

**EFFECT OF GLYCEROL AND SHEA BUTTER ON THE PROPERTIES OF  
FILMS PRODUCED FROM CHITOSAN AND *Borassus aethiopum* STARCH  
COMPOSITES**

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M.Tech/ SLS/2017/7008**

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**OCTOBER, 2021**

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**A THESIS SUBMITTED TO THE POST GRADUATE SHOOOL, FEDERAL  
UNIVERSITY OF TECHNOLOGY MINNA, NIGERIA IN PARTIAL  
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE  
DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY**

**OCTOBER, 2021**

## ABSTRACT

The concern in environmental waste management and toxicity of petroleum derived plastics has led to the search for renewable, nontoxic and biodegradable materials for packaging. Starch is the most studied and promising biopolymer for the production of biodegradable films, but this starch films have poor properties which can be improved by blending with other materials and plasticizers. *Borassus aethiopum* shoot starch locally known as “Muruci” is a promising film forming biopolymer with high amylose content. Completely randomized design was used in this research with four factors; *Borassus aethiopum* shoot starch (BS), chitosan (CHI), glycerol (GLY) and shea butter (SB). This study presents the evaluation of the effect of plasticizer types (GLY) and (SB) singularly and in combination with varying concentration over the range of 0 to 50 % w/w on *Borassus aethiopum* shoots starch/Chitosan films (BSCF). Solution casting technique was employed for the film preparation at 80°C. Starch from African Palmyra Palm (*Borassus aethiopum*) shoot has 20 % dry weight with amylose content of about 75.4 %. After series of different ratio combinations, the best films were formed from 2 %w/v of chitosan and starch composites in the presence of 30 %w/w GLY and 1 %w/w SB which were smooth and less transparent. Thickness, solubility in water, tensile strength (TS) and elongation at break (EAB) of BSCF were analyzed. Significant ( $p<0.05$ ) increase in the films TS and thickness was observed with the increase in solute concentration of starch and CHI, while the Solubility in water and EAB decreases. Plasticizers incorporation significantly ( $p<0.05$ ) increases the films thickness but lowered the film's TS. Presence of GLY increased the films solubility and EAB significantly ( $p<0.05$ ), but the introduction of SB significantly reduced the films thickness and solubility in water, singularly and in combination with GLY. Biodegradability test shows that the films with high chitosan concentration in the presence of SB had the longest degradation time (33 days). The transparency of BSCF is generally low and increase in the film opacity was observed with increase in solute concentrations of the starch. This study demonstrated that the BSCF in the presence of glycerol and low concentration of shear butter as plasticizers is a biodegradable film with the strength and barrier property needed for several applications such as in packaging.

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## LIST OF ABBREVIATIONS AND SYMBOLS

### Abbreviation

BSCF *Borassus aethiopum* starch/chitosan Film

GLY Glycerol

SB Shea butter

B.S *Borassus aethiopum* starch

CHI Chitosan

RH Relative humidity

TS Tensile strength

EAB Elongation at break

### Symbol

G Gram

L Liter

mL Milliliter

% Percentage

Mm Millimeter

MPa Megapascal

w/v weight to volume ratio

w/w weight to weight ratio

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background to the Study

The desire to protect the environment has led to the research in developing environmental friendly materials. Since the discovery of crude oil, there have been high rate of production of petroleum products with desired properties for both domestic and industrial uses, but with negative consequences to the environment and human life. Petroleum products are non-renewable, difficult to recycle and are not biodegradable; therefore the need to develop environmental friendly materials becomes paramount. The already known chemistry of biomaterials has helped the growing demand for the development of a range of biodegradable polymers in the emerging technologies such as tissue engineering, regenerative medicine, novel drug delivery systems, nanotechnology and implantable devices (Heller *et al.*, 2003).

Polymeric materials are either made from natural or synthetic polymers. Natural polymers are those from proteins, lipids and carbohydrate sources (Pascall *et al.*, 2013), while the synthetic ones are those from petrochemical sources such as polyolefins, polyvinyl chloride, polyamides, polyvinylidene chloride, polystyrene, polyethylene and polyimides. Due to the drawbacks in synthetic polymers such as toxicity and non-biodegradability, the use of biopolymers which can be degraded by enzymes could be implored to ameliorate these limitations (Shanta *et al.*, 2015). The world is driving towards the use of renewable natural materials with the sole aim of facing out toxic polymers in packaging. Polymers from carbohydrate source (polysaccharides) such as starch alginate, cellulose ethers, chitosan, carageenan, or pectins are renewable biopolymers which can be used for the manufacture of films with potential to improve

food shelf life and quality (Sothornvit and Krochta, 2001). They can also impart hardness, adhesiveness compactness, thickening quality, viscosity, and gel forming ability to a variety of films.

Films in the area of biosciences refer to materials made from polymers with the thickness generally not more than 0.3 mm (Sentrurk *et al.*, 2018). The thicker layers of polymeric materials are called sheets. Films made from renewable biopolymers have not yet found wide use in food packaging. Reasons behind this are that such biopolymers present problems when processed with traditional technologies as well as show inferior performances in terms of functional and structural properties (Mensitieri *et al.*, 2011). However, base formulations are widely being studied to improve their performance and processing (Verbeek, 2011). Amongst natural polymers, starch is one of the most promising of all on the basis of biodegradability, availability, renewability, non-toxic and affordability (Dang & Yoksan, 2015).

Starch and its major components, amylose and amylopectin are biopolymers, which are attractive raw materials for use as barrier in packaging materials. Starch is primarily derived from cereal grains. It is the major carbohydrate reserve in plant tubers and seed endosperm where it is found as granules, each generally containing several million amylopectin molecules in composition with much larger number of smaller amylose molecules (Stephen *et al.*, 2016). Amylose is responsible for the film forming properties of starch to replace plastic polymers either to used alone because of its low cost and renewability or in combination with other polysaccharides such as chitosan (Claudia, 2005). Starch often used in industrial foods. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, and it has good mechanical properties (Xu *et al.*, 2005).



However, wide application of starch film is limited by its mechanical properties and efficient barrier against low polarity compounds (Azeredo *et al.*, 2000). In order to overcome its shortcoming, Bourtoom and Chinnan (2008) blend rice starch with different chitosan to increase the tensile strength. A major component of biodegradable films is the plasticizer, as well as the film-forming polymer. Addition of a plasticizer agent as well as other polymeric materials such as chitosan is required to overcome some of the drawbacks with starch films (Bourtoom, 2008).

Chitosan is the deacetylated derivative of chitin; it is a natural polycationic linear polysaccharide (Zvezdova, 2010). It is composed of  $\beta$ -(1-4) linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer (Chandy *et al.*, 1990). The interesting characteristics of chitosan such as biocompatibility, low toxicity, low allergenicity and biodegradability allow it to be used in various applications (Kumar *et al.*, 2004). The reaction of chitosan is more versatile compared to that of cellulose due to the presence of  $-NH_2$  groups (Dutta *et al.*, 2004). Other naturally occurring polysaccharides such as cellulose, dextran, pectin, alginic acid are variably acidic or neutral, but chitosan is highly basic in nature. Due to this unique property, chitosan has several functional properties such as the ability to form films, chelate metal ion and optical structural characteristics (Kumar, 2010). Findings show that films produced from chitosan are brittle and not suitable for use in the dry state (Chang *et al.*, 2019). These properties of chitosan films can be overcome by incorporating other polysaccharides and plasticizers.

Plasticizer is a substance or material incorporated into another material (usually a plastic or an elastomer) to increase its flexibility, workability, or distensibility as defined by International Union of Pure and Applied Chemistry (IUPAC). A plasticizer

may reduce the viscosity, lower the temperature of a second-order transition, or lower the elastic modulus of the product (Sobral *et al.*, 2001). Plasticizers like glycerol, sorbitol and mannitol are polyols commonly used in preparation of edible films from hydrophilic materials like those from starch source. But the choice of plasticizer depends on the compatibility between plasticizer and polymer for effective plasticization. Carefully plasticized African palmyra palm (*Borassus aethiopum*) shoots starch blended with chitosan can form a desired film.

African Palmyra palm (*B. aethiopum*) is a palm tree having huge fan shaped leaves (Balami *et al.*, 2016). It is called *Giginya* in Hausa, *Agbonoloduin* Yoruba and *Ubiri* in Igbo (Umar *et al.*, 2015). The shoot of *B. aethiopum* is a well-known source of dietary carbohydrate in some parts of Northern Nigeria. It is obtained by burying the matured seeds of the plant in a pit and allowing it to germinate. The young germinating shoot or hypocotyls locally known as “Muruchior Gazari”, is usually harvested after 7 to 8 weeks of planting (Ali *et al.*, 2010). The study of plasticizer effect on the blend of chitosan and African palmyra palm (*Borassus aethiopum*) shoots starch will provide us with better understanding on how to control and improve the processing of films from this carbohydrate. It can further lead to commercialization of these polysaccharides based films for packaging.

## **1.2 Statement of the Research Problem**

As the plastic industry continuously grows, there are concerns in the area of environmental pollution, renewability and toxicity from the use of synthetic polymers in packaging. These petroleum based polymers are nonrenewable, non-biodegradable and also not easily recycled. Phthalates are the most used plasticizers in production of these

synthetic plastics which is toxic and have higher tendency to migrate into food (Sunny *et al.*, 2004), thus, the need for eco-friendly materials and safe plasticizers.

The search for renewable and eco-friendly material had led to the discovery of starch polymers which has excellent film forming ability with good chance of replacing synthetic polymers, but starches from common sources (cassava, rice, yam, potatoes and corn) are scarce and expensive due to their high demand for food and industrial use, therefore the need for cheaper and underutilized starch sources (Zhu, 2020).

Films from starch polymers have poor properties such as high water sensitivity and low mechanical properties which are not suitable for many applications (Han *et al.*, 2006, Shen *et al.*, 2010, Kuorwel *et al.*, 2013). Several researchers have experimented on the use of chitosan and plasticizers such as polyols (glycerol, sorbitol and manitol) and oils (peanut oil and soybean lecitin) to improve the low mechanical properties and high water solubility of starch films, but the high cost of some of these plasticizers (oils) due to high demand for consumption and other industrial uses is big hurdle to overcome, therefore the need for cheaper and under-utilized source of oils as plasticizers.

The use of this oil (shea butter) as plasticizer singularly and in combination with glycerol on starch films is yet to be established and the information on effects of shea butter and glycerol as plasticizers for the preparation of films from blends of chitosan and starch from *Borassus aethiopum* shoot composites is presently not readily available. Therefore, this investigation was designed to make a comparative study of the effects of glycerol and shea butter as plasticizers and their concentrations on films from blends of *Borassus aethiopum* shoot starch and chitosan, which could lead to low cost-effective films with excellent tensile strength and good water resistivity for commercial use.

### **1.3 Aim and Objectives**

#### **1.3.1 Aim**

The aim of this study was to investigate the effects of different concentrations of glycerol and shea butter as plasticizers on the properties of films produced from blends of chitosan and *Borassus aethiopum* shoot starch composites.

#### **1.3.2 Objectives**

The specific objectives are to;

- i. Determine starch yield of *Borassus aethiopum* shoot and amylose/amylopectin ratio.
- ii. Determine the properties of films produced from blends of chitosan and *Borassus aethiopum* shoot starch composites.
- iii. Determine the effect of glycerol and shear butter on the properties of films from blends of chitosan and *Borassus aethiopum* shoot starch composites.
- iv. Optimize the production of glycerol and shear butter plasticized films from blends of chitosan and *Borassus aethiopum* shoot starch composites.
- v. Determine the biodegradability of glycerol and shear butter plasticized films produced from blends of chitosan and *Borassus aethiopum* shoot starch composites.

### **1.4 Justification for the Study**

Abundance, renewability and eco-friendliness are the major factors that have led to the increased interest in starch-based films. Starch from *Borassus aethiopum* shoot is easily processed and preliminary examination of this starch shows that it has high amylose content, which is the prerequisite for excellent film forming ability. Currently, the starch from *Borassus aethiopum* shoot is not being deployed for any known industrial

uses, therefore making the starch affordable. The utilization of this starch would encourage its cultivation and subsequently improves farmer's income.

