical and locally sourced biodegradable alternative.

1.3 JUSTIFICATION OF THE STUDY

The justification for this study can be viewed from two angles: environmental and economic.

1.3.1 Environmental Justification

Plastic waste continues to accumulate in the environment with less than 10% being recycled worldwide (FAO, 2021). As plastics break down, they release micro-plastics and toxic additives that pollute soil and water (Zhao *et al.,* 2020). Nigeria contributes significantly to this problem due to population size and limited recycling capacity (Ojo *et al.,* 2022).

Biodegradable starch-based films offer a promising alternative. They break down naturally and pose fewer environmental risks (Awokoya *et al.,* 2020). Introducing kaolinite enhances film durability during use without preventing biodegradation afterward (Ibrahim *et al.,* 2019).

1.3.2 Economic Justification

Using cassava starch and kaolinite materials that Nigeria has in abundance, reduces the need for imported synthetic polymers (Ojo *et al.,* 2022). This supports local industries, strengthens agricultural and mineral value chains, and opens opportunities for small and medium-scale enterprises.

1.4 SCOPE OF THE WORK

This study covers the extraction of cassava starch, its chemical modification through acetylation, and the development and analysis of biodegradable films reinforced with kaolinite and plasticized with ethylene glycol.

The research focuses on optimizing film preparation, studying how fillers and plasticizers affect performance and evaluating mechanical, thermal, water absorption, HCl absorption, biodegradation properties, ethanol and base solubility.

Analysis of variance and regression model was carried out using R analytical tools on the bioplastic material.

1.4.1 Chemical Modification of Starch

Acetylation replaces some hydroxyl groups in starch with acetyl groups (Shogren and Willett, 2021), enhancing hydrophobicity and flexibility (Zhao *et al.,* 2020). The study aims to achieve a suitable degree of substitution.

1.4.2 Film Preparation

The synthesis of the starch acetate–kaolinite bioplastic film will involve solution casting and solvent evaporation techniques. The modified starch acetate will be plasticized with ethylene glycol to enhance film flexibility (Oluwole *et al.,* 2021). Kaolinite filler will be dispersed homogeneously within the polymer matrix using mechanical stirring to ensure even particle distribution and interfacial adhesion.

1.5 AIM AND OBJECTIVES

Aim: To synthesize and characterize biodegradable films derived from acetylated cassava starch, examining their thermal, mechanical properties**,** biodegradability**,** water absorption, HCl absorption, ethanol and base solubility of the films, as well as assessing the impact of plasticizers (ethylene glycol) and fillers (kaolinite) on the final properties, with the goal of optimizing the films for various practical applications.

Objectives:

1. To extract cassava starch from cassava roots using a standard method involving cleaning, peeling, grating, washing, and drying to obtain pure starch.
2. To modify the extracted cassava starch through acetylation.
3. To prepare biodegradable film from modified starch and various amount of plasticizer (ethylene glycol) and filler (Kaolinite).
4. To investigate the impact of varying concentrations of plasticizer **(**ethylene glycol) and filler (kaolinite) on the flexibility**,** strength**,** thermal stability, degradability ,water absorption, HCl absorption base and ethanol solubility of the films.
5. To carry out Anova and regression to model the interaction between tensile strength , elongation at break and moisture absorption.

1.6 LITERATURE REVIEW

1.6.1 Starch as a Renewable Polymer

Starch is one of the most abundant biopolymers available in nature, serving as a principal energy reserve in plants. It is composed of two polysaccharides: amylose, a mostly linear polymer of α-(1→4) glycosidic linkage, and amylopectin, a highly branched polymer with α-(1→6) linkages (Hoover, 2001). The ratio of amylose to amylopectin determines the physicochemical characteristics of starch, such as gelatinization temperature, film-forming ability, and mechanical strength.

Cassava starch**,** derived from *Manihot esculenta*, has been identified as one of the most promising raw materials for bioplastic production due to its high carbohydrate content, wide availability, and low cost (Averous, 2004). However, the inherent hydrophilicity and poor mechanical performance of native starch restrict its direct use in polymeric applications (Kaur *et al.,* 2012).

These limitations have motivated extensive research into chemical modification and filler reinforcement strategies to enhance its material performance and expand its application range (Bastioli, 2005).

1.6.2 Chemical Modification of Starch (Acetylation Process)

Chemical modification is one of the most effective ways of improving starch functionality. Acetylation introduces acetyl groups (–$COCH_3$) into hydroxyl sites on the glucose monomer, thereby decreasing intermolecular hydrogen bonding and improving flexibility, thermal stability, and water resistance (Shogren, 2003; Kaur *et al.,* 2012).

The acetylation process typically involves reacting starch with acetic anhydride in the presence of a catalyst such as sodium hydroxide or pyridine. The degree of substitution (DS), defined as the average number of hydroxyl groups replaced per glucose unit, determines the extent of modification and resultant properties (Jane, 1995).

At low DS values (0.01–0.2), the starch retains thermoplastic properties, whereas higher DS values yield more hydrophobic and less biodegradable products (Thiebaud *et al.,* 1997). Optimal acetylation balances flexibility and biodegradability to ensure environmental compatibility.

Starch acetate films exhibit enhanced transparency, flexibility, and moisture resistance compared to native starch films (Lawal, 2011). Moreover, acetylated starch demonstrates improved compatibility with inorganic fillers and polymeric additives due to its reduced polarity (Teixeira *et al.,* 2009).

1.6.3 Bioplastics: Definition, Types, and Environmental Relevance

Bioplastics are materials that are either biobased**,** biodegradable**,** or both. They can be produced from renewable biomass sources such as starch, cellulose, or vegetable oils

(Mohanty *et al.,* 2018). The major categories include starch-based polymers**,** polylactic acid (PLA)**,** polyhydroxyalkanoates (PHA)**,** and biopolyester blends**.**

Compared to conventional plastics, bioplastics decompose more rapidly under natural environmental conditions, thus reducing landfill accumulation and ocean pollution (Song *et al.,* 2009). Their development aligns with global sustainability initiatives and circular economy frameworks that prioritize resource renewability and end-of-life biodegradability (European Bioplastics, 2021).

Despite these advantages, commercial bioplastics face challenges such as high production costs and moderate mechanical performance compared to polyethylene or polypropylene. Research efforts therefore focus on developing cost-effective, high-performance bioplastic composites based on abundant natural polymers like starch (Lu *et al.,* 2014).

1.6.4 Starch-Based Bioplastics and Their Limitations

Starch-based bioplastics are among the earliest and most widely studied biodegradable materials. Their advantages include low cost, easy processability, and full biodegradability (Avella *et al.,* 2002). When plasticized with glycerol or sorbitol, starch forms flexible films suitable for short-term packaging (Jane, 1995).

However, the films remain sensitive to humidity and exhibit poor tensile strength and elongation at break, primarily due to the hydrophilic nature of starch (Bertuzzi *et al.,* 2007). This water sensitivity limits their use in moist environments.

To overcome these drawbacks, several strategies have been explored, including chemical modification (acetylation, oxidation, crosslinking), blending with other biopolymers (such as PLA or PVA), and reinforcement with inorganic fillers or nanomaterials (Thakur and Thakur, 2016). Among these, filler reinforcement offers a simple, scalable, and cost-effective approach to mechanical enhancement.

1.6.5 Role of Fillers in Biopolymer Composites (Kaolinite Focus)

Incorporation of fillers improves the strength, stiffness, and thermal stability of biopolymer films. Fillers such as kaolinite**,** provide reinforcement by forming interfacial interactions with polymer chains (Nath *et al.,* 2019).

Kaolinite, an aluminosilicate clay with a 1:1 layered structure ($Al_2Si_2O_5(OH)_4$), has hydroxyl groups on its surface that enable hydrogen bonding with hydroxyl or acetyl groups of starch acetate (Wang *et al.,* 2014). This interaction enhances the interfacial adhesion between the filler and matrix, leading to improved tensile strength, modulus, and thermal stability (Nguyen *et al., 2*018).

The effectiveness of kaolinite reinforcement depends on dispersion quality**,** filler loading and compatibility with the matrix (Zhou *et al.,* 2013). Excessive filler content can lead to agglomeration, which may reduce flexibility and homogeneity. Optimal loading levels (usually below 10 wt%) tend to achieve the best balance between stiffness and ductility (Adebowale *et al.,* 2019).

Moreover, kaolinite acts as a thermal barrier**,** retarding heat transfer and improving the decomposition temperature of the composite during TGA/DSC analysis (Nath *et al.*, 2019).

1.6.6 Characterization Techniques

Comprehensive characterization is essential to evaluate the physicochemical and mechanical performance of the bioplastic composites:

- Fourier Transform Infrared Spectroscopy (FTIR): Used to confirm acetylation of starch and the presence of characteristic carbonyl and C–O–C peaks (Lawal, 2011).
- Thermogravimetric Analysis (TGA): Determines the thermal degradation profile, indicating enhanced stability due to filler addition (Wang *et al.,* 2014).
- Differential Scanning Calorimetry (DSC): Measures glass transition and melting temperatures, reflecting polymer–filler compatibility (Teixeira *et al.,* 2009).

- Mechanical Testing: Evaluates tensile strength, elongation at break, and Young's modulus to assess film flexibility and toughness (Bertuzzi *et al.,* 2007).

1.6.7 Summary of Literature Gaps

Although research exists on starch-based bioplastics, few studies have examined cassava starch acetate reinforced with local kaolinite and ethylene glycol as plasticizer. Therefore, this study bridges that gap by synthesizing cassava starch acetate–kaolinite composites and conducting systematic TGA/DSC and mechanical analyses to establish relationships between composition, structure and performance.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

- Analytical weighing balance
- Magnetic stirrer with hot plate
- Water bath (set at 80°C for gelatinization)
- Thermometer (for temperature monitoring)
- Mold (for film casting)
- Spatula
- Oven
- Desiccator (for moisture prevention and sample preservation)
- Drying trays
- Airtight storage container
- Cassava root
- Mesh sieve (chiffon fabrics).

2.2 REAGENTS

- Concentrated sodium hydroxide
- Hydrochloric acid
- Acetic anhydride
- Ethylene glycol
- Kaolinite
- Ethanol
- Distilled water
- Phenolphthalein
- Isopropyl alcohol

2.3 METHODOLOGY

2.3.1 Sample Collections and Preparation

Cassava Starch Collection and Preparation

Cassava tuber was purchased from a farm in Ekosodin community Benin City. Preparation of cassava starch was carried out using the method described by Ezeoha and Ezenwanne (2013). The cassava tubers were mechanically grated after being manually peeled and cleaned with portable water. "Three times as much water as the shredded cassava was added to the mixture. A coarse sieve and a filter cloth were used to sieve and filter the mixture, respectively. The filtrate was given six hours to settle. Then the starch was combined with water once more and let to settle for a full day, then it was decanted manually, the dewatering wet starch, was oven-dried for four hours at a low temperature. This was done to ensure that the starch had the lowest possible moisture content.

2.3.2 Starch Yield

The starch yield from the extraction process was calculated using the Equation below.

$$\text{Starch yield (\%)} = \frac{weight\ of\ extracted\ starch\ (g)}{weight\ of\ cassava\ tubers} \text{ x } 100 \text{ ----------(1)}$$

2.3.3 Determination of Gelatinization Temperature, pH, and Moisture content of extracted starch

One gram of dried extracted starch was placed in a beaker filled with 10 ml of distilled water and subjected to heat treatments using a hot plate. While stirring and noting the temperature at which gel formed. The gelatinization temperature was recorded using a thermometer. The pH of the starch was recorded using a calibrated FP20 Mettler Toledo pH meter.

The moisture content was determined using the methodology proposed by Alobi *et al*., (2017). A weighed quantity of the starch was dried in an oven at 105^0C for 24 h. The dried sample was weighed, and the percentage moisture content was calculated using the Equation

Moisture content (%) = $\frac{initial-final\ weight}{initial\ weight}$ x 100 ----------(2)

2.3.4 Preparation of Acetylated Starch

Acetylated starch was obtained using the method reported by (Henry 2007) with some modification. Starch (20g) was dispersed in 100cm^3 of distilled water and then constantly stirred for 30 minutes.The slurry was adjusted to pH 8.0 with 3%NaOH, 1.2g of acetic anhydride was added to the slurry. After the addition of the acetic anhydride, the reaction was allowed to proceed for another five minutes. The pH of the slurry was adjusted to 4.5 with 0.5M HCl and filtered through whatman No 1 filter paper. The residue obtained was washed for four times with distilled water to remove completely some acids that may be present in the product and finally air dried at room temperature. The yield was calculated

yield (%) = $\frac{weight\ of\ modified\ starch\ (g)}{weight\ of\ native\ starch\ (g)}$ x 100 ----------(3)

2.3.5 Determination of Degree Substitution of Acetylated Starch:

The acetyl group (AG expressed as percentage on dry basis) and the degree of substitution (DS) of cassava starch were determined according (Mark and Mehltretter, 1972). A 5g of starch sample was weighed, transferred to a 250 ml conical flask and dispersed in 50 ml distilled water. Few drops of phenolphthalein indicator were added and titrated with 0.1N sodium hydroxideto permanent pink colour. Then 25.0 ml of 0.45N NaOH was added to it and shaken vigorously for half an hour. The stopper and neck of flask was flushed with little distilled water and then the excess alkali was titratedwith 0.2N HCl to disappearance of pink colour. A total of 25.0 ml of 0.45N NaOH was titrated as blank. Acetyl group and degree of substitution were calculated as follows:

% Acetyl = $\frac{(Blank-sample)ml\ x\ M(HCl)\ x\ 0.43\ x100}{weight\ of\ sample}$ ----------(4)

DS = $\frac{162\ X\ \%Acetyl}{4300-(42\ x\ \%Acetyl)}$----------(5)

2.3.6. Fourier Transform Infrared (FT-IR) spectroscopy determination of native starch and modified starch:

The FT-IR spectrum of native starch and modified starch were acquired on a Perkin Elmer FT-IR spectrophotometer (Perkin Elmer, Inc., MA, USA) using a potassium bromide (KBr) disc prepared from powered samples mixed with dry KBr. The spectra were recorded (16 scans) in the transparent mode from 4000 to 400 cm-1 (Bernardino-Nicanor*et al*., 2017)

2.3.7. Preparation of Biodegradable Plastic Film and Casting of films

The preparation was done following a refined modification of the method proposed by Nwaka *et al.,* (2025). 10 g of the acetylated starch powder was weighed in a beaker to which 100 mL of distilled water was added. It was stirred at 350 rpm for 10 min on a magnetic stirrer. Kaolinite powder was then added at different weight, 0g : 0% (w/w), 0.5g :5% (w/w), 1g: 10% (w/w) and stirred. Ethylene glycol was also added at different weight (2g, 3g, 4g, 5g) and stirred at 350 rpm for 15 min. The solution was heated at about 80 ºC to form gel with continuous stirring. The slurry was then poured onto a Mold, dried in a hot air oven at 50 ºC and stored at room temperature.

