Development of Avocado Seed Based Starch Binder for Industrial Application in Paper Packaging

Zereu Haftu Hiluf

A Thesis Submitted to

The Department of Chemical Engineering

School of Mechanical, Chemical and Materials Engineering

Presented in Partial Fulfillment of the Requirement of the Degree of Master's

In Chemical Engineering (Specialization in Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

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Adama, Ethiopia

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Advisor: Melaku Tesfaye (Associate Professor)

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DECLARATION

I hereby declare that this Master Thesis Entitled "**Development of Avocado Seed Based Starch Binder for Industrial Application in Paper Packaging**" is my original work. That is, it has not been submitted to any other university for. All sources of materials that are used for this have been duly acknowledged through citation.

_____	_____	_____
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RECOMMENDATION

I , the major advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "**Development of Avocado Seed Based Starch Binder for Industrial Application in Paper Packaging**" prepared under my guidance by Zereu Haftu submitted in partial fulfilment of the requirements for the degree of Master's of science in Chemical Engineering (Specialization in Process Engineering). There for, I recommended the submission of the revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

APPROVAL OF BOARD REVIEWERS

We, the advisors of the thesis entitled "**Development of Avocado Seed Based Starch Binder for Industrial Application in Paper Packaging**" and developed by Zereu Haftu, hereby certify that the recommendation n and suggestion made by the board of examiners are appropriately incorporated into the final version of the thesis.

Advisor	Signature	Date
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Zereu Haftu have read and evaluated the thesis entitled "**Development of Avocado Seed Based Starch Binder for Industrial Application in Paper Packaging**" and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemical Engineering (Specialization in Process Engineering).

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Finally, approval and acceptance of the thesis is contingent upon submission of its final Copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC)

Department Head	Signature	Date
School Dean	Signature	Date
Postgraduate Dean	Signature	Date

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LIST OF ACRONYMS AND ABBREVIATIONS

AS	Avocado Seed Starch
DO	Degree of Oxidation
FTIR	Fourier Transmission Infrared Spectroscopy
TGA	Thermogravimetric Analysis
XRD	X-ray diffraction
DSC	Differential scanning Calorimetry
SEM	Scanning electron microscope
mS	Modified Starch
nS	Native starch
RSM	Response Surface Methodology
CCD	Central composite design

ABSTRACT

One of the most common and renewable carbohydrate-based biopolymers in nature is starch which serves as a sustainable binder material (Sheikhi, 2018). Because starch and its derivatives are readily available, inexpensive, and entirely biodegradable, they are frequently utilised in the paper business. In this study the potential of avocado seed starch as a sustainable and cost-effective raw materials for the development of starch- based binders in the paper packaging industry was explored and paper was formulated and tested its quality. The functional modification to improve the properties of starch was done by oxidation method using CuSO4 as oxidative agent and H2O2 as catalyst. Response surface methodology (RSM) was used to study the optimum condition for The experiment was conducted using the Central composite design (CCD) method to study the effect of three independent variables: temperature, time and catalyst dosage on the degree of oxidation (DO). The result showed that the optimum conditions of DO were 60 °C, 90 min and 1% with DO 51.3%. The increased as reaction times and temperatures increased, but it reduced as catalyst dosage increased.The structures and properties of native starch and oxidized starches were characterized by Fourier Transform Infrared (FT-IR), DSC, Thermogravimetric (TGA) and differential thermal analysis (DTG), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The oxidation reduced the intrinsic viscosity and thermal stability of the oxidized starches, and could change the crystalline structures into amorphous states when the DO reached 51.3%. After the starch was oxidised, the gelation temperature was increased. All of these characteristics supported the idea that native starch undergo structural change as a result of the addition of carboxyl and carbonyl groups. Paper packaging was formulated and its qualities like tensile strength, burst, tear strength and porosity was tested. Corrugated cardboard was also produced then burst strength and crush strength was tested. This study findings suggests that modified avocado seed starch could be used as binder for both paper production and cardboard production.

Keywords: Starch, oxidation , degree of oxidation, binding, paper packaging, cardboard

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

The use of adhesives dates back to ancient times, with evidence of their use found in artifacts from 1500-1000BC (Adhesives & Application et al, 2017). The adhesive industry is one of those that is expanding most rapidly in the globe. 2015 recorded the production of about 20.2 million metric tonnes of adhesives valued at 64 billion dollars (Bandara, 2017).Global adhesive production is expected to reach 75.52 billion dollars in 2019 with a 4.5% annual growth rate. It has been applied to a variety of industries, such as paper bonding, wood processing, automotive, and aerospace. From these, the adhesive uses totaling 65% are attributed to the structural products industry (Bandara, 2017).

The two main types of adhesives used in structural applications at present are thermosetting and synthetic. The non-renewable petrochemical raw materials are the source of these synthetic and thermosetting adhesives (Adhesives & Application, 2017), But as technology has developed, people have started to realise that adhesives based on non-renewable petrochemicals may pose risks to public health, safety, and the environment. Moving towards a green, eco-friendly world requires the sustainable, effective use of plant-derived regenerable resources as substitutes for non-renewables (i.e., fossil resources) for the manufacture of renewable chemicals, materials, and fuels. Plant oils, carbohydrates, lignin, protein, and tannin are some of these renewable resources. which are low in cost (Bozell & Petersen, 2010; Gong et al., 2015 et al., Li et al., 2016).

For the application in bio-based adhesives, various types of Researches have been done on bio-based materials.. These Adhesives can be used to bond a different kinds of materials:- Metals, plastics, wood glass, ceramic, rubber and paper.

One of the most common and renewable carbohydrate-based biopolymers in nature is starch which serves as a sustainable binder material (Sheikhi, 2018). According to Salam, starch can increase the biodegradability and recyclability of paper products and is frequently used as a co-binder with synthetic materials (Salam, 2013, Bigel NazilAltay et al. 2021). In the pulp and paper business, starch is a versatile biopolymer that is highly utilised. This innovative role might make it possible to add more filler at higher levels,

which would lower costs and energy usage while preserving the essential characteristics of paper-based products. It is mentioned that other biopolymers, like those generated from cellulose and hemicellulose, can be useful substitutes in this regard in addition to starch or its derivatives (Ankerfors, Lindström & Söderberg, 2014 et al., T. Li et al., 2016).

It should be noted that the majority of the chemicals used in the paper industry today are derived from fossil fuels, and given the finite supply of fossil fuels and the substantial carbon footprints they leave behind, using renewable biological resources to create chemicals is a good way to meet the concept of sustainability. However, as environmental awareness and social media coverage develop, customers are becoming more aware of product labels and are actively seeking out greener items. Starch-based products that differ in composition, structure, and characteristics have been created to overcome these obstacles and enhance coating performance at the same time as lowering costs and having a smaller environmental impact (H. Li et al., 2019).

To enable the starch adhesive to be used in the field of binder for paper packaging, it needs further improvement by modifying the starch adhesives and providing a method for preparing a bio based starch adhesive. To clear up these and other shortcomings derivation of starch, such as etherification, esterification (Maribel Victoria Tupa et al., 2014), cross-linking (Yunchuan Zeng et al., 2016) grafting and oxidation are the modifications method. (Nguyen Thanh Tung et al., 2021). From the different methods, oxidation is one of the promising methods of modification of these polymers which has earned significant recognition recently.

Starch oxidation is a highly interesting and difficult process to study from a scientific and technological approach. The goal is to convert the hydroxyl groups in starch to carbonyl and carboxyl groups via partially oxidation. Compared to native starch, oxidised starch is far more soluble in water and is utilised in a variety of applications, including paper coating, textile sizing, and the food industry as a gelling agent (Z Tapio Salmi et al. 2016).

The major source of starch intake worldwide are cereal (rice, wheat, and maize) , root vegetables (potatoes and cassava) and other sources like sweet potato, sago, Avocado seed, mango seed and banana peel.

Fresh avocado fruit is eaten and utilised to make a variety of goods. However, due to the growing need for nutritious foods that include both macro and micronutrients including

proteins, fibre, and antioxidants, avocado consumption is rising quickly worldwide. Avocado by products are produced in enormous quantities as a result, giving enterprises eager to participate in sustainability with cost-effective and environmentally friendly production a chance. The avocado fruit seed, which makes up 15–16% of the fruit and contains roughly 29% starch, can therefore be used to obtain the avocado fruit by-product. This is an alternate source of the polysaccharides that are widely used in the food and food packaging sectors (Juliani Buchveitz Pires et al., 2024).

There for the research on the synthesis of Avocado seed (AS) starch based binders for the paper industry mainly focuses on the oxidation modification of Avocado seed starch properties to improve its suitability for papermaking. The research highlights the potential of AS starch based binders in meeting the needs of the paper industry, including environmental conservation, cost-effectiveness, and enhanced durability.

1.2. Problem of the statement

In Ethiopia, the paper packaging industry imports approximately 60% of its starch demand from other countries. The total value of oxidized starch import in to Ethiopia is $3,736,220, with an average import price of $0.63 per unit. Information gathered from the various manufacturers that were investigated makes it abundantly evident that these local PLCs that extract starches do provide 40% of the total starch that the various factories that were surveyed require. This suggests that Ethiopia's paper packaging sector depends a great deal on imported starch.

Because starch and its derivatives are abundantly available, inexpensive, and entirely biodegradable, they are used significantly in the paper business, but in Ethiopia 60% of its starch is imported and the rest domestic industries prepare starch from the edible things. Using avocado seed Waste as a source of starch offer a promising solution to this problem, but there is a lack of research on the modification, optimization and characterization of binders from avocado seed starch for the paper packaging industries.

Different literatures have focused on the extraction and characterization of starch from avocado seeds (AS) for potential applications in biodegradable films and food systems, but there is a lack of comprehensive research on the optimization of avocado seed starch for its use as a binding agent in sustainable paper production.

The current literature primarily emphasizes the extraction process and the potential applications of avocado seed starch, but there is a research gap in systematically optimizing the properties of avocado seed starch to tailor its properties for improved bindings performance in the context of paper manufacturing. This gap highlights the need for a more focused exploration of the modification and optimization of avocado seed starch to address its specific application as a binding agent in the production of eco-friendly paper.

1.3. Research Question

RQ1. Can it be possible to optimize extracted AS starch with parameters temperature, time and catalyst dosage which can be use as binder for paper production?

RQ2. What are the quality of paper and cardboard produced using modified AS starch as bindings?

RQ3. How does the addition of AS-based starch affect the mechanical strength, printability and surface properties of paper packaging?

Based on the above research questions the choices of the systems are framed as follows :

Avocado seed starch is selected as the main raw material due to :

- Its availability, low cost (westage) and desirable functional properties make it a suitable binder for paper packaging.

1.4. Objectives

1.4.1. General objective

This study focused on the development of Avocado seed based starch and its oxidation process optimization at different reaction temperature, time and catalyst dose for industrial application in paper packaging.

1.4.2. Specific objective

- ✓ To carry out the extraction and characterization of starch from avocado seed.
- ✓ To optimize the oxidation of avocado seed starch and achieve its desired functional properties at optimum temperature, time and catalyst dose using RSM.

- ✓ To validate the oxidized starch prepared at optimal condition using different characterization instruments.
- ✓ To conduct the paper quality tests for the suitability of starch binder in paper packaging application.
- ✓ To produce cardboard using the modified starch as binder and testing it's burst and crush qualities.

1.5. Significance of the study

The research holds significance in the context of developing sustainable and eco-friendly bindings for paper production. By utilizing avocado seed starch, which is a renewable and abundance, the research contributes to the advancement of environmentally friendly alternatives in the paper manufacturing industry. The modification and optimization of avocado seed starch for paper bindings can lead to the development of binding agent with enhanced performance characteristics. This can include improved adhesion, strength and durability, which can contribute to the quality of paper product. The abundance of avocado seeds and the potential for cost–effective extraction of starch make this research significant in terms of economic viability. By utilizing a by-product of the avocado industry, the study contributes to the development of environmental friendly and sustainable solution for paper production. The study represents a novel application of avocado seed starch in the context of paper production. While previous research has focused on the extraction and characterization of avocado seed starch for biodegradable films, the specific utilization of this starch as a bindings agent for paper production represents a unique and valuable contribution to the field.

1.6. Choice of system

According to the above research questions the selection of the systems was formed as follows: Avocado seeds were chosen for paper bindings due to their rich content of starch, which makes them a promising and sustainable source for bindings agents in paper production. Avocado seeds contain significant amounts of Carbohydrates, including starch, which can be extracted and optimized for use as a binding agent in various applications.

1.7. Scope of the study

The scopes of the thesis work that can be finished within the time and resource limits are as follows:

- ✓ Extracting starch and modifying it from AS.
- ✓ Optimizing parameters like reaction temperature, reaction time and catalyst dosage using RSM
- ✓ Characterising native starch and modified starch
- ✓ Producing paper and cardboard , then testing there qualities like tensile strength, burst, porosity, crush and tear strength.

To answer the above research questions, the scope of the study was focused on the laboratory scale Extraction, modification and optimization of starch based binder for paper packaging and producing of paper and cardboard, then studied it's quality.

CHAPTER TWO

2. LITRATURE REVIEW

2.1. Adhesive

Any non-metallic substance applied to one or both sides of two separate items that binds them together and prevents their separation is an adhesive, often known as glue, cement, mucilage, or paste (Adhesives & Application, 2017). High internal strength is also required for optimal adherence (cohesion). The type of bonding that takes place between the adhesive and the substrate determines the internal strength of the glue. Cohesion is determined by the strength of hydrogen bonds, London interactions, polar bonds, and covalent bonds. Adsorption, simple mechanical interlocking, diffusion, electrostatic contact, and weak-boundary layer are the most popular theories of adhesion. They can be classified as natural or synthetic based on their composition and origin.

Natural Adhesives

Natural adhesives are derived from naturally occurring materials, including plant resins, animal products, vegetable starches, and glues derived from minerals. Plant resins, animal hide and skin glues, and adhesives derived from minerals are a few types of natural adhesives. Comparing these adhesives to synthetic adhesives, they are often weaker and have a shorter lifespan (Gani et al., 2016). They are appropriate for uses like ordinary packing when reliability is not an issue. For a strong binding, natural adhesives usually need to be applied to both surfaces (Gani et al., 2016).

Synthetic Adhesives

Synthetic adhesives consist of pre polymers (oligomers) or polymers. They are typically based on synthetic resins and rubbers and are known for their versatility and strength. Examples of synthetic adhesives include polymers such as elastomers, thermoplastics, and thermosets Synthetic adhesives are efficient, hypo-allergenic, and odorless. They can be produced without exposing users to allergens and may only need to be applied to one of the surfaces, saving time during manufacturing(Anna Rudawska et al., 2012). Transitioning towards an environmentally friendly world necessitates the responsible and efficient utilization of plant-based resources that can be replenished, serving as substitutes for non-renewable sources (e.g., fossil fuels) in the production of renewable fuels, chemicals, and materials. Affordable and renewable sources like tannin, lignin, plant oils, proteins, and

starch play a crucial role in this endeavor (Ma et al., 2020). Nevertheless, the utilization of native starch for packaging materials is constrained by certain limitations, including its inadequate resistance to moisture, challenging processability due to high viscosity, brittleness, and incompatibility with hydrophobic polymers. To overcome these drawbacks, additional modifications to starch are necessary. These modifications aim to introduce hydrophobic characteristics and enhance mechanical strength and moisture resistance properties (Borsig et al., 2008).

2.1.1. Starch based adhesive

Currently, starch has gained significant importance and attention as a valuable biopolymer in various industries. This is primarily attributed to its abundant availability and cost-effectiveness. Native starch holds the potential to serve as a raw material for the production of thickening agents, stabilizers, emulsifiers, fuel ethanol, and bio-plastics. In the paper industry, starch finds extensive application as a sizing agent, enhancing the mechanical strength and film-forming properties of paper, paperboard, and textiles. By binding together the different components of the paper web, including fibers, pigments, and fillers, starch plays a crucial role in improving the overall quality of paper products (Y. Zhang et al., 2015).

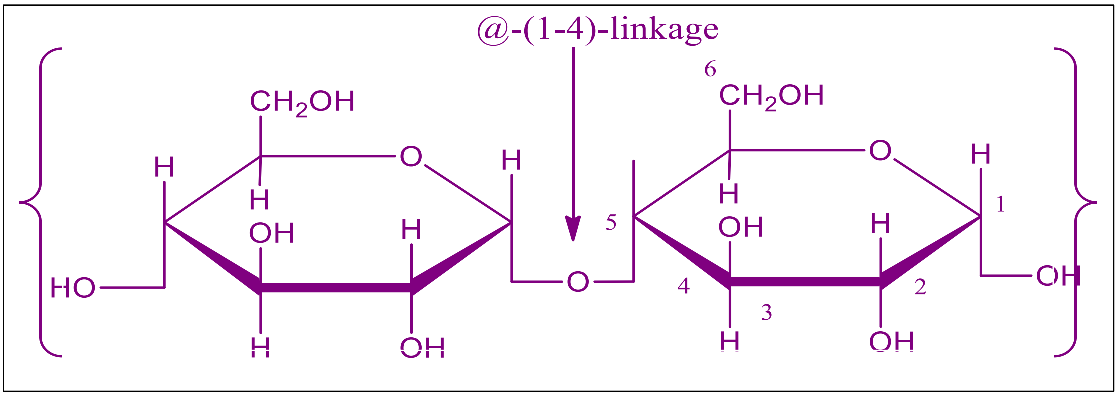
Food industry and other non-food businesses use starch in a variety of ways. Considering its abundance, starch is a natural polysaccharide macromolecule with potential use in starch-based adhesives.. Its comparatively good adhesion performance and lack of formaldehyde emissions to the environment have also led to substantial research and widespread application in the adhesives sector. However, it is not strong enough to bond structural products together. It's a green material that promises to replace adhesive elements derived from petrochemicals. Using alternative raw materials will be crucial because adhesive demand has increased recently (Chen et al., 2020).

The adhesive industry heavily depends on petroleum-based resources due to their favorable performance and affordability. However, the use of petroleum-based ingredients in adhesives can lead to the emission of carcinogenic compounds, posing significant health hazards to humans and compromising indoor air quality. As a result, there is an increasing demand to substitute the petrochemical-based raw materials utilized in structural adhesive formulations with renewable and environmentally friendly alternatives. One of the most prevalent green biopolymers, starch is utilised by a wide variety of plants that store energy,

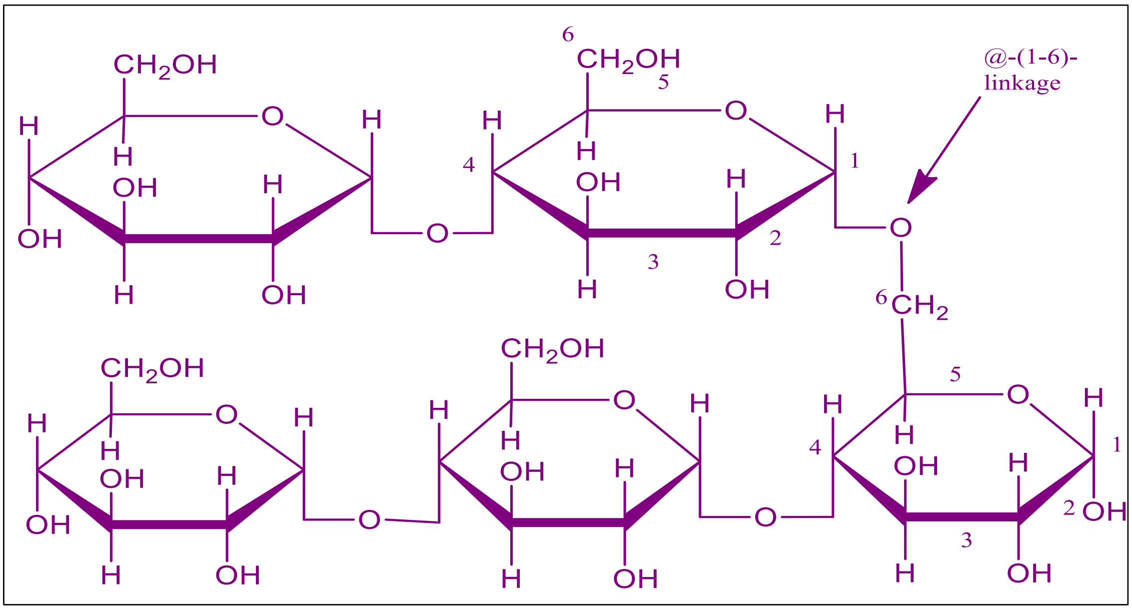
such as cereals, tubers, roots, fruits, and seeds (Adhesives & Application, 2017). However, in its natural state, neither its physical nor chemical characteristics make it better for the formulation of structural adhesives. Its degree of retrogradation is high and its shear and thermal stability are low. Furthermore, Zhang et al. (2015) noted that the natural form of starch is capable of syneresis that goes beyond the pastes' propensity to gel.

2.1.2. Structure of Starch

Starch, after cellulose, is the most widely distributed polysaccharide biomaterial. In plants, it serves as a vital energy storage compound (Haq et al., 2019). Starch is composed of two main components: amylopectin and amylose, both consisting of D-glucose units. Amylopectin is highly branched, with approximately one branch every 20 glucose units, while amylose has branches approximately every 200 glucose units. Figure 2.6 demonstrates that amylose (Am) is a linear molecule composed of α-(1-4)-D-glycoside linkages connecting glucose units. On the other hand, amylopectin (AmP), shown in Figure 2.7, consists of a linear α-1,4 chain with α-(1-6) D-glycoside bonds and has average molecular weights of 106 Da and 108 Da, respectively (Fabian et al., 2011).Starch is a simple carbohydrate that is found in great quantities in common foods including wheat, potatoes, maize rice, and cassava. It is a very prevalent carbohydrate in the human diet, made up of an enormous quantity of glucose units. It's one of the most adaptable biomaterials and has recently drawn more attention for use in a variety of industries, including the food, textile, cosmetic, plastic, adhesive, paper, and pharmaceutical (Mo & Omojola, 2013). This is because of its inherent superior physicochemical qualities, degradability, non-toxicity, compatibility, abundance, high caloric value, low cost, and ease of modification to different derivatives (Mo & Omojola, 2013).