The low mechanical properties of starch-based films led to the Introduction of chitosan which is compatible and provide necessary toughness and barrier properties to starch films. But the films from starch and chitosan blends are still brittle, therefore the need for plasticizers (glycerol and shea butter). Glycerol has often been used as a plasticizer since it is also compatible with amylose and as such, it improves the stiffness of starch films by decreasing intermolecular forces between amylose molecules. The introduction of glycerol to starch-based films is often accompanied with high film solubility which is a drawback and this drawback could be ameliorated by introducing oils such as shea butter. Shea butter is less expensive and presently used in cosmetic industries. Therefore its use as plasticizer in starch-based films could lead to increased cultivation of shea tree and oil production, which in turn could improve farmer's income generation.

This study was designed to promote the use of cost effective materials in the production of active biodegradable films for commercialization.

## **CHAPTER TWO**

### **2.0 LITERATURE REVIEW**

#### **2.1 Films**

In relation to packaging, films are thin layer materials made from polymers with varying degrees of strength, flexibility and thickness not more than 0.3mm. Polymers used for film production are either synthetic or natural, functioning as a barrier of mass transfer such as humidity, gas and light (Rosida *et al.*, 2018).

##### **2.1.1 Types of films**

Films are generally formed from polymers using different methods. The base material for film production differentiates various films. There are natural polymers which are found in nature generally from plants and animals sources. Examples are proteins, cellulose, starch and resins. There are also semi-synthetic polymers which are obtained from natural polymers by simple chemical treatment to change the physical properties of natural polymers like Starch and silicones. Another form are those from synthetic polymers which are fibers synthesized in laboratory by polymerization of simple chemical, example: Nylon, polyethene, polystyrene, synthetic rubber, PVC and Teflon (Kusum *et al.*, 2016).

#### **2.2 Polymers**

The word ‘polymer’ is derived from Greek words, poly which means ‘many’ and meros means ‘parts or units of high molecular masses. Each molecule consists of a very large number of single structural units joined together in a regular manner by covalent bonds. Polymers are the giant molecules of high molecular weight called macromolecules which are formed by linking together a large number of small molecules, called

monomers. The process by which monomers combine to form polymer is known as polymerization (Gowariker *et al.*, 2005).

### **2.2.1 Synthetic polymer**

These are man-made molecules arising from repeated end to end addition of single molecules called monomers. Some examples of synthetic polymers are; polyethylene, polypropylene, polychloroethylene, polyvinylchloride, polychloropren, neoprene, polymethyl methacrylate, polytetraflouroethylene, polyacrylonitrile and polystyrene (Kusum *et al.*, 2016). Which can keep growing by sequential addition of more monomer until some termination reaction such as the combination of two free radicals occurs. Synthetic polymers are gradually being replaced by biodegradable materials especially those derived from natural resources, due to their ability to biodegrade.

### **2.2.2 Natural polymer**

Polymers found in nature generally from plants such as, (cellulose, hemicellulose, glucomannan, agar, starch, pectin, inulin, rosin, guar gum, locust bean gum, gum acacia, karaya gum, gum Tragacanth, aloe verage) and animals sources(chitin, alginates, carageenans, psyllium, xanthum gum) are referred to as natural polymers (Kulkarni *et al.*, 2017). They have found applications in application in pharmaceutical formulations, manufacture of solid implants, films, beads , nanoparticles, inhalable, micro particles and injectable systems as well as viscous liquid formulations (Chamarthy *et al.*, 2008, Alonso *et al.*, 2009). Films from natural polymeric materials are generally biodegradable.

## **2.3 Biodergradable Polymers**

Biodegradable polymers are polymers that can be reduced by the actions of microorganisms. They are polymers produced from renewable sources. Biopolymers are manufactured from plant raw materials, in the first place, but in recent times and of animal sources. Their main feature is their biodegradability. They are classified in many ways such as, chemical structure, origin, methods of synthesis, application and cost-effectiveness (Davidovic and Savic, 2010).

Polymers from renewable resources are different from synthetic polymers because their synthesis is induced intentionally. Conventional polymers are not biodegradable because of long chains of molecules that are too big and too well connected to each other. Unlike conventional, polymers made from natural plant materials from wheat, potato or corn starches have molecules that are easily microbiologically degradable. Large hectares of soil is needed for the cultivation of this plants, at the same time, it means the destruction of large areas of forest/rainforest for the production of biodegradable materials (Goodship and Ogar, 2004). The natural origin of natural polymers provides the inherent biodegradable ability since enzyme, polymerase activities produce a natural polymer, and there is depolymerase capable of catalyzing the decomposition of the polymer (Scholz & Khemani, 2006).

### **2.3.1 Classification of biodegradable polymers**

The traditional way of dealing with biodegradable packaging materials is divided into three generations based on historical background.

#### **2.3.1.1 *First generation***

The first generation of material was used for shopping bags, consisting of synthetic polymers such as low density polyethylene (LDPE-low density polyethylene) with a



proportion of 5–15 % starch fillers and pro-oxidizing and auto oxidative additives. Later these materials decompose or bio-fragment into smaller molecules that are not biodegradable. Such materials have created a very bad image of bio materials especially for consumers who were convinced that they played in terms of biodegradability (Chiellini, 2008). Low density polyethylene-LDPE produced in 1933 by Imperial Chemical Industries (ICI) using high pressure process via free radical polymerization. Its production uses the same methods today. It is estimated that about 5.7 %wt of LDPE can be recycled. LDPE is in the range defined by the density of 0.910 to 0.940 g/cm<sup>3</sup>. Non-reactive at room temperature, except in the action of strong oxidizing agents and some solvents cause swelling. Excellent resistance to acids, alcohols, esters, and a base, followed by resistance to various aldehydes, ketones and vegetable oils, and low is resistant to halogen hydrocarbons. They are stable up to a temperature of 80°C. It is produced in transparent or opaque variations, and is quite flexible and tough, but also fragile. It is used for general purposes (packaging for juices) or for industrial purposes (corrosion resistant materials and welding machine (Malpass, 2010).

#### **2.3.1.2 Second generation**

The second generation of biomaterials comprises a mixture of pre-gelatinized starch (40–70 %) and low density polyethylene (LDPE) with the addition of the hydrophilic copolymer such as ethylene acrylic acid, polyvinyl alcohol and vinyl acetate, which are used to compact (Mohammadpour *et al.*, 2019, Han, 2005). Complete degradation of starch takes 40 days and the degradation of the whole of the above-mentioned film lasts 2–3 years.

### **2.3.1.3 Third generation**

The third generation of the material fully consists of biomaterials and can be divided into three main categories according to the origin and production methods:

- i. Polymers extracted and isolated directly from biomass
- ii. Polymers produced by classical chemical synthesis and bio-monomers
- iii. Polymers obtained directly from natural or genetically modified organisms (Chiellini, 2008).

### **2.3.1.4 Polymers extracted and isolated directly from biomass**

This category of biopolymers is most present on the market. Polymers of this category are obtained from plants, marine and domestic animals. Examples are polysaccharides such as cellulose, chitin and starch, whey protein, casein, collagen, soy protein, myofibrillar proteins of animal muscle, etc., can be used alone or as a mixture with synthetic polyesters such as polylactic acid (PLA). The most prevalent category that is used in food packaging is cellulose-based paper. There appears also regenerated cellulose film (cellophane paper) and cellulose acetate. Hemi-cellulose, the second most abundant plant polymer in the world, is in its infancy as a biomaterial research for food packaging (Grondahl *et al.*, 2006).

Generally, polysaccharide films are made from starch, alginate, cellulose ethers, chitosan, carrageenan, or pectins and exhibit good gas barrier properties. Linear structure of some of these polysaccharides, for example, cellulose (1,4-b-d-glucan), amylose (a component of starch, 1,4-a-d-glucan), chitosan (1,4-b-d-glucosamine polymer), renders their films tough, flexible and transparent. Their films are resistant to fats and oils (Tharanathan *et al.*, 2003). However, due to their hydrophilic nature, they are poor water vapor barriers. Among polysaccharide and biopolymers in general,

starch is considered to be one of the most promising materials for use in biodegradable plastics (Ma *et al.*, 2009).

Different approaches have been carried out to use this polysaccharide as a natural biopolymer for the production of biodegradable thermoplastics and different types of starch were processed in blend systems with natural plasticizers and commercial fibers by conventional extrusion and injection molding techniques (Van *et al.*, 1997).

#### ***2.3.1.5 Polymers produced by conventional chemical synthesis of bio-monomers***

It is possible to get large range bio-polyesters by chemical synthesis. In theory, all previous packaging materials can be replaced by new types derived from renewable monomers, but the question of economic viability (Kobayashi, 2010, Berezina and Martelli, 2014). The most famous of these groups biopolymer is polylactic acid (PLA-poly lactide). PLA is biodegradable thermoplastic linear polyester, for its properties similar to polystyrene. The raw material for obtaining the lactic acid is obtained by fermentation of glucose or starch from another source (Wackett, 2008). The most common way to the PLA is a ringopening polymerization of lactide with various metal catalysts (typically tin (II)-ethyl hexanoate) in solution or as a suspension. The reaction metal catalyst often causes racemization of PLA, reducing stereospecificity with respect to the starting material. This procedure should be carried out at a temperature lower than 200 °C because above this temperature there is a formation of low molecular weight materials (Bordes *et al.*, 2009, Tharanathan *et al.*, 2003). Direct condensation is carried out in steps, where the lactic acid in the first oligomerizes PLA oligomers. Thereafter, the polycondensation is carried out in solution or the melt where the short oligomeric units combine to give a high molecular weight polymer chain. Removing the water using vacuum or azeotropic distillation favors to polycondensation during

transesterification. Even higher molecular weight can be obtained by careful crystallisation of the crude polymer from the melt. Polymerization of the mixture of L- and D-lactide typically leads to the synthesis of poly-DL-lactide (PDLLA), which is amorphous. The use of a catalyst can result in PLA exhibiting crystallinity. PLA is mainly processed into thermoformed pads and containers for packing and serving food, films, transparencies and bottles and other packaging blown, but also mixed with other materials to improve their excellent water vapor-permeability which is important in the packaging of fresh food, which is necessary for the water vapor which evaporates quickly while reducing disturbance of the packaging. Is largely processed into thermoformed pads and containers for packing and serving food, but also mixed with other materials to improve their mechanical properties (Seglina *et al.*, 2009).

#### ***2.3.1.6 Polymers obtained directly from natural or genetically modified organisms***

Many bacteria accumulate these polymers as a source of energy and as a carbon reserve. This group includes polyhydroxyalkanoates (PHAs) and bacterial cellulose (Verlinden *et al.*, 2007). PHAs are polyesters that are part of the living organism structure, hydrophobic and insoluble in water. Their characteristics are most associated with the properties of the monomer building blocks of which a wide variety of different biopolymer can be synthesized by microbial fermentation. Enzyme PHA-polymerase catalyzes the reaction of polymerization HA in the PHA within the cell. PHA, which synthesizes type *Alcaligenes*, *Azotobacter*, *Bacillus*, *Halobacterium*, *Rhizobium* and many other micro-organisms can be produced in large quantities biotechnologically, renewable substrate, using fermentation and known physical and chemical processes extracted from biomass after production (Koller *et al.*, 2010). Depending on the bacteria and the carbon source, the polyhydroxyalkanoate may be manufactured from rigid brittle to plastic to rubber-like polymer having similar properties such as propylene and

polyethylene (Zivkovic, 2009). The most common is the use of derivative polyhydroxybutyrate labeled PHB. PHB is biodegradable polyester linear prepared by bacterial fermentation of sugar or lipid. It can be used for food packaging, cosmetics and pharmaceutical products, as well as in agriculture. The aerobic conditions are completely degraded into water and carbon dioxide. Biodegradation in favorable conditions takes 5–6 weeks (Botana *et al.*, 2010). Apart from renewable, biodegradable plastics can be produced from synthetic polymers by using bacteria. The bacterium *Pseudomonas putida* converts styrene monomer in the polyhydroxyalkanoate (PHA), biodegradable plastic which has a wide range of applications. PHA is water insoluble, biodegradable material and compostable whose improvement works intensively before its commercialization (Chiellini, 2008).

## **2.4 Film Manufacturing Processes**

There are many different types of film materials as there are various forms of manufacturing processes and technologies. Each having its own advantages and disadvantages, it is important to distinguish between them, and know the impact that they will ultimately have on the film. With every combination of material type, and processing method, a unique set of characteristics will be produced as described by National research council (1994).