2.3.8 Experimental Design for Biodegradable plastic Film Formulation

Table 2.1: Bioplastic Film Formulation

	ETHYLENE GLYCOL (plasticizer)	**FILLER (kaolinite)**
starch acetate	2g	0g
		0.5g
		1.0g
	3g	0g
		0.5g
		1.0g
	4g	0g
		0.5g
		1.0g
	5g	0g
		0.5g
		1.0g

2.4 Properties and Characteristics of Prepared Bioplastic Film and Chemical Properties of Biodegradable Plastic Film; all carried out using the method proposed by Nwaka *et al.*, (2025) with slight modifications.

2.4.1 Water Absorption Resistance

The water absorption resistance of the prepared bioplastic films was evaluated by soaking them in water at room temperature for one hour, drying them with cotton pieces, and weighing them. The percentage of water absorption was calculated

Water absorption (%) = $\frac{wet\ \ weight - dry\ weight}{dry\ weight}$ x 100 ----------(6)

2.4.2 Acid Absorption Resistance

For acid absorption resistance, the bioplastic films were soaked in 1M hydrochloric acid solution, and their weights were recorded after one hour. The percentage of acid absorption was calculated

Acid Absorption (%) = $\frac{wet\ \ weight - dry\ weight}{dry\ weight}$ x 100 ----------(7)

2.4.3 Base Absorption Resistance

The base absorption resistance was tested by soaking the films in 1M sodium hydroxide solution and weighing them at regular intervals. The percentage of base absorption was calculated

Base Absorption (%) = $\frac{wet\ \ weight - dry\ weight}{dry\ weight}$ x 100 ----------(8)

2.4.4 Moisture Absorption Resistance

Moisture absorption resistance was determined by drying the bioplastic films in a desiccator until a constant weight (W1) was achieved, then placing them in a normal atmosphere for 24 hours, and weighing them again (W2). The percentage of moisture absorption was calculated

Moisture Absorption (%) = $\frac{W2 - w1}{w2}$ x 100 ----------(9)

2.4.5 Solvent Solubility Test

The solubility test involved placing 0.3 g of bioplastic film pieces in test tubes containing 3 ml of ethanol and observing solubility at 30 °C after one hour.

2.5 BIODEGRADABILITY TEST

The biodegradability property was tested using the soil burial method. Bioplastic films (3 inches by 3 inches) were weighed (W1), buried in moist soil at a 3-inch depth for one week, and reweighed (W2). The percent weight loss was calculated

(%) Weight loss = $\frac{W1-W2}{W1}$ x 100 ----------(10)

2.6 THICKNESS OF THE FILM

The thickness of the bioplastic was observed using the micrometer screw gauge. Each sample was recorded at five different points. The mean value was recorded as the thickness of the bioplastic.

2.7 MECHANICAL PROPERTIES

The mechanical properties of the composites were evaluated using standardized testing methods. Tensile strength and elongation at break were determined using the Tensile Strength Test Machine TM 2101-T7**,** following ASTM D638, with a maximum force of 10kN, the percentage elongation was also calculated,

$\%Elongation = (L-Lo)/Lo \times 100$ ----------(11)

where L is the final length and L0 is the initial length. Tensile Strength, Elongation at Break, Ultimate Elongation, Toughness, Yield Point, is obtained from the stress – strain curve.

2.8 THERMOGRAVIMETRIC/DIFFERENTIAL THERMAL ANALYSIS (TGA/DTA)

TGA/DTA was conducted using the PerkinElmer TGA 4000 (Netherlands) to assess the thermal stability and decomposition behavior of the bioplastic film.” This test provided insights into the materials' resistance to heat and the efficiency of the composite’s formulation and filler reinforcement.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL COMPOSITION OF EXTRACTED CASSAVA STARCH

Table 3.1: Yield, Moisture and the pH of the Extracted Cassava

%yield	%moisture	pH	Gelatinization temperature ^{0}C
62.3	12.3	6.0	65

The physicochemical properties of the extracted cassava starch are presented in Table 3.1. The starch yield was 62.3%, indicating efficient extraction and minimal processing losses. “A moisture content of 12.3% was recorded, which lies within the acceptable range for starch storage stability (typically below 13%) (Chisenga *et al.*, 2019), suggesting that the starch can be preserved without significant microbial growth or deterioration. The pH value of 6.0 indicates a slightly acidic nature, consistent with most native starches obtained from plant sources. This mild acidity can affect starch retrogradation and influence its suitability for further modification.

The gelatinization temperature of 65 °C represents the point at which starch granules begin to swell and lose their crystalline structure when heated in water. This value falls within the typical range of (60–70) °C for cassava starch (Santos *et al.*, 2016), confirming normal starch behavior and good thermal stability. Gelatinization temperature is an important indicator of starch quality, reflecting the degree of crystallinity and the strength of hydrogen bonding within the granules. The observed value therefore suggests that the extracted cassava starch has balanced structural integrity, making it suitable for further chemical modifications such as acetylation.

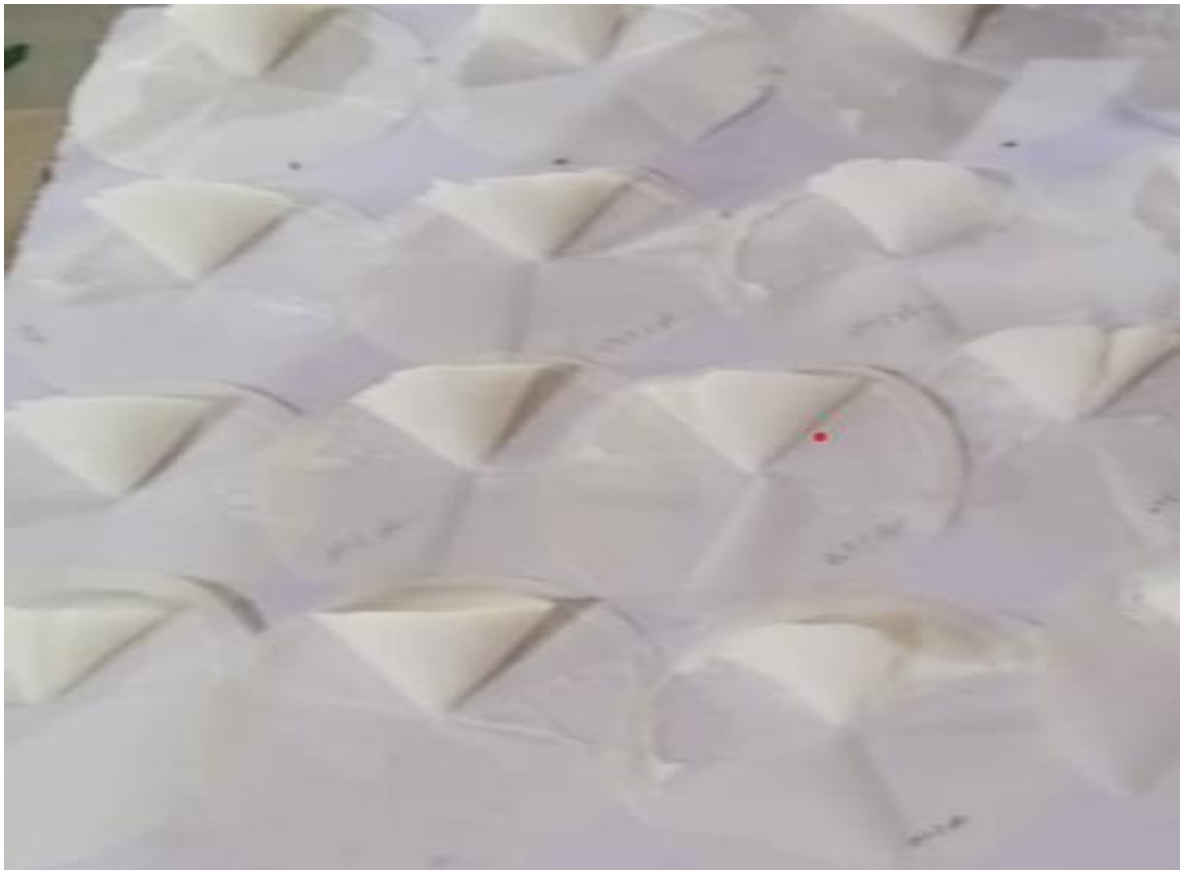

Plate 3.0: Acetylated starch

3.2 FTIR ANALYSIS

The various functional groups of the developed bioplastic films were analyzed using FTIR, as shown in Fig. 3.0, and 3.1. The FTIR graph observed the characteristic peaks of extracted and modified starch both were observed, confirming the modification process.

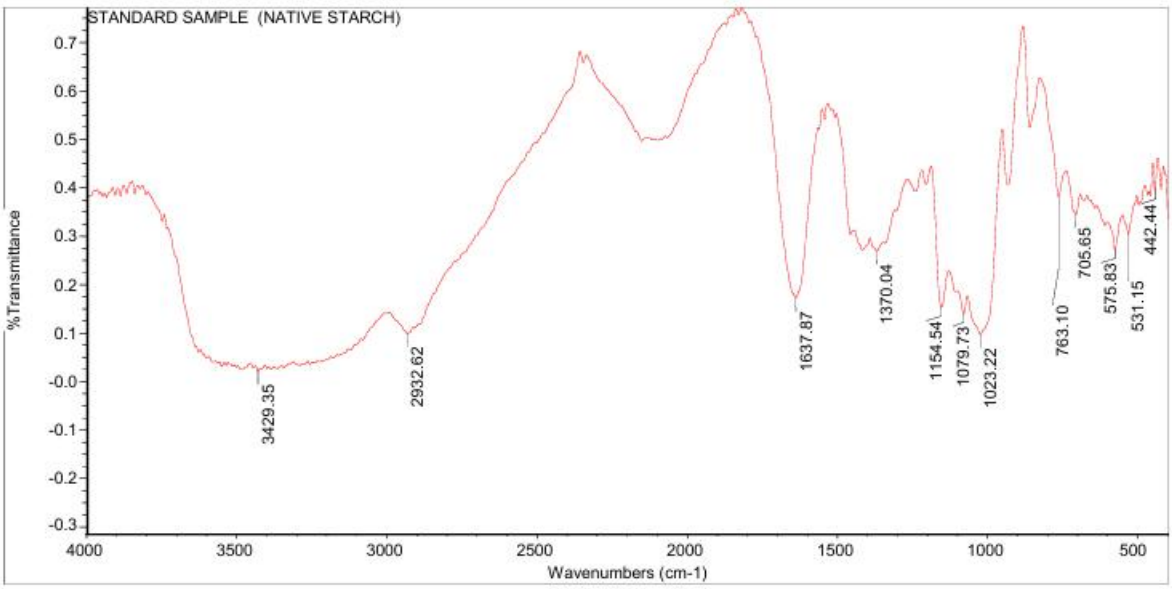

Figure 3.0: FTIR for Native Starch

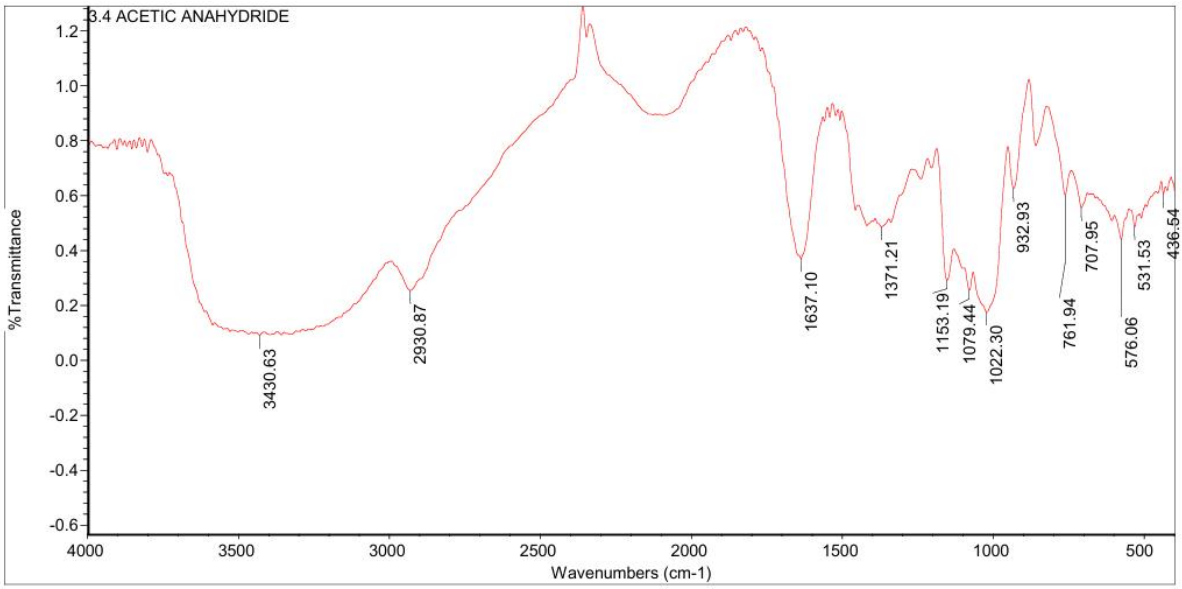

Figure 3.1: FTIR for Acetylated Starch

DISCUSSION

Fourier Transform Infrared (FTIR) spectroscopy was used to identify and confirm the functional groups present in the native and chemically modified starch samples. The spectra for native acetylated starch are presented in Figures 3.0 and 3.1 respectively. Distinct differences in absorption bands among the samples provide evidence of successful chemical modification of the starch molecules.