(A)



(B)

Figure 2.1: (A) Amylose , and (B) Amylopectin:

2.1.3. Function of Starch as a Renewable Resource

Starch, despite its numerous industrial applications, faces limitations due to certain properties it exhibits. These limitations include insolubility in cold water, a decrease in viscosity and thickening ability when subjected to heat. Furthermore, the industrial use of starch is constrained by its low flexibility in the formation of films, low shear resistance, inadequate temperature resistance, poor stability and processing tolerance, as well as limited retention of tensile properties in wet environments (Singh et al., 2007).

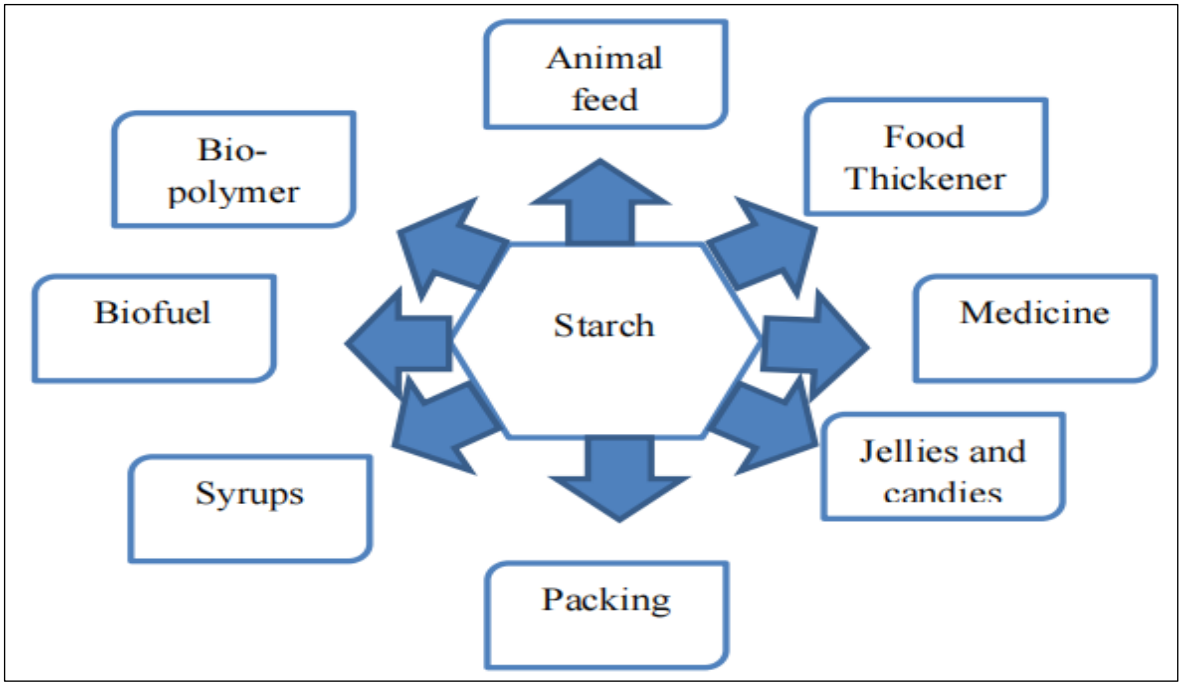


Figure 2.2: Uses of starch

On the other hand, starch serves a multitude of functions, such as coating, sizing, stabilising, texturizing, gelling, film formation, encapsulating, water retention, and extending shelf life (Tam et al., 2021).

Starch as Thickener and Stabilizer

Because stabilisers can enhance thickness and sensory qualities, they are important ingredients in manufactured dairy products. Stabilisers: the least expensive category, comprising the most often used ones, include both synthetic and plant-based stabilisers (Rengsutthi & Charoenrein, 2011). Corn starch was investigated by (Rengsutthi & Charoenrein, 2011) as a thickening and stabilising agent in stew sauce.

Film Formation of Starch

Starch films present several advantages due to their abundant availability in nature. These advantages include their low thickness, flexibility, and transparency. Consequently, it increases resistance against humidity by employing starch sheets. Many techniques can be applied, such as starch modifying processes such starch crosslinking (Sharma et al., 2021).

Starch as a Gelling Agent

When dissolved in a fluid state as a colloidal mixture, gelling agents are the agents that shape or produce gels with a weakly strong internal structure. Various reports have been published regarding the gelling properties of different starches (Sharma et al., 2021).

Starch as a Renewable Resource for Bio-Adhesive

Renewability, biodegradability, and affordability are among the advantages of using starch as a raw material in the creation of adhesives. Grain or root crops such as sweet potatoes, cassava, wheat, yams, and avocado seeds provide starch.Research on enset has been relatively low compared to other cultivated starch source plants (Sharma et al., 2021)

2.2. Modification of starch

The chemical and physical properties of starch play a vital role in its utilization across various industries, such as textiles, food, paper, and adhesives. To enhance its properties, several modification approaches can be employed. These include esterification, etherification, crosslinking, grafting, oxidation, and the use of bio-based coupling agents. These modification methods enable the customization and improvement of starch to meet specific application requirements (Watcharakitti et al., 2023).

2.2.1. Modification of starch by Esterification method

Esterification stands as a highly effective approach in the chemical synthesis of starch alterations. This process commonly involves the utilization of alcohols and carboxylic acids, alongside catalysts like potassium carbonate, sodium hydroxide, and sodium hydrogen phosphate (Junistia et al., 2008). Alternatively, acid catalysts such as p-toluene sulfuric acid, hydrochloric acid, and sulfuric acid can also be employed (Watcharakitti et al., 2023)..

2.2.2. Modification of starch by oxidation method

For over a century and a half, the paper industry has relied on sodium hypochlorite oxidation to enhance the mechanical and film-forming properties of paper, paperboard, and textiles. In this context, oxidized starches are widely employed as sizing agents. According to Tolvanen et al. (2009), oxidized starches work by binding the fibers, pigments, and fillers present in the paper web. This utilization of oxidized starches leads to improved strength and printability of the paper (Parovuori et al., 1995). During the process, the hydroxyl groups on starch molecules undergo oxidation, first transforming into carbonyl

groups and then into carboxyl groups. The degree of oxidation is determined by the quantity of carboxyl and carbonyl groups present in the oxidized starches.

Wurzburg (1986) states that this form of oxidation primarily affects the hydroxyl groups located at positions C-2, C-3, and C-6 of starch molecules. El-Sheikh et al. (2010) highlight that oxidized starches are highly desirable for industries such as textiles, laundry finishing, drilling, casting, and binding materials. This is due to the incorporation of carbonyl and carboxyl functional groups into the amylose and amylopectin chains of starch. These industries can effectively employ oxidized starches for purposes such as surface sizing and film formation. A comprehensive and recent review of research is available, which covers the use of various oxidizing agents such as ozone, hydrogen peroxide, and sodium hypochlorite to facilitate starch oxidation from different sources.

Oxidation with sodium hypochlorite

In the commercial production of oxidized starches, sodium hypochlorite is commonly used as the oxidizing agent because of its easy availability and its well-known impact on starch properties. Various factors influence the degree of hypochlorite oxidation, including pH (Sangseethong et al., 2009), reaction temperature (Dias et al., 2011), reaction time, concentration of sodium hypochlorite (Sánchez-Rivera et al., 2005), molecular structure of the starch, and the source of the starch (Kuakpetoon & Wang, 2001). These factors play a significant role in determining the extent of oxidation achieved during the process.

When amylose and amylopectin polymers are subjected to sodium hypochlorite, the hydroxyl groups within these polymers undergo oxidation, resulting in the formation of carbonyl and carboxyl groups. Higher levels of oxidation may also lead to some depolymerization of amylose and amylopectin. The presence of carbonyl and/or carboxyl groups directly impacts the physicochemical properties of oxidized starches. The quantity of these functional groups determines the suitability of the starches for various industrial applications (Tolvanen et al., 2009).

Oxidation with ozone

Hydrogen peroxide and sodium hypochlorite are among the most extensively studied chemical oxidizing agents. However, their oxidation processes can generate wastewater and potentially leave unwanted residues in food products (An & King, 2009). In contrast, ozone oxidation is considered a cleaner process that requires minimal additional

purification stepsThe study will utilize oxidation using hydrogen peroxide as the oxidizing agent and MnO2 as the catalyst. This choice is based on the fact that ozone oxidation produces carbon dioxide, water, inorganic ions, and less hazardous byproducts as end products (Tolvanen et al., 2009). The decision to use hydrogen peroxide and MnO2 as the oxidizing system in this study is supported by previous research conducted by Chan and colleagues (2011) and Amorim, Leo, and Moreira (2009).

Oxidation with hydrogen peroxide

While sodium hypochlorite is more commonly used in commercial settings, there have been numerous studies documenting the oxidation of starches from various sources using hydrogen peroxide. Unlike sodium hypochlorite, hydrogen peroxide does not produce harmful byproducts as it rapidly decomposes into oxygen and water. This makes hydrogen peroxide a preferable choice with minimal environmental impact. Hydrogen peroxide oxidation is the most extensively researched chlorine-free oxidation method, as highlighted by Sangseethong et al. (2010).

The mechanism of starch oxidation using hydrogen peroxide becomes highly intricate when a metal catalyst is present. It is believed that the oxidation occurs through a chain reaction involving radicals. In the presence of a metal catalyst, hydrogen peroxide undergoes rapid decomposition, producing hydroxyl radicals (•OH). These highly reactive radicals react with carbohydrates, particularly sugar rings, by extracting hydrogen from the C-H groups. This process generates new radicals known as R•CHOH. During peroxide oxidation, the predominant formation is the carbonyl functional group, while the production of carboxyl groups is comparatively lower (Sangseethong et al., 2010).

Based on studies conducted by Tolvanen et al. (2009) and Zhang et al. (2012), hydrogen peroxide oxidation typically results in degrees of oxidation below 2.78% (w/w). However, other research has demonstrated the ability to achieve higher levels of oxidation using hydrogen peroxide (Zhang et al., 2009). Metal ions are often employed as catalysts in hydrogen peroxide oxidation methods to increase the abundance of functional groups in modified starch. According to Parovuori et al. (1995), the efficiency of metal ion catalysts can be ranked as follows: Cu > Fe > WO4.

According to research done by Parovuori et al. (1995), starch oxidation in the presence of UV light is affected by pH (3.5, 7.0, and 9.0), reaction time (2.0, 4.0, 6.8 and 10.0), and hydrogen peroxide concentration (1, 2, 3, and 4 mol/0.42 units of starch anhydroglucose).

The highest carbonyl and carboxyl concentrations were found in oxidised starches made in an acidic pH with a long reaction period and a high hydrogen peroxide concentration.(Y. Zhang et al., 2012) evaluated the efficiency of corn starch oxidation using different concentrations of the catalyst CuSO4.

Tolvanen et al. (2009) used iron tetrasulfophthalocyanine (FePcS) as a catalyst to oxidise potato starch with hydrogen peroxide. According to the researchers, the production of carboxyl and carbonyl groups was preferred at pH 10.0 and pH 8.4, respectively. according to Liu et al. (2014). Using cross-linked starch rather than native starch promotes the introduction of carboxyl groups by facilitating the oxidation reaction. According to Liu et al. (2014), less oxidant was needed to modify cross-linked maize starch to a given degree than native maize starch. In order to provide a more exposed internal structure after drying, cross-linking may stop starch molecules from rearranging. Oxidant molecules would find it easier to enter the inner structures of starch granules with such an exposed structure, which would promote oxidation.

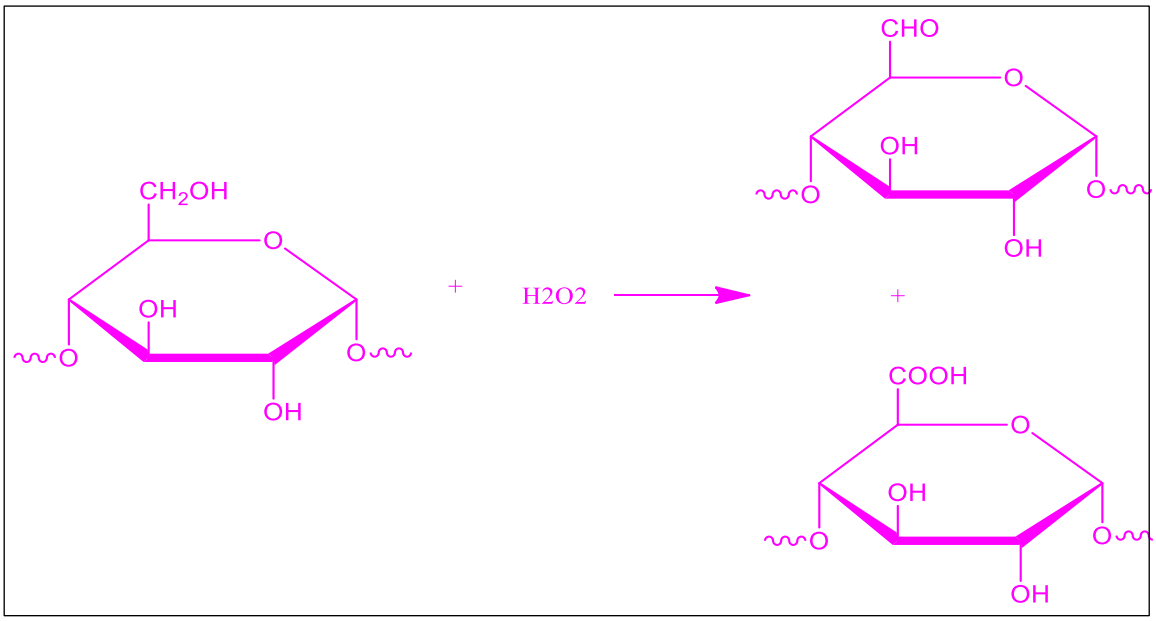


Figure 2.3: Oxidation of starch with hydrogen peroxide

In a study conducted by Zhang et al. (2009), corn starch was subjected to oxidation using varying concentrations of hydrogen peroxide. This process resulted in oxidized starches with degrees of oxidation ranging from 0.096 to 0.554. The authors noted that these oxidized starches exhibited high levels of aldehyde groups and could be utilized in the production of thermoplastic starch, which is an environmentally friendly material.

Another investigation carried out by El-Sheikh et al. (2010) focused on the oxidation of rice starch. In this study, hydrogen peroxide with a concentration of 2.7% and UV irradiation as a photo-initiator were employed. The researchers found that higher levels of carbonyl and carboxyl groups were observed at reaction temperatures of 60 and 70 °C and a starch-to-water ratio was 1:8.

There is a limited number of studies that directly compare oxidized starches prepared using different oxidants. In a study conducted by Sangseethong et al. (2010), the authors specifically compared the effects of sodium hypochlorite and hydrogen peroxide as oxidizing agents. They oxidized cassava starch using both agents at a pH of 10.0 and varied the reaction times, specifically setting them at 30, 60, 120, and 300 minutes.The oxidation process using sodium hypochlorite was observed to promote the creation of carboxyl groups, whereas the oxidation process using hydrogen peroxide favored the formation of carbonyl groups. Both sodium hypochlorite and hydrogen peroxide resulted in oxidized starches with comparable viscosities and molecular size distributions, but hydrogen peroxide required less time to achieve these characteristics. Furthermore, the cooled gels formed from starches oxidized by hydrogen peroxide required greater firmness compared to gels formed from starches oxidized with sodium hypochlorite.

Table 2.1: Related studies on the starch oxidation

Result	Starch powder	Oxidizing agent	catalyst	Reaction condition	Wash solvent	ref
Oxidized starch	10g	H_2O_2	$FeSO_4$	3h, 45 ^{o}c	Distilled water	(Liu et al., 2014)
Oxidized starch	10g	H_2O_2	$FeSO_4$	1.5h, 30 ^{o}c	Distilled water	(Tillayeva & Sharipov, 2023)
Oxidized starch	20g	H_2O_2	$FeSO_4$	5h, 50 ^{o}c	Distilled Water	(Karić et al., 2020)
Oxidized starch	50g	H_2O_2	-	24h, 25 ^{o}c	Distilled water	(S. D. Zhang et al., 2009)

2.2.3. Modification of starch by cross-linking

Crosslinking is employed to enhance the resistance of starch against conditions such as intense shear forces, high temperatures, and low pH levels. When there is a need for a thick and stable starch paste, particularly for dispersal in environments where it will encounter elevated temperatures, shear stresses, or low pH levels, cross-linked starches are employed. Cross-linked starches play a crucial role in maintaining the uniformity of food products by effectively suspending other ingredients. They exhibit increased durability and resistance to breakage compared to unmodified starch. Various types of cross-linked starches are utilized for this purpose, such as sorghum starch, waxy maize starch, waxy wheat starch, and amylopectin potato starch (FatimaZahra Semlali Aouragh Hassani, 2022). Cross-linked starches find extensive application in various food products, particularly those with acidic characteristics like pizza sauce or barbecue sauce. This is because the modified starch exhibits improved resistance to acidity compared to unmodified starch. Additionally, cross-linked starches are used in the preparation of pie fillings, puddings, bread, gravies,baby salad dressings, foods, soups, retort foods, and in aseptic processing (Science, 2022).

Table 2.2: Related studies on modification of starch

Modification method	Added functional groups	Structural change of starch	Properties change	Reference
Acetylation	hydroxyl group and acetyl group	It retards the crystallization in starch granules	It increases the viscosity and swelling capacity of granules while preventing the formation of intra molecular hydrogen bonds. It lessens irrationality	Thirumdas R et al.(2017)
Hydroxyethylation	addition of an ethyl hydroxyl group to the starch.	Changes the granular structure.	Increases the capacity of some anticancer and other drugs to bind to their targets.	Paleos CM. et al. (2018)
Hydroxypropylation	Addition of hydroxyl propyl group on the starch.	It damages the hydrogen bonds between and within molecules of starch, weakening its granular structure. In amorphous regions, it enhances the motional freedom.	It raises the peak viscosity, enzymatic digestibility, swelling power, water binding capacity, and solubility.	Lee HL. Et al. (2011)
Oxidation	addition of carboxyl and carbonyl groups to natural starch	The starch undergoes depolymerization, which delays the process of recrystallization because carbonyl and carboxyl groups are incorporated.	It decreases the starch's dispersion viscosity, increases the stability, clarity, and biding qualities.	Boukhalfa, F. et al. (2018)

2.3. Overview of Avocado

Avocado (Persea americana Mill) is widely consumed globally, primarily due to its desirable fatty acid profile and appealing sensory properties. These characteristics are key factors driving its cultivation and commercialization (Mariana D. Salazar-Irrazabal, 2023). Fresh avocado fruits are consumed as a standalone food and are also utilized in the production of various goods. Avocado consumption is experiencing a significant surge worldwide, driven by the growing demand for nutritious food rich in essential macro and micronutrients like fiber, antioxidants, and proteins. The by-product of the avocado fruit, specifically its seed, is a valuable resource, comprising approximately 15-16% of the fruit and containing around 29% starch content. This makes it an alternative and abundant source of this polysaccharide, which is widely utilized in the food and food packaging industries (Juliani Buchveitz Pires et al., 2019).

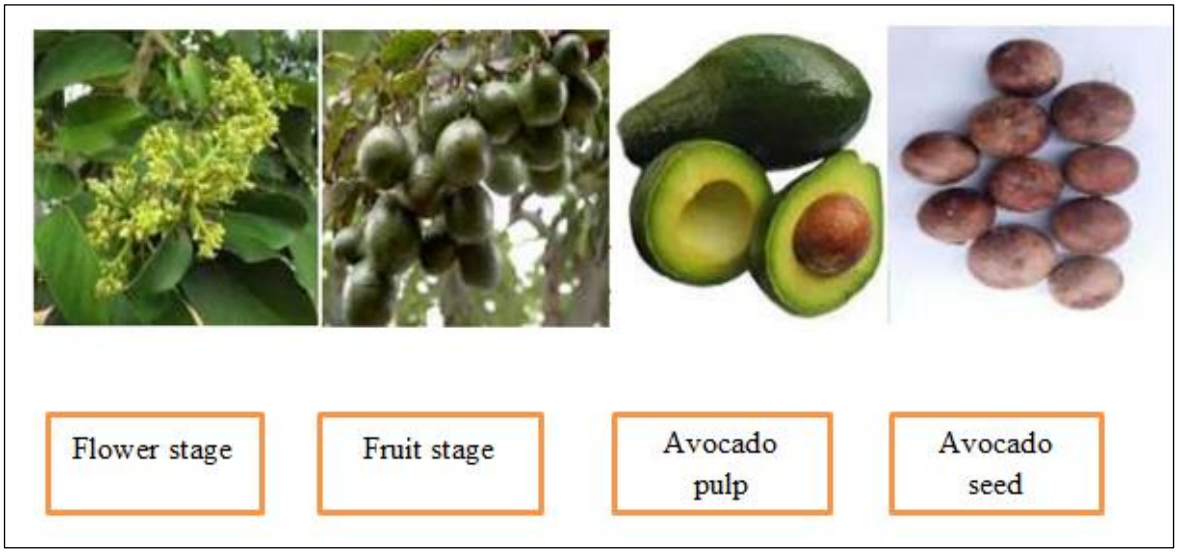


Figure 2.4: Avocado fruit

During the industrial processing of avocados, significant quantities of peels and seeds are generated as by-products. Despite being commonly discarded as waste, avocado seeds are actually a valuable source of nutrients. The seed, also known as the pit, serves as the storage organ of the plant and contains a wealth of important nutritional compounds. Untreated avocado waste, including seeds and leaves, can lead to an increase in the population of insects and rodents. However, it is worth noting that avocado waste contains a variety of beneficial chemicals. As a result, there is potential for extracting and utilizing these seeds and leaves to generate valuable income for the avocado industry. The avocado

seed accounts for approximately 13-18% of the total fruit size and serves as a source of phytochemicals (Setyawan et al., 2021).