### **2.4.1 Methods of film manufacturing**

Methods used for the production of biodegradable films are divided into two main groups; that is the wet and dry processes. The wet process needs solvents for the solution and dispersion of the polymer onto a flat surface, followed by drying in controlled condition for the removal of the solvent and formation of the film. The procedure is energy consuming, which are adequate for laboratories but not for the

industrial scale (Mellinas *et al.*, 2016). The production of films by dry methods includes the extrusion, injection, blow molding and heat pressing processes as those most commonly used (Han, 2005, Silva *et al.*, 2013). The combination of efficiency and high productivity provided by these thermal processes have induced the increase of their application for active edible-films manufacturing (Lopez *et al.*, 2014). But, the high temperatures used in the dry processing could affect the presence and concentration of some active compounds on the films, while the addition of plasticizers is sometimes necessary to decrease the glass transition temperature for polymer matrices. The method selected for the production of active edible films could affect and modify the final properties of the material (Suhag *et al.*, 2020).

#### **2.4.2. Wet processing**

##### **2.4.2.1 Cast film**

Cast film is manufactured by expelling the melted polymer through a flat die or slot, forming a thin sheet or film. After poring, it is attached to the surface of a chilled (rotating) roller so that it is cooled extremely fast. It is the surface of the rotating roller that gives cast film its characteristic smooth and clear appearance. While cast film can be produced at much higher line speeds, there is higher waste along with little orientation in the cross direction (Siemann, 2005).

Casting method is the easiest for the production of biodegradable films, but it is a batch procedure used in a very small-scale. Nevertheless, a continuous casting method (knife-coating or tape-casting) can be used at the industrial scale (Mohamed *et al.*, 2020), since the film-forming suspension is prepared on continuous carrier-tapes with effective control of thickness. The formed film is dried by heat conduction, convection or radiation in short times. Tape casting is a suitable technology to scale up the

production of cassava starch based edible films. In general, the wet process could be divided into four steps: dispersion or gelatinization, homogenization (in the case of emulsions or mixtures), casting and drying. Several factors can influence the film behavior and should be optimized depending on the polymer matrix: the solvent, plasticizer, method for the granules disruption, temperature and time (Jiménez *et al.*, 2012). If different hydrocolloids or other non-miscible components are added, a mixing step is also required to obtain stable emulsions and homogenous film forming solutions. Homogenization is currently performed with new homogenizer devices, such as rotor-stator or Brabender viscometers, to induce high pressures that could enhance the disruption and interactions between all components (Maniglia *et al.*, 2014, Matsakidou *et al.*, 2013, Shojaei *et al.*, 2014).

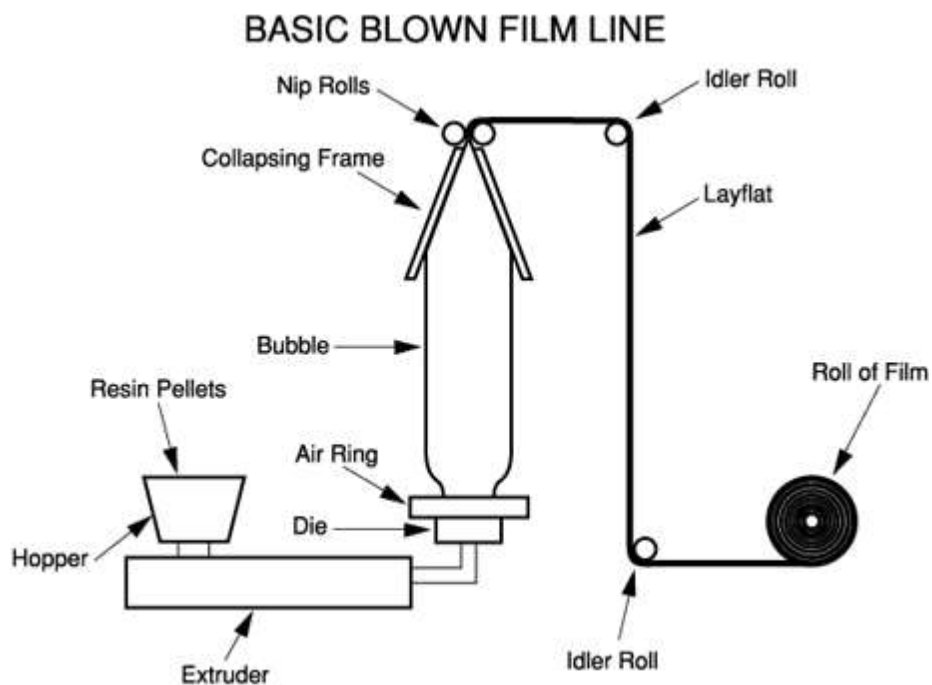
Furthermore, all components of the film forming solutions should be homogeneously dispersed in the solvents to produce edible films without phase separation. Emulsifiers can be added to avoid this situation, even when using incompatible compounds (Shen *et al.*, 2015). The addition of food-grade plasticizers rich in hydroxyl groups, such as glycerol, sorbitol or polyethylene-glycol (PEG) in concentrations between 15 and 30 wt% reduce the polymers rigidity and glass transition temperature, enhancing the distribution of the film-forming solutions. Essential oils (EO) extracted from plants have been used in the last years as active compounds for edible films manufacturing (Bahram *et al.*, 2014).

Casting is mostly used for the direct incorporation of plasticizers into film-forming solutions by the low temperatures used for the homogenization and drying steps. For example, cinnamon plasticizers increased the antimicrobial activity of whey protein concentrate as well as the water vapor permeability and water solubility (Bahram *et al.*, 2014).

### 2.4.3 Dry processing

#### 2.4.3.1 *Blown film*

Blown film manufacturing involves using a jet of air to blow the (melted) plastic polymer through a circular blown film die. The melted plastic then forms a continuous tube, which is inflated, flattened by rollers, and cut to length resulting in double thickness film. Blown film is one of two main processes that are used in the manufacture of film products since it is efficient (little polymer is needed to produce a lot of film) and used in a wide variety of applications (Erin, 2009).

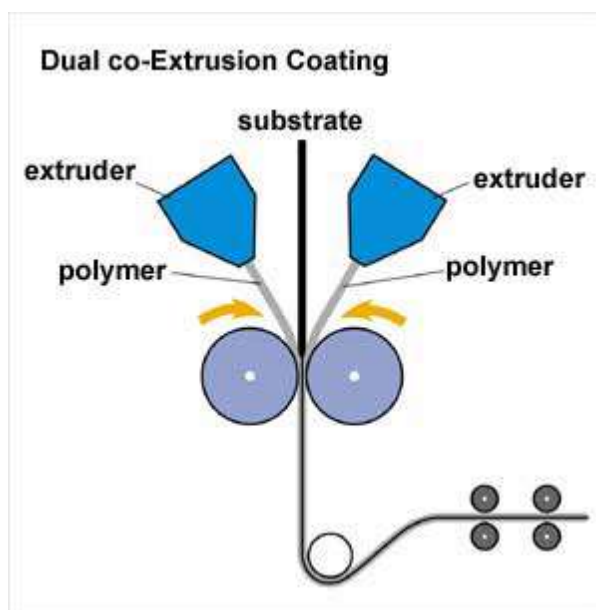


**Figure 2.1: Blown Film Manufacturing Diagram** (Erin, 2009)



#### 2.4.3.2 Coextrusion

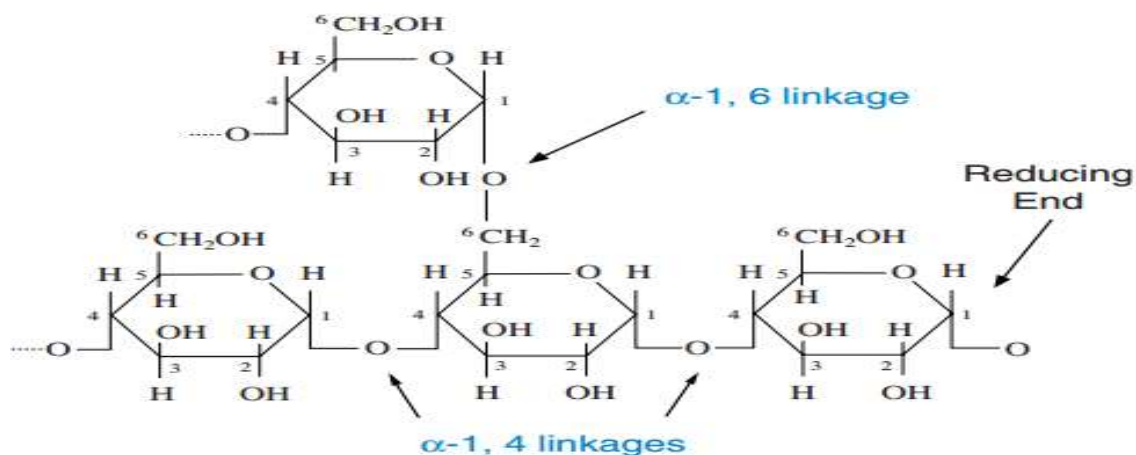
Barrier packaging would not be what it is today had it not been for the discovery of the multilayer structure (MLS). Prior to the development of coextrusion technology, multilayer films were produced by laminating thin plastic layers together. While this process of lamination worked, it was found to be slow and not very efficient. Coextrusion involves combining two or more layers of melted plastic into a single extruded web (Erin, 2009).



**Figure 2.2: Coextrusion Manufacturing Diagram** (Erin, 2009)

### 2.5 Starch

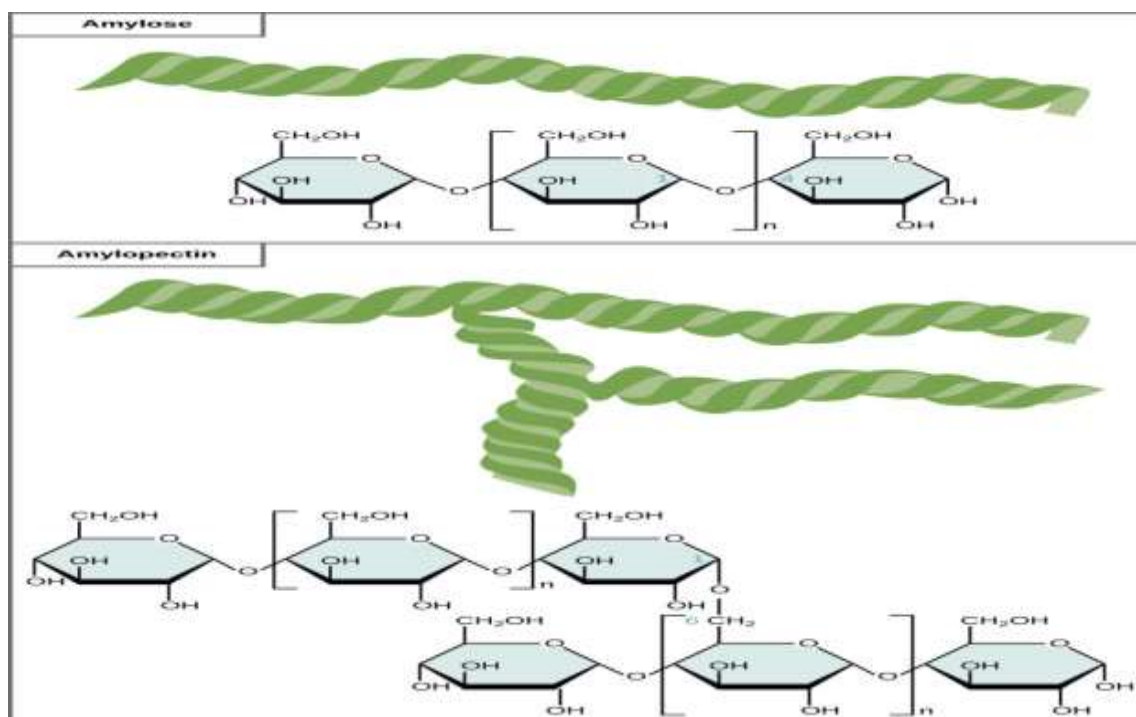
Starch is a polysaccharide produced by all green plants as an energy store and source of carbon (Smith, 2001). It is the most abundant carbohydrate reserve in plants and it is found in fruits, leaves, flowers, seeds, stems and roots (Alcazar and Merirels, 2015). Starch occurs in the form of granules (starch grains) and are formed by the linkage of glucose units at  $\alpha$ -1,4 and  $\alpha$ -1,6 carbon atoms (Kulkarni *et al.*, 2017).