The native starch spectrum (Figure 3.0) exhibited characteristic absorption bands typical of polysaccharides. A broad band around 3400 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups involved in intra- and intermolecular hydrogen bonding. The weak band near 2920 cm^{-1} arises from C–H stretching vibrations of methylene groups, while the strong absorption in the 1150–1000 cm^{-1} region represents C–O–C and C–O stretching of the glycosidic linkages. A weak band around 1640 cm^{-1} is attributed to bound water molecules.

The acetylated starch spectrum (Figure 3.1) displayed a distinct absorption peak around 1740 cm^{-1}, which is attributed to the carbonyl (C=O) stretching vibration of ester groups, confirming acetylation. An additional peak at 1230–1260 cm^{-1} corresponds to C–O stretching of the acetyl ester linkage. The observed decrease and narrowing of the O–H stretching band intensity around 3400 cm^{-1} reflects the replacement of hydroxyl groups by acetyl moieties, reducing intermolecular hydrogen bonding. Slight intensity variations in the 1000–1150 cm^{-1} region also indicate modifications to the starch backbone."

Comparative analysis of the spectra clearly demonstrates that the acetylation chemical modifications were successfully achieved.

3.3 DEGREE OF SUBSTITUTION (DOS) PERCENTAGE YIELD AND GELATINIZATION TEMPERATURE OF MODIFIED STARCH

Table 3.2: Degree of substitution (DOS) percentage yield and gelatinization temperature of modified starch

Sample	Percentage yield	DOS	Gelatinization temp ^{0}C
Starch acetate	61	0.34	51

DISCUSSION

The chemical modification of native starch through acetylation was successfully achieved, as reflected in the percentage yield, degree of substitution, and observed thermal and structural changes (Gelatinization).

The percentage yield serves as a direct indication of the efficiency of the modification process. In this study, the recovered yields were 61% for starch. These value, although slightly below the theoretical maximum, are consistent with the expected recovery range for chemically modified starches. The slight loss in yield can be attributed to multiple factors such as material loss during washing and filtration, dissolution of low molecular fragments in the reaction medium, and partial hydrolysis of starch granules under alkaline conditions. This outcome shows that while some starch chains were degraded or solubilized, the main polymer backbone remained intact, resulting in a reasonable balance between modification efficiency and product recovery.

The DOS values obtained were 0.34 for starch acetate, representing moderate substitution levels. These results imply that approximately one in every ten hydroxyl groups was successfully replaced by acetyl groups. A moderate DOS of this nature is desirable because it introduces sufficient functional changes to improve the starch's physical and chemical behavior without completely destroying its granular structure or crystalline order. Excessive substitution would make the starch too soluble and reduce its film-forming and structural integrity, while too little substitution might not significantly alter its performance. Therefore,

the DOS values reported here suggest that the modification reactions were well controlled and effective in tuning the starch properties toward the desired balance of hydrophilicity, flexibility, and processability.

The moderate DOS values directly influenced several key material properties. The substitution of hydroxyl groups by acetyl groups reduces the extensive hydrogen bonding network that holds the starch granules together, resulting in a looser, more open structure. This structural change increases water affinity, swelling power, and solubility. The introduction of acetyl groups enhances the hydrophilic–hydrophobic balance, improving film flexibility while slightly reducing intermolecular cohesion. These molecular alterations explain the improved gelatinization and swelling behaviours observed for the modified starches.

One of the outcomes of the modification process was the reduction in gelatinization temperature. The native starch gelatinized at 65 °C, whereas the acetylated starches gelatinized at approximately 51 °C. The reduction in gelatinization temperature indicates that the structural order of the starch granules was partially disrupted by chemical substitution. Gelatinization involves the unwinding of double helices and the breakdown of crystalline regions within the granules as they absorb water and swell. In native starch, these regions are stabilized by strong hydrogen bonds between hydroxyl groups. Substitution with acetyl groups weakens these interactions, making the crystalline zones less stable and easier to disrupt. Consequently, less heat energy is required to achieve gelatinization. The increased hydrophilicity and partial fragmentation of the granules further facilitate this process, allowing water to penetrate the structure more easily and trigger gelatinization at a lower temperature.

3.4 BIOPLASTIC FORMULATION; COLOUR AND THICKNESS.

Table 3.3: Bioplastic Formulation; Colour and Thickness

	ETHYLENE GLYCOL (plasticizer) (g)	FILLER (kaolinite) (g)	Colour	Thickness (mm)
Starch Acetate	2	0	White	0.413
		0.5	Milky	0.512
		1.0	Milky	0.518
	3	0	White	0.412
		0.5	Milky	0.520
		1.0	Milky	0.525
	4	0	White	0.420
		0.5	Milky	0.521
		1.0	Milky	0.522
	5	0	White	0.421
		0.5	Milky	0.530
		1.0	Milky	0.531

Discussion

The bioplastic formulations, made from starch acetate, show that adding ethylene glycol (plasticizer) and kaolinite (filler) affects both the colour and thickness of the films. As the amount of ethylene glycol and kaolinite increases,the colour shifts from white to milky, indicating changes in the material's structure. The thickness of the films also increases with more ethylene glycol and filler, likely because ethylene glycol adds flexibility and kaolinite enhances structural integrity. These variations suggest that both play a key role in customizing the bioplastic's properties for different applications. Overall, ethylene glycol and kaolinite can be used to fine-tune the physical characteristics of bioplastics for specific uses.

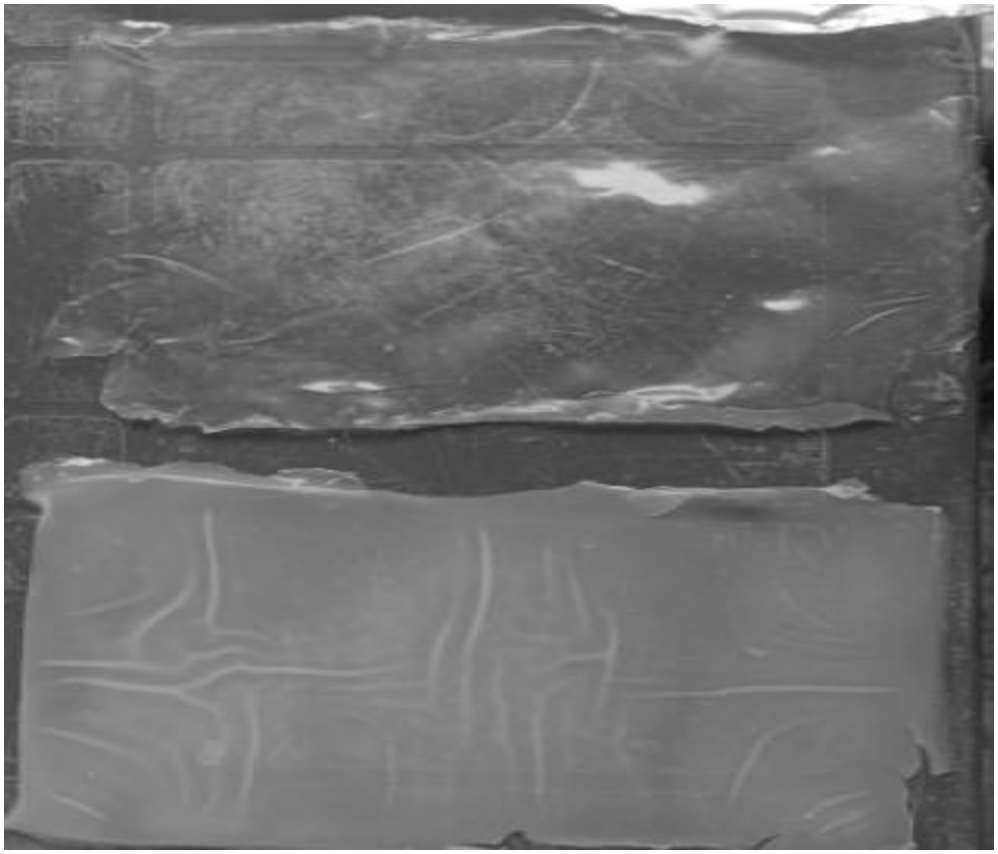

Plate 3.1: Biofilm

3.5 ABSORPTION AND SOLUBILITY TEST ON PLASTICS FILMS.

Table 3.4: Absorption and solubility test on plastics films

	ETHYLENE GLYCOL (plasticizer) (g)	FILLER (kaolinite) (g)	% Water absorption	% Moisture absorption	% 1M HCl absorption	Solubility in base (1M NaOH)	% Ethanol absorption
Starch Acetate	2	0	20.0	9.0	20.0	Soluble	Insoluble
		0.5	33.3	7.5	26.6	Soluble	Insoluble
		1.0	25.0	7.0	23.8	Soluble	Insoluble
	3	0	26.5	20.1	28.4	Soluble	Insoluble
		0.5	28.5	10.3	29.5	Soluble	Insoluble
		1.0	24.3	10.0	24.1	Soluble	Insoluble
	4	0	31.3	28.0	35.5	Soluble	Insoluble
		0.5	28.9	17.0	36.1	Soluble	insoluble
		1.0	28.3	12.0	29.4	Soluble	Insoluble
	5	0	38.3	25.0	43.0	Soluble	Insoluble
		0.5	38.5	21.4	37.2	Soluble	Insoluble
		1.0	32.1	14.0	33.3	Soluble	Insoluble

Discussion

The absorption and solubility behaviour of the starch-acetate bioplastic films were significantly influenced by the combined effects of ethylene-glycol plasticizer and kaolinite filler. Overall, increasing the plasticizer concentration from 2 g to 5 g resulted in higher water absorption, moisture uptake and acid absorption, while the incorporation of 0.5–1.0 g kaolinite produced a moderating effect on most of these properties. Water absorption ranged between 20.0% and 38.5%, increasing progressively with plasticizer loading. This behaviour reflects the hydrophilizing effect of polyol plasticizers, which disrupt intermolecular hydrogen bonding in starch-derived matrices, increase chain mobility and expose more hydrophilic groups that interact with water molecules (García *et al.,* 2015; Müller *et al*., 2017). As a result, higher ethylene-glycol content creates a more open polymer structure that facilitates water diffusion. In contrast, kaolinite incorporation generally reduced water uptake at each plasticizer level because the filler's layered silicate structure increases tortuosity and reduces accessible sorption pathways. The barrier effect provided by clay minerals in starch-based composites has been widely documented (Carvalho *et al.,* 2014; Rhim and Ng, 2007).

Moisture absorption followed a similar pattern, ranging from 7.0% to 28.0% and increasing with plasticizer concentration. Higher ethylene-glycol levels promote interaction with atmospheric humidity due to increased mobility of hydroxyl-rich polymer chains, an effect consistent with earlier findings in plasticized starch films (Talja *et al*., 2008). The presence of kaolinite reduced moisture uptake in most formulations because the filler improves structural compactness and reduces vapour permeability. Acid absorption (1 M HCl) also increased with plasticizer concentration, reaching up to 43.0% at 5 g plasticizer. Starch acetate contains ester linkages susceptible to acid-catalysed hydrolysis, and increased free volume in plasticized matrices facilitates acid penetration and accelerates partial degradation (Arvanitoyannis, 1999; Thakhiew *et al*., 2013). Although kaolinite slightly restricted

diffusion at lower plasticizer levels, its influence diminished when plasticizer concentrations were high, suggesting that swelling and hydrolytic effects dominated transport behaviour in strongly plasticized matrices.

All samples were soluble in 1 M NaOH regardless of plasticizer or filler content. This universal solubility is attributed to alkaline saponification of acetate ester bonds, resulting in rapid chemical degradation of the polymer network. Reports on esterified starch systems similarly indicate that structural reinforcement does not prevent alkaline cleavage of ester linkages (Liu *et al.*, 2009). Conversely, the films remained fully insoluble in ethanol and showed negligible ethanol absorption. This outcome reflects the hydrophilic nature of starch-acetate materials, which interact preferentially with aqueous polar solvents rather than with lower-polarity organic solvents such as ethanol. Comparable behaviours have been reported in modified starch films and polysaccharide-based plastic materials (Bertuzzi *et al*., 2012; Juma *et al*., 2019).

Overall, the results highlight a balance between two opposing material effects: the plasticizer-induced relaxation and hydrophilicity versus filler-induced reinforcement and diffusion resistance. Ethylene glycol enhances flexibility but increases water sensitivity and chemical reactivity, while kaolinite improves dimensional stability and reduces moisture and water interaction. However, neither additive prevents alkaline degradation due to the intrinsic susceptibility of starch-acetate ester bonds to saponification. This combined behaviour ultimately determines the functional stability and potential application range of the formulated bioplastic films.