2.3.1. Avocado seed extraction methods

Several techniques can be used to extract starch from AS for use in paper binding. The wet approach and the use of sodium metabisulphite solution are two of the extraction techniques. Using the wet process, the avocado seeds are blended with water and then filtered to extract the starch. Araújo et al. (2018) have reported that the application of sodium metabisulphite solution during the soaking treatment of avocado seeds has the ability to inhibit the browning reaction and enhance the extracted starch's properties, leading to an increase in the starch yield , degree of whiteness, and starch content. These techniques are used to extract high-quality starch from avocado seeds for uses like the creation of active and biodegradable films (Martins et al., 2022). The research was conducted using the wet process extraction method .

2.3.2. Composition of Avocado seed (Persea-Americana mill)

The table below shows the avocado seed's nutritional content according to the sample's wet weight basis.

Table 2.3: Nutritional composition of Avocado seed

Cultivar	Moisture	Minerals	Lipids	Fibres	Proteins	Reference
Hass	14.55	2.81	3.32	3.97	0.14	(Calleja-Agius et al., 2021)
Utz	9.44	2.79	6.32	4.24	3.09	(Syariah & Ilmu, n.d.)
Hass	54.45	1.29	14.7	-	2.19	(Syariah & Ilmu, n.d.)

2.4. Starch and its derivatives for paper coatings

Paper's biodegradability, light weight, and recyclability make it a great material for a variety of uses. As the paper business has grown, a lot of focus has been placed on improving the qualities of paper in an economical way. Surface coating is a very adaptable technique that can be used to conceal unwanted variation and contamination, enhance

qualities, or achieve specific functionality (Khwaldia et al., 2010). In fact, during the past few decades, the paper industry has made extensive use of surface coating to enhance the qualities of paper and accomplish particular functionalities (Andersson, 2008).

Starch plays a significant role in the paper industry due to its abundance and cost-effectiveness, making it suitable for widespread application. Globally, the paper industry is one of the primary non-food sectors that extensively utilizes starch. Within the paper industry, surface application is the predominant use of starch. This includes surface sizing, functional coating, pigment coating and other similar applications.

2.4.1. Starch derivatives as coating binders

A type of paper known as coated paper is made up of a coating layer and a base paper. In the coating process, the base paper is coated with colour to improve its characteristics. Pigments, binders, and other additives make up the majority of coating colour. According to Li et al. (2019), binders are the second most common component in the coating colour, behind pigments. The binders might be synthetic latex, like styrene-butadiene, poly(vinylacetate), and polyacrylates, or they can be natural materials like starch and protein. Starch is a cheap natural binder when compared to synthetic latex. The insolubility of native starch in cold water, its propensity to retrograde, and its loss of viscosity and thickening power during cooking and storage limit its applicability. This means that starch needs to be modified.

Modified starch can be added to improve water retention, inhibit pigment flocculation, and modify rheology. According to Chen et al., elongation at break falls as the starch content increases, but ultimate tensile strength and elastic modulus can also rise. On the other hand, adding too much starch to the coating formulation can increase fracture area, reduce picking resistance, and slow down the drying rate (Du et al., 2014; Hallajisani et al., 2011). Finding a balance between cost and performance is the essence of true art. Li, H., and others, 2019.

2.4.2. Starch derivatives for surface sizing

Surface sizing is the process of applying size agents to the paper web during production, typically done online. The primary objective of surface sizing is to enhance the paper's surface strength, printability, and water resistance. By applying size agents, the paper's surface properties are improved, allowing for better ink absorption, reduced feathering, and improved resistance to water penetration.

Modified starch as sizing agents

Among sizing agents, starch and its derivatives are most commonly utilised. Low viscosity of starch solution is achieved through surface scaling, which necessitates native starch modification. For surface sizing, a variety of modified starches are utilised, including carboxyl methyl starch, cationic starch, enzyme-degraded starch, oxidised starch, acid-degraded starch, and starch ester (Andersson, 2008). For both financial and environmental considerations, oxidised starch is a better option for surface sizing than other modified starches (H. Li et al., 2019).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. The Study Sites Description

Developing and characterization of starch based binder from AS for paper packaging industrial application was performed in Chemical Engineering Lab of Adama Science and Technology University (ASTU) and the paper was produced at Ethiopia pulp and paper industry laboratory which found in Wenji. Some characterizations was performed in different places due to the lack of some instruments. SEM characterization was performed in the chemistry laboratory in ASTU, TGA and XRD characterization was carried out in Materials Science Engineering Laboratory, FT-IR was performed in Addis Ababa Science and Technology University (ASTU), DSC was performed in Bahir Dar University (BDU) and the Paper quality test was conducted in Ethiopia pulp and paper industry laboratory.

3.2. Materials and Chemicals

Sample collection : Avocado seed was collected from a local fruit juice shop in Adama.

Chemicals: All the chemicals that was used in this study are Sodium hydroxide (NaOH), Hydrogen peroxide (H_2O_2) for starch oxidation, pH adjuster hydrochloric acid (HCl), $NaHSO_3$, ethanol, phenolphthalein, acetic acid and distilled water was used throughout the experiment. The chemicals like H_2O_2, HCl, acetic acid and phenolphthalein was taken from Adama Science and Technology University from the department of chemical engineering lab and the chemicals like NaOH , $NaHSO_3$, Ethanol and $CuSO_4 \cdot 5H_2O$ was purchased commercially from local chemical markets.

Equipment: The major materials that was used in this study are blender and mortar, sieve, PH meter, Analytical balance, magnetic stirrer, beaker, flask and measuring cylinder, heater, oven, magnetic stirrer, Fourier transforms infrared spectroscopy analysis (FTIR),furnace, X-ray Diffraction (XRD), thermos gravimetric analysis (TGA), Differential scanning Calorimetry analysis (DSC), Scanning Electron Microscopy (SEM) , and UV –Vis.

3.3. Methodology

3.3.1. Extraction of starch from Avocado seed

The extraction process of starch from raw AS involves several steps. Initially, the AS were thoroughly washed to remove any surface debris, followed by peeling. The peeled seed was then cut into approximately 1 cm cubes and these were further reduced in size using a mechanical shredder for a duration of 5 minutes, resulting in uniformly crushed AS. A suspension was prepared by combining 10% (w/v) crushed AS with tap water, then suspension was then subjected to filtration using a 150 µm sieve, and the resulting filtrate was kept for 3 hours. To ensure accuracy and reliability, each treatment was replicated three times. The filtrate was allowed to settle, leading to the formation of a solid layer composed of starch. The supernatant was decanted, and the starch-containing sediment undergoes at least two additional washes before being dried. The sediment was dried in an oven at 40°C for 48 hours. Finally, the dried sediment was processed into starch powder using a ball mill.

$$\text{Starch yield (\%)} = \frac{\text{dry weight of extracted starch (g)} * 100}{\text{dry weight of raw materials (g)}}$$

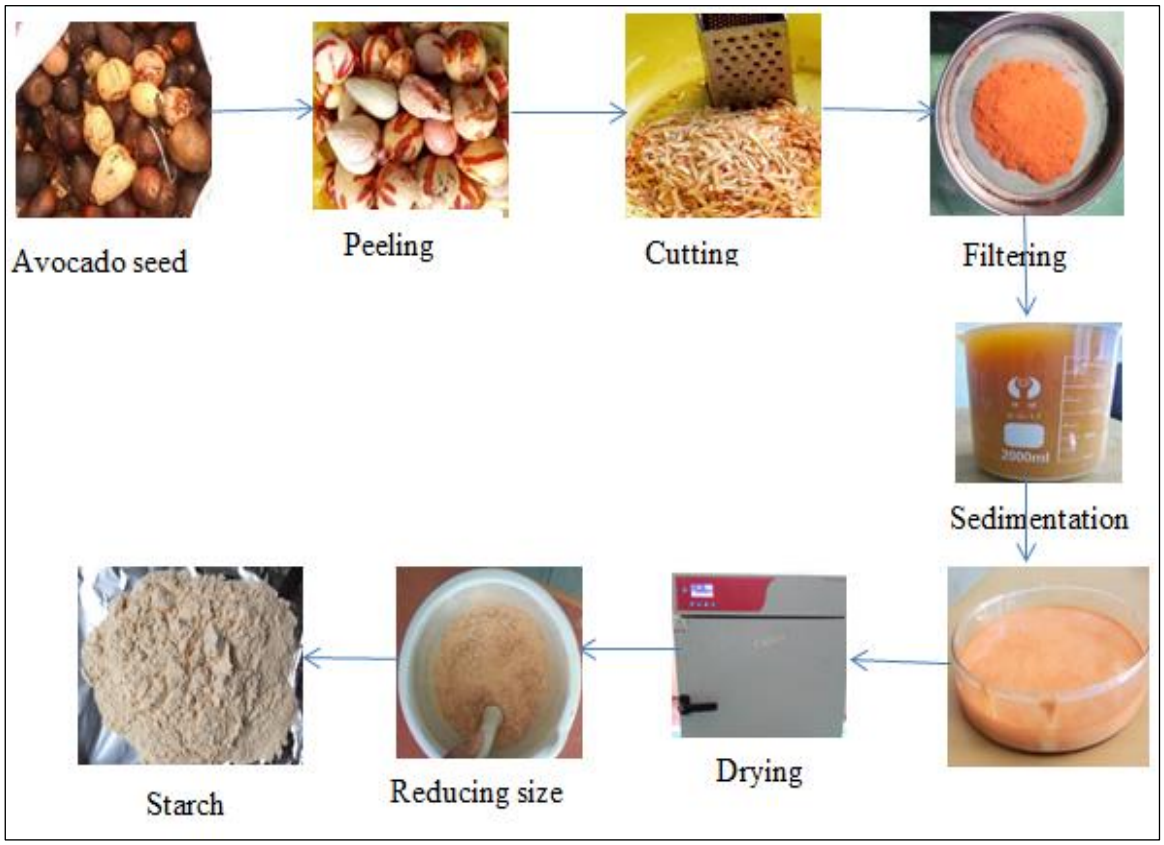


Figure 3.1: Experimental process to extract starch from Avocado seed

3.3.2. Modification of Avocado seed Starch

The oxidation procedure was adapted based on the method described by Wang et al. (2007). To prepare the starch slurry, 10 g of starch and 100 ml of distilled water were added to a 250 ml round bottom flask. The flask was equipped with a magnetic rod and a heating device such as a hot plate. The mixture was heated to 80°C while being gently stirred to facilitate the gelatinization of the starch. This process typically took approximately 30 minutes. After that, the temperature was decreased to reaction temperatures ranging from 30^{o}C-40^{o}C. The $CuSO_4 \cdot 5H_2O$ was dissolved in 10ml distilled water before being added to the starch then it was first added to the starch solution and the pH was then adjusted to 9 by the addition of NaOH (concentration of 1N). When the pH had reached the value of 9, was followed by the addition of H_2O_2 in half an hour and the pH was maintained constant by continuous addition of NaOH. The addition of Hydrogen peroxide was repeated 6 times, every 10 minutes, until having reached a total amount of 2.5% by weight of dry starch and the residual peroxide was neutralized by adding a small excess of During the oxidation process, the pH was kept constant by continuously adding NaOH. The addition of hydrogen peroxide (H_2O_2) was performed six times, with each addition occurring every 10 minutes. The total amount of H_2O_2 added reached 2.5% by weight of the dry starch. To neutralize the residual peroxide, a small excess of starch sodium metabisulfite ($Na_2S_2O_5$) was added and Following the oxidation step, the oxidized starch was washed using ethanol. The resulting starch slurry was then filtered and dried in an oven at 50°C for a period of 24 hours. The catalyst loadings for starch oxidation varied from 0.1% to 4% based on the weight of the AS starch. The reaction time for AS starch oxidation typically ranged between 30 minutes to 150 minutes and The temperature range for AS starch oxidation was between 50°C and 70°C.

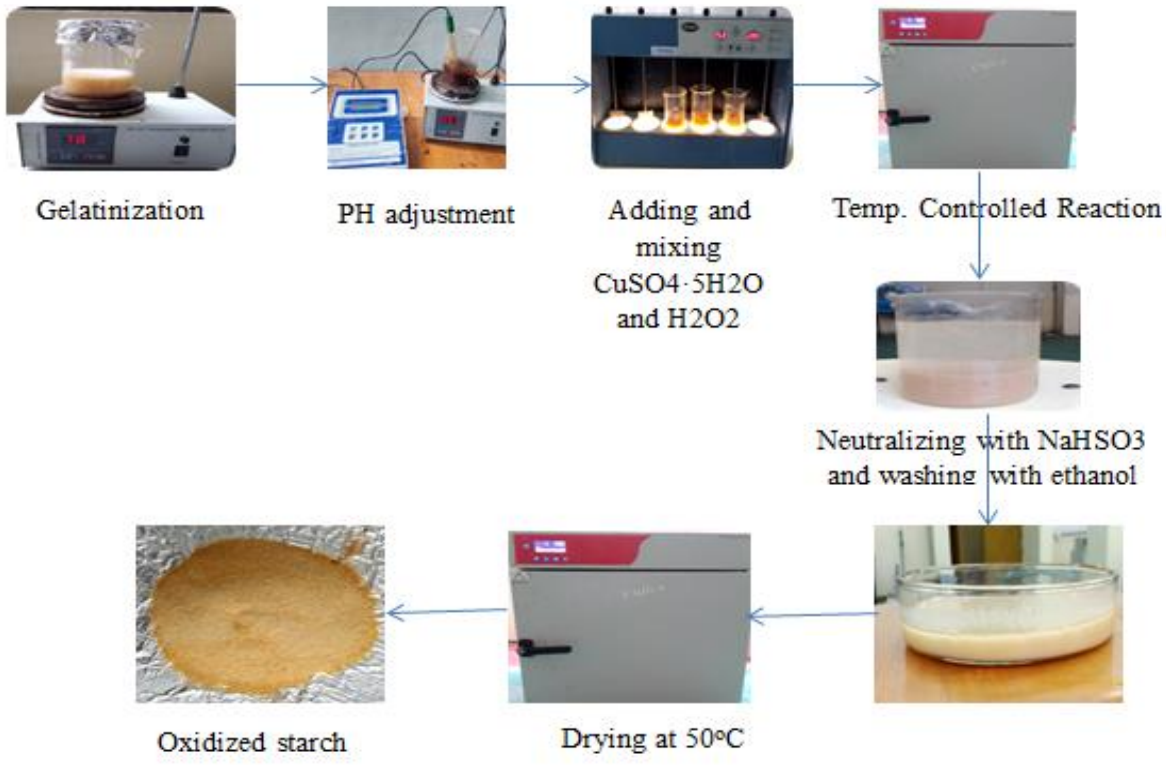


Figure 3.2: Process of modification of AS starch

3.3.3. Determining degree of oxidation

The degree of oxidation of the starch was quantified by measuring the number of total carboxyl and carbonyl groups per 100 glucose units. In previous studies on oxidized starch by (Kuakpetoon & Wang, 2006), the contents of carbonyl groups and carboxyl groups were determined separately using two different analytical methods. The content of carboxyl groups was determined by titrating a sample solution with a standard NaOH solution, while the carbonyl group content was determined by reacting the carbonyl groups with a hydroxylamine reagent and then back-titrating with an HCl solution. To simplify the process, a modified method based on the literature (Kuakpetoon & Wang, 2006; Wing & Willett, 1997) was developed to determine the total degree of oxidation of the oxidized starch. The procedure was began by taking a dry sample of 0.1 g and mixing it with 50 ml of distilled water. Subsequently, 10 ml of a 0.1 M NaOH solution was added. To ensure complete solubility, the slurry was heated until it reached boiling point. Once the solution had cooled down, its pH was adjusted to a value below 7.0 using 10 ml of 0.15 M HCl aqueous solution. Due to water evaporation during the heating process, additional water was added to maintain the original solution volume. The solution was then heated to boil for another one minute, and the resulting mixture was rapidly back-titrated with 0.10 M

NaOH until the phenolphthalein end-point was reached. Each sample was tested three times.

$$DO = CNaOHVNaOH – CHClVHCl / m/162 \times 100\%$$

where CNaOH and CHCl represented the concentrations of NaOH and HCl solutions, respectively; VNaOH and VHCl were the volumes of used NaOH and HCl solutions; m was the weight of dry or oxidized starch DO of the samples.

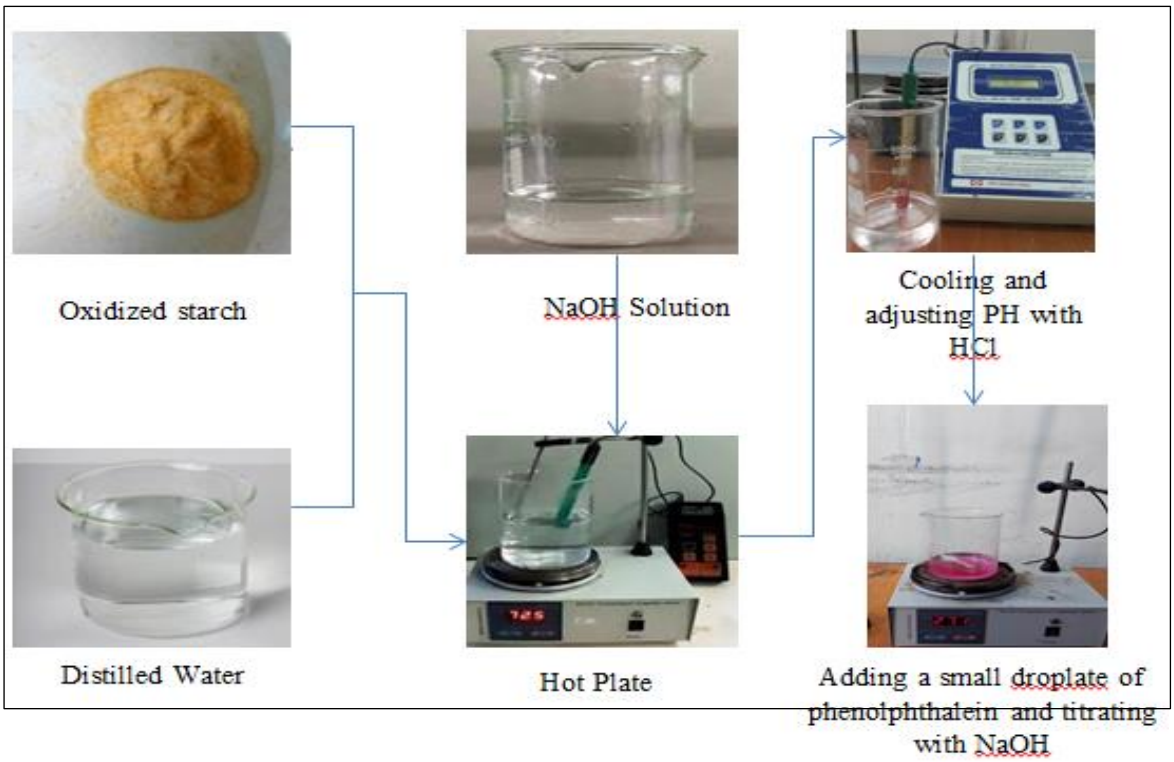


Figure 3.3: Experimental procedure to Determining degree of oxidation

3.4. Experimental Design

Response Surface Methodology (RSM) is an important statistical and mathematical tool for optimization. The value of the experiment was needed to determine the factor of the three operating variables like temperature, time and catalyst dosage for optimized starch from AS starch for paper binding with high qualities. Version 13 of Design Expert software was utilised to plan the experiments and evaluate the outcomes. Central composite was used to modified starch. Three process variables (temperature, time and catalyst dosage) were studied at three levels as shown Table 3.1. Analysis of variance was used to examine the influences and effects of each factor, level, and interaction on the responses degree of oxidation (ANOVA).

Table 3.1: Level of Each Factor

S.no	Factor	Unit	Level		
			Minimum	Middle	Maximum
1	Temperature	^{o}C	50	60	70
2	Time	min	30	90	150
3	Catalyst dosage	%	1	2.5	4

Table 3.2: Central composite design (CCD) for optimization of process parameters affecting degree of oxidation during reaction conditions

Run	Factor 1 A:temperature C	Factor 2 B: time Min	Factor 3 C:catalyst dosage (g)	Response 1 Degree of Oxidation (%)
1	60	30	2.5	
2	70	30	1	
3	70	30	4	
4	50	30	4	
5	60	90	2.5	
6	50	150	1	
7	60	90	1	
8	60	90	2.5	
9	60	90	2.5	
10	70	150	1	
11	60	90	2.5	
12	70	150	4	
13	60	90	2.5	
14	60	90	2.5	
15	60	150	2.5	
16	50	150	4	
17	60	90	4	
18	50	30	1	
19	50	90	2.5	
20	70	90	2.5	

3.5. Characterisation of Native and Modified Avocado Seed Starch

3.5.1. Proximate analysis of Avocado seed starch

Moisture content determination

The moisture content in the avocado starch was analyzed based on the method described by Alemu et al. (2022). Initially, 3 grams of avocado starch were measured and placed in a crucible. The crucible containing the sample was then subjected to drying in an oven at 105°C for a duration of 12 hours. Subsequently, the dried sample was allowed to cool inside a desiccator until its final weight was determined. The percentage of moisture content in the avocado starch was calculated using the following equation:

$$\text{MC}(\%) = \frac{W1-W2}{W1} X100$$

where MC = moisture content, W1= initial weight (g), and W2 = final weight (g)

PH Determination

The pH of the starch solution was measured using a method described in the study by Alemu et al. (2022). To begin, 5 grams of starch were added to 20 milliliters of distilled water, and the mixture was stirred vigorously for a duration of 5 minutes. The resulting starch slurry was then tested using a pH meter electrode, and the pH value of the mixture was determined..