**Figure 2.3:  $\alpha$ -1,4 and  $\alpha$ -1,6 Glycosidic Bonds of Starch** (Kulkarni *et al.*, 2017).

### 2.5.1 Starch composition

Starch is composed of amylose (non-branched) and amylopectin (branched) polymers at varying degrees due to glucose polymerization (Zobel, 1988 and French, 1984). Starch's structural units, amylose and amylopectin, are shown in the Figure below.



**Figure 2.4: Starch Amylose/Amylopectin Helical Structure** (And and Han, 2005)

Normal starch consists of about 75 % amylopectin and 25 % amylose; waxy starches consist of mainly amylopectin and 0 to 8 % amylose; and high-amylose starches consist of 40 to 70 % amylose, which is the key component involved in water absorption, swelling and gelation of starch in food and processing of material (Hoseney, 1986).

Major source of starch are cereals (40 to 90 %), roots (30 to 70 %), tubers (65 to 85 %), legumes (25 to 50 %) and some immature fruits like oranges, bananas or mangos, which contain approximately 70 % of starch by dry weight (Santana, 2014). Starch from cereals contain little portion of lipid molecules in their structures in the form of phospholipids and free fatty acids; which are found to be associated with the amylose fraction (Tester *et al.*, 2004). The lipid complexes in some starch granules within the helices formed by amylose chains are observed as a hydrophobic nucleus. The complexes vary between 0.15 to 0.55 % of the amylose fraction of starches from cereals. Lipids in starch granules, though in small fraction, can significantly reduce the swelling capacity of the starch paste.

The film forming ability of starch is due to the repeated long linkage of glucose subunits. Plants have different percentages of amylose/ amylopectin ratios. Plants with higher amylose contents produces better films (Curá *et al.*, 1995, Hood *et al.*, 1982). The strength of films from starch are generally increased by incorporation of chitosan, though they are both brittle in dry state. Some starch source and their amylose contents in percentage are tabulated below.

**Table 2.1: Starch Source and their Amylose Contents** (Patindol *et al.*, 2015)

Species	Amylose (% w/w)
Wheat	37.5±1.0 (Hung <i>et al.</i> , 2007)
Barley	38.4-44.1 (Morrison <i>et al.</i> , 1984)
Sorghum	23.7-27.6 (Boudries <i>et al.</i> , 2009)
Rice	21-25 (Patindol <i>et al.</i> , 2015)
Corn	28.5 Nuwamanya <i>et al.</i> , 2011)
Potato	29.3±0.2 (Yuan <i>et al.</i> , 2007)
Cassava	23.7±0.1 (Yuan <i>et al.</i> , 2007)
Turmeric	48±3 (Braga <i>et al.</i> , 2006)
Ginger	34±2 (Braga <i>et al.</i> , 2006)

### 2.5.2 Properties of starch

Starch is known to go through various notable transformations and exhibit various properties and physical structures. Native granular starch, after being soaked in water, can absorb up to 30% by weight of moisture. The water absorbed is present in the amorphous region of the starch granule. This process is reversible, and water can be evaporated on drying at a temperature below gelatinization. The heating of the starch granules in the presence of water leads to the loss of starch double helical crystalline structure (Kulkarni *et al.*, 2017). The process is known as gelatinization. This process is irreversible and each starch has its own characteristics gelatinization temperature. The continuous heating of starch granules in excess water leads to the development of viscosity and become a paste (Nuwamanya *et al.*, 2011). The viscosity increases as temperature decreases and the starch molecules develop a network.

### 2.5.3 Solubility and swelling power of starch

Generally, starch is not water soluble because of granules is too large to form a solution. Depending on the source, starch has a relative high density, about 1.45-1.64 g/cm<sup>3</sup> (And and Han, 2005). Starch granules absorb some water in suspension at room temperature, but the amount of swelling is in the intact granules. The progressive

heating of starch to a critical temperature allows swelling, losing the polarization crosses, the process known as gelatinization.

#### **2.5.4 Retrogradation of starch**

During freezing and chilling under low energy input, more hydrogen bonding may occur resulting in further tightening of structure with loss of water holding capacity. This process occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. At early phases, two or more starch chains may form a simple juncture point that may then develop into more extensive orderly regions (Nawapat, 2012). This unique characteristics of starch helps in formation of bond in a linear manner, giving rise to a better film formation for industrial uses in both edible coating and packaging

#### **2.6 African Palmyra Palms (*B. aethiopum*) Shoot**

African Palmyra palm (*B. aethiopum*) is a dioecious tree and can grow as high as 20m on average and 1m in diameter (Muller, 1988). In Nigeria, it is called *Giginya* in Hausa, *Agbonolodu* in Yoruba and *Ubiri* in Igbo (Umar *et al.*, 2015). The shoots from this tree are called '*Muruci*' in Hausa.



**Figure 2.5: African Palmyra Palmtree and Shoot (*Muruci*)**

The shoot of *B. aethiopum* (Figure 2.5) is a well-known source of dietary carbohydrate in some parts of Northern Nigeria. It is obtained by burying the matured seeds of the plant in a pit and allowing it to germinate. The young germinating shoot or hypocotyls is usually harvested after 7 to 8 weeks of planting (Ali *et al.*, 2010). Rural people in Northern Nigeria consume the germinating shoot either raw or boiled and claimed that it enhances libido in women and aphrodisiac in men (Akinniyia *et al.*, 2010). The shoot of *B. aethiopum* plant on dry weight basis has high concentration of carbohydrate (83.00 %), crude fibre (3.96 %) and low fat (1.49 %) (Ali *et al.*, 2010).

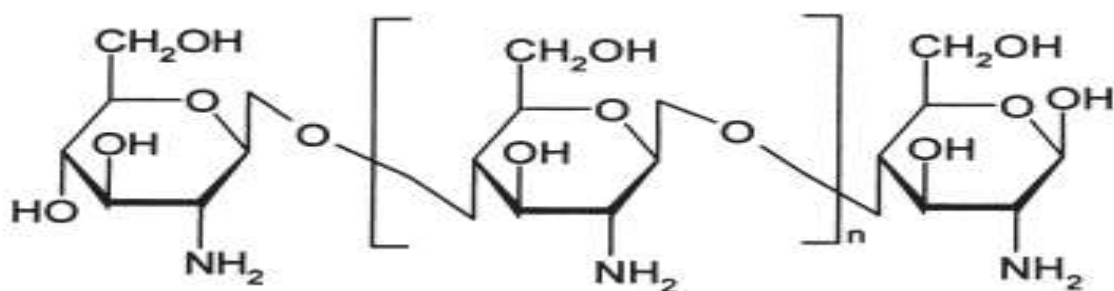
The plant is widely cultivated in some states of northern Nigeria and its shoots has been reported to be a potential source of starch in Cote d'Ivoire which is an important raw material in industry (Mazumdar, 2004). However, due to a lack of information on potential value-added chain products that can originate from the plant's shoot, as well as physiological and biochemical processes that occur within the plant such as fermentation and rotting, most of it is wasted after harvest. Reduction in postharvest

losses of this shoot will lead to higher economic growth as increased earnings from this plant will be achieved. The starch content of this plant presents a prospect for processing it into powdered starches though the shoots from *Burassus aethiopum* tree is not recognized as a common source of industrial starch (Dufour *et al.*, 1996).

Physicochemical properties such as gelatinization temperature and amylose and amylopectin ratios of starches are the basic materials in industries involved in the production of super cooked starches such as pasta and noodles. Lack of meaningful information on the functional properties of the starches is one of the limiting factors for industrial application of non-conventional starch such as “*Muruci*” (Riley *et al.*, 2006).

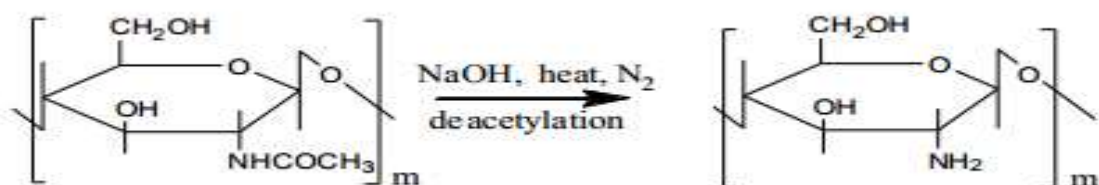
## **2.7. Chitosan**

Chitosan is a natural linear polycationic polysaccharide derived from partial deacetylation of chitin. It was purportedly first discovered by Rouget in 1859 while he was experimenting with chemical and thermal manipulation of the natural fiber chitin. It is composed of  $\beta$ -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer (Venkatesan *et al.*, 2010). Chitosan is a special material due to its cationic nature. Most polysaccharides are usually either neutral or negatively charged in an acidic environment allowing the formation of electrostatic complexes or multilayer structures with other negatively charged natural or synthetic polymers (Kumar *et al.*, 2004).



**Figure 2.6: Chemical structure of chitosan** (Kimberly *et al.*, 2003).

After deacetylation of chitin with sodium hydroxide, the resulting chitosan becomes soluble in acid typifying the form present in the stomach. Once solubilized, the chitosan forms a gel-like substance that binds lipids in the gastrointestinal tract, subsequently resulting in their fecal elimination (Macchi, 1996). The electrostatically charged chitosan is poorly absorbed systemically but capable of binding to lipids and prevent their digestion. The positively charged amino groups on the chitosan molecule bind to the negatively charged carboxylic groups of free fatty acids. This electromagnetic bond appears to be stronger than those observed in other dietary fibers. These interactions give rise to the formation of hydrophobic bonds between chitosan and neutral fats such as cholesterol and triglycerides (Macchi, 1996).



**Figure 2.7: Schematic Representation of the Conversion of Chitin into Chitosan** (Shanta *et al.*, 2015)

Chitosan and its derivatives are versatile biomaterials due to their diverse bioactivities, biodegradability non-toxicity, biocompatibility and low-allergenicity. They have good



mechanical properties such as high surface area, porosity, tensile strength and conductivity (Elsabee *et al.*, 2009). The nature of chitosan allows the easy molding into different forms and shapes (Chameettachal *et al.*, 2015). Films from blends of chitosan and starch have shown great improvements in the areas of strength and barrier properties when properly plasticized.

### **2.7.1 Films from chitosan**

Chitosan is one of the most used biopolymers in the production of active edible films. Mixing of chitosan with protein directly changes the film structures significantly, leading to the reinforcement of the chitosan matrices, improving their barrier, antimicrobial and antioxidant properties (Arancibia *et al.*, 2015). Edible films of chitosan with gelatin or methylcellulose have been developed to improve mechanical and barrier properties, while showing antimicrobial activity against Gram-positive bacteria. In addition, chitosan/gelatin films exhibited antioxidant activity monitored by  $\beta$ -carotene bleaching and DPPH radical scavenging (Hosseini *et al.*, 2016).

### **2.7.2 Chemical properties of chitosan**

This biopolymer has a number of chemical properties that make it suitable for several biomedical applications. Some of them are listed below (Dutta *et al.*, 2004). Chitosan is a linear polyamine, It has reactive amino groups ( $-\text{NH}_2$ ), there is availability of reactive hydroxyl groups ( $-\text{OH}$ ), it has chelating ability for many transitional metal ions (Shanta *et al.*, 2015)

### **2.7.3 Biological properties of chitosan**

Besides the various chemical properties, chitosan shows diverse biological properties (Venter *et al.*, 2006, Younes and Rinaudo, 2015). It is Biocompatible, safe and non-toxic, biodegradable to normal body constituents, binds to mammalian and microbial

cells aggressively, regenerative effect on connective gum tissue, accelerates the formation of osteoblast, responsible for bone formation, haemostatic (causes stop bleeding), fungistatic (inhibiting the growth of fungi), spermicidal (birth control), antitumor or anticancer (inhibiting the growth of tumor or cells), anticholesteremic (cholesterol lowering agent), central nervous system depressant (slow down the brain activity), immunoadjuvant (involved in the improvement of immune response (Younes and Rinaudo, 2015)).