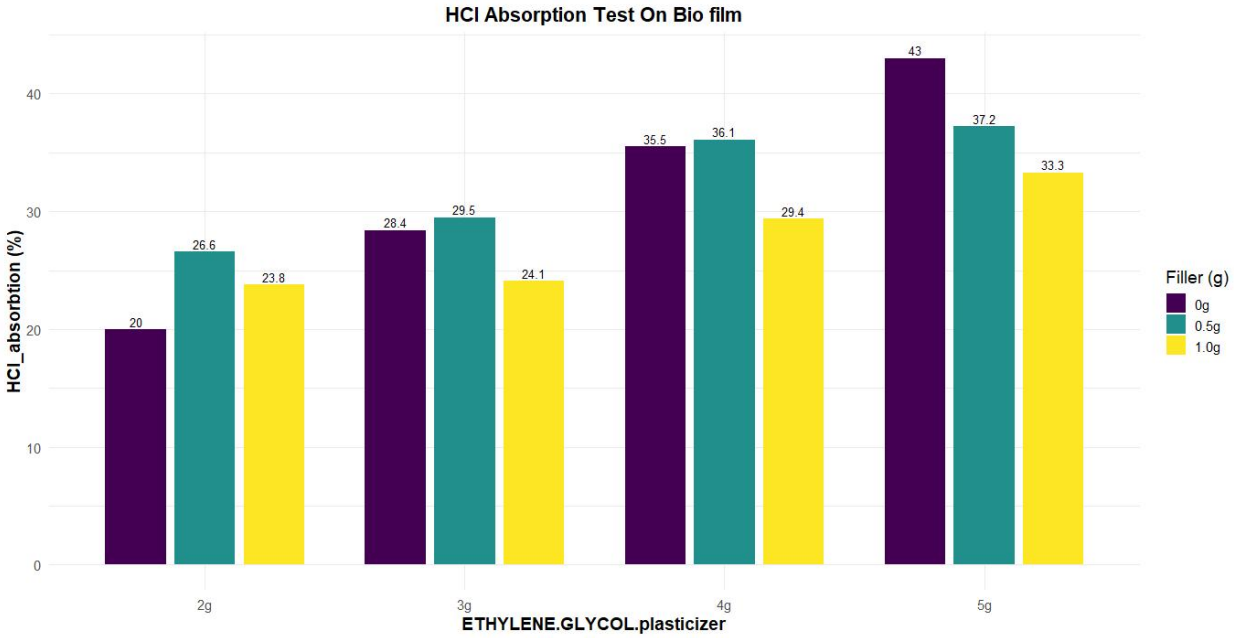

Figure 3.2: HCl Absorption Test on Bio Film

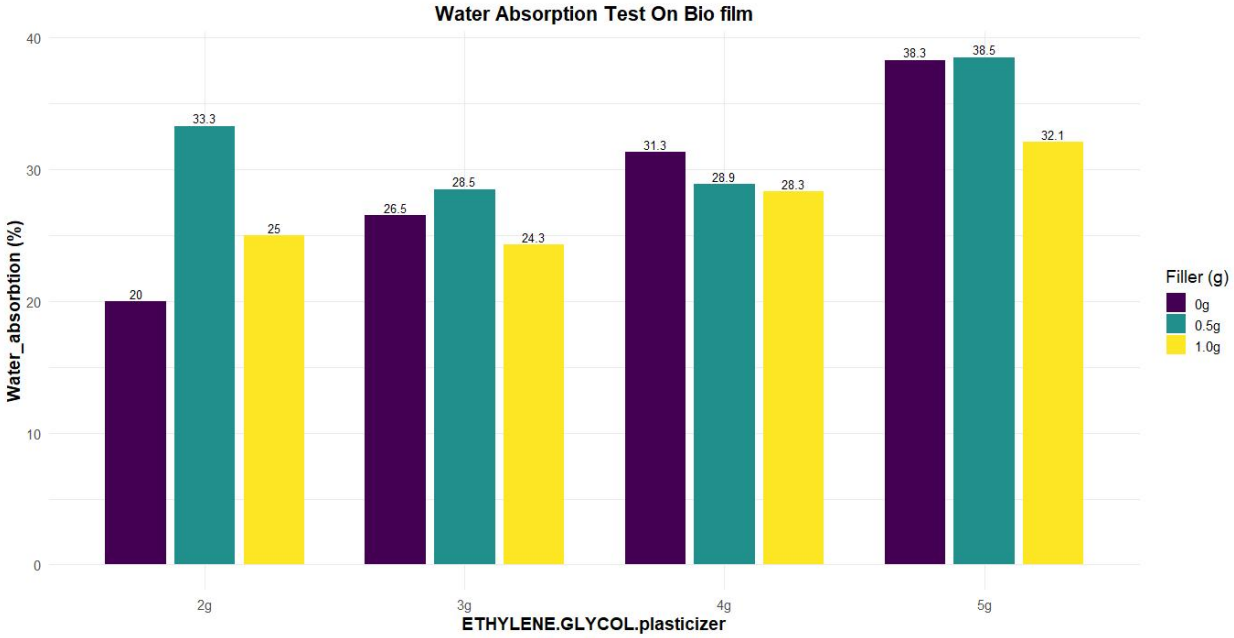

Figure 3.3: Water Absorption Test on Bio film

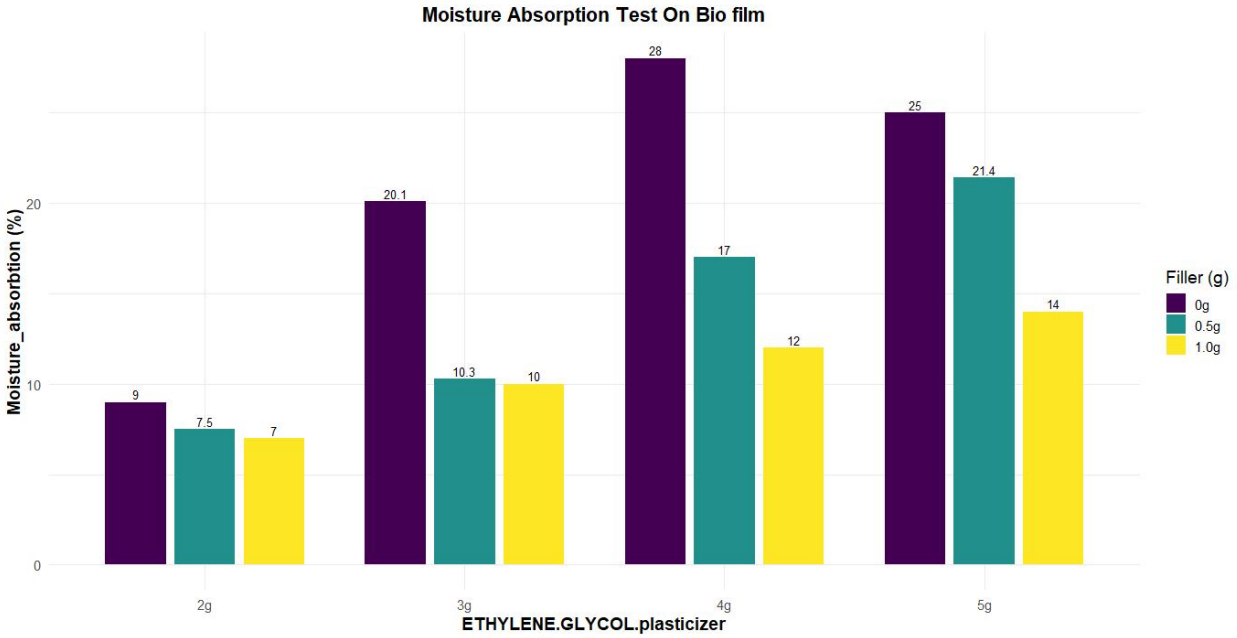

Figure 3.4: Moisture Absorption Test on Bio Film

Table 3.5: Soil Burial Degradability test

	ETHYLENE GLYCOL (plasticizer)	FILLER (kaolinite)	% degradability Week 1	% degradability Week 2	% degradability Week 3	% degradability Week 4
Starch Acetate	2g	0g	22.5	43.0	63.0	Degraded
		0.5g	17.0	32.0	52.0	Degraded
		1.0g	10.0	20.1	40.1	Degraded
	3g	0g	25.5	49.0	69.0	Degraded
		0.5g	18.6	35.2	55.2	Degraded
		1.0g	12.0	22.0	52.0	Degraded
	4g	0g	31.0	58.0	78.0	Degraded
		0.5g	22.0	42.0	62.0	Degraded
		1.0g	17.0	32.0	52.0	Degraded
	5g	0g	34.0	61.0	81.0	Degraded
		0.5g	26.5	50.0	70.0	Degraded
		1.0g	23.5	45.0	65.0	Degraded

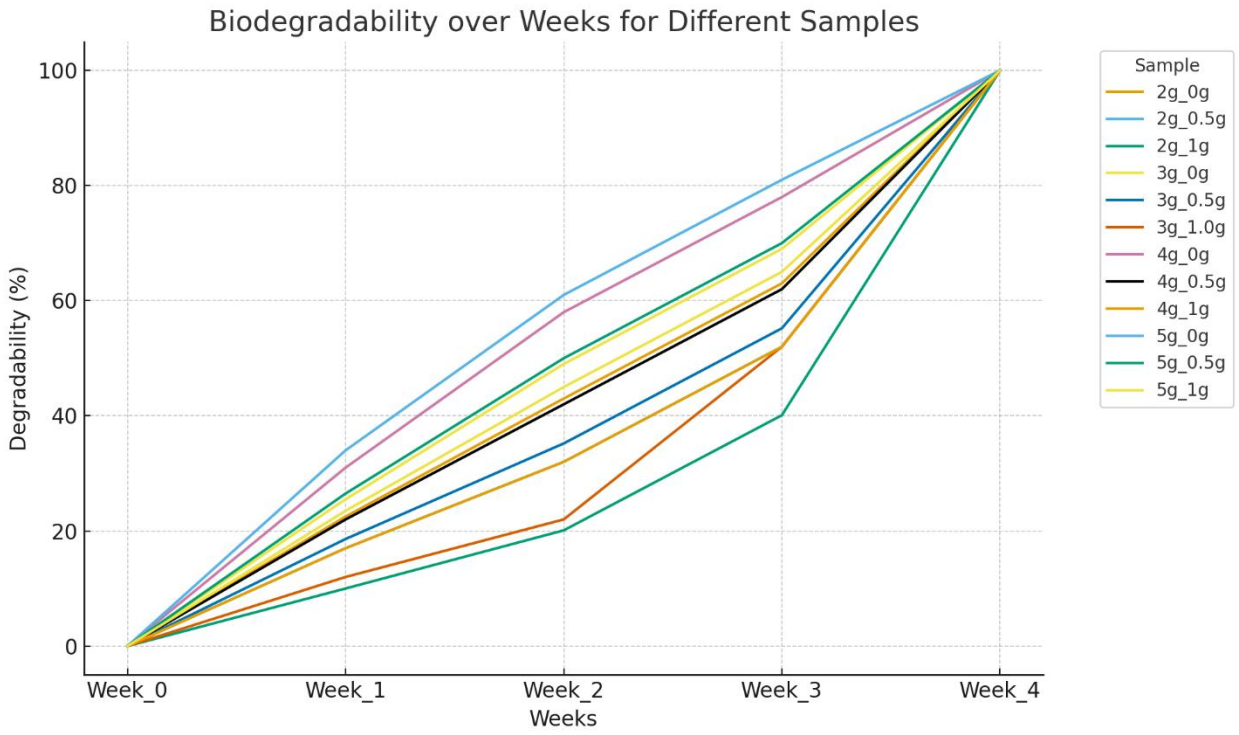

Figure 3.5: Biodegradability Over Weeks for Different Samples

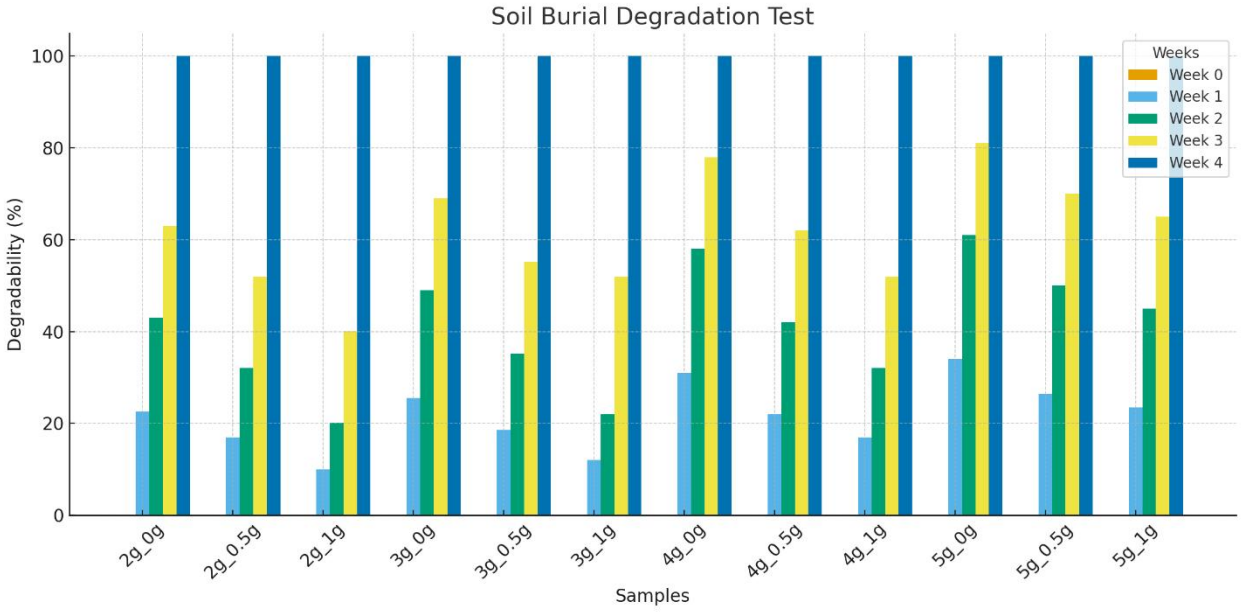

Figure 3.6: Biogradability Bar Chart

Discussion

Findings from the Soil Burial Degradation Test (as illustrated in the bar chart and table 3.5), indicated that all the samples exhibited increased biodegradability over the four weeks, with some samples achieving 100% degradability by Week 4.