Yield of Starch

The starch yield was calculated by measuring the quantity of starch obtained from the avocado seed kernel and expressing it as a percentage, using the following equation:

$$\text{Y}(\%) = \frac{Wi\ x\ 100}{Ws}$$

where Y = yield of starch, Wi = weight of isolated starch (g), and Ws = weight of seed (g).

Determination of Ash Content

The ash content of the avocado starch was analyzed using the procedure outlined in the AOAC (Association of Official Analytical Chemists) 1984 method. Initially, 5 grams of avocado starch were placed in a dried crucible, which was then heated in a furnace at a temperature of 540°C for a duration of three hours. Subsequently, the crucible was allowed to cool in a desiccator, and its weight was recorded to determine the weight of the ash remaining after incineration. The ash content was calculated using the following equation:

$$AC\ (\%) = \frac{Wa}{Wd} X100$$

where AC = ash content, Wa= weight of ash (g), and Wd = weight of dry starch (g).

Determination of Crude Fat

The fat content of the samples was determined using the Soxhlet extractor method described by Oladebeye et al. (2009). Each sample was initially weighed (W1), and the filter paper used was also weighed (W2). The sample was carefully placed on the filter paper, and the combined weight of the filter paper and sample was recorded (W3). The filter paper containing the samples was then inserted into the extraction unit, and petroleum hexane (at a temperature range of 40-60°C) was used to extract the samples for a duration of 5 hours. After the extraction process, the filter papers were removed, cooled in a desiccator, and then dried for approximately 30 minutes at 100°C. The resulting weight was recorded as (W4). Subsequently, the crude fat percentage was calculated as follows:

$$\%\ \text{Crude fat} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

$$\%\ \text{Crude fat} = \frac{W3 - W4}{W1} \times 100$$

Determination of Amylose Content

The method described by Tessema, A. (2021) was utilized to assess the percentage of amylose in starch using a colorimetric assay. To prepare the standard stock solution, 0.1 g of amylose was dissolved in 1 mL of ethanol and combined with 9 mL of 1 N NaOH in a volumetric flask. The flask's contents were thoroughly mixed, and the flask was sealed with a stopper. The sample was then subjected to a boiling water bath for 10 minutes to gelatinize the starch. After removing the samples from the water bath, they were allowed to cool down to room temperature. Once cooled, the samples were thoroughly shaken and filled with distilled water until reaching the appropriate level. Next, a series of steps were followed to develop a dark blue color. Initially, 1 mL of 1 N acetic acid and 2 mL of iodine solution (consisting of 2.0 g KI in 100 mL of de-ionized water and 0.2 g of I2) were added to the samples. The solution was then filled to the volume of 100 mL with distilled water, resulting in the development of a dark blue color. A portion of this solution was poured into another 100 mL volumetric flask to create standard amylose solutions containing 50%, 40%, 30%, 20%, and 10%.

To measure the absorbance at 600 nm, a UV Spectrophotometer (UV-1800, Shimadzu, Japan) was employed. The sample was shaken for 20 minutes before the absorbance measurement took place. For the blank solution, a mixture of 1 mL of ethanol and 9 mL of NaOH was boiled and then filled up to the mark with distilled water. Subsequently, 5 mL of this solution was transferred to a 100 mL volumetric flask. To this flask, 2 mL of iodine solution and 1 mL of 1 N acetic acid were added, followed by filling the flask to the recommended level. This process allowed for the calibration of the spectrophotometer at 600 nm. Additionally, absorbance measurements of pure starch amylose solutions were taken at the same wavelength. By utilizing the concentrations and absorbance values of known mixtures of amylose solutions, the amylose content of the starch was determined using a standard calibration curve. The experiment was conducted in triplicate, and the mean values were calculated.

$$\text{Amp (\%)} = 100 - \text{Am (\%)}$$

where Am = amylose content and Amp = amylopectin content

Determination of Swelling Power and Solubility of Starch:

The method described in reference (Torruco-Uco & Betancur-Ancona, 2007) was followed to assess the swelling power and solubility of the starch. A 2 g sample of starch was placed in a centrifuge tube along with 60 ml of distilled water. The mixture was then heated at 70°C in a water bath for 1 hour, with periodic shaking every 10 minutes. After cooling to room temperature, the mixture was centrifuged at 1200 rpm for 15 minutes. The liquid portion (supernatant) was carefully collected in a clean tray that had been pre-weighed, while the swollen starch sediment was weighed separately. The supernatant was evaporated and dried in an oven at 100°C for 3 hours until a constant weight was reached. The swelling power and solubility were calculated using the following equations:

$$Sp(\%) = \frac{Ww}{Wd}\ x\ 100$$

$$\text{S(\%)} = \frac{Wds}{Wd}\ x100$$

where Ww =weight of the wet sediment (g), Sp = swelling power, S = solubility, Wds = weight of the dry supernatant (g), and Wd = weight of the dry starch (g).

3.5.2. Fourier transforms infrared spectroscopy analysis (FTIR)

A small amount of starch powder was delicately applied onto the surface of a diamond crystal pellet using a press tip flap system, which was operated under a hydraulic press. The FTIR scan covered a transmittance range of 4000 to 400 cm−1 wave numbers. The spectrum was recorded under ambient conditions.

3.5.3. X-ray diffraction analysis (XRD)

An XRD (X-ray Diffraction) scan was conducted using a scanning speed of 2°/min. The XRD instrument operated at 40 kV with Cu radiation and a current of 40 mA. The angular range (2θ) covered in the scan was from 5° to 55°, and the measurement mode employed was continuous scanning. The samples used in the XRD analysis were dried starch powder.

3.5.4. Thermo gravimetric analysis (TGA)

Thermogravimetric studies (TGA) were performed using a nickel-calibrated equipment, specifically the Perkin-Elmer TGA located in Norwalk, CT. Sample weights ranging from 3 to 8 milligrams were placed in the balance system of the TGA instrument. The samples were then heated in a nitrogen environment, and the temperature was increased at a rate of 20°C per minute. The heating process was carried out within the temperature range of 30 to 700°C.

3.5.5. Differential scanning Calorimetry analysis (DSC)

According to the methodology described by Reddy et al. (2015), the starch sample weighing 5 mg was mixed with 15 ml of distilled water. The resulting starch slurry was stirred thoroughly to ensure a homogeneous solution, and it was allowed to stand for 1 hour before analysis. For the analysis, the starch sample was placed in a sample pan holder. An empty, sealed sample holder was used as a reference. The pan containing the sample was then subjected to heating from 20 to 200°C at a heating rate of 10°C per minute. The heating process was carried out under an inert nitrogen (N_2) gas atmosphere with a purge rate of 50 ml per minute. During the heating process, various thermal characteristic parameters were determined, including the onset temperature (To), gelatinization enthalpy (H), conclusion temperature (Tc), and peak temperature (Tp). These parameters provide valuable insights into the behavior and properties of the starch sample during the heating process.

3.5.6. Scanning electron microscopy (SEM) analysis

Using SEM (Hitachi S-3000N, Tokyo, Japan), the morphologies of native and modified AS starch was studied. Before any testing, the sample was vacuum-coated with platinum to make it conductive, and it was then fixed on the SEM stub using double-sided adhesive tapes. To generate clear images, scanning electron photomicrographs was taken at different magnifications.

3.5.7. Determination of Gelatinization Temperature

1.5g of oxidized starch sample was dissolved in a beaker with 10ml of distilled water, mixture was stirred, a thermometer inserted and beaker placed in a water bath. The solution was stirred continuously until its color change and thickened. This was the gel point and the temperature at this point was read off as the gelatinization temperature.

The gelatinization temperature was determined by dissolving 1.5g of oxidized starch sample in a beaker containing 10ml of distilled water. The mixture was stirred while a thermometer was inserted, and the beaker was then placed in a water bath. Continuous stirring was performed until the solution underwent a color change and thickened. At this stage, known as the gel point, the temperature was recorded as the gelatinization temperature.

3.6. Paper production and quality test

3.6.1. Paper production with oxidized starch

The process was started by making a solution of 80g of modified starch and 620ml of water and gelatinized the solution by heating to the appropriate temperature range of 70.2^{o}C and cooled down. The pulp fiber solution was also prepared by mixing 400g of pulp and 23L of water for 20min and from this solution, freeness test was done by taking 1,200ml of solution and mixed with 880ml of water to control beating time. After that 4,000ml (4L) of water was added to the prepared second solution, then from this solution 400ml was taken and this was mixed with 15ml of gelatinized starch. After the starch evenly distributed throughout the suspension, the mixture of starch and pulp fiber was poured in to a sheet molding machine which is suitable surface to form sheet of paper and the water was drained. As the water drained through the machine, the fibres and starch bonded together and formed a coherent sheet. After the sheet formed, excess water was removed from the paper sheet by pressing it. The pressing was also helped to improve the

bonding and sizing efficiency. The pressed paper sheet was transferred to a drying section oven and finally after completely dried the paper was evaluated it's overall quality and sizing properties including tensile strength, porosity, burst strength and tear strength .

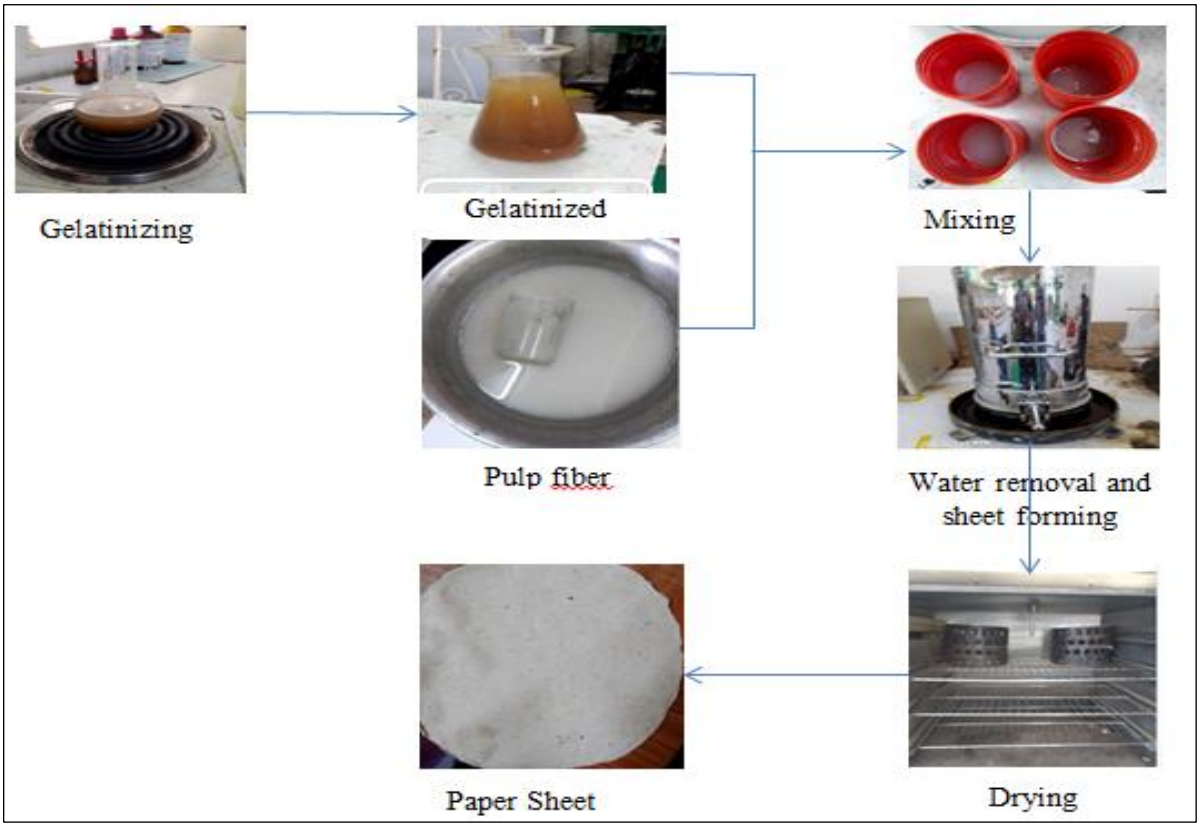


Figure 3.4: : process of paper production

3.6.2. Tensile strength

The tensile strength of paper refers to its ability to resist breaking or elongation under tension. It is a measure of the maximum force or stress that a paper specimen can withstand before it breaks. The tensile strength of paper was measured using a tensile tester (XLW-B, China). The paper sheet was cut in to a rectangular- shaped specimen. The end of the specimen was securely clamped in to the grips of the testing machine then steady and continuous force was applied to the specimen in the longitudinal direction and the cross-head of the testing machine moves at a constant speed. The testing machine measures and records the force applied to the specimen and the corresponding elongation or extension of the specimen. Finally the tensile strength was calculated by:-

$$\text{Tensile strength} = \frac{7.4 \ x \ 200{,}000}{3 \ x \ base \ weight}$$

3.6.3.Burst strength and Burst factor

Burst strength is a measure of the pressure that paper can withstand before it tears. This property is particularly important for paper packaging. To determine burst strength, the maximum hydrostatic pressure needed to rupture a paper sample is measured by gradually increasing the applied pressure. A bursting tester (PN-BSM600, China) was used to conduct the burst resistance test.

$$\text{Burst factor} = \frac{\text{burst strength x } 100}{\text{base weight}}$$

3.6.4.Tear strength and tear factor

Tearing strength is a crucial characteristic of paper as it provides insights into its behavior in different scenarios. It is particularly useful in evaluating the ability of paper to run smoothly on web-based processes, ensuring the quality of newsprint, and assessing the toughness of packaging papers that need to withstand shocks and impacts. By measuring tearing strength, we can gather valuable information about the paper's durability and its suitability for specific applications.Tear resistance was tested with tearing tester with cutting paper as 63 mm × 75 mm and the pendulum was set up with the blade attachment so that it was in the starting position, away from the specimen. Then the pendulum was released and allowed it to swing and strike the specimen with the blade. The blade was tear the specimen further along the initial tear line and the force required to tear the specimen further was measured and recorded.

$$\text{Tear factor} = \frac{tear\ strength\ x\ 100}{base\ weight}$$

3.6.5. Porosity

Porosity is a crucial physical characteristic that plays a significant role in determining the texture and quality of a material (Cui, Huang, & Liu, 2017). It refers to the presence of empty spaces within a material, which can be found both between its particles and within its overall volume. The porosity value indicates the amount of fluid that can be contained within the material, making it particularly relevant in the context of paper production. Paper is made up of randomly intertwined fibers, resulting in a structure with varying degrees of porosity. This porosity determines the paper's ability to absorb and allow the passage of both liquids and gases, making it a highly important property for its practical use. Paper is indeed a highly porous material, with air accounting for as much as 70% of its composition. Porosity plays a vital role in various types of paper, including Printing Paper,

Laminating Paper, Filter Paper, Cigarette Paper, Bag Paper, Anti-tarnish Paper, and Label Paper. Porosity refers to the measurement of the interconnected air voids present in a sheet, both vertically and horizontally. It provides an indication of the sheet's absorptivity, determining its ability to accept ink or water. Porosity can also be a significant factor in vacuum feeding operations on a printing press, as it affects the paper's ability to feed smoothly through the machinery. Therefore, porosity is a critical factor to consider in the production and application of different types of paper.The porosity of a paper sample was measured to assess its air permeability, employing the Gurley porosity test as per the TAPPI T460om-15 standard. In this test, a prepared specimen of the paper was securely clamped onto the lower clamping ring of the Gurley densometer apparatus. The upper clamping ring was then subjected to a calibrated pressure, which caused a specific volume of air to pass through the paper. The time taken for the air to flow through the specimen was carefully measured using a stopwatch. To ensure accuracy, three replicate measurements were performed. The average time recorded from these measurements was then compared to the standard to evaluate the porosity of the paper sample. The Gurley porosity test provides valuable information about the paper's air permeability, which can be essential for various applications where air flow or ventilation is a critical factor.

3.7. Cardboard production

The process was started by making two solutions of mS and water in two different tanks. The first solution was prepared by mixing 69g of mS with 238ml of water in the first thank. The second solution was also prepared by mixing 47g of starch with 238ml of water. After that NaOH solution was done by mixing 2.5g of NaOH with 15ml of water and this solution was added to the second solution and was mixed well, finally this solution and the first prepared solution was mixed. The final solution was very viscous because of the addition of NaOH solution, so additional 400ml of water was added and viscosity was measured with viscometer to be 40 s/m^2. Finally this solution of starch was used as surface sizing to produce cardboard.

To produce cardboard 3 main bodies were needed, two of them are called liners which are used as the bottom and top cover parts for smooth surface, printing and protections the card board and the third one was the flute paper , which refers to the wavey layer sandwiched between two flat liner boards, it address strength and rigidity to the box structure while providing cushioning and protection for its content. The process was started by fluting the produced paper. Starch was applied to the single sheet of paper

which was used as the bottom cover then the flute paper brought in to contact under the influence of heat and pressure to form a strong bond between the two layers, creating a single-face corrugated paper. In a new operation, additional starch adhesive was applied to the exposed ends of the grooves of the single-faced corrugated board. A second strip of cover was then applied to the adhesive-coated single-face board and glued under the influence of heat and pressure, using a machine to produce the final double-face or double-wall corrugated board. The starch adhesive played a crucial role in binding the corrugated paper (flute) to the liner board, creating a strong and durable corrugated cardboard material. then burst and crushing was tested.

Sizing the liner and the flute

Card board

Figure.3.5: Image of corrugated cardboard

3.7.1. Burst strength test

The burst strength test is an important quality control measure for corrugated packaging to ensure it can withstand the rigors of shipping and handling without failing. The cardboard material was cut out a square or rectangular piece of cardboard, ensuring it is free from any visible defects or irregularities. The specimen was placed on the rubber diaphragm of the burst strength tester. The tester then applied hydraulic pressure to the sample through the diaphragm until it burst and at this point the pressure was recorded, this pressure value is the burst strength of the cardboard.

3.7.2. Crush strength test

The crush strength test is an important quality control measure for corrugated packaging to ensure it can withstand the rigors of shipping and handling without failing. A rectangular sample of corrugated cardboard was prepared, larger than clamp of the crush tester. The dimensions of the sample were important to ensure it does no slip during testing. The

sample was placed in between the compression plates of the crush tester. The plates were designed to apply a uniform compressive force to the sample. The crush tester was applied a controlled compressive force to the sample at a specific rate until it failed or predetermined deformation. The maximum force at which the sample failed or deformed significantly was recorded as the crush strength. The value was used to determine the material's ability to withstand compressive loads.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1. Characterization of native Avocado seed starch

4.1.1. Proximate analysis of native Avocado seed starch

Moisture Content: Moisture content is an important factor in determining how much water and other volatile components will be absorbed from all kinds of raw materials and other finished goods. The starch moisture percentage of the extracted avocado seed was determined to be 14.1%. This substantially exceeds the values that were previously studied by (Sartika et al., 2018). The starch is resistant to bacterial or fungal destruction, even at room temperature, with a moisture content of 14.1%.

pH: Measurements of the pH are essential for determining how basic or acidic a substance is. The starch's pH was determined to be 5.34 based on the experimental result, which is consistent with other studies' findings. (Mo & Omojola, 2013).

Yield: The observed starch yield of 17.2% was discovered to be somewhat less than that of 19% stated yield (Macena, Jessica Franco Freitas Arruda De Souza et al., 2020) but significantly differed from 24.4% reported yields by (Bahru et al., 2019).

Ash Content: The starch's ash level was discovered to be 0.56%, a significant departure from the 0.23% reported amount (Sartika et al., 2018) however lower than the amount stated in another study (Ginting et al., 2018). The small starch burning with inorganic substances could be the cause of this ash content (Arvanitoyannis & Kassaveti, 2004). This suggests that the starch's impurities are negligible.

Amylose and Amylopectin Content: The amylose and amylopectin content, as well as the granule size, influence starch properties such as water absorption, gelatinisation and pasting, and susceptibility to enzymatic attack. UV spectrophotometry was used to identify amylose content of avocado seed starch, and absorbance of the standard sample was measured at 600 nm. Based on the recorded characteristic absorbance, the amount of amylose content was determined to be 19.6 %, which is comparable to reported research (Alemu et al., 2022).

The amylopectin content of the starch was obtained to be 80.4% by subtracting the amylose content from 100%. The variation of amylose and amylopectin contents of avocado starch might be significantly affected by various factors, such as botanical genotypes, soil type, climatic conditions, and harvesting time of cultivation.

Table 4.1 : Proximate analysis of Avocado seed starch

Raw material	**Moisture (%)**	**PH**	**Yield (%)**	**Ash (%)**	**Crude fat (%)**	**Am and AmP (%)**
Avocado seed starch	14.1	5.34	17.2	0.56	0.63	19.6 and 80.4

Swelling Power and Solubility:

The capacity of water abssorption (WAC), the ability to swell (SP), and solubility are directly affected by temperature increases and are corelated. The swelling power of avocado starch, which is the amount of water it can absorb per unit of starch, is approximately 6 grams, slightly different from the value reported by Chel-Guerrero et al. The ability of the starch to retain water is responsible for its swelling power through hydrogen bonding. When heated in excess water, the crystalline structure of the starch becomes disrupted, possibly due to the disruption of hydrogen bonds, as well as changes in amylose and amylopectin content. The solubility of avocado starch was determined to be approximately 21.3 ± 0.02%, which indicates the amount of soluble starch that leaches out of swollen granules.