## **2.8. Plasticizers**

International Union of Pure and Applied Chemistry (IUPAC) developed a universally accepted definition for a plasticizer in 1951 as a substance or material incorporated in a material (usually a plastic or an elastomer) to increase its flexibility, workability, or distensibility. The uses of plasticizers have increased greatly worldwide (Wilkes *et al.*, 1999). The effectiveness of a plasticizer depends on thorough mixing for proper incorporation into then polymer matrix. This is obtained by heating and mixing until either the resin dissolves in the plasticizer or the plasticizer dissolves in the resin. The plasticized material is then molded or shaped into a desired product and cooled. Different plasticizers will exhibit different characteristics in both the ease with which they form the plasticized material and in the resulting mechanical and physical properties of the flexible product (Melissa *et al.*, 2011). These substances (plasticizers) reduce the tension of deformation, hardness, density, viscosity and electrostatic charge of a polymer, at the same time as increasing the polymer chain flexibility, resistance to fracture and dielectric constant (Rosen *et al.*, 1993). Other properties are also affected, such as degree of crystallinity, optical clarity, electric conductivity, fire behavior and resistance to biological degradation, amongst other physical properties (Bialecka *et al.*, 2007).

The mechanistic explanation of plasticization assumes that the plasticizer molecules are not permanently bound to the polymer molecules but are free to self-associate and to associate with the molecules at certain sites such as amorphous sites (Choi and Park, 2004). Plasticizer interactions with the parent molecules are weak, due to the dynamic exchange process whereby, as one plasticizer molecule becomes attached at a site or center, it is readily dislodged and replaced by another. Plasticizers of different compositions yield different plasticization effects because of the differences in the strengths of the plasticizer polymer and plasticizer-plasticizer interactions.

Hydrophilic compounds, such as polyols (GLY and sorbitol) are commonly used in starch films (Garcia *et al.*, 2000, Bergo *et al.*, 2008). However, some sugars, surfactants, amino acids and fatty acids could also be employed to improve the fluid's mechanical and barrier properties. However, a limiting barrier to the development of starch materials is the brittle nature of blends containing high concentrations of starch. The re-crystallization of starch restrains starch from coming into real practical use, because it easily becomes rigid and brittle during long-term storage, and therefore loses its value in use (Huang *et al.*, 2005). Overcoming the brittleness of starch while achieving full biodegradability in blends, can be accomplished with the addition of biodegradable plasticizers. An ideal plasticizer for starch-based materials should impart flexibility and suppress retrogradation to thermoplastic starch (TPS) during aging (Ma *et al.*, 2004). The crystallization of GLY-containing potato starch plastic sheets revealed a significant influence on their mechanical properties. The changes in crystallinity were clearly related to the initial amount of plasticizer and moisture migration during aging. The differences in material properties could be attributed to the formation of an entangled starch matrix and by starch chain-to-chain associations that are related to plasticizer content (Van *et al.*, 1997). Ethanolamine is a novel plasticizer that can be

used for thermoplastic starch processing, destroying the native starch granules and making them come into a uniform continuous phase. Ethanolamine plasticized thermoplastic starch (ETPS) could restrain the re-crystallization of traditional thermoplastic starch plasticized by GLY (GTPS), improving their mechanical properties and thermal stability (Huang *et al.*, 2005).

### **2.8.1 Characteristics of plasticizer**

Plasticizers generally have high boiling point with average molecular weights of between 300 and 600, and linear or cyclic carbon chains (14 to 40 carbons) (Donhowe and Fennema 1993, Wilson *et al.*, 1995). The low molecular size of a plasticizer allows it to occupy intermolecular spaces between polymer chains, reducing secondary forces among them. In the same way, these molecules change the three-dimensional molecular organization of polymers, reducing the energy required for molecular motion and the formation of hydrogen bonding between the chains. The degree of plasticity of polymers is largely dependent on the chemical structure of the plasticizer, including chemical composition, molecular weight and functional groups. A change in the type and level of a plasticizer will affect the properties of the final flexible product (Rahman *et al.*, 2004, Cao *et al.*, 2009).

Compatibility between plasticizer and polymer is of major significance for effective plasticization and various parameters can indicate this feature, including polarity, hydrogen bonding, dielectric constant and solubility parameters (Choi *et al.*, 2004, Van *et al.*, 2003). Other important factor is solvation, as plasticizers with solubility parameters close to those of the polymer require less energy to fuse or solvate the polymer. The temperature of fusion or gelation is related to the solvation strength of the plasticizer and to the size of its molecule (Rahman *et al.*, 2004). Permanence is related

to volatility and resistance to migration and extraction in water, solvents and oils. Therefore, the plasticizer should have low vapor pressure and a low rate of diffusion in the polymer (Wilson *et al.*, 1995). The ease or difficulty of processing plastic can be significantly influenced by the plasticizer type and concentration as well as other formulating additives. So, as they do not only modify the physical properties of polymers but can also improve processing characteristics, plasticizers can also be considered as processing additive. Plasticizers can influence processing by inducing lower viscosity, faster filler incorporation, easier dispersion, lower power demand and less heat generation during processing, better flow, improved release and enhanced building tack. For example, as a property modifier, plasticizers can reduce the second order transition temperature and the elasticity modulus, as a result cold flexibility is improved. The softening effect of plasticizers leads mostly to improve processing through easier filler incorporation and dispersion, lower processing temperatures and better flow properties (Zweifel *et al.*, 2009).

In biopolymer-based films and coatings production, plasticizers are also essential additives since they can improve flexibility and handling of films, maintain integrity and avoid pores and cracks in the polymeric matrix (Garcia *et al.*, 2000). Incompatibility is commonly evidenced by phase separation between the biopolymer and plasticizer, presented in the form of exudated drops on the surface of the product immediately after its blending or during final product application (Wilson *et al.*, 1995). For example, plasticizer addition improved starch-based coatings performance by increasing barrier properties to water vapor, maintaining the selective gaseous permeability. GLY and sorbitol showed to be compatible with amylose and improved mechanical properties of films, by decreasing intermolecular attraction and interfering with the amylose packing (Garcia *et al.*, 2000).

## **2.8.2 Classes of plasticizer**

### **2.8.2.1 *External or internal plasticizer***

Plasticizers can either be external or internal based on functions and applications. External plasticizers are low volatile substances that are added to polymers. In this case, plasticizer molecules interact with polymer chains, but are not chemically attached to them by primary bonds and can be lost by evaporation, migration or extraction (Rahman *et al.*, 2004). On the other hand, internal plasticizers are inherent parts of the polymer molecules and become part of the product, which can be either co-polymerized into the polymer structure or reacted with the original polymer (Pielichowski *et al.*, 2006, Choi *et al.*, 2004). Internal plasticizers generally have bulky structures that provide polymers with more space to move around and prevent polymers from coming close together. Therefore, they soften polymers by lowering the T<sub>g</sub> and, thus, reducing elastic modulus. For both types, although more pronounced for internal plasticizers, a strong temperature dependence of material properties is observed (Bialecka, *et al.*, 2007). The benefit of using external plasticizers, compared to internal ones, is the chance to select the right substance depending on the desired product properties (Sothornvit and Krochta, 2005).

### **2.8.2.2 *Primary and secondary plasticizer***

Plasticizers can also be classified as primary and secondary (Godwin *et al.*, 2008). If a polymer is soluble in a plasticizer at a high concentration of the polymer, it is said to be a primary plasticizer. This type of plasticizers are used as the sole plasticizer or as the main element of the plasticizer, they should gel the polymer rapidly in the normal processing temperature range and should not exude from the plasticized material. Secondary plasticizers, on the other hand, have lower gelation capacity and limited

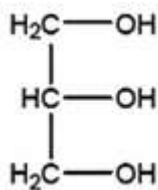
compatibility with the polymer, they are typically blended with primary plasticizers, to improve product properties or reduce the cost (Chanda *et al.*, 2008).

### **2.8.2.3 Water soluble and water insoluble plasticizer**

Plasticizers, for biopolymer-based films, can be divided into water soluble and water insoluble (Siepmann *et al.*, 1998). The type and the amount of plasticizer strongly affect the film formation from polymeric aqueous dispersions (Johnson *et al.*, 1991). Hydrophilic plasticizers dissolve in the aqueous medium when they are added to polymer dispersions and if added in high concentration they can lead to an increase in water diffusion in the polymer. In contrast, hydrophobic plasticizers may close the micro-voids in the film, leading to a decrease in water uptake. However, water insoluble plasticizers can cause phase separation leading to flexibility losses or yet to the formation of discontinuity zones during film drying. As a consequence, water vapor permeability rates are increased. Complete uptake of insoluble plasticizer by the polymer can be achieved by an optimum stirring rate of the polymeric dispersion with the plasticizer (Bodmeier *et al.*, 1997).

## **2.9 Glycerol**

Glycerol is a simple polyol compound which is colorless, odorless, viscous liquid with sweet tasting and non-toxic (Perry *et al.*, 1997). Its IUPAC nomenclature is propane-1,2,3-triol. It is also commercially known as glycerin, (1,2,3-propanetriol), trihydroxypropane, glyceritol or glycidic alcohol (Rahmat *et al.*, 2010). Glycerol is the main component of triglycerides, found in vegetable oil, animal fat, or crude oil (Foglia *et al.*, 1998, Choi, 2008). Glycerol is a liquid containing three hydrophilic hydroxyl groups that are responsible for it being hygroscopic and its solubility in water (Blieck *et al.*, 2005).



**Figure 2.8: Structure of Glycerol** (Mario *et al.*, 2008)

Glycerin is completely miscible in many substances such as alcohol (methyl, ethyl, isopropyl, n-butyl, isobutyl, secondary butyl, and tertiary amyl); ethylene glycol, propylene glycol, trimethylene glycol monomethyl ether and phenol (Chung *et al.*, 2007) Solubility of glycerin in acetone is 5 % by weight; in ethyl acetate it's 9 %. It's slightly soluble in dioxane and ethyl and partially insoluble in superior alcohol, fatty acids and hydrocarbonate, a well as in chlorinated solvents such as hexane, benzene, and chloroform. Owing to the presence of three hydroxyl groups, glycerol is miscible with water and it is hygroscopic in nature. At normal temperature, it remains a viscous liquid even at 100 % concentration without crystallizing. At low temperatures, concentrated glycerin solutions tend to super cool as high viscosity fluid. Aqueous glycerin solutions (at different concentrations) tend to have lower viscosity (Chung *et al.*, 2007 and Blieck *et al.*, 2005). At low temperatures glycerin tends to super cool instead of crystallize. Aqueous glycerin solutions resist freezing and are used as antifreeze in cooling systems. Glycerin does not oxidize in the atmosphere in normal conditions, but can be easily oxidized by other oxidants. Glycerin solutions need inhibitors when they are exposed to heat and in contact with ferrous metal or copper since the salts contained in these materials can catalyze oxidation (Soares *et al.*, 2006). It is used in medical and pharmaceutical preparations, mainly as a means of improving smoothness, providing lubrication and as a humectant, that is as a hygroscopic substance which keeps the preparation moist. Glycerol helps to maintain texture and adds humectancy, controls water activity and prolongs shelf life in a host of



applications. It is also widely used as a laxative and, based on the same induced hyperosmotic effect, in cough syrups (elixirs) and expectorants. In personal care products glycerol serves as an emollient, humectant, solvent, and lubricant in an enormous variety of products, including toothpaste, where its good solubility and taste giving an edge over sorbitol (Mario *et al.*, 2008).