- Week 0: All samples initially had 0% biodegradability, which is expected as no degradation would have occurred before the soil burial test began.
- Week 1 to Week 4: A steady increase in degradability is observed across all samples, with the biodegradability percentages escalating as the weeks progressed. Notably, the samples with higher ethylene glycol content (such as 5g) exhibited a quicker degradation rate compared to those with lower ethylene glycol content (like 2g).
- Influence of Ethylene Glycol: The results suggest that as the amount of ethylene glycol increased, the degradability of the bioplastic films improved. This could be due to the fact that plasticizers like ethylene glycol enhance the flexibility of the polymer chains, making the films more susceptible to microbial attack. For instance, the 5g samples (regardless of kaolinite content) displayed high biodegradability, especially at Week 4, compared to the 2g samples, which had relatively lower biodegradability.
- Influence of Kaolinite Filler: The presence of kaolinite as a filler also affected the biodegradability. While all the samples reached 100% biodegradability by Week 4, the 0g kaolinite samples exhibited higher biodegradability rates in the earlier weeks compared to the 0.5g and 1g kaolinite samples. This could suggest that the filler might slightly hinder the microbial access to the bioplastic matrix, as the presence of kaolinite may add rigidity to the film, thus slowing down the degradation process in the initial stages.

3.6 MECHANICAL PROPERTIES

Table 3.6: Mechanical properties

Starch acetate film		Tensile Strength (MPa)	Elongation at Break (%)	Yield Point (MPa)	Toughness (MJ/m^3)
2g Ethylene Glycol	0g Filler	6.62	2.83	0.92	0.58
	0.5g Filler	3.05	8.66	0.65	0.60
	1g Filler	10.74	4.48	1.30	0.62
3g Ethylene Glycol	0g Filler	2.00	3.09	0.38	0.63
	0.5g Filler	4.40	5.15	0.49	0.65
	1g Filler	10.05	4.60	1.80	0.67
4g Ethylene Glycol	0g Filler	0.91	20	0.37	0.70
	1.5g Filler	1.38	13.5	0.55	0.71
	1g Filler	1.67	17	0.47	0.73
5g Ethylene Glycol	0g Filler	0.79	25	0.27	0.78
	0.5g Filler	1.79	22	0.87	0.80
	1g Filler	2.36	20	1.25	0.82

The mechanical properties of the starch acetate films, as influenced by varying amounts of ethylene glycol (2g, 3g, 4g, 5g) and filler (0g, 0.5g, 1g), demonstrate distinct trends in tensile strength, elongation at break, yield point, and toughness.

Without Filler: As ethylene glycol content increases from 2g to 5g, the material exhibits decreasing tensile strength and yield point, while elongation at break and toughness initially improve, reaching their peak at 5g ethylene glycol. However, the material becomes weaker and less rigid with higher ethylene glycol concentrations.

With Filler: The addition of filler leads to significant improvements in tensile strength, yield point, and toughness across all glycol concentrations, reversing the negative effects seen without filler. However, elongation at break decreases as filler content increases, particularly at higher glycol concentrations, reflecting a reduction in material flexibility with increasing glycol and filler levels. The findings suggest that a balance between ethylene glycol and filler content is necessary to optimize the material's mechanical properties, depending on the desired application.

This combination demonstrates the potential of starch acetate-based bioplastics as a viable alternative for environmentally friendly packaging materials that meet the mechanical demands of

the industry while remaining biodegradable (Jumaidin *et al.,* 2024; Mbey *et al*., 2012; Meité *et al*., 2018).