4.1.2. Fourier transforms infrared spectroscopy (FTIR) analysis of native starch

Figure 4.1 displays the FTIR spectra of the samples. In the case of avocado seed starch, the spectra covered the range from 4000 to 400 cm−1. The peaks below 800 cm−1 correspond to the skeletal mode vibration of the glucose pyranose ring. Additionally, the peaks observed within the 800 to 1500 cm−1 range indicate the characteristic vibration of glucose molecules. Notably, avocado seed starch exhibits distinctive bands, with a peak observed at 2922 cm−1, which can be attributed to the symmetric stretching of the C-H bond. The band at 1634 cm−1 exhibited the O-H bending vibration of absorbed water molecules, particularly in the amorphous region of Starch . Characteristic peak intensities observed in

the region of 800–1500 cm−1 at 1338, 1150, 1075, 994, 1147, 921.13, and 858cm−1 , which all indicates the C-C bond stretching and C-H group bending vibrations.

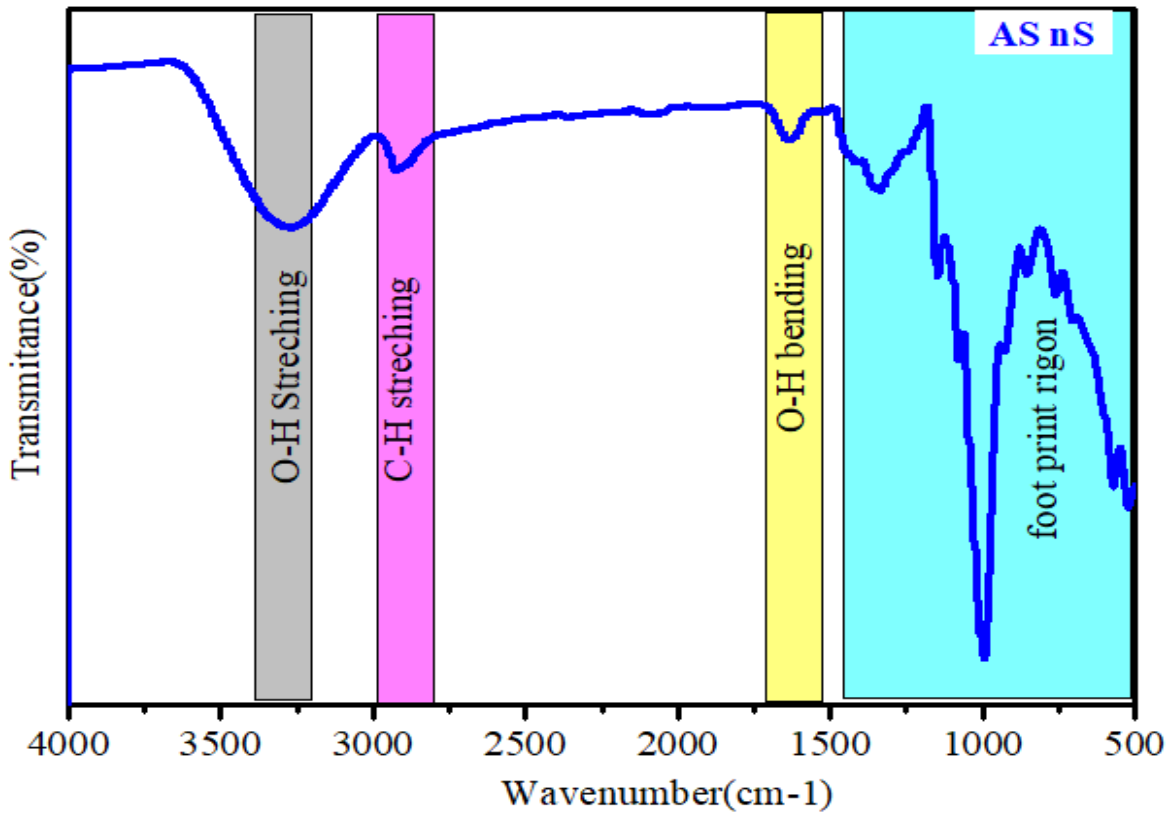


Figure 4.1: FTIR curves of the native starch

4.1.3. X-ray diffraction (XRD) analysis of native starch

The XRD spectrum provides information about the relative crystallinity, crystal patterns, and diffraction peaks. The presence of amylopectin and the type of cellulose present in the starch determine the specific diffraction peaks observed. Based on the XRD pattern, starch can exhibit three different crystallinity types: A, B, and C.Type A starch is characterized by strong peaks at 15^{o} and 23^{o}, along with imperfect diffraction at 17^{o} and 18^{o}. Type B starch displays strong peaks in the 17^{o} region, along with weak diffraction peaks at 15^{o}, 20^{o}, 22^{o}, and 24^{o}, as well as special diffraction peaks around 5.6^{o}. Type C starch represents a combination of types A and B, featuring strong peaks in the 17^{o} and 23^{o} regions, as well as several smaller peaks in the 5^{o} and 15^{o} regions (Cai & Wei, 2013). These crystal patterns and diffraction peaks provide insights into the structural characteristics of the starch samples.

Based on the figure 4.2, it can be seen that the crystalline diffraction pattern of avocado seed starch is a type B pattern. This is because the strongest peaks are at 17^{o} and some small peaks are exhibited strong diffraction 2θ-peak values of 5.64°, 17.30°, 19.62° and 22.3°. Then there is a peak on 5.6^{o} which is a special character of the type B pattern. The shape of the diffraction pattern of avocado seed starch is much sharper. The relative higher crystallinity of the avocado seed starch might be due to rich amylopectin chain-based crystallites and the purification steps applied.

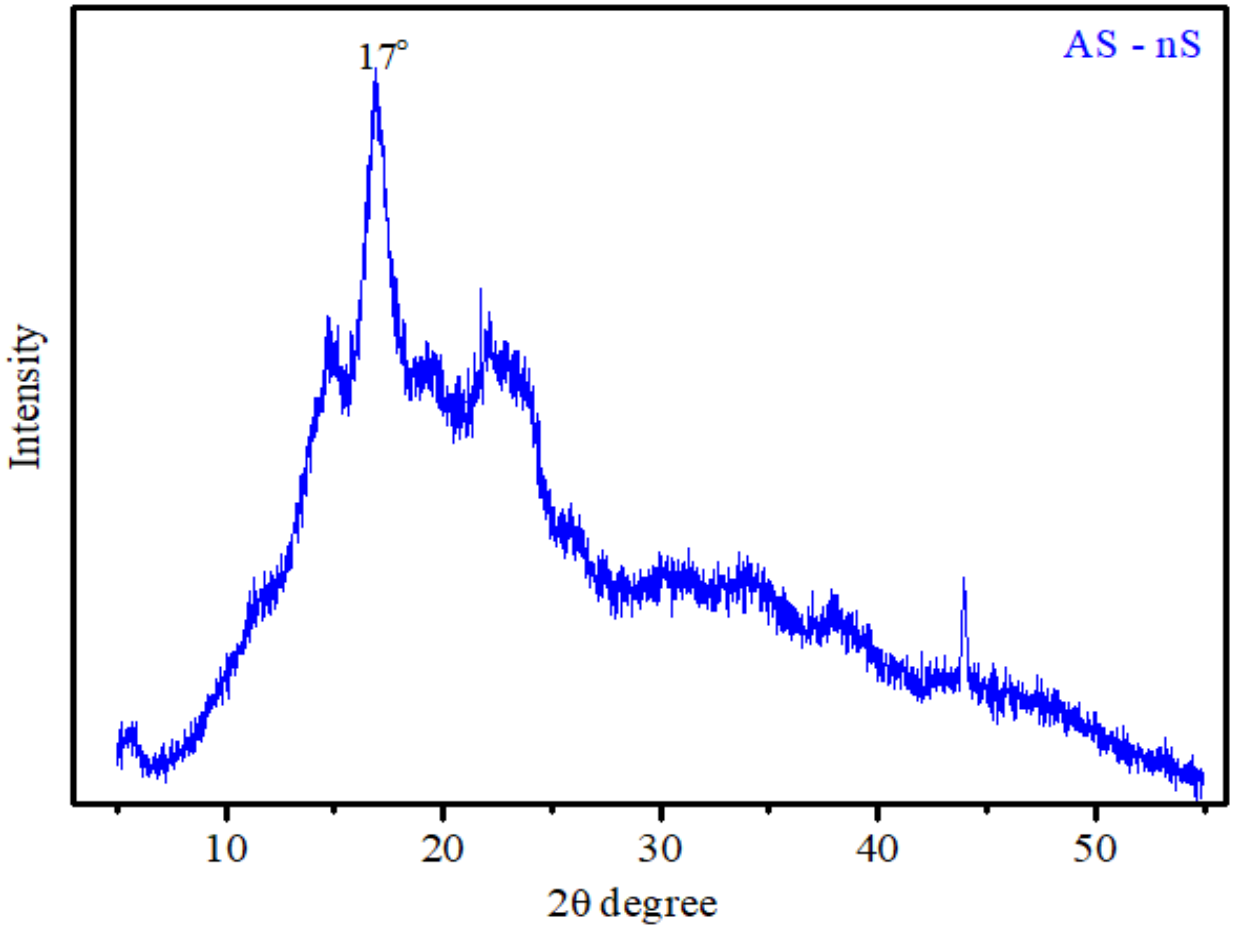


Figure 4.2: XRD curve of native starch

4.1.4. Thermo gravimetric (TGA) analysis of native starch

Thermogravimetric analysis was used to evaluate the avocado seed starch's thermal stability (TGA). Figure 4.3 below shows the avocado seed starch TGA data. In general, there were four phases of weight loss. The first loss (13.44%) was noted in a temperature range of 25 ℃-73.33℃ and is explained by the release of volatile components that were physically adsorbed and hydrogen-bonded water that stayed in the starch even after preconditioning. The second loss (60.94%) was attributed to the degradation of starch and

occurred in the temperature range of 255-331.43℃. The range of 331.43°C was where the maximum decomposition occurred, as the DTG curve in Figure 4.3 illustrates. Water was the primary by-product of the intermolecular and intramolecular dehydration events that cause starch molecules to decomposition. The temperature range of 331.4 -342.3°C showed the third weight loss (23.1%), while the temperature range of 342.3°C–520.33°C observed the fourth weight loss (1.23%), which was attributed to carbonisation and the generation of ash from degraded monomers.

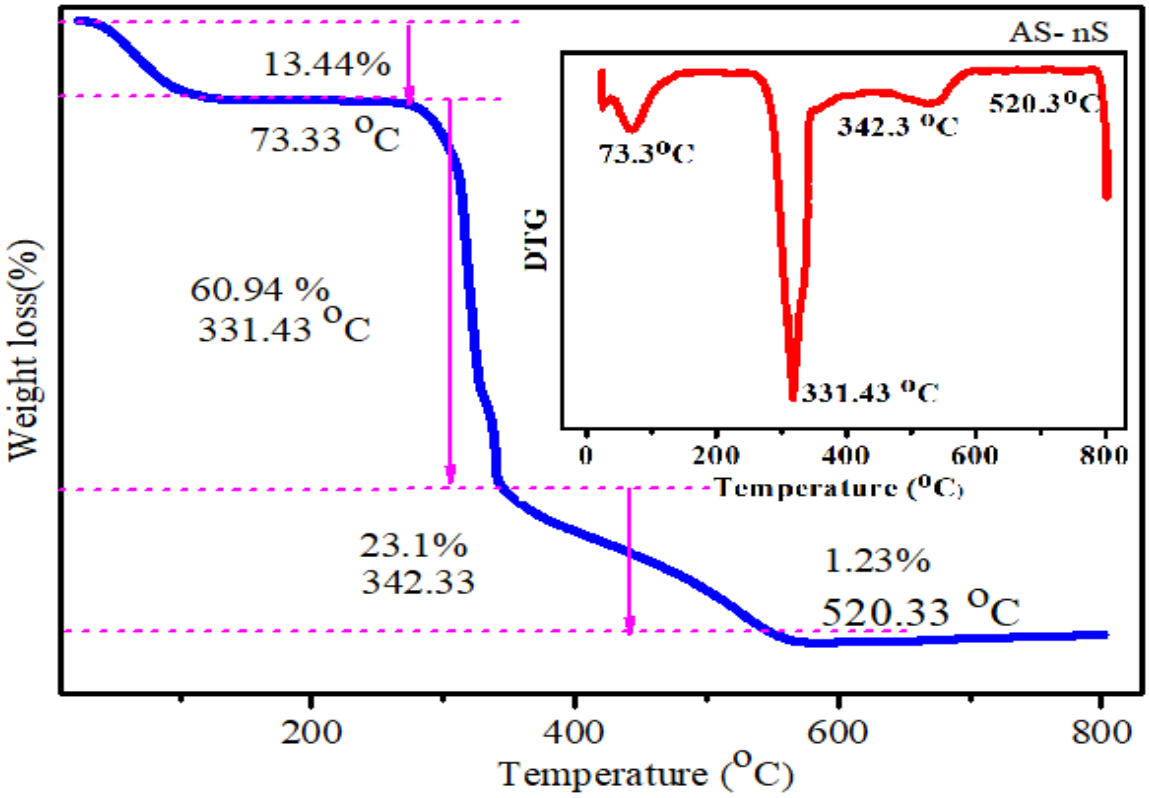


Figure 4.3: TGA curve of native starch

4.1.5. Differential scanning Calorimetry (DSC) analysis of native starch

Table 4.2 below contains data from the gelatinization endothermic curve and gelatinization temperature, as well as the DSC of AS starches analysed at a starch-de-ionized water ratio 1:3. Three categories apply at these temperatures: onset (at), peak (Tp), and conclusion (Tc) of gelatinization. The process of gelatinization involves starch granules transitioning from a semi-crystalline structure to an amorphous form. According to Vermeylen et al. (2004), the distribution of the starch granule size range is determined by the heat stability of the crystalline structure, which is revealed by the gelatinization temperature. Heating starch with distilled water helps to achieve this change. The temperature at which samples start to gelatinize is known as the onset temperature; at peak temperature, gelatinization

reaches its highest intensity; and at conclusion temperature, gelatinization ends. According to the AS starch thermograms, the temperature at which the reaction was began 62.33 °C, the peak temperature was reached at 68.67°C, and the conclusion temperature at 74.08°C. The degree to which the molecules in starch granules are arranged is directly correlated with these temperatures. The degree of gelatinization has been found to be influenced by the starch content and the architecture of crystalline and amorphous regions. These observations lead to the conclusion that this AS starch is more crystallinity than AS starch that has been previously described. This could be connected to raw AS root's botanic origin.

. Table 4.2: Thermal properties for the avocado starch

Sample	**To (oC)**	**Tp (oC)**	**Tc (oC)**	**Tc − To (oC)**	**ΔH (J/g**
Starch	61.33	68.67	74.08	12.75	13.45

To = onset temperature, Tp = peak temperature, Tc = conclusion temperature, ΔH = gelatinisation enthalpy.

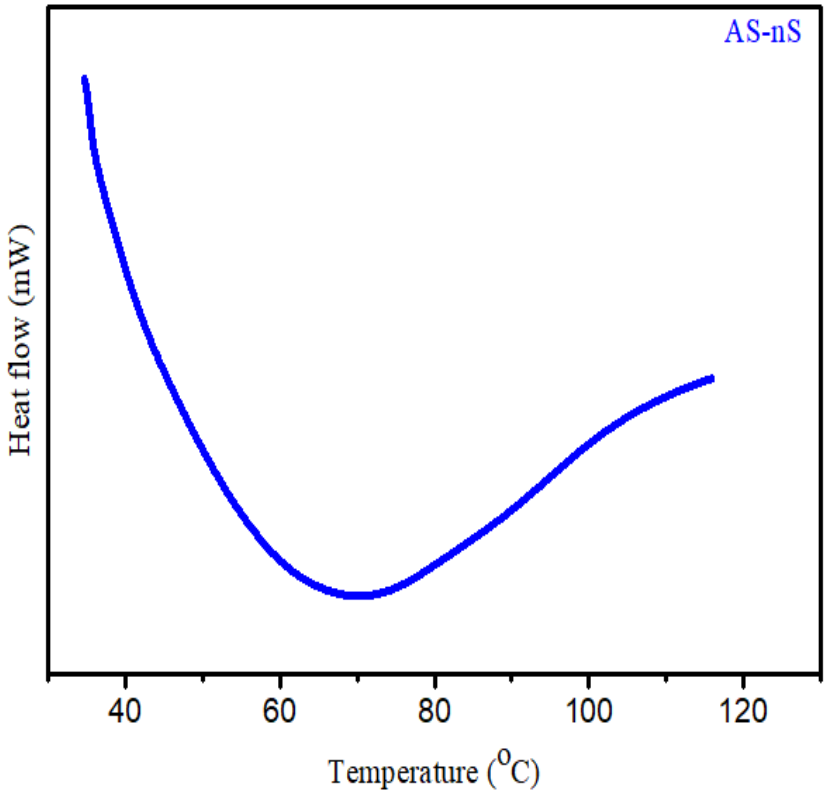


Figure 4.4: DSC curve of AS native starch

4.1.6. Scanning electron microscopy (SEM) analysis of native starch

SEM morphology of avocado starch is shown in Figure 4.5. The morphology of the starch, on the other hand, has a smooth surface and granular structure. SEM study revealed that granular morphology was mostly oval and ellipsoid, some elongated, with a very smooth surface and no cracks or surface pores. The nature of granular morphology may be attributed to the biological origin and biochemistry of the amyloplastic physiology of the plant (Yu et al., 1998).

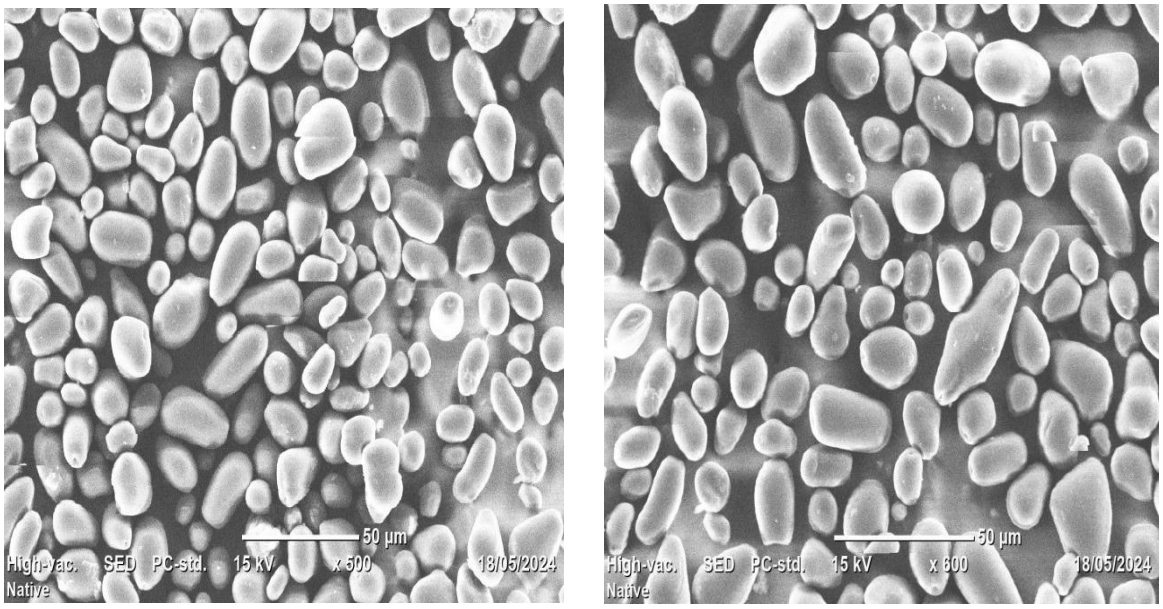


Figure 4.5: Scanning electron microscopy (SEM)

4.2. Determining Degree of Oxidation

In this work, mathematical models were developed for the performance namely the degree of oxidation. Response surface methodology Approach was used to determine the optimum degree of oxidation parameters that were got higher degree of oxidation from modified AS starch. This study focused on three key factors: reaction temperature, reaction time and catalyst loading. In order to examine the effect of the parameters on the Degree of oxidation, a second–order polynomial response surface can be fitted in to the following equation.

$$Y= \beta0+\beta1X1+\beta2X2+\beta11X21+\beta22X22+\beta12X1X2$$

Where Y is the response(Y=Degree of oxidation); β0 is a constant; β1 and β2 represent the regression co-efficient; while β11 and β22 are the quadratic coefficients: and β12 is the interaction coefficient.

4.2.1. Design experiment result

Table.4.3: Experimental result of modified starch

Run	Factor 1 A:temperature C	Factor 2 B: time min	Factor 3 C:catalyst dosage (g)	Response 1 Degree of Oxidation (%)
1	60	30	2.5	32.3
2	70	30	1	37.5
3	70	30	4	30.5
4	50	30	4	27.4
5	60	90	2.5	47.1
6	50	150	1	50.2
7	60	90	1	51.3
8	60	90	2.5	49.6
9	60	90	2.5	42.1
10	70	150	1	44.2
11	60	90	2.5	48.5
12	70	150	4	40.7
13	60	90	2.5	46
14	60	90	2.5	46.8
15	60	150	2.5	38.9
16	50	150	4	29.2
17	60	90	4	44.1
18	50	30	1	35.6
19	50	90	2.5	34.4
20	70	90	2.5	44.7

4.2.2.ANOVA for quadratic model

Analysis of Variance

The experimental results of the influence of three factors, temperature time, and catalyst dosage were designed using Central composite design (CCD) of ANOVA. As shown in Table 4.4 below, As can be seen from the **p-values** of the model coefficients, the values of

temperature, time, and catalyst dosage are much less than 0.0001. This indicated that all factors have a significant effect in determining the model.