## **2.10 Shea Butter**

Shea butter (*Vitellaria paradoxa*) is a versatile plant fat extracted from kernels of shea nuts, seeds of shea trees (Nahm, 2011). Shea butter, rich in unsaturated fatty acids undergoes hydrolytic and oxidative degradation during postharvest processing and storage, resulting in inconsistent and degraded quality and limited shelf-life. Shea butter has long been used in the West African countries, dating back to ancient Egypt based on the record that during the Cleopatra's Egypt, caravans carried clay jars of valuable shea butter for cosmetic uses (Goreja, 2004). The *V. paradoxa* nuts/seeds are usually processed into shea oil that constitutes an important source of fat. The shea butter fat can also be used in soap making, cosmetic and traditional medicine in many rural areas (Alandar, 2004). Shea butter is a triglyceride (fat) derived mainly from stearic acid, palmitic, linoleic and oleic acid (Okullo *et al.*, 2010). It is a complex fat that in addition to many nonsaponifiable components melts at body temperature. In some African countries, shea butter is used for cooking oil, as a waterproofing wax, for hairdressing, for candle-making, and as an ingredient in medicinal ointments (Goreja, 2004). It is used by makers of traditional African percussion instruments to increase the durability of wood. Some of the isolated chemical constituents are reported to have anti-inflammatory emollient and humectant properties (Akihisa *et al.*, 2010)

### **2.10.1 Role of plasticizers on the properties of films**

Plasticizers are high boiling point liquids with average molecular weights ranging from 300 to 600 and linear or cyclic carbon chains 14–40 carbons (Donhowe and Fennema, 1993). A plasticizer's small molecular size allows it to fill intermolecular gaps between polymer chains, lowering secondary forces between them. Similarly, these compounds alter the three-dimensional molecular architecture of polymers, lowering the energy required for molecular motion and the creation of hydrogen bonds between chains. As a result, Wypych (2004) observes an increase in free volume and, as a result, in molecular mobility. Thus, the degree of plasticity of polymers is largely dependent on the chemical structure of the plasticizer, including chemical composition, molecular weight and functional groups (Snijkers *et al.*, 2004). A change in the type and level of a plasticizer will affect the properties of the final flexible film (Rahman and Brazel, 2004).

Plasticizer-polymer compatibility is critical for effective plasticization, and several factors, such as polarity, hydrogen bonding, dielectric constant, and solubility parameters, might indicate this feature, (Choi and Park, 2004). The plasticizer type and concentration, as well as other formulation ingredients, can have a major impact on the ease or difficulty of manufacturing biodegradable films. As a result, they can improve not only the physical qualities of polymers but also their processing features.

Dehydration of biopolymer structures results in strong cohesive films that usually necessitate the addition of plasticizers. The inclusion of a plasticizer reduces intermolecular pressures along polymer chains, improving flexibility and chain mobility. These are used to improve film flexibility, reduce brittleness, and prevent shrinkage during handling and storage, (Bordes *et al.*, 2009). Several investigations, however, have found that plasticizers have a negative impact on the properties of edible

films. The majority of these refer to an increase in gas, solute, and water vapor permeability, while the decrease in cohesion primarily affects mechanical qualities. The characteristics of films, based on biopolymers, depend therefore on an equilibrium between the degree of cross linking of the polymer matrix (sometimes necessary to reduce the solubility in water, but induces brittleness) and the addition of plasticizers for better workability (da Silva *et al.*, 2009). Above a critical concentration, the plasticizer can exceed the compatibility limit with the biopolymer, and phase separation with plasticizer exclusion is usually observed (Yang and Paulson, 2000). Usually biopolymers and plasticizers are typically hygroscopic; the moisture content of the film is altered by environmental conditions. Furthermore, water is the primary solvent in natural biopolymer technology. Its molecules lower the T<sub>g</sub> and increase the free volume of biomaterials, making them plasticizers. In addition to water, the most commonly used plasticizers are polyols, mono-, di- and oligosaccharides. Polyols have been found to be particularly effective for use in plasticized hydrophilic polymers (Zhang and Han, 2006). Glycerol (GLY) was, thus, nearly systematically incorporated in most of the hydrocolloid films (Cuq *et al.*, 1997). GLY is indeed a highly hygroscopic molecule generally added to film-forming solutions to prevent film brittleness (Karbowski *et al.*, 2006). Recently, many studies have focused on the use of polyols such as GLY (Galdeano *et al.*, 2009), ethylene glycol, fatty acids (Jongjareonrak *et al.*, 2006); monosaccharides (glucose, mannose, fructose, sucrose) (Veiga-Santos *et al.*, 2007); ethanolamine (EA), urea, triethanolamine (TEA), vegetable oils; lecithin; waxes (Garcia *et al.*, 2000); amino acids, surfactants and water (Kristo and Biliaderis, 2006), as plasticizers of biodegradable films.

## 2.11 Blending

Different macromolecules blending by either a direct way or associated with co-drying processes leads to films with better control of their final properties. Studies show that blending of different matrices to produce films has synergistic effects on the final products (Arancibia *et al.*, 2015, Hosseini *et al.*, 2016). Blending in a direct form consists of an initial preparation stage of individual polymer solutions such as starch polymers with the subsequent homogenization and casting. It shows some advantages, since the co-drying method requires strict conditions, such as narrow concentration range and good compatibility (Pan *et al.*, 2014).

Chitosan is one of the most used biopolymers in the production of active edible films. Mixing of chitosan with starch composites changes the film structures significantly, leading to the reinforcement of the chitosan matrices, improving their barrier properties (Arancibia *et al.*, 2015). Edible films of chitosan with gelatin or methylcellulose have been developed to improve mechanical and barrier properties, while showing antimicrobial activity against Gram-positive bacteria. In addition, chitosan/gelatin films exhibited antioxidant activity monitored by  $\beta$ -carotene bleaching and DPPH radical scavenging (Hosseini *et al.*, 2016).

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1. Experimental Materials

##### 3.1.1 Sample collection

The shoots of *Buorassus aethiopum* plant were purchased from the site of cultivation at Kaltungo, Gombe South, Gombe state in North Eastern Nigeria. It was bagged and transported to Minna before processing.

Chitosan (95.0 % deacetylation degree and molecular mass of 1100 kDa) from Yuhuan Ocean Biochemistry Co. Ltd. (Zhejiang, China) was purchased from Agrimedik Ltd. Lagos (Nigeria).

The plasticizers, Glycerol (99.0 % purity) and shea butter were purchased from Multichem Industries Limited. Lagos, Nigeria.

#### 3.2 Methods

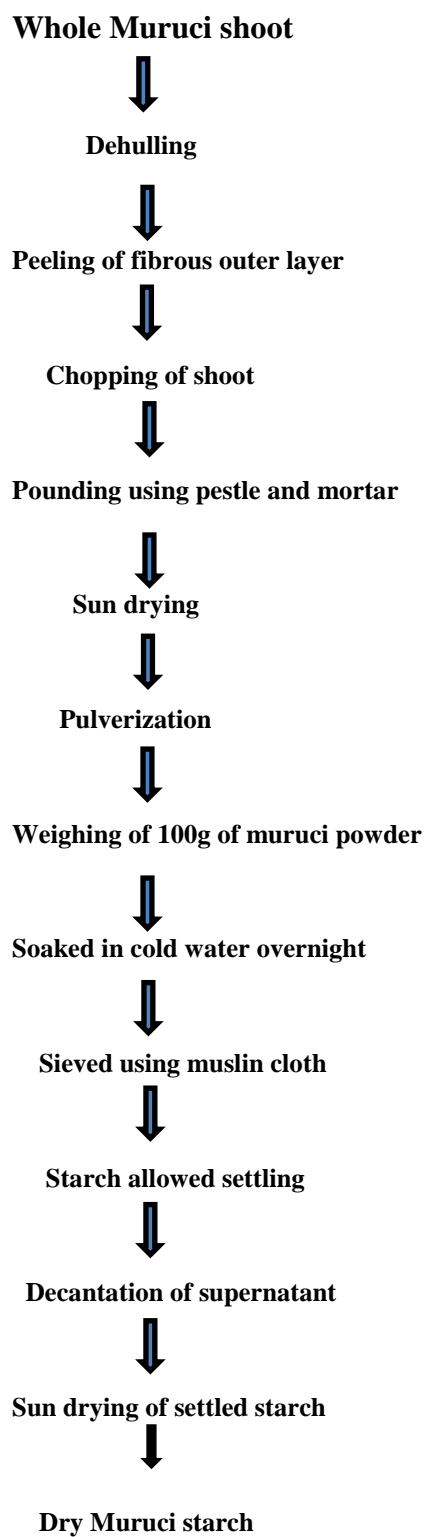
##### 3.2.1 *Borassus aethiopum* shoot starch extraction

The extraction of starch from *Borassus aethiopum* shoot was manually processed using knife, mortar and pestle.

###### 3.2.1.1 Procedure

The shoots of *Borassus aethiopum* were dehulled and the outer fibrous layers were peeled, chopped into smaller pieces, pounded with mortar and pestle then dried. The dried sample was pulverized into powder using blending machine and sieved with 4.75 mm sieve, soaked with distilled water for 5 minutes and filtered using muslin clothe. The filtrate was allowed to settle for six hours before decantation and drying.

### **3.2.1.2 Extraction flowchart.**



**Figure 3.1: Muruci Starch Extraction Flowchart**



**Plate I: *Burassu aethiopum* Shoot (A), Shoot Pounded form (B), Dried from (C) and Starch Powder (D)**

### 3.3 Starch Yield

One hundred grams of *Borassus aethiopum* shoot starch powder was weighed and soaked in cold water overnight and sieved using muslin cloth. The starch was allowed to settle and the supernatant was decanted. The starch was dried and stored in plastic covered container for further analysis. The starch yield was calculated in percentage using the equation below.

$$\% \text{ Yield} = \frac{\text{Final weight of dried extract}}{\text{initial weight of milled starch}} \times 100 \quad (3.1)$$

### 3.4 Determination of Amylose and Amylopectin

The amylose content was determined using iodine colorimetric method, which is based on the transmission of light through a color complex of amylose when reacted with iodine, according to Martínez and Cuevas 1989 with slight modification by Almeida *et al.* (2010).

#### 3.4.1 Procedure

A total of 100 mg of *Borassus aethitopum* was homogenized with 1 mL of 95 % ethanol and 9mL of 1 M NaOH. The sample was heated for 10 min in a boiling-water bath to gelatinize the starch. After cooling at room temperature, it was transferred into a volumetric flask and the volume was made up to 100 mL with water. Then 1 ml of 1 M acetic acid and 2 mL of iodine solution (0.2 % I<sub>2</sub>, 2 % KI) were added. A 5mL of the solution was transferred to a 100 mL volumetric flask and allowed to stand for 10 min. Spectrophotometric quantification was performed at 620 nm. Two determinations were made on separate test portions taken from the same sample in each of the replicates. The apparent amylose content was calculated using an equation obtained from the standard curve as shown below.

$$y = 0.0168x + 0.2138 \quad (3.2)$$

$$y = \text{absorbance} = 1.480$$

$$x = \text{amylose concentration}$$

Therefore;

$$1.480 = 0.0168x + 0.2138$$

$$x = \frac{1.480 - 0.2138}{0.0168}$$



### **3.5. Film Preparation**

Chitosan/*Borassus aethiopum* shoot starch based films were prepared by conventional solution-casting technique as reported by Shojaee *et al.* (2013) with slight modification.

#### **3.5.1 Procedure**

Chitosan/*Borassus aethiopum* shoot starch films (CBSF) were prepared by conventional solution-casting technique. Starch solution with concentration of 1 and 2 % w/v were prepared by dispersing *Borassus aethiopum* shoot starch, in distilled water and heating the mixtures on hot plates with stirring until they gelatinized. The chitosan solutions (2 % w/v) were prepared by dispersing (0.5, 1 and 2 %) of chitosan in 100 mL of 1 % acetic acid. Chitosan blend film was prepared by mixing 100 mL of 2 % starch solutions with 100 mL of 2 % chitosan solution. Glycerol and shea butter 0, 30 and 50 % w/w were added under continuous stirring on a magnetic hot plate for further 10 min and 50 mL of the film-forming dispersion (FFDs) was poured into a Petri dish of 9 cm in diameter and oven dry at 45°C for 12 hours. Dried films were peeled off from the glass plates and stored for evaluation.

### **3.6 Experimental Design**

The factors that were employed in this experiment are *Borassus aethiopum* starch (1-2 %), Chitosan (0-2 % w/w), Glycerol (0-50 % w/w) and Shear butter oil (0-50 % w/w). The films from 1 and 2 % starch were analyzed in the absence of chitosan and plasticizers. Then films from incorporation of 1 and 2 % chitosan to the starch were also analyzed in the absence of plasticizers. Finally, films prepared from the introduction of 0 to 50 % w/w glycerol and shear butter to 1 and 2 % starch and chitosan separately and in combination were analyzed.

### 3.7 Film Thickness

The thicknesses of the films were measured with a handheld micrometer with sensitivity of 0.001 mm. The Measurements were made at different points (at least five random locations) and the results were reported as mean for each film.

### 3.8 Solubility in Water

The solubility of film samples was determined according to the method of Solubility test as proposed by Gontard *et al.* (1993) with slight modification by Ahmad *et al.* (2012).