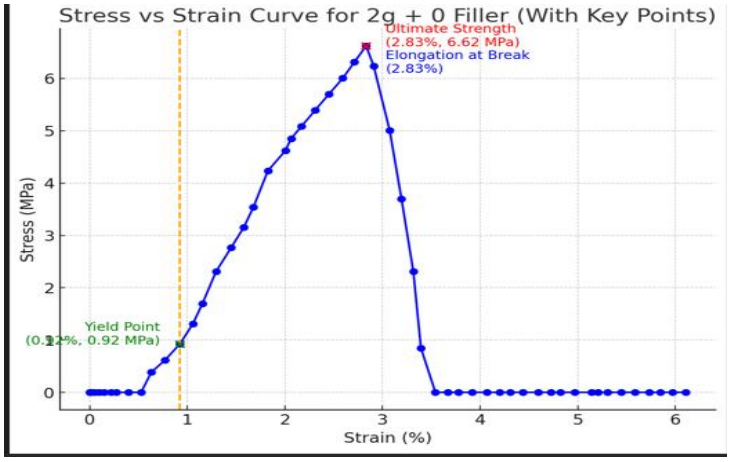

Figure 3.6: Stress strain curve for 2 g ethylene glycol and 0 g kaolinite

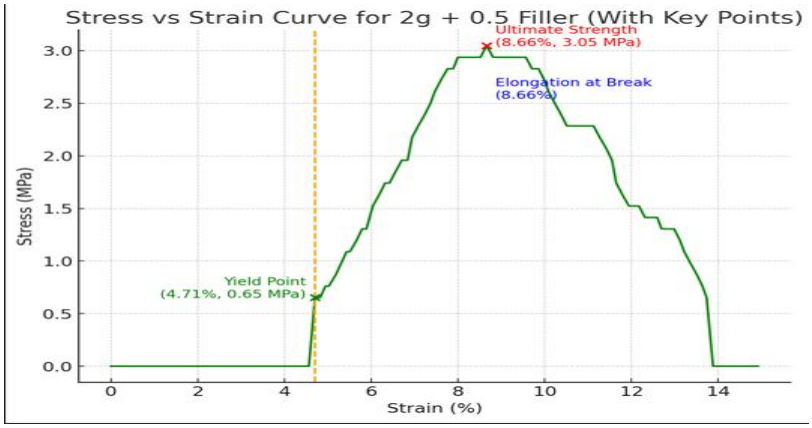

Figure 3.7: Stress strain curve for 2 g ethylene glycol and 0.5 g kaolinite

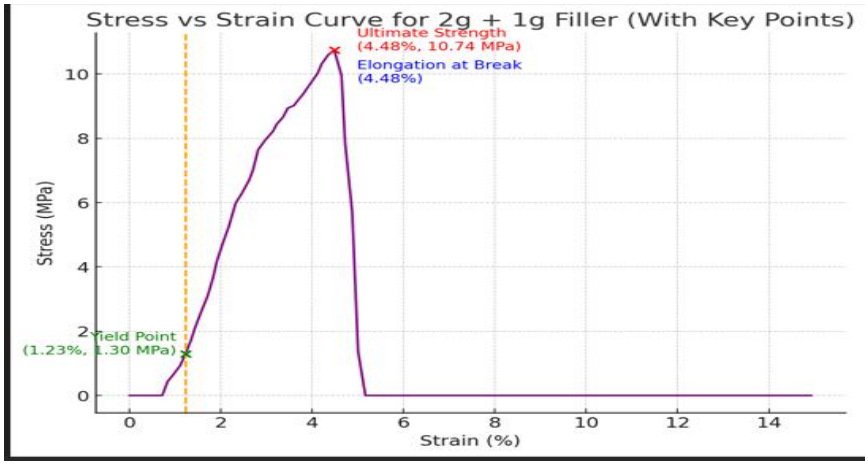

Figure 3.8: Stress strain curve for 2 g ethylene glycol and 1 g kaolinite

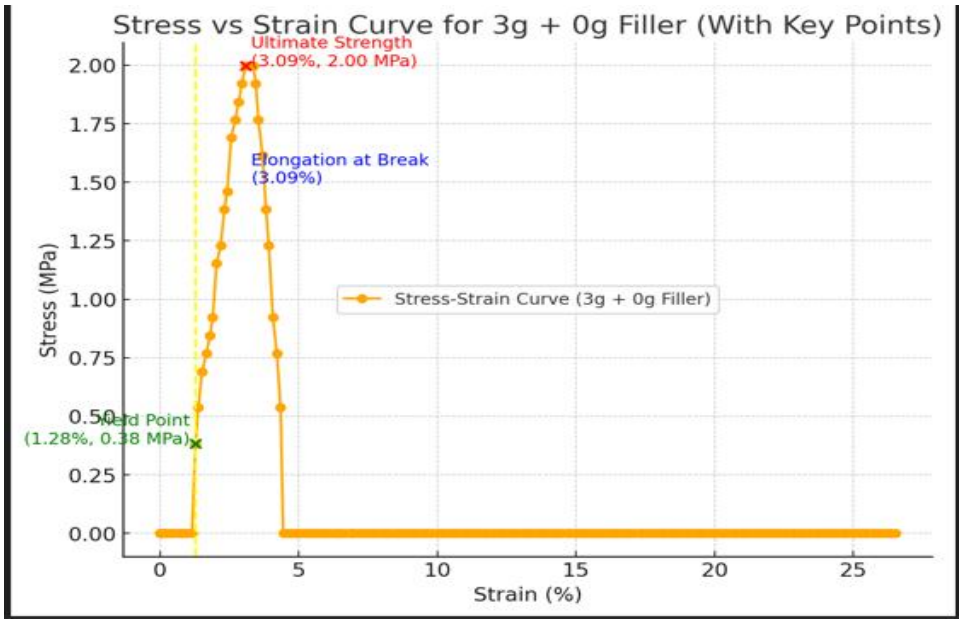

Figure 3.9: Stress strain curve for 3 g ethylene glycol and 0 g kaolinite

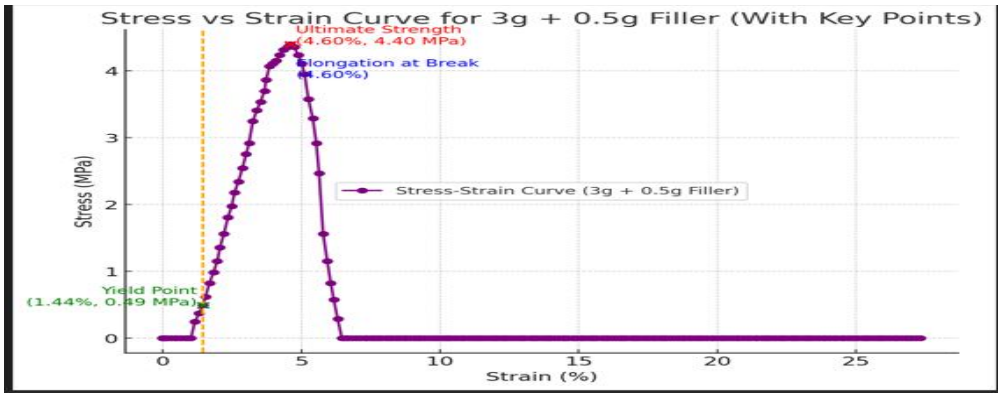

Figure 3.10: Stress strain curve for 3 g ethylene glycol and 0.5 g kaolinite

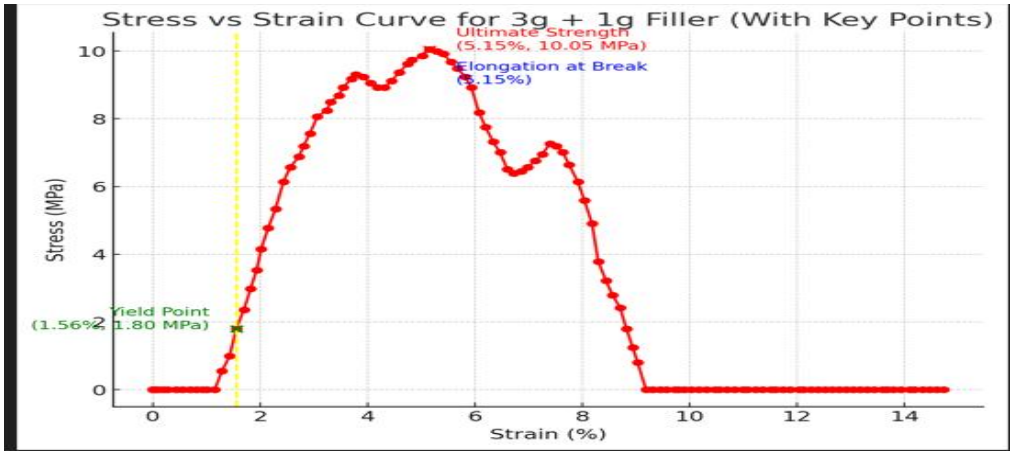

Figure 3.11: Stress strain curve for 3 g ethylene glycol and 1 g kaolinite

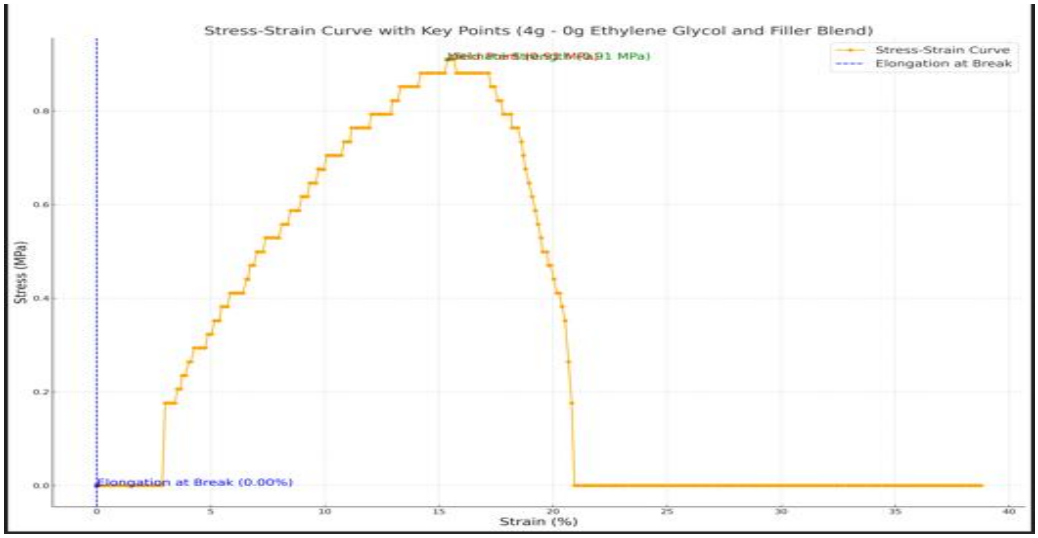

Figure 3.12: Stress strain curve for 4 g ethylene glycol and 0 g kaolinite

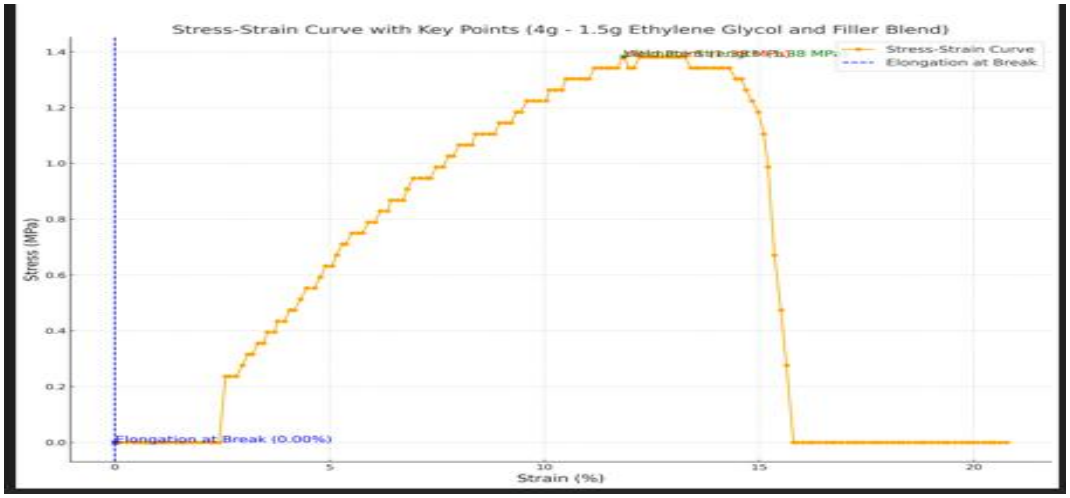

Figure 3.13: Stress strain curve for 4 g ethylene glycol and 0.5 g kaolinite

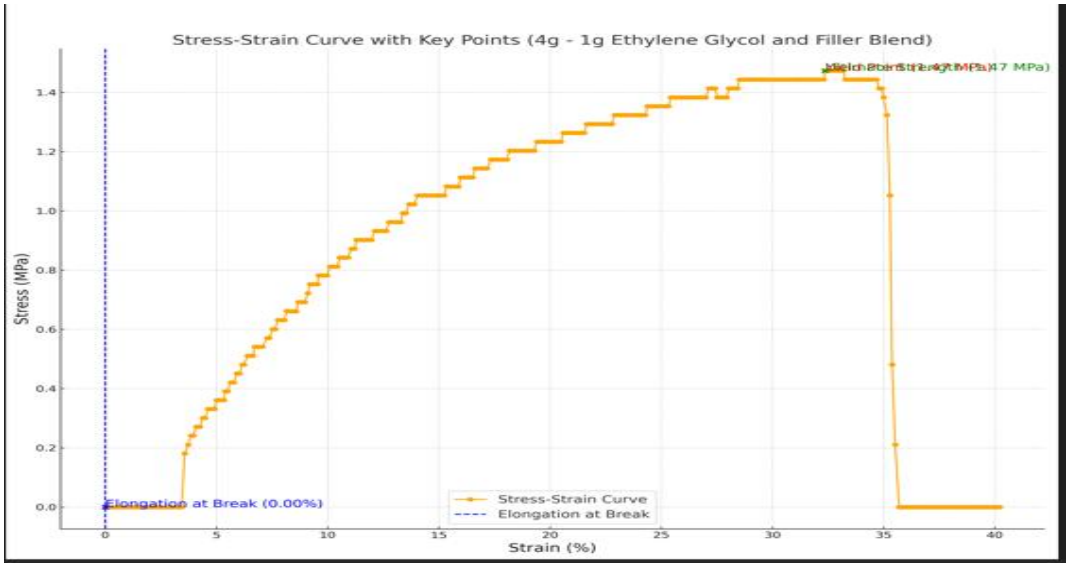

Figure 3.14: Stress strain curve for 4g ethylene glycol and 1 g kaolinite

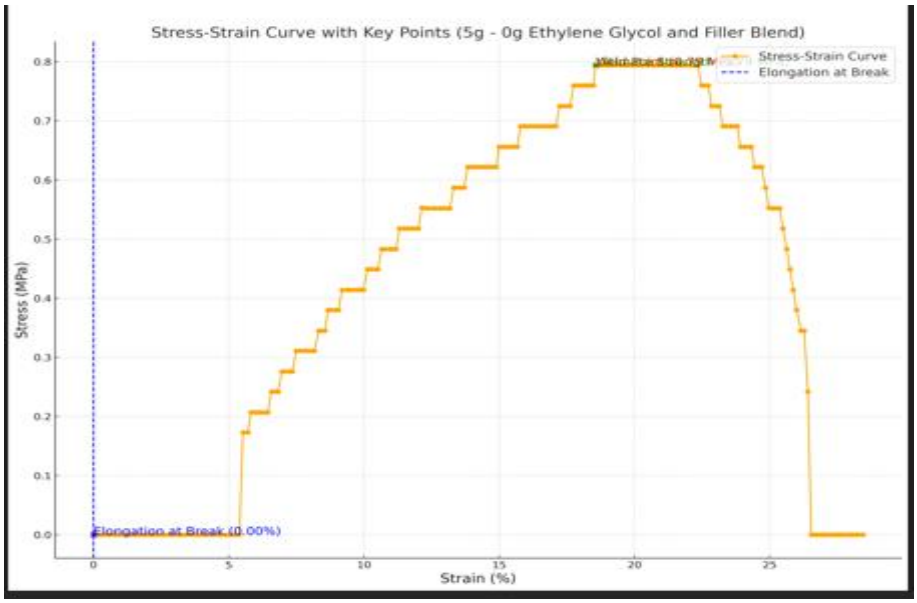

Figure 3.15: Stress strain curve for 5 g ethylene glycol and 0 g kaolinite

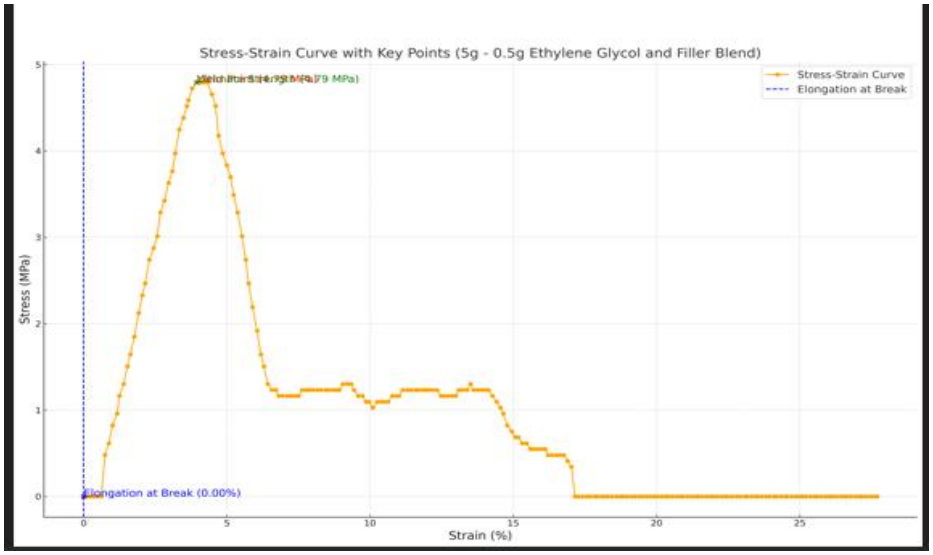

Figure 3.16: Stress strain curve for 5 g ethylene glycol and 0.5 g kaolinite

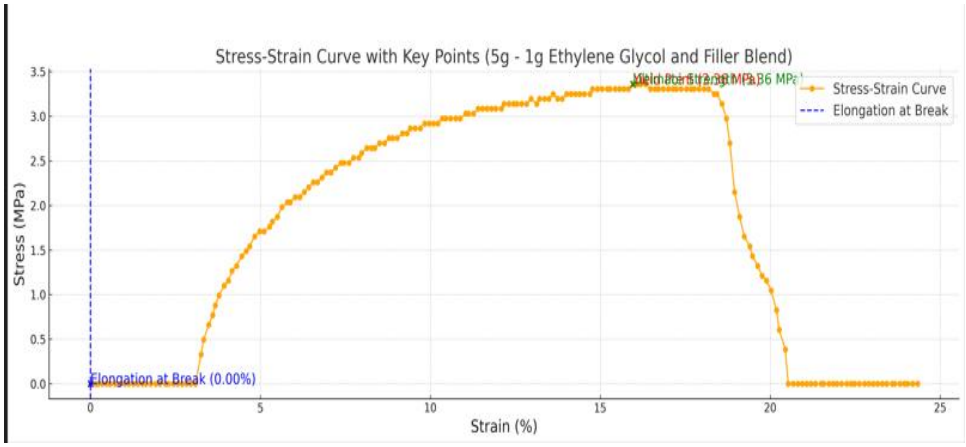

Figure 3.17: Stress strain curve for 5g ethylene glycol and 1 g kaolinite

3.7 TGA/DTA FOR STARCH ACETATE BIOPLASTIC

The TGA/DTA analysis provides valuable insights into the thermal stability and behavior of starch acetate-based bioplastic films, which is crucial for their application in biodegradable packaging, medical devices, and other fields requiring good thermal performance.

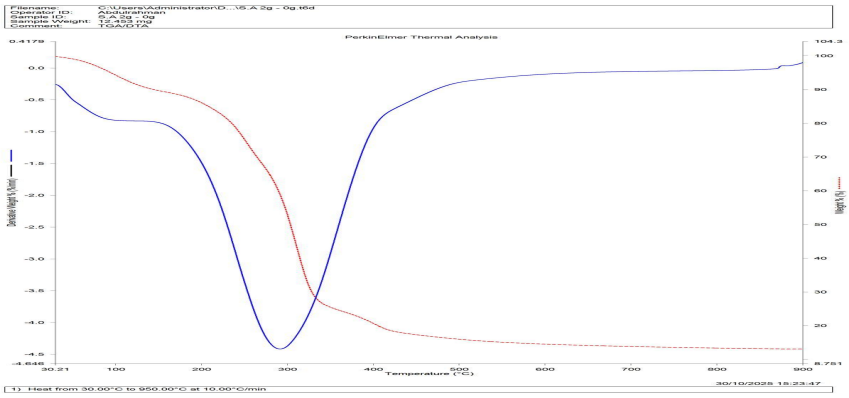

Figure 3.18: TGA/DTA Curve 2 g ethylene glycol and 0 g kaolinite.

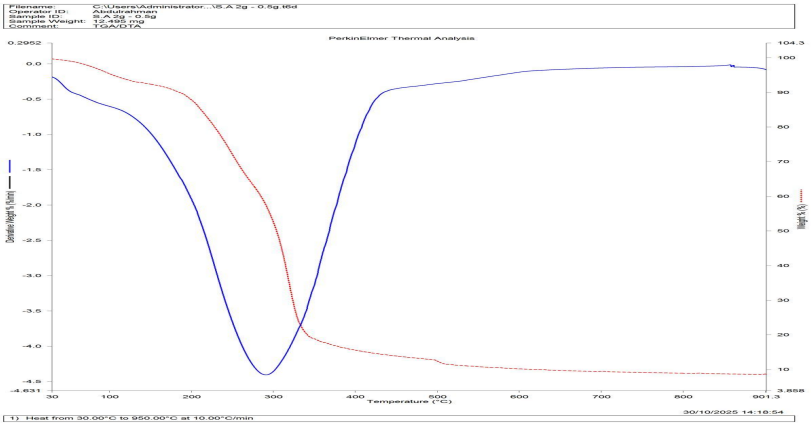

Figure 3.19: TGA/DTA Curve 2 g ethylene glycol and 0.5 g kaolinite.

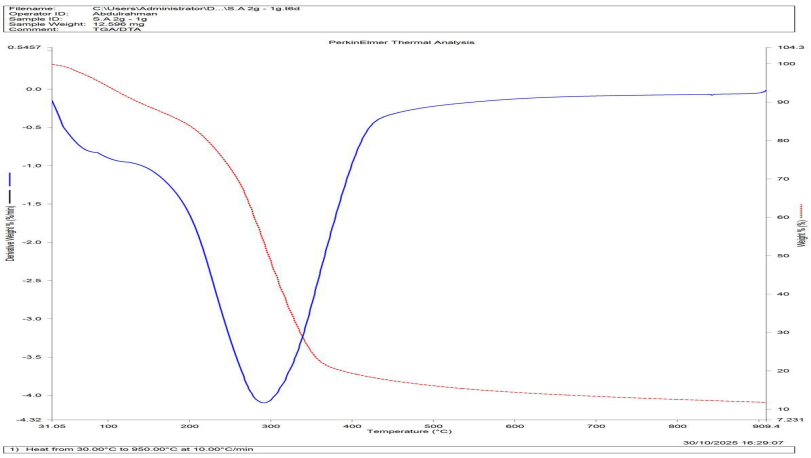

Figure 3.20: TGA/DTA Curve 2 g ethylene glycol and 1 g kaolinite.

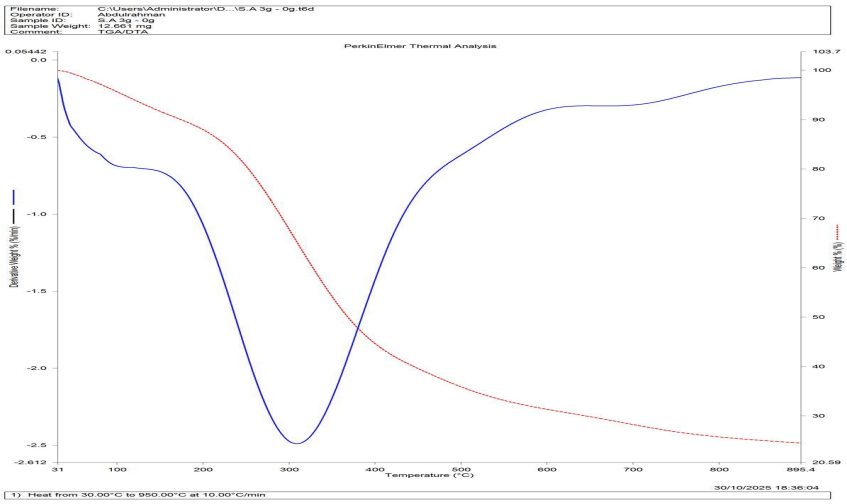

Figure 3.21: TGA/DTA Curve 3 g ethylene glycol and 0 g kaolinite.