The **Model F-value** of 18.7 suggests that the model is significant which have only 0.01% possibility that an F-value could occur in case of noise.

P-**Values** fewer than 0.0500 show model terms are significant model terms in this instance are A, B, C, AB, AC, BC, A^2, B^2, and C^2. The model expressions are considered not significant if the values exceed 0.1000. Another useful statistic here was the one labeled "Adeq Precision". This is a kind of signal-to-noise ratio that measures the ratio of a range of variation in the predicted response to an estimate of the standard error of the predictions and is obtained by subtracting the minimum predicted value from the maximum predicted value and then dividing by the average standard deviation of a prediction.

Based on table 4.5, The Predicted R^2 of 0.8304 and the Adjusted R^2 of 0.8938 for the ab Bove model are reasonably in agreement; that is, the difference is less than 0.2. Adeq Precision measures the ratio of signal to noise. Ideally, the ratio should be higher than 4. A sufficient signal is indicated by the ratio of 15.546. The design area can be navigated with the help of this model.

Table 4.4: Analysis of Variance (ANOVA)

Source	**Sum of squares**	**df**	**Means square**	**F-valus**	**P values**	
Model	935.13	9	103.90	18.77	<0.001	Significant
A- temperature	128.16	1	128.16	23.15	0.0007	
B- time	62.00	1	62.00	11.20	0.0074	
C-catalyst dosage	101.76	1	101.76	18.38	0.0016	
AB	30.03	1	30.03	5.42	0.0421	
AC	1.71	1	1.71	0.301	0.5905	
BC	4.06	1	4.06	0.736	0.4118	
A^2	71.91	1	71.91	12.99	0.0048	
B^2	225.91	1	225.91	40.80	<0.001	
C^2	25.35	1	25.35	4.58	0.0580	
Residual	55.36	10	5.54			
Luck of Fit	21.90	5	4.38	0.652	0.6542	not significant
Pure Error	33.47	5	6.69			
Cor total	990.49	19				

The following table shows the result of Fit Statistics .

Table 4.5: Fit Statistics

Std. Dev.	**2.35**	**R^2**	**0.9441**
Mean	40.30	Adjusted R^2	0.8938
C.V. %	5.84	Predicted R^2	0.8304
		Adeq Precision	15.5457

Assuming that all other factors remain constant, the coefficient estimate in Table 4.6 shows the anticipated change in response for each unit change in factor value. The average of all the runs' responses is the intercept in an orthogonal design. Using the factor settings as a guide, the coefficients adjust the average. VIFs of 1 indicate orthogonal factors; multi-colinearity is indicated by VIFs larger than 1. More severe factor correlations are indicated by higher VIF values. Tolerable VIFs are generally less than 10.

Final Equation with Coded Factors

Yield = **+45.88+ 3.56A + 2.49B+ -0.319C+ 1.94AB+ 0.4625 AC+ 0.7125BC+ -5.11A^2 + --9.06B^2+3.04 C^2**

Where A Represents Temperature B Time and C Represents catalyst dosage

From this quadratic regression model equation, it was observed that the linear variables A, B, and C,C2 and the interaction variables AC, have a positive effect on the oxidation of starch where as other terms have a negative effect.

For certain amounts of each element, predictions regarding the reaction can be made using the equation expressed in terms of coded factors. The factors' high values are automatically typed as+1, and their low levels are coded as -1. By comparing the factor coefficients, the coded equation helps determine the factors' respective impacts.

Table 4.6: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	45.88	1	0.8089	44.07	47.68	1.0000
A-Temperature	3.58	1	0.7441	1.92	5.24	1.0000
B-Time	2.49	1	0.7441	0.8321	4.15	1.0000
C-catalyst dosage	-3.19	1	0.7441	-4.85	-1.53	1.0000
AB	1.94	1	0.8319	0.0839	3.79	1.0000
AC	0.4625	1	0.8319	-1.39	2.32	1.0000
BC	0.7125	1	0.8319	-1.14	2.57	1.0000
A^2	-5.11	1	1.42	-8.28	-1.95	1.82
B^2	-9.06	1	1.42	-12.23	-5.90	1.82
C^2	3.04	1	1.42	-0.1251	6.20	1.82

4.2.3. Optimization of the responses

In order to find the optimum value of each independent variables, four statistical metrics such as the larger regression coefficient (R2), smaller lack of fit F-value, lower p-value and higher sum squares have been used. The response optimization was used to identify the combination of independent variables particularly temperature, time, and catalyst dosage with response degree of oxidation. The goal of the process was to obtain maximum DO. According to the result obtained from optimizer Design-Expert version 11 software the maximum DO achieved 51.3 % by using 60^{O}C, 90 min, and 1% of catalyst dosage.

4.2.4. Diagnostics and Contour Plots

The residual plots are the main notes that offer the usual range of residual plots for checking assumptions such as Normality and constant variance. The Box-Cox plot for power transformations helps us decide whether it could improve the model's fit by measuring the response on a different scale, e.g., by using the log of the response values.

Plots of leverage and influence statistics shows the influence of individual data points on the fitted model.

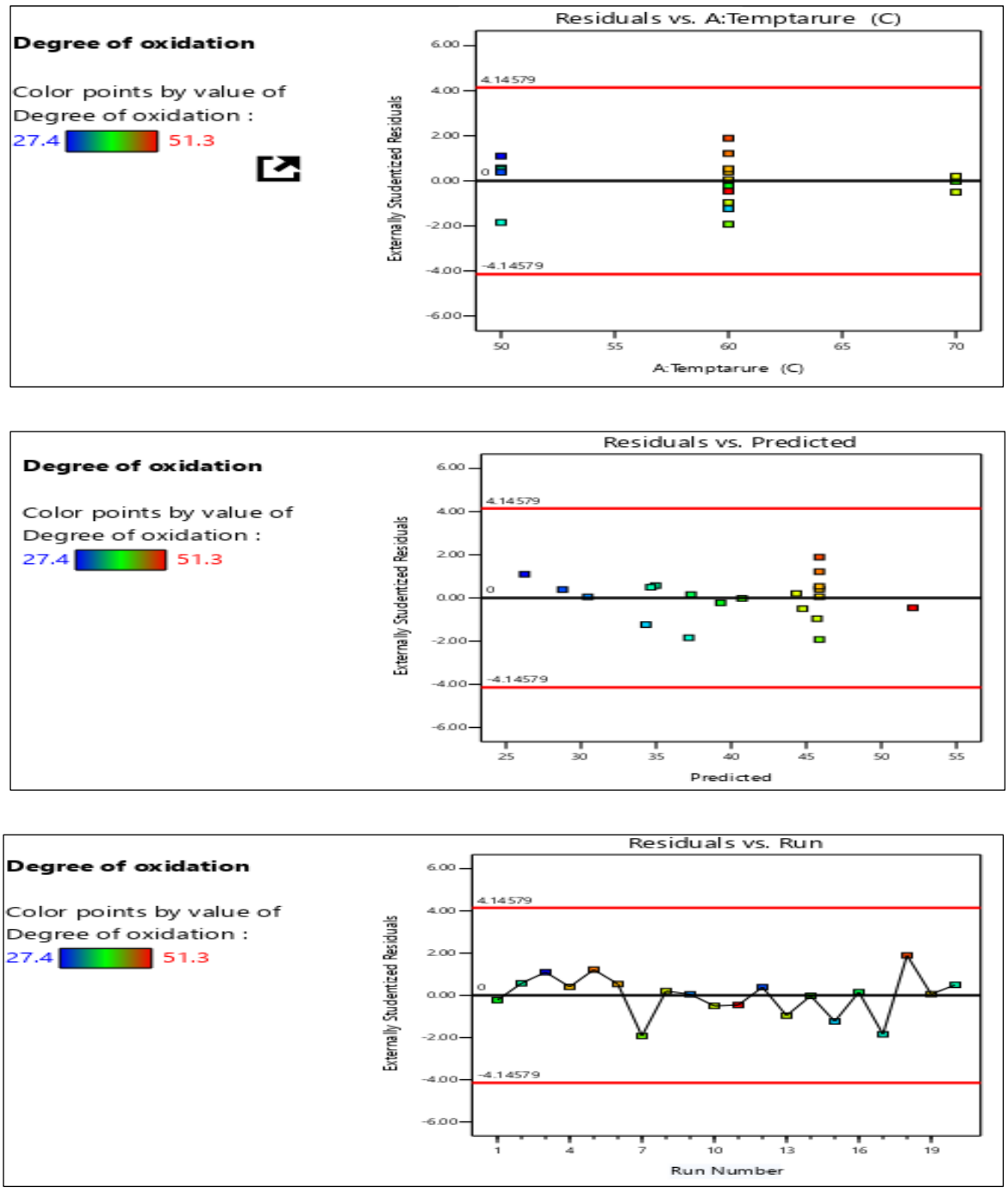


Figure 4.6: Diagnostics Graphs in terms of Coded Factors

The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure 4.7. This plot, therefore, clarifies the performance of the correlation in an evident way.

As a result, the experimental data, in which every point is extremely close to the line of perfect fit, was extremely accurately described by the regression model equation. This result suggests that the association between the three process factors and the degree of oxidation was successfully established.

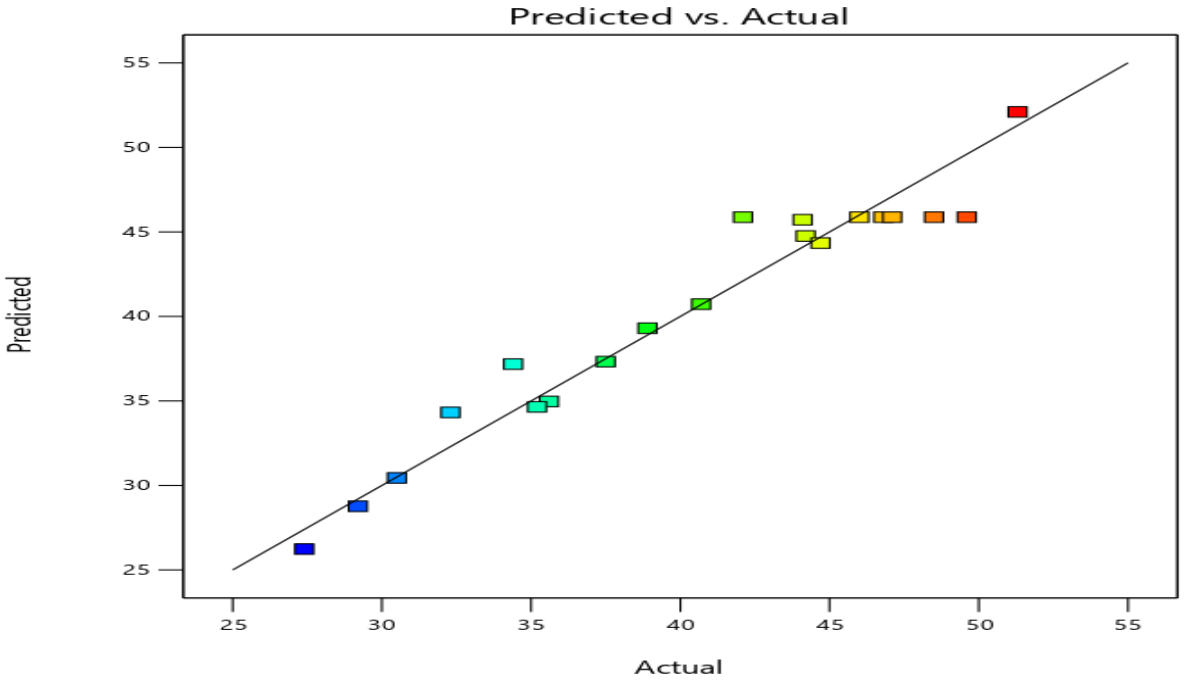


Figure 4.7: Actual versus Predicted Value

Table 4.7: Diagnostics Case Statistics

Run	Actual Value	Predicted Value	Residual	Leverage	Internally Studentizdd Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS
1	38.90	39.30	-0.491	0.491	-0.239	-0.239	0.006	-0.224
2	35.60	34.97	0.6330	0.793	0.592	0.592	0.134	1.119
3	27.40	26.24	1.16	0.793	1.087	1.087	0.453	2.150(1)
4	46.80	45.88	0.9245	0.118	0.418	0.418	0.002	0.147
5	48.50	45.88	2.62	0.118	1.188	1.188	0.019	0.445
6	47.10	45.88	1.22	0.118	0.554	0.554	0.004	0.196
7	42.10	45.88	-3.78	0.118	-1.709	-1.709	0.039	-0.705
8	44.70	45.88	0.3582	0.491	0.213	0.203	0.004	0.199
9	30.50	30.45	0.0530	0.793	0.049	0.049	0.001	0.092
10	44.20	44.76	-0.5570	0.793	-0.521	-0.501	0.104	-0.981
11	51.30	52.10	-0.5570	0.491	-0.478	-0.458	0.022	-0.450
12	29.20	28.77	0.4330	0.793	0.405	0.387	0.063	0.758
13	44.10	45.72	-1.62	0.491	-0.966	-0.962	0.090	-0.945
14	40.70	40.73	-0.0270	0.793	-0.025	-0.024	0.0000	-0.047
15	32.30	34.32	-2.02	0.491	-1.204	-1.236	0.140	-1.213
16	37.50	37.33	-0.1730	0.793	0.162	0.154	0.010	0.301
17	34.40	37.18	-2.78	0.491	-1.657	-1.846	0.265	-1.812
18	49.60	45.88	3.72	0.118	1.686	1.890	0.038	0.692
19	46.00	45.88	0.1245	0.118	0.056	0.053	0.000	0.020
20	35.20	34.65	0.5530	0.793	0.517	0.497	0.102	0.973

(1) Exceeds limits.

4.2.5. The Effects of Interaction of Process Variables on the DO

The influence of interaction between independent process variables on DO was investigated using response surface plots, as shown below. The result revealed that the interaction of temperature, time, as well as the catalyst dosage, has the most substantial ($p<0.0001$) influence on the DO, followed by temperature with a catalyst dosage.

Effect of Temperature with Time on DO

The interaction plot was used to show the effect of changing one control variable with changes in a second control. The interaction effect of all factors could also determine from ANOVA in Table 4.6. From Figure 4.8 below, the interaction effect of temperature and time on the DO shows that when both the temperature and time increase, the DO also increased and reached optimal value. After the optimal point, the DO decreased.

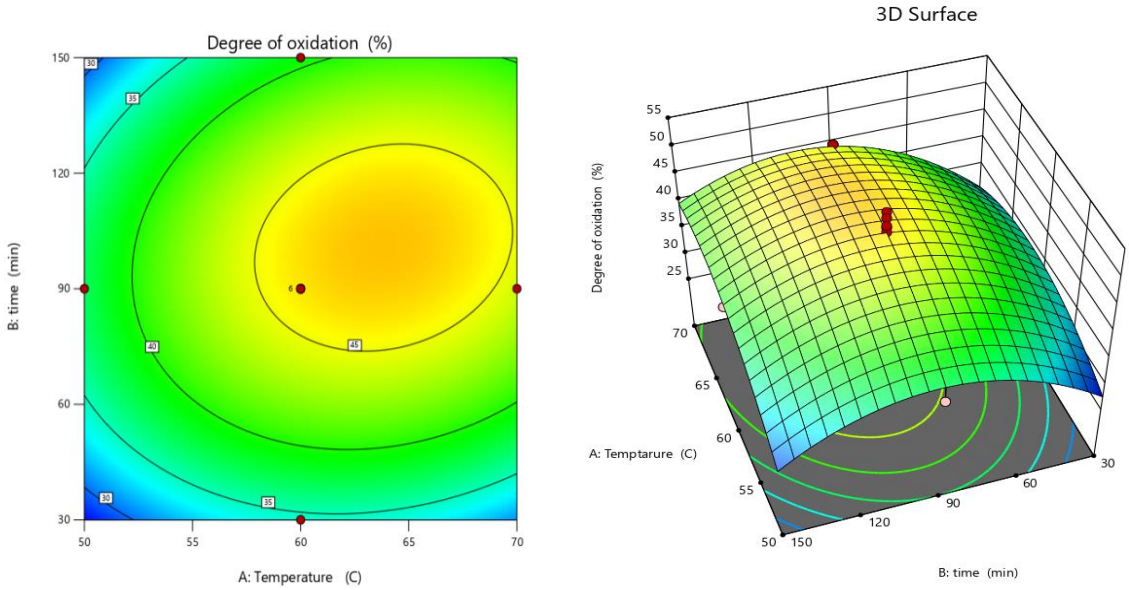


Figure 4.8: Contour and 3-D plot for effect of Temperature and Time

Effect of catalyst dosage with time on DO

From Figure 4.9 below, the interaction effect of catalyst dosage and time on the DO shows that, when the catalyst dosage increased DO decreased but when time increased DO also increased and reached optimal. After the optimal point, the DO decreased with time.

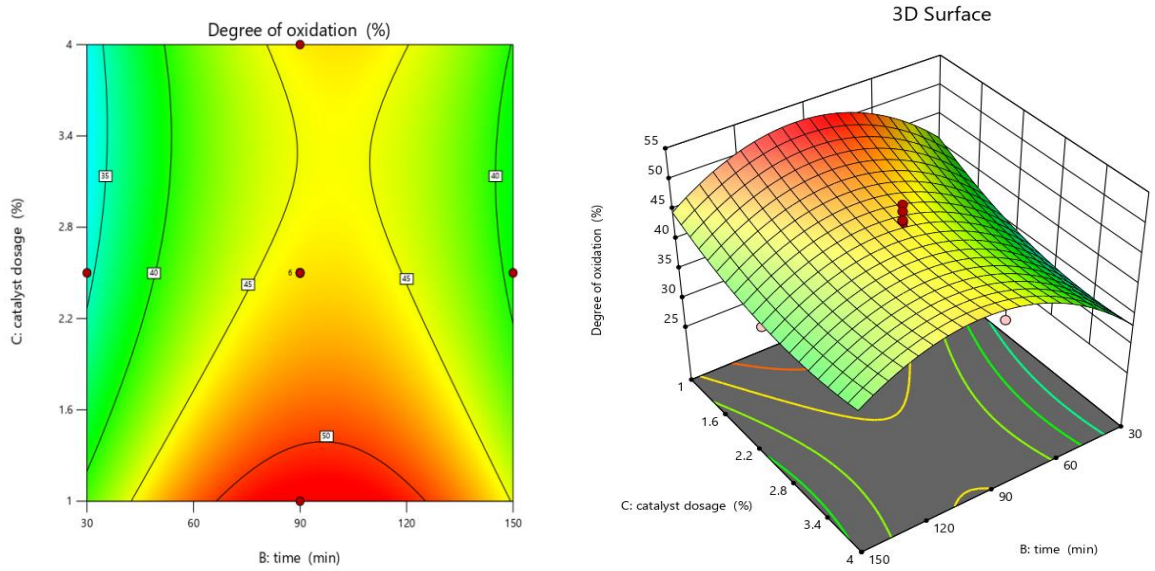


Figure 4.9: Contour and 3-D plot for effect of catalyst dosage and Time

Effect of catalyst dosage with temperature

The interaction plot was used to show the effect of changing one control variable with changes in a second control. The interaction effect of all factors could also determine from ANOVA in Table 4.4. From Figure 4.11 below, the interaction effect of catalyst dosage and temperature on the DO shows that when catalyst dosage increased DO decreased but when the temperature increased the DO also increased and reached optimal value. After the optimal point, the DO slightly decreased with temperature.

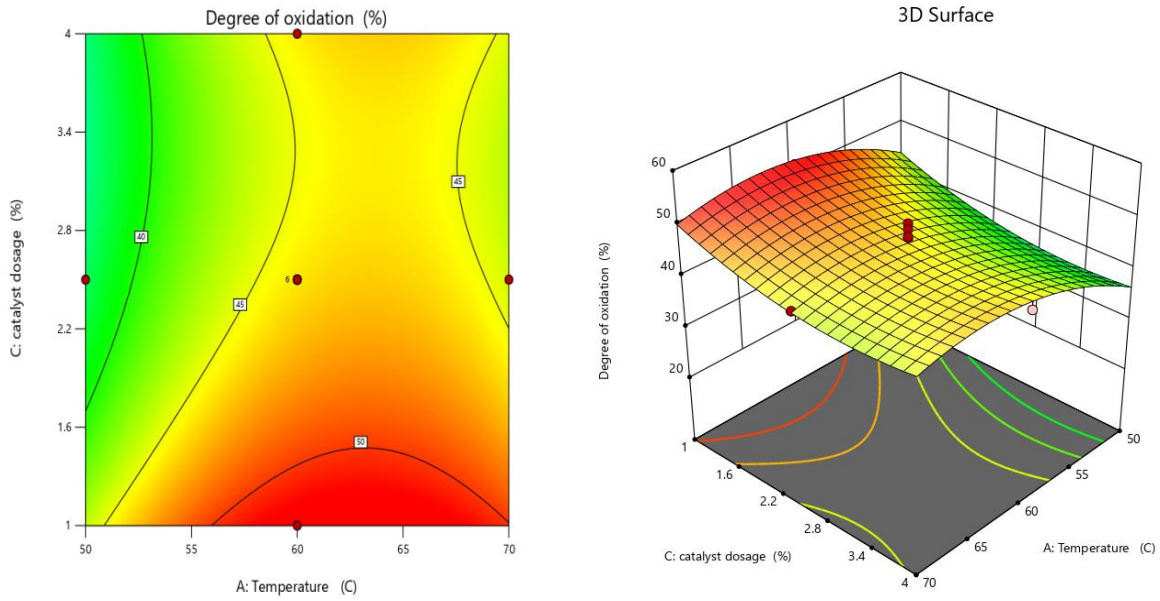


Figure 4.10: Contour and 3-D plot for effect of catalyst dosage and temperature

Numerical Optimization

One of the primary objectives of the present study was to find the optimum process parameters for maximizing the quantity of DO. The process variables such temperature, time and catalyst dosage have been optimized using CCD and their output values are executed using Design-expert software 11. In the optimizing process, the temperature, time and catalyst dosage are a set of process parameters that should be "in range" while the DO, need to be "maximized". Table 4.8 shows the summary of factors responses and goals and the corresponding set of specific objectives that was optimized .The table 4.9 exhibits the desired combinations of process parameters that would provide the highest responses by using Numerical optimization. Numerical optimization was used to optimize any combination of one or more goals. The goals may be apply either factors or responses. The model capable of predicting the maximum DO, the optimum values of the process variables were the operating process variables are putting on the range.