#### 3.8.1 Procedure

Samples (3x2 cm) in size were dried at 105°C for 24 h and weighed ( $w_i$ ). Each sample was then placed in 50 mL of distilled water at 175 rpm in a rotary shaker, at 25°C for 24h. The solution was filtered, and the residues remained on the filter paper were dried in an oven at 105°C for 24 h after which the samples were weighed to determine the dry matter soluble in water ( $w_f$ ). The solubility in water was expressed as a percentage of solubilized material, calculated from the results in triplicate.

$$S = \frac{w_i - w_f}{w_i} \times 100 \quad (3.3)$$

Where,

$w_i$  = initial mass (g)

$w_f$  = final mass (g).

### 3.9 Mechanical Properties

The mechanical properties tested include tensile strength (TS) and elongation at break (ELB). The film samples were evaluated according to ASTM standard method D882, 2001 by Testometric Machine as described by Dashipour *et al.* (2015).

### **3.10.1 Procedure**

Film strips (1.5×4.5 cm) were cut from each of the samples prior to testing and were equilibrated at 25°C (53 %RH) in desiccators containing Mg(NO<sub>3</sub>)<sub>2</sub> saturated solutions for 48h. Film strips were mounted between the grips. Separation of 50mm was used and the crosshead speed was set at 10 mm/min with 500 N load cells. A microcomputer was used to record the stress–strain curves of each film tested.

### **3.10 Biodegradation Test**

Biodegradation behavior of blends of chitosan and *Borassus aethiopum* shoot starch composites in the presence of plasticizers was studied by ascertaining the time taken for the complete decomposition of various blends during the soil burial test.

#### **3.10.1 Procedure**

2.5cm piece of various blends were buried in loamy soil and covered with a rectangular transparent plastic and were monitored daily.

### **3.11 Data Analysis**

The data were analyzed statistically using one factor analysis of variance (ANOVA). SPSS statistical software. P-value less than 0.05 were considered to be significantly different.

### **3.12 Film Optimization**

The experimental design, optimization and regression analysis of *Burassus aethiopum* shoot starch/chitosan Film (BSCF) was carried out using Design expert 11.0 software.

### **3.13 Optical Properties**

The transparency of the films was determined by measuring their light absorption at wavelength of 600 nm using a UV-1800 visible spectrophotometer Shimadzu, according to the method described by Maran *et al.* (2013). The film specimens were cut

into strips (1x4cm) and placed directly in the spectrophotometer test cell. Air was used as reference. Opacity was expressed as absorbance units per thickness unit.

## CHAPTER FOUR

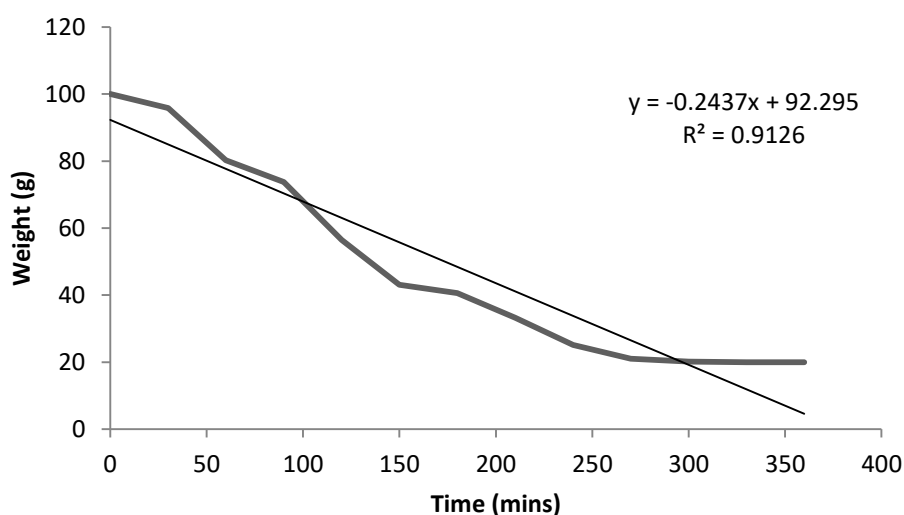
### 4.0

### RESULTS AND DISCUSSION

#### 4.1 Results

##### 4.1.1 Starch yield from *Borassus aethiopum* shoot

The results of starch yield and the rate at which *Borassus aethiopum* shoot starch dried to a constant weight is shown in Figure 4.1. Moist *Borassus aethiopum* shoot starch yielded 20 % dry weight after 360 minutes at 100°C. Weight lost was notable after 30 minutes and constant weight was observed after 330 minutes of drying and weighing. The slope shows the rate of weight lost is at 24 %.



**Figure 4.1: Rate of Drying of *Borassus aethiopum* Shoot Starch**

The amylase content in the starch was 75.4 % while the amylopectin ratio is 24.6 % as shown in Table 4.0.

**Table 4.0: Starch Yield and Amylose/Amylopectin ratio of *Borassus aethiopum* Shoot**

Starch yield (%)	Amylose (%)	A mylopectin (%)
20	75.4	24.6

#### 4.1.2 Properties of films produced from the composites of *Borassus aethiopum* shoot starch and chitosan blends

Properties of films from blends of chitosan and *Borassus aethiopum* shoot starch in the absence of plasticizers (glycerol and shea butter) are shown in Table 4.1.

**Table 4.1: Properties of Films Prepared from 1-2 %w/v of *Borassus aethiopum* Shoot Starch and Varying Concentrations of Chitosan Blend**

BS (g)	CHI (g)	GLY (w/w %)	SB (w/w %)	Thickness (mm)	Solubility (%)	Elongation at Break (%)	Tensile Strength (MPa)
1	0	0	0	0.043±0.001 <sup>a</sup>	30.40±0.013 <sup>f</sup>	29.15±0.002 <sup>e</sup>	1.52±0.006 <sup>a</sup>
1	0.5	0	0	0.081±0.003 <sup>b</sup>	11.90±0.004 <sup>d</sup>	34.14±0.003 <sup>f</sup>	3.55±0.016 <sup>b</sup>
1	1	0	0	0.092±0.001 <sup>c</sup>	06.81±0.011 <sup>b</sup>	20.89±0.012 <sup>d</sup>	5.02±0.001 <sup>d</sup>
2	0	0	0	0.095±0.001 <sup>d</sup>	25.91±0.014 <sup>e</sup>	09.34±0.005 <sup>b</sup>	4.31±0.031 <sup>c</sup>
2	1	0	0	0.163±0.002 <sup>e</sup>	10.91±0.008 <sup>c</sup>	17.02±0.011 <sup>c</sup>	6.46±0.016 <sup>e</sup>
2	2	0	0	0.205±0.005 <sup>f</sup>	5.11±0.011 <sup>a</sup>	9.02±0.001 <sup>a</sup>	7.94±0.021 <sup>f</sup>

**Values are Mean ± Standard Error of Mean of quintuplicate determination for film's thickness and triplicate determination for the remaining parameters. Values with different alphabets within a sample are significant (p<0.05) along the column**

The films prepared from 1 %w/v of *Borassus aethiopum* shoot starch in the absence of chitosan and plasticizers are relatively transparent and the thickness was measured to be 0.043±0.001mm. The films were 30.40±0.013 % soluble in water with 29.15±0.002 % elongation at break and 1.52±0.006 Mpa tensile strength. Introduction of 0.5 w/w of dissolved chitosan to 1 %w/v of *Borassus aethiopum* shoot starch produced films with 0.81±0.003 mm thickness, which is significantly (p<0.05) higher compared to the films

without chitosan. The films elongation at break and tensile strength were significantly higher  $34.14 \pm 0.003$  % and  $3.55 \pm 0.016$  Mpa respectively, but there was a significant reduction in the film's solubility in water  $11.90 \pm 0.004$  % as recorded in Table 4.1

Significant ( $p < 0.05$ ) increase in film thickness from  $0.043 \pm 0.001$  to  $0.205 \pm 0.005$  mm and tensile strength from  $1.52 \pm 0.006$  to  $7.94 \pm 0.021$  MPa was observed as the concentrations of the starch and chitosan increases from 1 to 2% respectively. The films solubility in water significantly decreased from  $30.40 \pm 0.013$  to  $5.11 \pm 0.011$  % and the elongation at break also decreased from  $34.14 \pm 0.003$  to  $9.02 \pm 0.001$  % as the ratio of chitosan increased to 2 %w/w.

#### **4.1.3 Effects of glycerol and shea butter on some properties of films produced from the composites of *Borassus aethiopum* shoot starch and chitosan blends**

Preliminary studies of the effect glycerol at lower concentration ( $< 30$  %) on *Borassus aethiopum* starch and chitosan blends present low plasticization effect, therefore the choice of 30 %w/w as the base glycerol concentration. The results of the effects of glycerol and shea butter concentrations on the properties of films produce from blends of chitosan and *Borassus aethiopum* shoot starch at 1 %w/v are shown in Table 4.2.

**Table 4.2: Effects of Glycerol and Shea Butter as Plasticizers on Films Prepared from 1 %w/v *Borassus aethiopum* Shoot Starch and 0.5-1 %w/w Chitosan**

BS (g)	CHI (g)	GLY (w/w %)	SB (w/w %)	Thickness (mm)	Solubility (%)	Elongation at Break (%)	Tensile Strength (MPa)
1	0	30	0	0.055±0.001 <sup>c</sup>	34.30±0.014 <sup>i</sup>	44.12±0.0915 <sup>i</sup>	1.44±0.001 <sup>a</sup>
1	0	0	30	0.051±0.002 <sup>a</sup>	10.15±0.005 <sup>f</sup>	9.05±0.105 <sup>a</sup>	1.44±0.001 <sup>a</sup>
1	0	30	30	0.052±0.011 <sup>b</sup>	10.90±0.050 <sup>g</sup>	42.68±0.001 <sup>h</sup>	1.54±0.130 <sup>b</sup>
1	0.5	30	0	0.089±0.002 <sup>f</sup>	12.01±0.005 <sup>h</sup>	39.12±0.010 <sup>g</sup>	3.22±0.110 <sup>d</sup>
1	0.5	0	30	0.080±0.003 <sup>d</sup>	9.35±0.003 <sup>d</sup>	32.45±0.005 <sup>e</sup>	3.91±0.001 <sup>e</sup>
1	0.5	30	30	0.081±0.001 <sup>e</sup>	9.91±0.015 <sup>e</sup>	38.12±0.006 <sup>f</sup>	3.13±0.010 <sup>c</sup>
1	1	30	0	0.094±0.010 <sup>h</sup>	8.81±0.305 <sup>c</sup>	29.79±0.452 <sup>d</sup>	4.93±0.051 <sup>h</sup>
1	1	0	30	0.093±0.003 <sup>g</sup>	3.99±0.015 <sup>a</sup>	25.11±0.017 <sup>b</sup>	4.46±0.001 <sup>g</sup>
1	1	30	30	0.094±0.012 <sup>h</sup>	4.35±0.052 <sup>b</sup>	28.45±0.110 <sup>c</sup>	4.21±0.023 <sup>f</sup>

**Values are Mean ± Standard Error of Mean of quintuplicate determination for film's thickness and triplicate determination for the remaining parameters. Values with different alphabets within a sample are significant (p<0.05) along the column**

Significant increase in films thickness, solubility in water and elongation at break was observed in the presence of 30 %w/w glycerol but the tensile strength was significantly reduced. The introduction of 30 %w/w shea butter to 1 %w/v *Borassus aethiopum* starch resulted to a significant decrease in the film's thickness, solubility in water and elongation at break 0.051±0.002 mm, 10.15±0.005 % and 9.05±105 % respectively but



no significant change in the films tensile strength as recorded in Table 4.2. The combinatory effect of 30 %w/w glycerol and shea butter on 1 %w/v *Borassus aethiopun* starch films resulted to a significant higher film thickness, solubility in water, elongation at break and tensile strength when compared to the 1 %w/v films plasticized with only shea butter.

The introduction of 0.5 %w/w chitosan to 1 %w/v *Borassus aethiopun* starch in the presence of glycerol resulted to films with increased thickness and tensile strength  $0.089\pm0.002$ mm and  $3.22\pm0.110$  MPa, but lower water solubility and elongation at break  $12.01\pm0.005$  % and  $39.12\pm0.010$  % respectively when compared with glycerol plasticized film in the absence of chitosan recorded in Table 4.2.