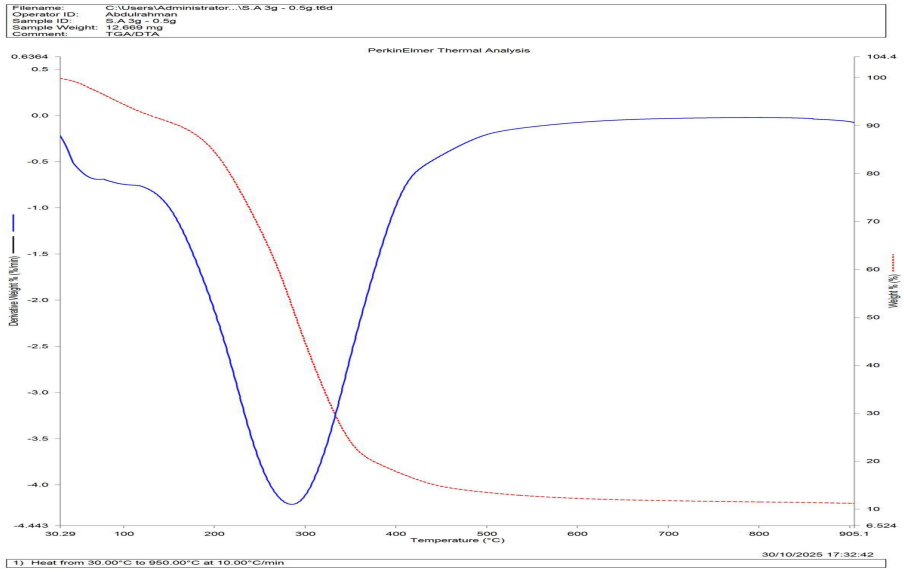

Figure 3.22: TGA/DTA Curve 3 ethylene glycol and 0.5 g kaolinite.

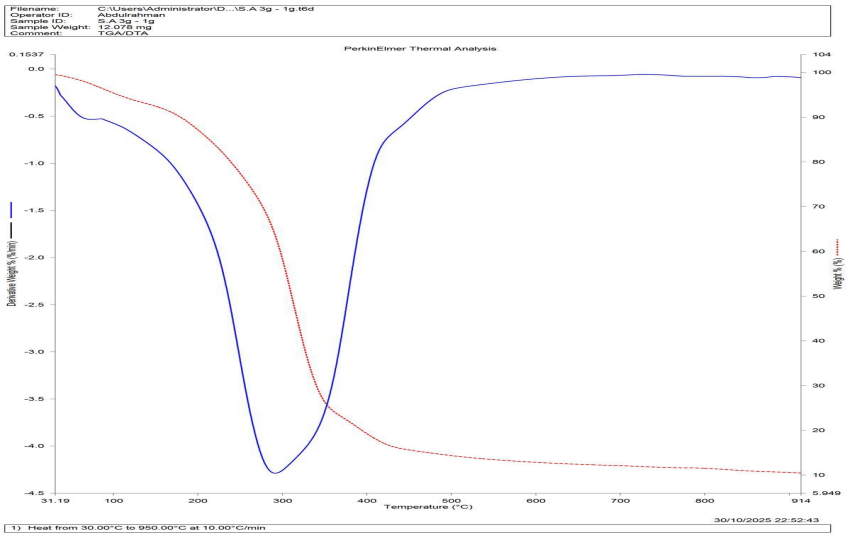

Figure 3.23: TGA/DTA Curve 3g ethylene glycol and 1 g kaolinite.

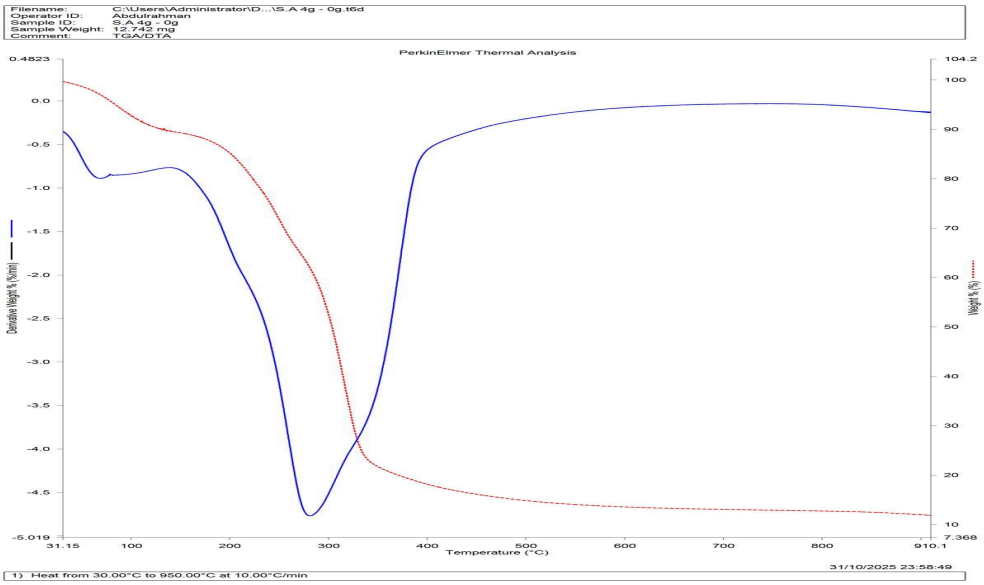

Figure 3.24: TGA/DTA Curve 4 g ethylene glycol and 0 g kaolinite.

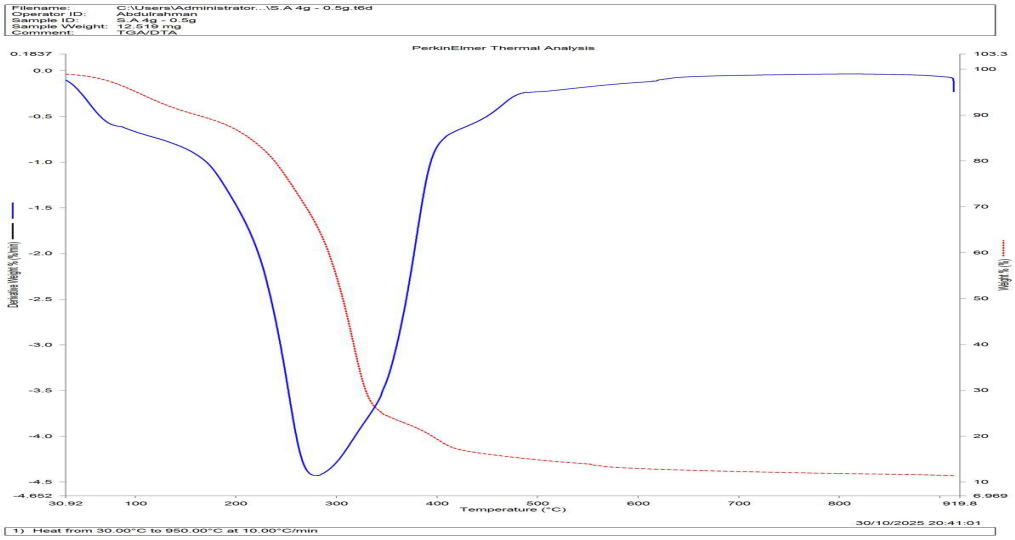

Figure 3.25: TGA/DTA Curve 4 g ethylene glycol and 0.5 g kaolinite.

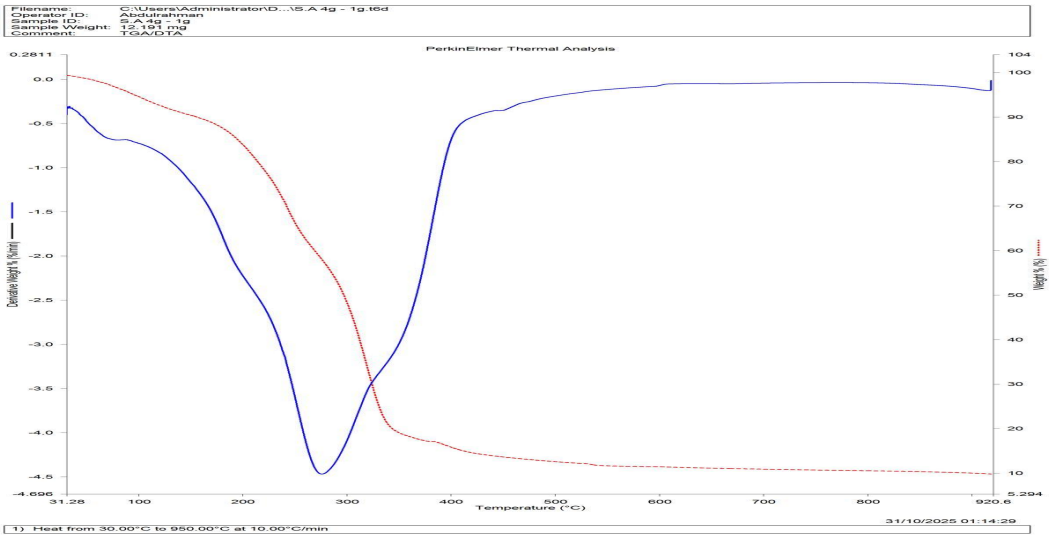

Figure 3.26: TGA/DTA Curve 4 g ethylene glycol and 1 g kaolinite.

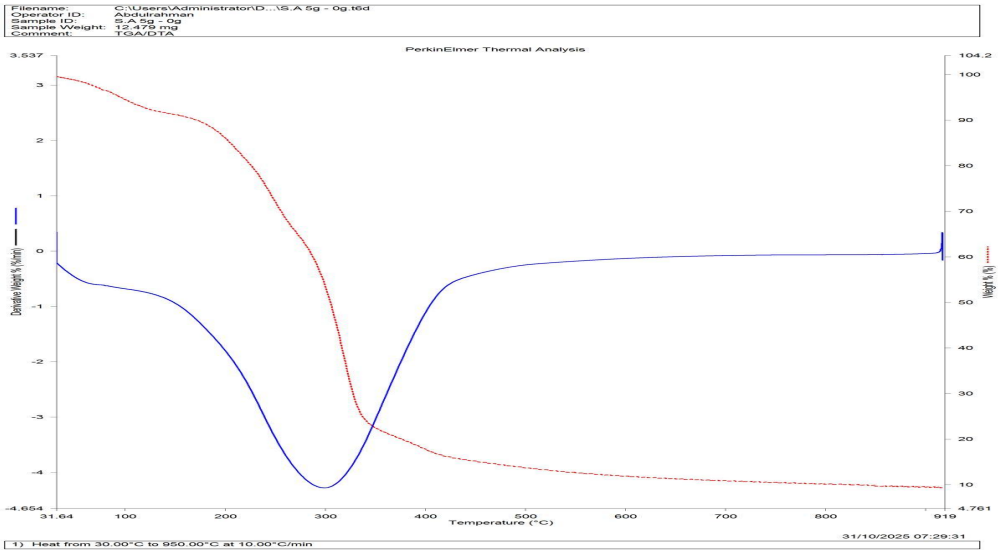

Figure 3.27: TGA/DTA Curve 5 g ethylene glycol and 0 g kaolinite.

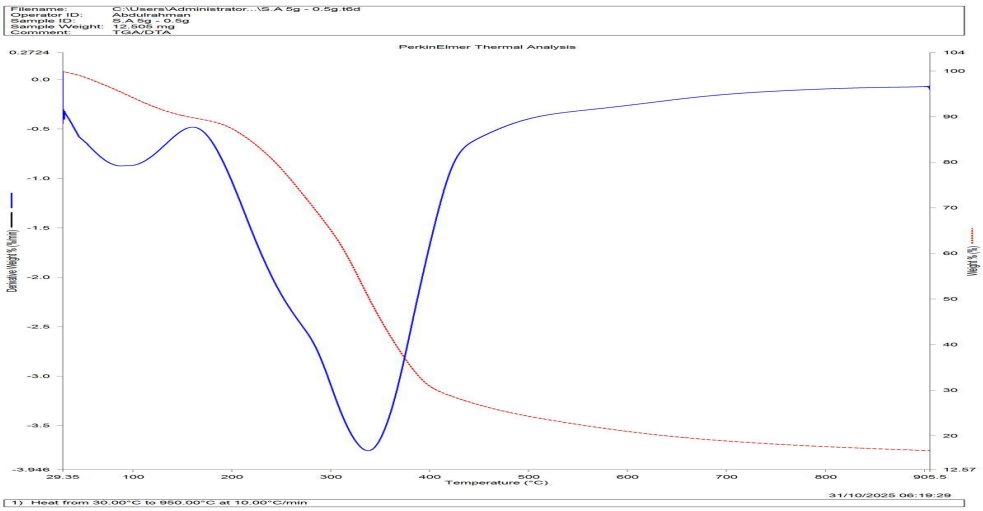

Figure 3.28: TGA/DTA Curve 5 g ethylene glycol and 0.5 g kaolinite.