Table 4.8: Optimization Constraints

Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**
A- :Temperature	In range	50	70	1	1
B- Time	In range	30	150	1	1
C- Catalyst dosage	In range	1	4	1	1
Degree of oxidation	maximum	27.4	51.3	1	1

By using the numerical optimization criteria in Table 4.9, the Design expert 11 solution was obtained. The possible solution for this model with the given factors that change the amount of the produced DO is shown in Table 4.9. The optimum possible solutions are presented in table 4.9.

Table 4.9: Numerical Optimization Solution

Number	**Temperature**	**Time**	**Catalyst dosage**	**Degree of oxidation**	**Desirability**	
1	64.881	105.690	1.022	52.366	1.000	selected

The desirability scale, which runs from 0 to 1, indicates how close an answer is to its ideal value. The desirability of a response is 0 if it falls inside the unacceptable intervals and 1 if it reaches its ideal value or falls inside the ideal intervals. Based on the above analysis best local maximum for DO 52.366% was found at temperature 64.881%, time 105.69 min and catalyst dosage 1.022 and the value of desirability obtained was 100%.

4.3. Oxidation Modification of Starch

The oxidation of starch primarily involves the conversion of hydroxyl groups to carbonyl and carboxyl groups, which modifies the functional properties of starch. This process can be carried out using various oxidizing agents like sodium hypochlorite, hydrogen per oxide and ozone. Such oxidation occurs primarily on the hydroxyl groups at the C-2, C-3 and C-6 positions. In this study, it was focused on the oxidation reaction of starch with hydrogen oxide as oxidizing agent and CuSO4(5H2O) catalyst (Muflihati et al., 2019). Oxidation happens as a result of a radical chain reaction. Hydrogen peroxide breaks down quickly in the presence of a metal catalyst to produce hydroxyl radicals (OH). By removing hydrogen atoms from C-H groups on sugar rings, these extremely potent free radicals react with carbohydrates quickly to produce new radicals (R- CHOH) (Fig4.12). Peroxide oxidation is the primary process that forms the carbonyl functional group; carboxyl groups are created in lower quantities during this process (Sangseethong et al.,2010)

In the hydrogen peroxide oxidation reaction hydro peroxide anion is formed,

$$H_2O_2 + Cu^{+2} \longrightarrow \bullet OH + OH^- + Cu^{+3} \quad (1)$$

The hydroxyl radical formed oxidizes an alcohol group on the glucose unit in the starch molecules, forming a radical,

$$RCH_2OH + \bullet OH \longrightarrow R\bullet CHOH + H_2O \quad (2)$$

The starch radicals then reacts with the $Cu^{+3,}$ forming an aldehyde group (carbonyl group)

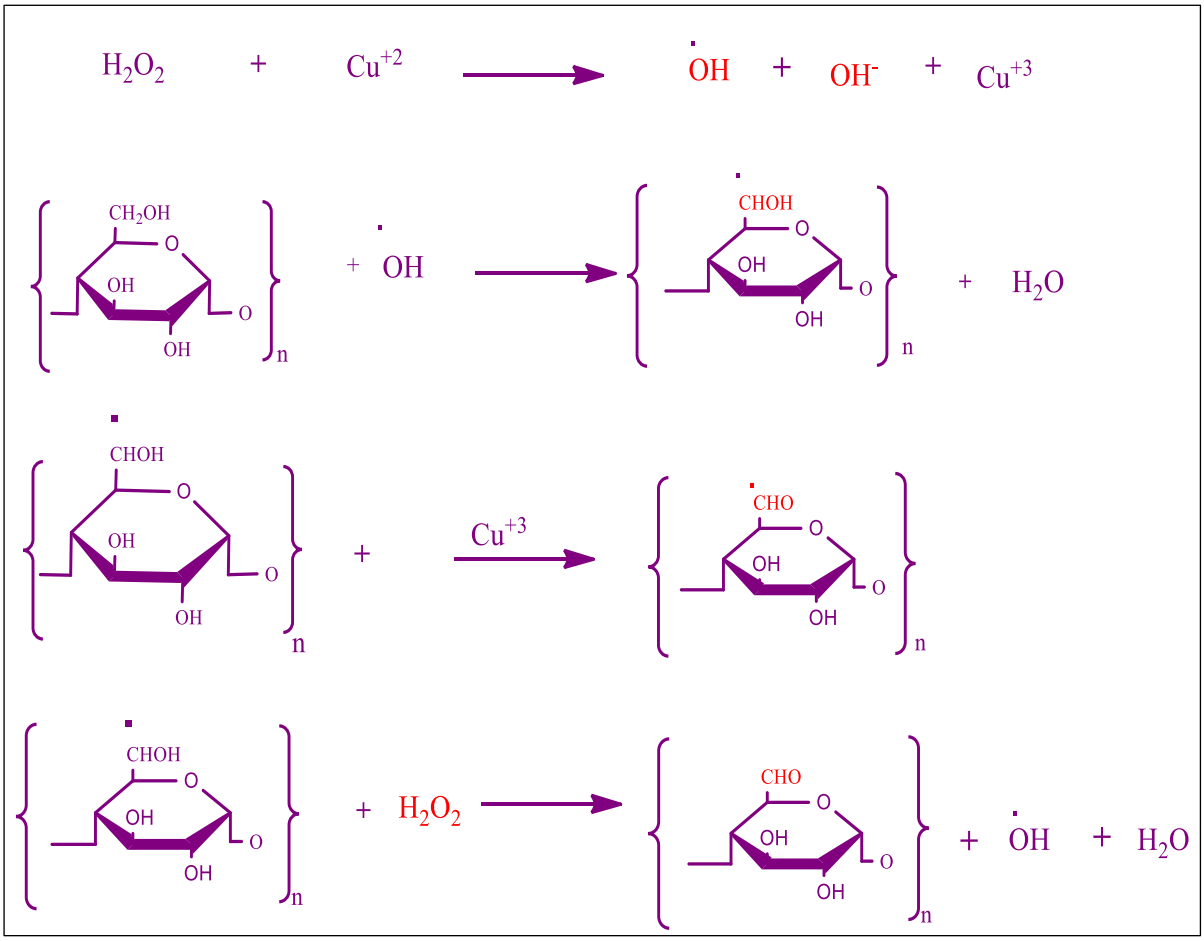
$$R\bullet CHOH + Cu^{+3} \longrightarrow RCHO + H^+ + Cu^{+2} \quad (3)$$

The starch radicals can be react with another H_2O_2 molecules, further oxidizing the starch:

$$R.CHOH + H_2O_2 \longrightarrow RCHO + OH + H_2O\ (4) \quad (4)$$

The carbonyl functional group is primarily formed during peroxide oxidation and carboxyl groups are formed during this process.

This treatment leads to the introduction of carboxyl and carbonyl groups within the starch molecules. Oxidized starches tend to have reduced viscosity, swelling power, retrogradations tendency, and pasting temperature these newly formed functional groups play a significant role in impeding the retrogradations process, which refers to the tendency of starch molecules to re associate and form a gel-like structure upon cooling. By hindering retrogradations, the presence of carboxyl and carbonyl groups enhances the stability of the modified starch, making it less prone to gel formation and suitable for various applications (Zhang et al., 2017). This leads to the starch's morphological, crystalline, gelatinization, and gelatinization enthalpy properties to alter. The FTIR, SEM, TGA/DTG, and XRD techniques were used to examine the changes in AS with varying degrees of oxidation.



Fingur.4.11: Reactions mechanism of oxidation starch

4.4. Characterization of Modified Avocado Seed Starch

4.4.1. Fourier transforms infrared spectroscopy analysis (FTIR)

Oxidation leads to the change of hydroxyl groups into carbonyl and carboxyl groups in the starch molecules. The introduction of carbonyl groups could be confirmed by FT-IR spectroscopy. The FTIR spectra of native and oxidized starches with different degrees of oxidation are shown in Fig4.12. The spectra show that native and oxidized starches have similar profiles. In the fundamental region, the absorption peaks at 3325 cm−1 and 2925 cm−1 are from the –OH and –CH2 stretching vibration of the glucose unit. The absorption at 1650 cm−1 is a typical band residing in the spectra of starch and its derivative, which is attributed to an H2O bending vibration (Luo, Huang, Fu, Zhang, & Yu, 2009; Wang et al., 2007); numbers of hydroxyl groups in starch molecules lead to the absorption of water. Compared with native starch, new absorption band at 1700 cm−1 can be seen in spectrum (DO = 51.3%), and it is assigned to the C=O stretching vibration (Hui, Qi-he, Ming-liang, Qiong, & Guo-qing, 2009; Kweon, Choi, Kim, & Lim, 2001; Para, 2004). The results indicate that native starch was successfully oxidized by hydrogen peroxide, and hydroxyl groups were changed to carbonyl and/or carboxyl groups.

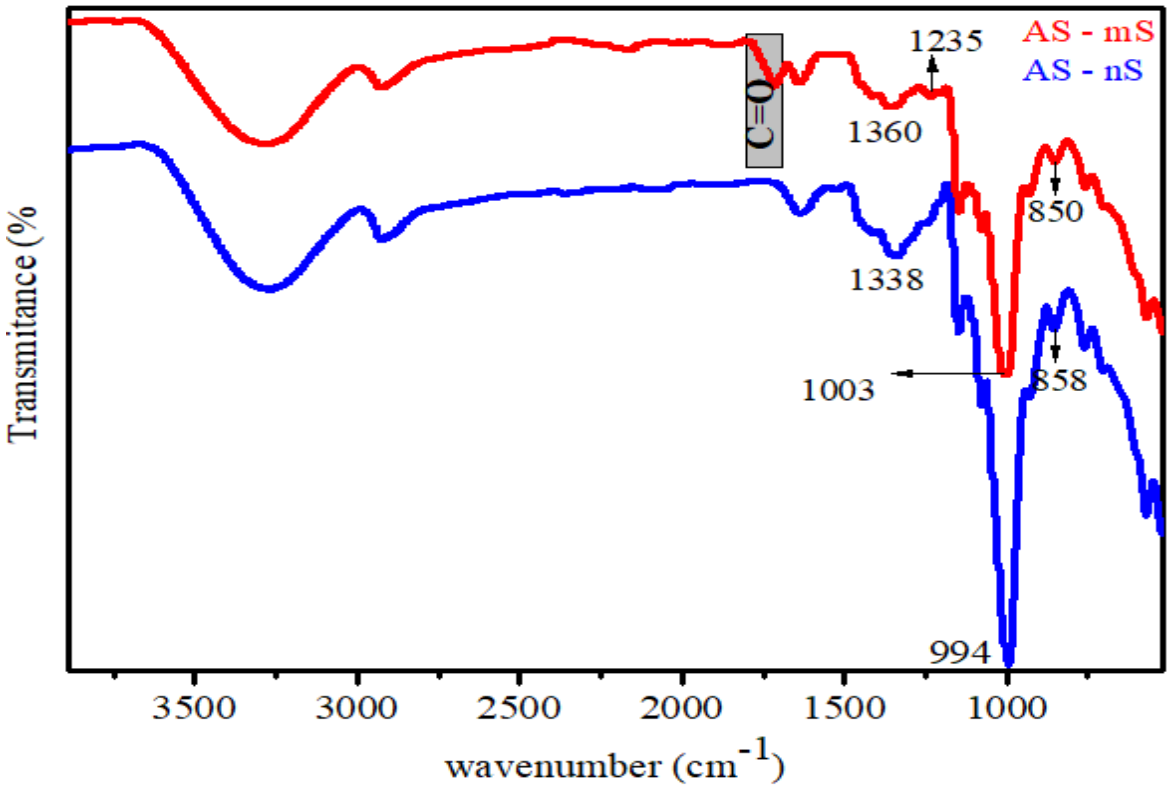
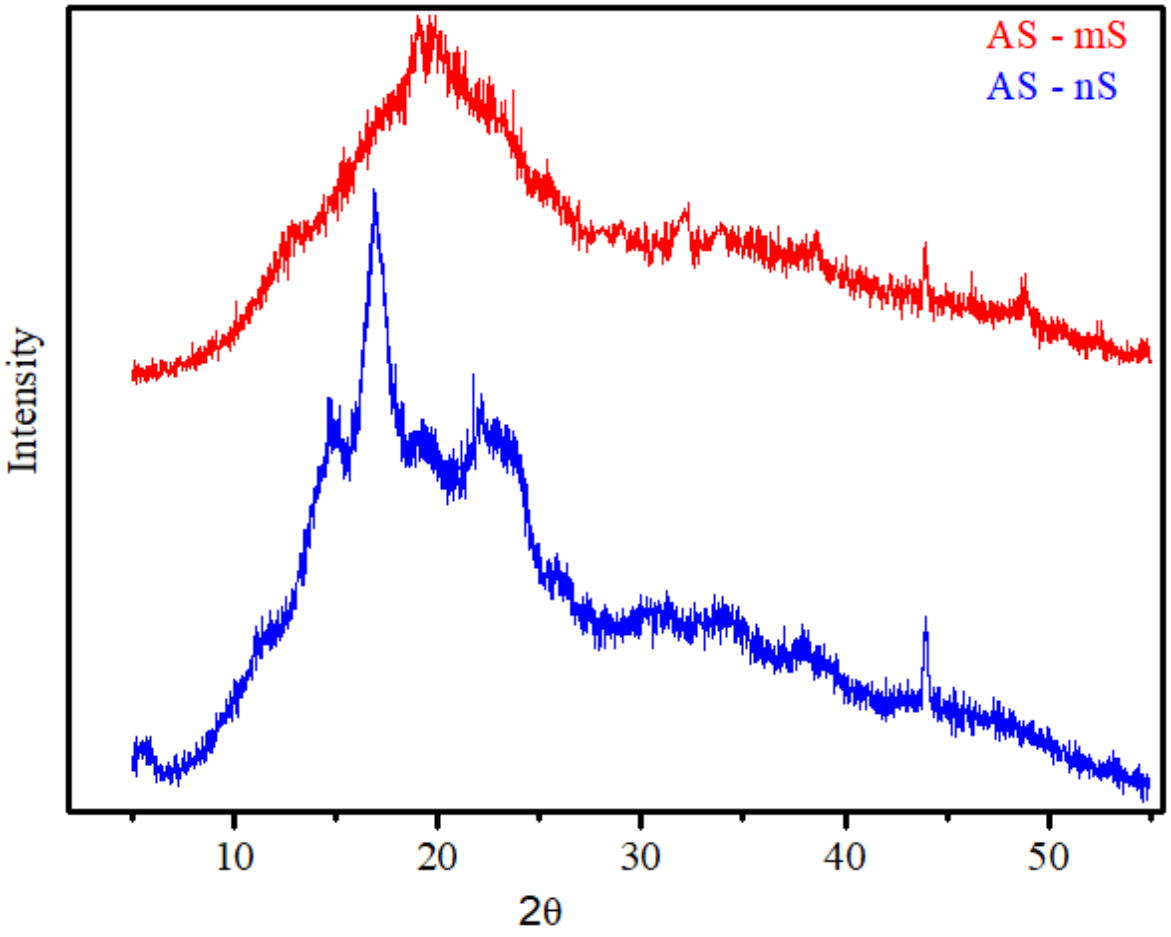


Fig.4.12: FTIR curves of both native and oxidized starch

4.4.2. X-ray diffraction analysis (XRD)

X-ray diffraction (XRD) measurements were conducted to investigate the impact of oxidation on the crystallinity of starch. Figure 4.13 illustrates the X-ray diffraction spectra of both native and oxidized AS (avocado seed) starches. As stated by Cai and Wei (2013), the native AS starch displayed a typical B-type pattern. This pattern is characterized by distinct diffraction peaks at 17° (2θ) and a few characteristic peaks at 5.64°, 17.30°, 19.62°, and 22.3°. These peaks are indicative of the crystalline structure of the starch.The purpose of comparing the XRD spectra of native and oxidized AS starches is likely to determine whether oxidation influenced the crystallinity pattern and the presence or intensity of the diffraction peaks. When the starch is oxidized, the crystalline structure is changed. The carboxyl and/or carbonyl groups play an important role in stabilizing the linear amylose molecules and minimizing retrogradation. For oxidized starch with a DO of 51.3%, as described in Fig. 13, the sharp peaks were disappeared except one small peak, meaning that the original crystal structure of the nS is subjected to a great change: a portion of original crystalline structure is destroyed during the reaction and exhibited an amorphous phase.

However, the outcomes differ from what (Chávez-Murillo et al., 2008) reported. They discovered that the oxidized starches with varying oxidation levels showed no changes in the XRD patter. The primary cause could be because the reactions primarily takes place in the amorphous lamellae and the starch granules were not destroyed by gelatinization. To release all of the hydroxyl groups in the amylose and amylopectin chains, starch was gelatinized prior to the reaction in this research.When starch is oxidized by hydrogen peroxide, the molecular chain de-polymerizes with the increase of oxidation and the type of crystal structure is changed (Li & Vasanthan, 2003). The oxidized starch they produced has too low DO and the oxidation didn't alter the crystal structure of the starch granule. This suggests that oxidation can impact the crystalline structure of starch, potentially converting it in to an amorphous state.



Figur4.13: XRD corves of both native and oxidized starch

4.4.3. Thermogravimetric analysis (TGA)

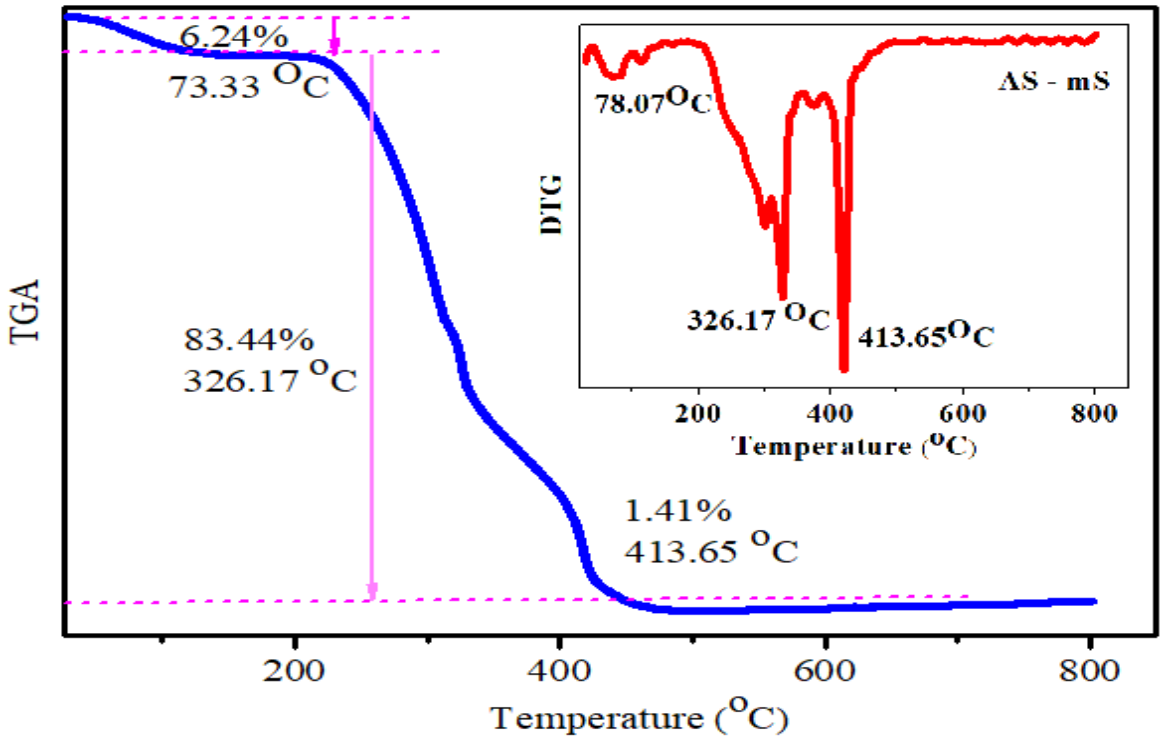
The thermal gravimetric analysis of native starch and modified starch is shown in Figure.4.14 with decomposition peaks as a function of temperature. According to TGA result *n*S and *m*S shows higher degradation peaks in four and three loss of mass steps respectively. These degradation peaks were seen in the DTG curve. The initial weight loss of 13.44% for native starch was noted in the temperature range of 25-73.33℃. This is explained by the release of physically adsorbed volatile components and hydrogen-bonded water that persisted in the starch even after preconditioning. The second weight loss (60.94%) was attributed to starch decomposition and occurred in the temperature range of 73.33 to 331.43℃. The maximum decomposition was found in the range of 331.43°C, as the DTG curve in Fig. 4.14 B illustrates. Water is the primary product range of starch decomposition, which occurs as a result of inter- and intramolecular dehydration processes of starch molecules. The temperature range of 331.4 - 342.3℃ observed the third weight loss (23.1%), while the temperature range of 342.3℃ - 520.33℃ showed the fourth weight

loss (1.23%), which was caused by carbonisation and the creation of ash from decomposition monomers.

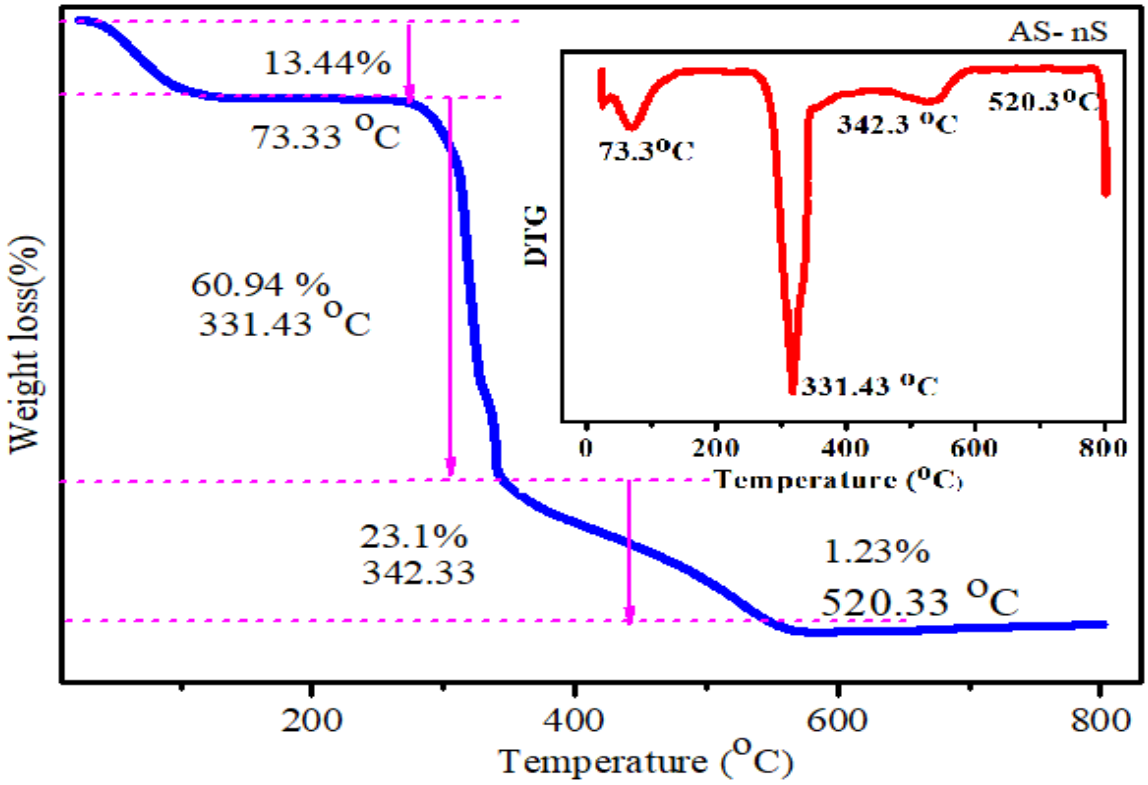
The mS samples with DO in the fig.4.14 A showed three mass losses. The first decomposition peak shows a 6.24% weight loss in the temperature 78.07℃. This decomposition is attributed to starch dehydration, the one attributed to the condensation of remaining hydroxyl molecules with water as a main product of decomposition. The second and the major peak has a weight loss of 83.44%, which is in the temperature of 78.07 - 326.17℃. The third decomposition was 1.41% in the Temperature range of 326.17 - 413.65 and the maximum decomposition occurred at 326.17℃ . This analysis is used to define a suitable temperature for using this nS and mS for other applications. The maximum decomposition Temperature for ns was 331.43℃ where as the maximum decomposition of oxidized starch was 326.17℃. The modified starch sample was shown to be more thermally stable than native starch, as confirmed by this finding. The minimal quantity of hydroxyl groups (OH) that remained in the modified starch molecules was the cause of the increase in thermal stability. The enhanced heat stability was also brought about by a rise in molecular weight and covalent bonding brought about by the oxidation of hydroxyl groups (OH).

Table.4.10: Thermogravimetric parameters of the native starch and the oxidized starches

	Tmax (oC)	**Weight loss (%)**
Native starch	331.43	60.94
Oxidized starch (51.3%)	326.17	83.44



(A)

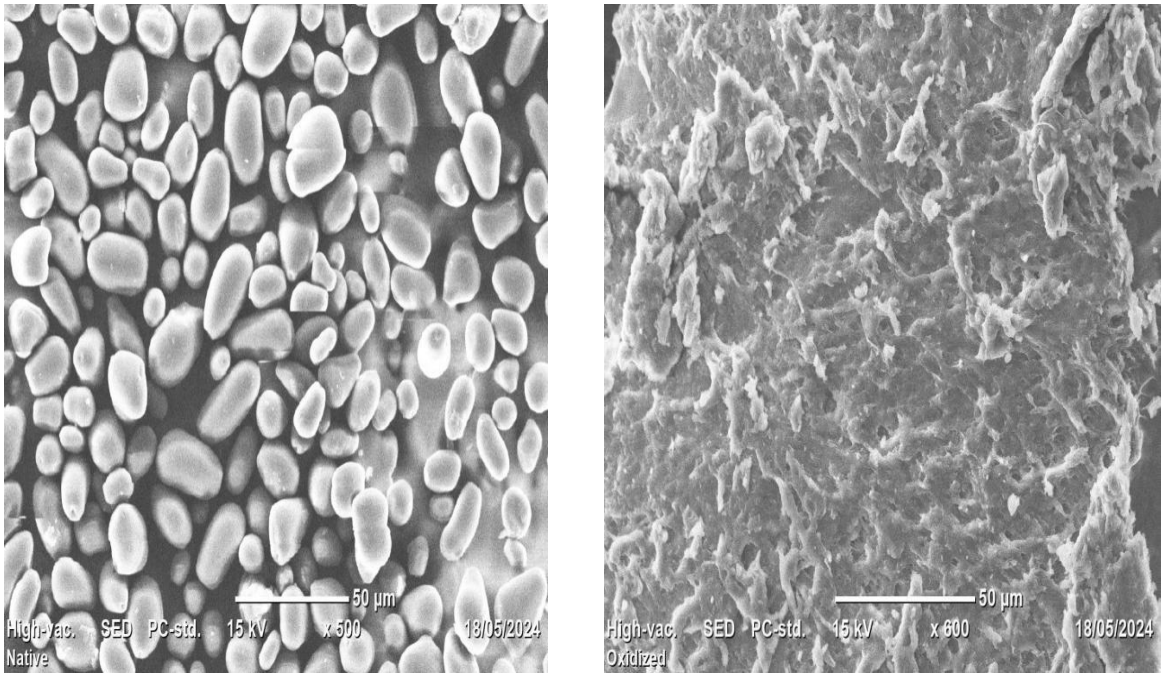


(B)

Figur4. 14: TGA and DTG curves of (A). modified starch (B). native starchs

4.4.4. Morphological analysis of modified avocado seed starch

The scanning electron micrographs (SEM) of the native and Oxidized AS starch granules are shown in Fig. 4.15. The SEM technique was applied in order to evaluate whether the Modification treatment was changed the structure of starch granules significantly, compared with Native AS starch. The native AS starch observed ellipsoid and oval shapes. The microphotograph of highly oxidised AS starch can be found in Figure 4.15 (B). There is an obvious structural difference between native and oxidised AS starch based on the shape of the starch granule. Furthermore, when oxidation occurs, the starch granules begin to rupture and develop various-sized cracks on their surfaces. The oxidized AS starch granules in fig 4.15(B) seem more swollen or distorted, with irregular shapes and disrupted (rough) surfaces, which is slightly different from the previous work of Zang et al., (2020) . This is because of the introduction of carboxyl groups. Consequently, the findings indicate that the chemical oxidation processes have an impact on both the interior and external components of the starch granules, resulting in a notable decrease in the crystallinity of the starch. Significant alterations in the sizes and forms of the starch granules were brought about by the addition of carboxyl groups, which are not limited to surface modification.



(A) (B)

Figure 4.15 : Morphology of (A), Native starch and (B), Modified starch

4.4.5. Determination of gelation temperature of oxidized starch

1.5g of starch sample was dissolved in a beaker with 10ml of distilled water and stirred the mixture until the starch was uniformly dispersed in water. Reliable and accurate thermometer was inserted in to the starch suspension with out touching the side or bottom of the beaker. Gradually increased the temperature and continuously monitored the temperature using the thermometer while stirring the mixture to prevent clumping and ensure even heating. As the temperature rised, it was observed that the starch suspension was formed a gel-like structure and the temperature read at which gelation occurred was at 75.45^{o}C. This result showed that the gelation temperature of the oxidized starch was more than that of native starch.

4.5. Paper quality test

4.5.1. Burst strength and Burst factor

The burst strength of the paper was measured to be 2.9g/m^2 and the standard is 2.5g/m^2. This impressive burst strength indicated that the paper was highly resistance to bursting, making it suitable for demanding application such as industrial use. The burst factor was also calculated as:-

$$\text{Burst factor} = \frac{\text{burst strength x 100}}{\text{base weight}}$$

$$\text{Burst factor} = \frac{2.9 \text{ x } 100}{132}$$

Burst factor = 2.19 where as the burst factor of the industry standard is 1.9.

4.5.2.Tensile strength test

The maximum force at the point of rupture was recorded as the tensile strength. The paper sample exhibited a tensile strength of 3868N/m^2 while the industry standard was 3905N/m^2 so it approaches the industry standard, indicating its suitability for paper application.

4.5.3.Tear strength and tear factor

The tear strength was determined to be 160 mN while the standard was 158 mN this indicates that the paper has relatively high tear strength, suggesting good resistance to tearing force. This result implys the paper is suitable for applications where tear resistance is crucial, such as packaging materials. Tear factor was also calculated as:-

$$\text{Tear factor} = \frac{\text{tear strength x 100}}{\text{base weight}}$$

$$\text{Tear factor} = \frac{160 \text{ x } 100}{132}$$

Tear factor = 121.2 mNm^2/ gm and tear factor of the standard is 121.5mNm^2/gm

4.5.4. Porosity

The time taken for the air to pass through the specimen was measured using stopwatch. Three replicate measurements were performed to ensure accuracy. The average time recorded was 19 seconds where as the standard is 16 seconds. This indicates that the paper possesses a moderate porosity, allowing air to flow through at a relatively slower rate. The porosity value suggests that the paper is suitable for application where controlled air permeability is desired, such as in packaging papers.

Table.4.11: summary of the paper quality test

Test parameters	**Unit**	**Sample paper test**	**Standard paper test**
Base weight	kg/m^2	132	123-132
Burst	g/m^2	2.9	2.5
Burst factor	___	2.19	1.9
Tensile strength	N/m^2	3868	3905
Tear strength	mN	160	158
Tear factor	mNm^2/gm	121.2	121.5
porosity	Sec	19	16

4.6. Cardboard production

4.6.1. Burst strength

The burst test result indicates that the cardboard sample had a high resistance to bursting, with a burst strength of 8 kg/cm^2 where as the standard is 7.8kg/cm^2. This information is crucial for determining the suitability of the cardboard for packaging applications, where it is subjected to stresses various stresses during handling, storage and transportation.

4.6.2.Crush strength test

The crush strength of the corrugated cardboard sample was determined to be 26 Lb while the standard is 25 Lb. based on the crush strength test result , it can be concluded that the starch can be used for surface sizing to produce corrugated cardboard.

Table.4.12: summary of the cardboard test

	unit	**Sample cardboard test**	**Standard**
Burst	Kg/cm^2	8	7.8
Crust	Lb	26	25

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

This research focused on extracting, characterising and structural modification of Avocado Seed starch for paper binding applications. Starch was extracted and characterized from Avocado seed. The SEM analysis of native starch confirmed that most of its granules were mostly oval and ellipsoid some elongated, with a very smooth surface and no cracks or surface pores. When examined using XRD, it revealed a prominent peak at 17o of 2θ, indicating B-type starch. The greater gelatinization temperature (84.8 oC) in the DSC analysis suggests that the starch from avocado seeds has a higher degree of crystallinity. For native avocado seed starches, FTIR analysis showed a broad bands at (3010–3600) cm-1, which represent the stretching vibrations of O–H groups. When examined by TGA/DTG, the temperature of maximum weight loss rate (Tmax) of native starch occurred at 331.43℃, and the range of highest decomposition was 73.33 - 331.43℃. These characteristics of avocado seed starch leads to conclude that it could be modified for usage as an effective substitute in binding starch products for paper packaging.

Avocado seed starch was oxidized by the reaction of native starch with H_2O_2 and $CuSO_4.5H_2O$ and the degree of oxidation was optimized using RSM. To examine the impact of three independent variables temperature, time, and catalyst dosage on the DO and the optimum DO was found to be 51.3% at 60 ^{O}C, 90 min and 1% of temperature, time and catalyst dosage, respectively. The DO was increased with increasing temperature and reaction time and decreased after the optimum value but DO decreased with increasing catalyst dosage. The structural features of a portion of oxidative starch with a DO of 51.3% were examined following modification. The native starch had significant physiochemical property changes as a result of structural changes. As a result of the oxidised group being introduced on native starch, the FT-IR spectra confirmed that the peak of the carbonyl group (C=O) occurred at 1700 cm-1. The crystal structure of the native starch progressively disappeared and was replaced by an amorphous structure, as confirmed by X-ray diffraction (XRD). The starch shape varied significantly, as seen by the scanning electron microscopy (SEM) images, and as DO increased, the smooth surface of the starch granules changed to a rough surface. Based on the TGA/DTG experiment results, the DO determines the thermal stability of oxidised starch. The oxidised starch with a high DO had a thermal stability of 326.17°C and 83.44% weight loss. As oxidized groups substitute the

native starch's hydroxyl (O-H) groups, the thermal stability of starch oxidation increases. The gelatinization temperature of the oxidized starch was recorded at 75.45^{o}C which is more than native starch. Finally these properties of modified starch concludes it as a potential candidate for the paper packaging binding starch products.

Paper was also produced by combining the pulp with the modified starch which acts as a binding agent and tested it's quality like tensile strength, burst, tear strength and porosity while the result were 3868N/m, 2.9g/m^2, 160mN and 19sec respectively. Cardboard was also produced using modified AS starch as binding purpose and burst strength and crush strength was tested.according to the results it can be concluded that modified AS starch can be used as an input for paper packaging.

5.2. Recommendation

A study carried out on the extraction and structural modification of avocado seed starch for use in paper binding. With the necessary binding characterisation, the modified starch showed promising properties, and the produced paper had good paper quality. Some suggestions might be made for further research projects based on the current study: Based on the current study, some recommendations can be made for future studies:

- ✓ Further studies were needed to identify and characterize the specific pigment responsible for the colour in avocado seed. By understanding the composition and properties of these pigments, researchers can diverse strategies to effectively target and remove them during the extraction process.
- ✓ Further works on modification of starch was recommended by optimizing parameters like PH, concentration of H_2O_2 and mixing ratio.
- ✓ In this study, the functional groups that were found in the oxidized starch are investigated through FTIR, but for further structural investigation NMR has to be done.
- ✓ Assessment of the costs and feasibility of avocado seed extraction and modification for binding paper packaging should be done.
- ✓ Further research was recommended on the addition of other source of starch to avocado seed starch for paper binding, it could be explored to increase the paper quality and contribute to the development of sustainable and effective binder formulations for paper industry.
- ✓ In this study, the concentration of the pulp and starch solution was according to the industry standard, but formulating the paper by varying the concentration of the starch was recommended.

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APPENDIXES

Appendix1: Picture of some laboratory

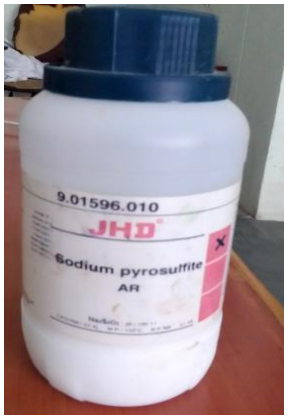
Appendix2: picture of card board

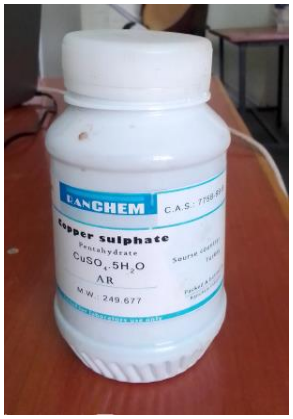
Appendix 2: Picture of some works on the paper formulation






Appendix 3: Some Chemicals used in the laboratory

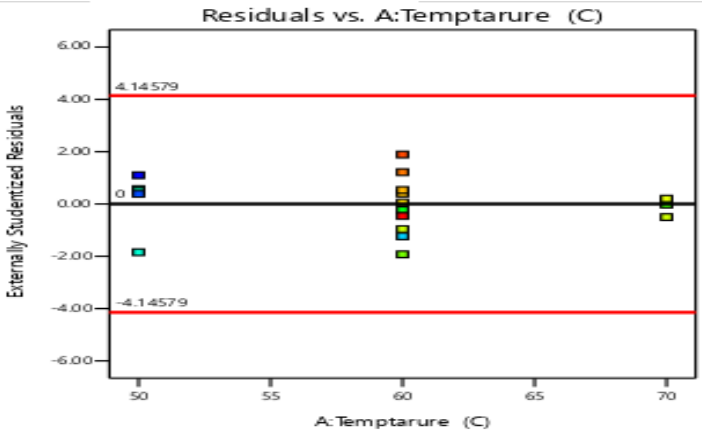




Appendix 4: Diagnostics Graphs in terms of Coded Factors

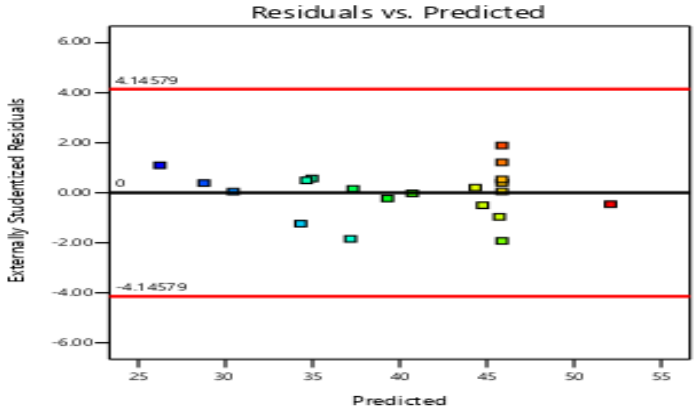
Degree of oxidation

Color points by value of
Degree of oxidation :
27.4 51.3



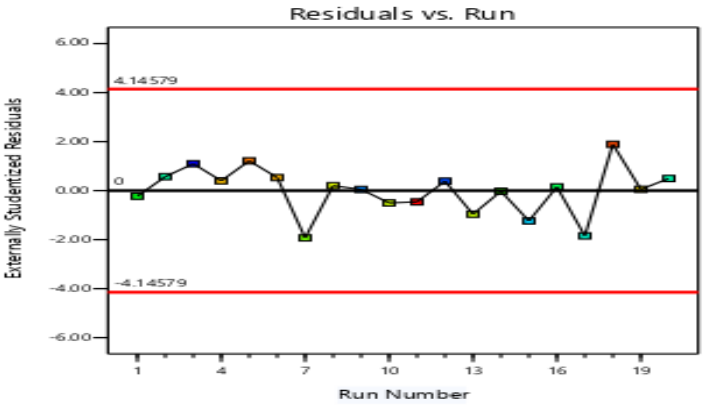
Degree of oxidation

Color points by value of
Degree of oxidation :
27.4 51.3

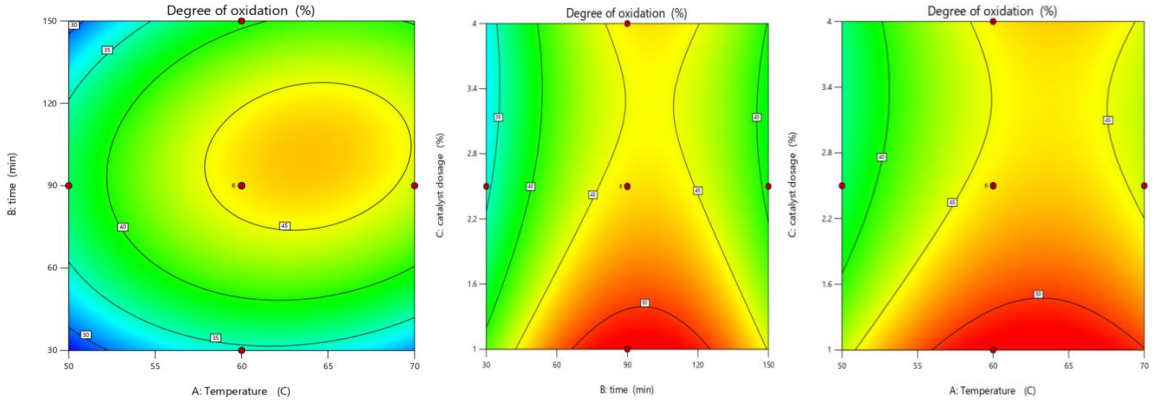


Degree of oxidation

Color points by value of
Degree of oxidation :
27.4 51.3



Appendix 5: Contour plots



Appendix 5: Testers for both cardboard and produced paper