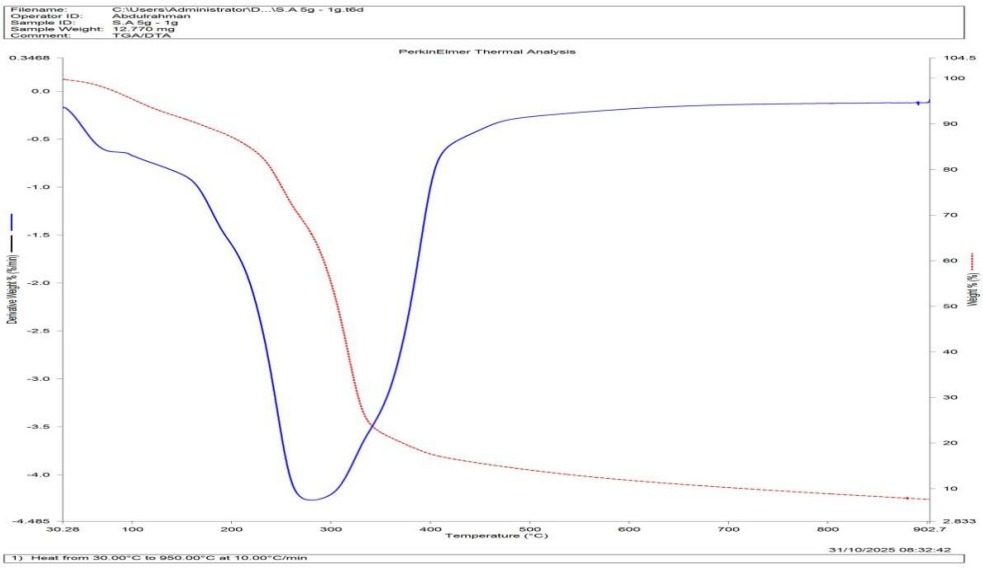

Figure 3.29: TGA/DTA Curve 5 g ethylene glycol and 1 g kaolinite.

Table 3.7: TGA/DTA FOR STARCH ACETATE BIOPLASTIC

Ethylene glycol Content (g)	Kaolinite Content (g)	Onset Temperature (°C)	Max Degradation Temperature (°C)	Tg (°C)	Thermal Stability
2g	0g	180	240	89	Low
	0.5g	200	250	82	Moderate
	1g	220	255	85	High
3g	0g	150	220	77	Low
	0.5g	200	185	72	Moderate
	1g	220	221	75	High
4g	0g	55	200	71	Low
	0.5g	70	250	66	High
	1g	75	260	68	Very high
5g	0g	55	170	60	Very low
	0.5g	65	200	56	Moderate
	1g	70	210	57	High

The TGA/DTA results for starch acetate bioplastics show that **ethylene glycol** and **kaolinite filler** both influence the material's thermal properties, including **Onset Temperature**, **Maximum Degradation Temperature**, and **Glass Transition Temperature (Tg)**.

Ethylene glycol primarily acts as a plasticizer, reducing the bioplastic's thermal stability. As the ethylene glycol content increases, the **Onset Temperature** decreases, from 180°C at 2g to 55°C at 5g. Similarly, the **Maximum Degradation Temperature** also decreases, from 220°C at 2g to 170°C at 5g, indicating that higher ethylene glycol concentrations make the material more susceptible to degradation at lower temperatures. Additionally, the **Glass Transition Temperature (Tg)** decreases with increasing ethylene glycol, from 89°C at 2g to 60°C at 5g, which suggests that higher glycol concentrations make the bioplastic more flexible by reducing its rigidity.

Kaolinite filler has the opposite effect, acting as a stabilizing agent. The addition of kaolinite increases the **Onset Temperature** across all ethylene glycol concentrations, with the onset temperature rising from 67°C to 77°C at 2g ethylene glycol when kaolinite content increases from 0g to 1g. This trend is consistent at higher ethylene glycol levels, indicating that

kaolinite enhances the thermal stability of the bioplastic. Similarly, the **Maximum Degradation Temperature** improves with kaolinite, especially at higher ethylene glycol concentrations, such as from 185°C to 221°C at 3g ethylene glycol with 1g kaolinite. This shows that kaolinite improves the bioplastic's resistance to thermal degradation. However, the **Tg** is slightly reduced with the addition of kaolinite, particularly at lower filler concentrations, although it shows a slight increase at higher filler levels, indicating some reinforcement of rigidity.

In summary, **ethylene glycol** decreases the thermal stability and rigidity of the starch acetate bioplastic, making it more flexible but also less resistant to heat degradation. In contrast, **kaolinite filler** improves thermal stability and degradation resistance, with its presence enhancing the bioplastic's ability to withstand higher temperatures. The combination of these two factors allows for optimization of the material's thermal properties, offering a balance between flexibility and rigidity depending on the desired application.

3.8 Analysis of Variance (ANOVA) for Tensile Strength: Influence of EB Percentage and Moisture Percentage.

3.8.1 ANOVA Model

fox_Data <- aov(Tensile ~ Elox * MOI)

> summary(fox_Data)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Elox	**1**	**60.41**	**60.41**	**12.332**	**0.00794 ****
MOI	**1**	**14.38**	**14.38**	**2.936**	**0.12496**
Elox:MOI	**1**	**19.27**	**19.27**	**3.935**	**0.08259 .**
Residuals	**8**	**39.19**	**4.90**		

Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

A two-way Analysis of Variance (ANOVA) was conducted to evaluate the effects of Elox, MOI, and their interaction (Elox × MOI) on the tensile strength of the developed films. The results show that Elox had a statistically significant main effect on tensile strength, $F(1, 8) = 12.33$, $p = 0.0079$, indicating that variations in Elox levels produced meaningful changes in tensile properties. This suggests that Elox is a critical formulation factor influencing polymer chain interactions and the resulting mechanical performance.

In contrast, MOI did not exhibit a significant main effect, $F(1,8) = 2.94$, $p = 0.125$, implying that changes in MOI levels alone did not produce substantial differences in tensile strength.

The interaction effect (Elox × MOI) approached statistical significance, $F(1,8) = 3.94$, $p = 0.083$, indicating a marginal interaction. Although not statistically significant at $\alpha = 0.05$, the trend suggests that the influence of Elox on tensile strength may depend on the level of MOI,

and vice versa. This emerging interaction implies that certain combinations of Elox and MOI could synergistically enhance or reduce tensile strength, even if the individual factors alone are not significant.

Overall, the ANOVA indicates that Elox is the dominant factor, while MOI plays a lesser independent role but may contribute jointly through its interaction with Elox. These findings emphasize the importance of formulation optimization involving both factors, particularly when the goal is to tailor the mechanical integrity of biodegradable films.

3.8.2 Regression Model

summary(foy_data)

Call:
lm(formula = Tensile ~ MOI + Elox, data = NUD_data)

Residuals:
Min 1Q Median 3Q Max
-2.9664 -1.8706 -0.0616 1.1349 4.0030

Coefficients:
Estimate Std. Error t value Pr(>|t|)
(Intercept) 9.0245 1.7962 5.024 0.000715 *
MOI -0.2146 0.1442 -1.488 0.170912
Elox -0.1615 0.1233 -1.310 0.222536**

Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

Residual standard error: 2.549 on 9 degrees of freedom
Multiple R-squared: 0.5613, Adjusted R-squared: 0.4638
F-statistic: 5.757 on 2 and 9 DF, p-value: 0.02454

A multiple linear regression analysis was performed to determine how MOI and Elox contribute to the tensile strength of the developed biodegradable films. The overall regression model was statistically significant, $F(2, 9) = 5.76$, $p = 0.0245$, indicating that the combined influence of MOI and Elox accounts for a meaningful proportion of the variation observed in

tensile strength. The model explained approximately 56.1% of the total variability ($R^2 = 0.5613$), with an adjusted R^2 of 0.4638, suggesting that the predictors offer a moderately strong explanation of the mechanical behavior of the films.

Although the overall model was significant, the individual regression coefficients for MOI ($\beta = -0.2146$, $p = 0.171$) and Elox ($\beta = -0.1615$, $p = 0.223$) were not statistically significant at the 5% level. This outcome implies that when both predictors are considered simultaneously, neither MOI nor Elox independently exerts a strong enough influence to significantly predict tensile strength on its own. The negative coefficients indicate a general trend where increases in either factor are associated with slight reductions in tensile strength, but the magnitude of these changes is not strong enough to be considered statistically meaningful.

Taken together, the regression results suggest that the tensile performance of the films is influenced more by the combined formulation environment rather than by MOI or Elox acting independently. This aligns with the complex interactions typically observed in polymer-filler and plasticizer-modified starch matrices, where mechanical behavior is often governed by network interactions, compatibility effects, and molecular mobility rather than by single-factor contributions. The significant overall model, despite the non-significance of individual predictors, reinforces the idea that the collective interplay of formulation variables better explains the film's tensile response than any individual component.

These findings complement your ANOVA results, which showed a significant main effect for Elox and a marginal interaction effect between Elox and MOI. Together, both analyses highlight that the mechanical strength of the films is not controlled by isolated variables but by the synergistic behavior of the formulation components, consistent with the structural complexity of biodegradable starch-based materials.

The fitted regression model describing the relationship between tensile strength and the predictors is:

Tensile = 9.0245 – 0.2146(MOI) – 0.1615(Elox).

3.9 CONCLUSION

This study successfully developed biodegradable packaging films from acetylated cassava starch reinforced with kaolinite and plasticized with ethylene glycol. The extraction and modification processes produced high-quality starch, with a yield of 62.3%, acceptable moisture content, and a reduced gelatinization temperature following acetylation. FTIR analysis confirmed the introduction of acetyl groups, indicating that the chemical modification was effective and appropriate for improving the performance of starch-based films.

Film characterization revealed that ethylene glycol and kaolinite significantly influenced the mechanical, structural, and barrier properties of the resulting bioplastics. Ethylene glycol enhanced flexibility, while kaolinite improved rigidity, tensile strength, and thermal stability. Absorption and solubility tests demonstrated that higher plasticizer content increased water, moisture, and acid absorption, whereas kaolinite reduced these effects by improving film compactness and reducing diffusion pathways. All films, however, remained vulnerable to alkaline degradation, consistent with the chemistry of acetate-based polymers.

Mechanical analysis showed that the best overall performance, balancing strength, flexibility, and toughness was achieved at 5 g ethylene glycol combined with 0.5–1 g kaolinite, making this formulation the most suitable for practical biodegradable packaging. Statistical analyses supported these findings: ANOVA revealed a significant effect of ethylene glycol on tensile properties, and regression modeling confirmed that the combined predictor model was significant, even though individual variables alone were not strong predictors. These

outcomes suggest that mechanical behavior arises from the synergistic interaction between plasticizer and filler rather than from single components acting independently.

Biodegradability tests further confirmed that the films decomposed readily under soil conditions, reinforcing their suitability as sustainable alternatives to petroleum-based plastics. All results shows that acetylated cassava starch–kaolinite composites represent a promising, locally sourced, environmentally friendly material system capable of addressing Nigeria's growing need for biodegradable packaging solutions.

3.10 FINDINGS

The findings of this study are:

1. **Starch Modification and Characterization:** The study successfully developed biodegradable packaging films from acetylated cassava starch reinforced with kaolinite and plasticized with ethylene glycol. The acetylation process resulted in high-quality starch with a yield of 62.3%, acceptable moisture content, and a reduced gelatinization temperature. FTIR analysis confirmed the introduction of acetyl groups, indicating effective chemical modification.
2. **Effect of Ethylene Glycol and Kaolinite:** The presence of ethylene glycol and kaolinite significantly influenced the mechanical, structural, and barrier properties of the films. Ethylene glycol enhanced the flexibility of the films, while kaolinite improved their rigidity, tensile strength, and thermal stability.
3. **Water Absorption and Solubility:** The films demonstrated increased water, moisture, and acid absorption with higher plasticizer content. However, kaolinite reduced these absorption effects by improving the film's compactness and reducing diffusion pathways.

4. **Alkaline Degradation**: All films showed vulnerability to alkaline degradation, which is typical for acetate-based polymers, indicating the need for further improvements in their alkaline resistance.
5. **Mechanical Performance:** The best mechanical performance, characterized by a balance of strength, flexibility, and toughness, was achieved with 5 g of ethylene glycol and 0.5–1 g of kaolinite. This combination was found to be the most suitable formulation for practical biodegradable packaging applications.
6. **Statistical Analysis**: ANOVA revealed a significant effect of ethylene glycol on the tensile properties, and regression modeling confirmed that the combined effect of ethylene glycol and kaolinite was a significant predictor of the films' performance, while individual variables alone were not strong predictors.
7. **Biodegradability:** Biodegradability tests confirmed that the films decomposed readily under soil conditions, highlighting their potential as sustainable alternatives to petroleum-based plastics.

3.11 RECOMMENDATION FOR FUTURE STUDIES

1. Scale-Up and Industrial Testing:

   Future studies should explore pilot-scale production to assess the films' performance under real manufacturing conditions. This will help determine industrial feasibility and cost implications for large-scale packaging applications.

2. Optimization of Filler and Plasticizer Levels:

   Although the 5 g ethylene glycol + 0.5–1 g kaolinite formulation performed best, further optimization using response surface methodology (RSM) or other design-of-experiment (DOE) tools could refine mechanical and barrier properties.

3. Surface Coatings for Moisture Resistance:

   Since the films still exhibit moderate water and moisture absorption, applying

biodegradable surface coatings (e.g., chitosan, beeswax, or cellulose derivatives) may enhance moisture resistance without compromising biodegradability.

4. Enhancing Thermal Stability:

   Blending starch acetate with other biopolymers or nanoparticles such as PLA, PHA, or nanocellulose could further improve thermal properties for packaging applications requiring higher heat tolerance.

5. Extended Biodegradation Studies:

   Longer-term degradation studies in soil, compost, and aquatic environments should be conducted to better understand the environmental behavior and breakdown mechanisms of the films.

6. Exploration of Other Natural Fillers:

   Locally available fillers such as bentonite, rice husk ash, or coconut fiber may offer unique reinforcement advantages and should be investigated as alternatives or supplements to kaolinite.

7. Barrier Property Improvements:

   To meet food packaging standards, future work should measure and enhance oxygen and water vapor transmission rates using improved polymer–filler interactions or multilayer film structures.

8. Toxicological and Safety Assessment:

   Before commercialization, it is recommended to perform migration, toxicity, and food-contact safety tests to ensure that the bioplastics are safe for direct use in food packaging.

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