Presence of 30 %w/w shea butter resulted to a significant decrease in the films thickness, solubility in water and elongation at break  $0.080\pm0.003$  mm,  $09.35\pm0.003$  % and  $32.45\pm0.005$  % respectively but the tensile strength  $3.91\pm0.001$  MPa significantly increased when compared to the films plasticized with only glycerol as recorded in Table 4.2.

The combined effect of 30 % glycerol and shea butter on 0.5/1 %w/v chitosan and *Borassus aethiopum* starch blends resulted to a significant increase in the films thickness, solubility in water, and elongation at break  $0.081\pm0.001$  mm,  $09.91\pm0.015$  % and  $38.12\pm0.006$  % respectively, but the tensile strength was significantly lower  $0.313\pm0.010$  Mpa compared to films with shea butter or glycerol as lone plasticizer, as reported in Table 4.2.

As the ratio of chitosan to *Borassus aethiopun* starch increases (1/1 %w/v) in the presence of 30 % glycerol, the films thickness and tensile strength significantly increased  $0.094\pm0.010$  mm and  $4.93\pm0.051$  MPa but solubility in water and elongation

at break were significantly lower when compared with films developed from 0.5 %w/v chitosan in 1 %w/v *Borassus aethiopum* starch. The elongation at break was also reduced to  $29.79 \pm 0.45$  % significantly but the tensile strength increased when compared with films from 0.5/1 % chitosan and starch respectively.

In the presence of 30 %w/w shea butter, blends of chitosan to *Borassus aethiopum* starch (1/1 %w/v) resulted to films with significantly reduced thickness, solubility in water, elongation at break and tensile strength  $0.093 \pm 0.003$  mm,  $03.99 \pm 0.015$  %,  $25.11 \pm 0.017$  % and  $4.46 \pm 0.001$  Mpa respectively when compared with glycerol plasticized film of the same composition in Table 4.2. Increased film thickness, solubility in water and elongation at break was observed when 30 %w/w of glycerol/shear butter was introduced, but the tensile strength was significantly reduced.

The results of the effects of glycerol and shea butter concentrations on the properties of films produce from 2 %w/v of *Borassus aethiopum shoot* starch are shown in Table 4.3.

**Table 4.3: Effect of Glycerol and Shea Butter Concentrations on the Properties of Films Prepared from 2 %w/v *Borassus aethiopum* Shoot Starch**

BS (g)	CH il(g)	GLY (w/w %)	SB (w/w %)	Thickness (mm)	Solubility (%)	Elongation at Break (%)	Tensile Strength (MPa)
2	0	30	0	0.095±0.007 <sup>e</sup>	26.01±0.092 <sup>e</sup>	26.09±0.001 <sup>f</sup>	4.22±0.021 <sup>e</sup>
2	0	0	30	0.092±0.003 <sup>b</sup>	10.51±0.001 <sup>b</sup>	24.49±0.001 <sup>d</sup>	3.95±0.061 <sup>b</sup>
2	0	30	30	0.093±0.003 <sup>c</sup>	10.81±0.801 <sup>c</sup>	22.60±0.031 <sup>b</sup>	4.30±0.131 <sup>f</sup>
2	0	50	0	0.096±0.004 <sup>d</sup>	26.91±0.030 <sup>f</sup>	24.73±0.011 <sup>e</sup>	4.19±0.031 <sup>c</sup>
2	0	0	50	0.077±0.010 <sup>a</sup>	5.21±0.011 <sup>a</sup>	22.20±0.003 <sup>a</sup>	3.61±0.018 <sup>a</sup>
2	0	50	50	0.095±0.047 <sup>f</sup>	11.12±1.003 <sup>d</sup>	23.72±1.303 <sup>c</sup>	4.21±0.047 <sup>d</sup>

**Values are Mean ± Standard Error of Mean of quintuplicate determination for film's thickness and triplicate determination for the remaining parameters. Values with different alphabets within a sample are significant (p<0.05) along the column**

Generally, as the solute concentration of *Borassus aethiopun* starch increases (2 %w/v), the thickness of the film also increases, which also reduced the transparency of the films as shown in plate II. The introduction of 30 %w/w glycerol in Table 4.3 resulted to a significant increase in the films thickness, solubility in water and elongation at break 0.095±0.007 mm, 26.01±0.092 % and 26.09±0.001 % respectively but a significant decrease in tensile strength 4.22±0.021 MPa when compared to the 2 %w/v starch without glycerol in Table 4.1. Introduction of 30 %w/w shea butter shows a significant reduction in the films thickness, solubility in water, elongation at break and tensile strength.

As the concentration of glycerol was increased to 50 %w/w of 2 % starch, film thickness, the elongation at break and solubility significantly increases but the tensile strength reduced. Significant decrees in the film thickness was observed (0.077±0.010 mm) as the concentration of shea butter increases to 50 %w/w. solubility, elongation at

break and tensile strength also reduced significantly, as recorded in Table 4.3. The combined effect of 50 %w/w of glycerol and shea butter resulted to a significant increase in the films thickness, solubility, elongation at break and tensile strength 0.095±0.047 mm, 11.12±1.003 %, 23.72±1.303 % and 4.21±0.047 MPa, respectively as shown in Table 4.3.

The results of the effects of glycerol and shea butter concentrations on the properties of films produce from blends of 1 % chitosan and 2 % *Borassus aethiopum* shoot starch are shown in Table 4.4.

**Table 4.4: Effect of Glycerol and Shea Butter as Plasticizers on the Properties of Films Prepared from Blends of 2 %w/v *Borassus aethiopum* Shoot Starch and 1 %w/w of Chitosan**

BS (g)	CHi (g)	GLy (w/w )	SB (w/w )	Thickness (mm)	Solubility (%)	Elongation at Break (%)	Tensile Strength (MPa)
2	1	30	0	0.163±0.001 <sup>c</sup>	15.91±0.008 <sup>f</sup>	17.12±1.011 <sup>d</sup>	6.45±0.008 <sup>e</sup>
2	1	0	30	0.161±0.002 <sup>a</sup>	8.65±0.105 <sup>c</sup>	16.98±0.001 <sup>b</sup>	6.35±0.005 <sup>b</sup>
2	1	30	30	0.165±0.002 <sup>d</sup>	10.62±1.001 <sup>d</sup>	16.99±0.001 <sup>b</sup>	6.41±0.081 <sup>d</sup>
2	1	50	0	0.172±0.008 <sup>f</sup>	13.41±0.001 <sup>e</sup>	17.43±0.051 <sup>c</sup>	6.15±0.085 <sup>a</sup>
2	1	0	50	0.161±0.010 <sup>b</sup>	5.41±0.005 <sup>a</sup>	16.65±0.041 <sup>a</sup>	6.39±0.011 <sup>c</sup>
2	1	50	50	0.165±0.070 <sup>c</sup>	6.68±0.021 <sup>b</sup>	16.69±0.023 <sup>a</sup>	6.41±0.091 <sup>d</sup>

**Values are Mean ± Standard Error of Mean of quintuplicate determination for film's thickness and triplicate determination for the remaining parameters. Values with different alphabets within a sample are significant (p<0.05) along the column**

Films from blends of 2 %w/v *Borassus aethiopum* shoot starch and 1 %w/w chitosan in the presence of 30 %w/w glycerol, resulted to a significant increase in thickness,

solubility in water and elongation at break  $0.163 \pm 1.001$  mm,  $15.91 \pm 0.008$  % and  $17.12 \pm 1.0116$  % respectively, but significant reduction in the films tensile strength was observed when compared with the same films without glycerol in Table 4.1

The introduction of 30 %w/w shea butter led to a significant lower film thickness, solubility, tensile strength and elongation at break as indicated in Table 4.4. As the concentration of glycerol increases (50 %w/w), the films thickness and elongation at break significantly increased but the tensile strength decreased significantly as recorded in Table 4.4. Presence of 50 %w/w of shea butter resulted to a significant reduction of the film's thickness, solubility in water and elongation at break but the tensile strength was significantly higher.

The results of the effects of glycerol and shea butter concentrations on the properties of films produce from blends of 2 % chitosan and *Borassus aethiopum* shoot starch are shown in Table 4.5.

**Table 4.5: Effect of Glycerol and Shea Butter Concentration on the Properties of Films Prepared from Blend of 2 %w/w Chitosan and *Borassus aethiopum* Shoot Starch Composites**

BS (g)	CHi (g)	GLy (w/w %)	SB (w/w %)	Thicknes s (mm)	Solubility (%)	Elongation at Break (%)	Tensile Strength (MPa)
2	2	30	0	0.206±0.005 <sup>c</sup>	5.50±0.010 <sup>f</sup>	13.22±0.002 <sup>a</sup>	7.66±0.007 <sup>b</sup>
2	2	0	30	0.204±0.013 <sup>b</sup>	4.11±0.010 <sup>c</sup>	13.80±0.010 <sup>e</sup>	7.60±0.001 <sup>a</sup>
2	2	30	30	0.204±0.012 <sup>b</sup>	4.72±0.001 <sup>d</sup>	13.86±0.044 <sup>f</sup>	7.71±0.010 <sup>d</sup>
2	2	50	0	0.208±0.001 <sup>d</sup>	5.41±0.010 <sup>e</sup>	16.37±0.001 <sup>b</sup>	7.69±0.030 <sup>c</sup>
2	2	0	50	0.203±0.009 <sup>a</sup>	3.41±0.001 <sup>a</sup>	13.56±0.011 <sup>c</sup>	7.86±0.101 <sup>f</sup>
2	2	50	50	0.209±0.101 <sup>e</sup>	3.91±0.001 <sup>b</sup>	13.78±0.003 <sup>d</sup>	7.82±0.021 <sup>e</sup>

**Values are Mean ± Standard Error of Mean of quintuplicate determination for film's thickness and triplicate determination for the remaining parameters. Values with different alphabets within a sample are significant (p<0.05) along the column**

The introduction of 2 %w/w chitosan to 2 %w/v *Borassus aethiopum* starch in the presence of 30 % glycerol resulted to a significant increase in films thickness and tensile strength 0.206±0.005 mm and 7.66±0.007 MPa respectively, but a lower solubility in water and elongation at break as recorded in Table 4.5.

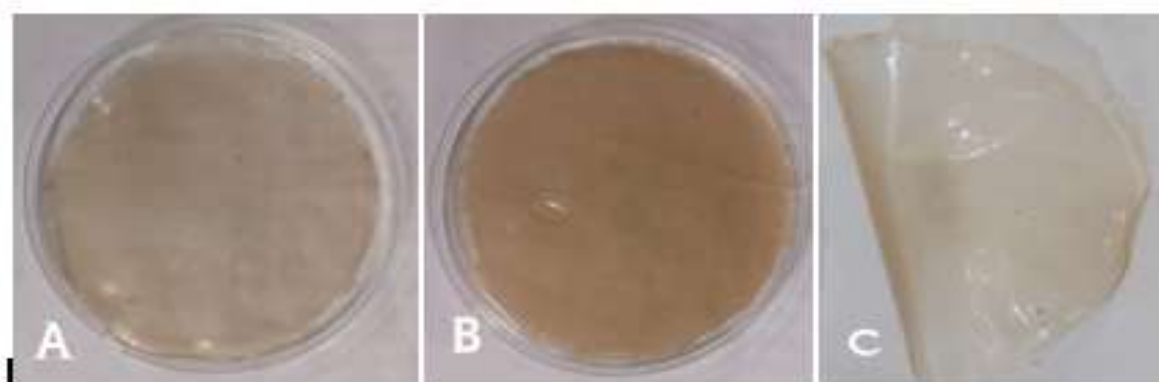
The incorporations of shea butter (30 %w/w) resulted to a significant reduction in the films thickness, solubility and tensile strength, but an increased elongation at break.

Increase of the glycerol volume to 50 %w/w, resulted to a significant higher film thickness, solubility in water and elongation at break 0.208±0.001 mm, 5.41±0.010 % and 16.37±0.001 % respectively, but a reduced tensile strength. The introduction of 50 %w/w of shea butter resulted to a significantly lower film thickness and solubility but significantly higher elongation at break and tensile strength as recorded in Table 4.5. Combined effect of 50 % glycerol and shea butter shows a significant higher film

thickness and tensile strength, but a lower elongation at break and solubility in water when compared with the same film composition plasticized with 30 % glycerol and shea reported in Table 4.5.

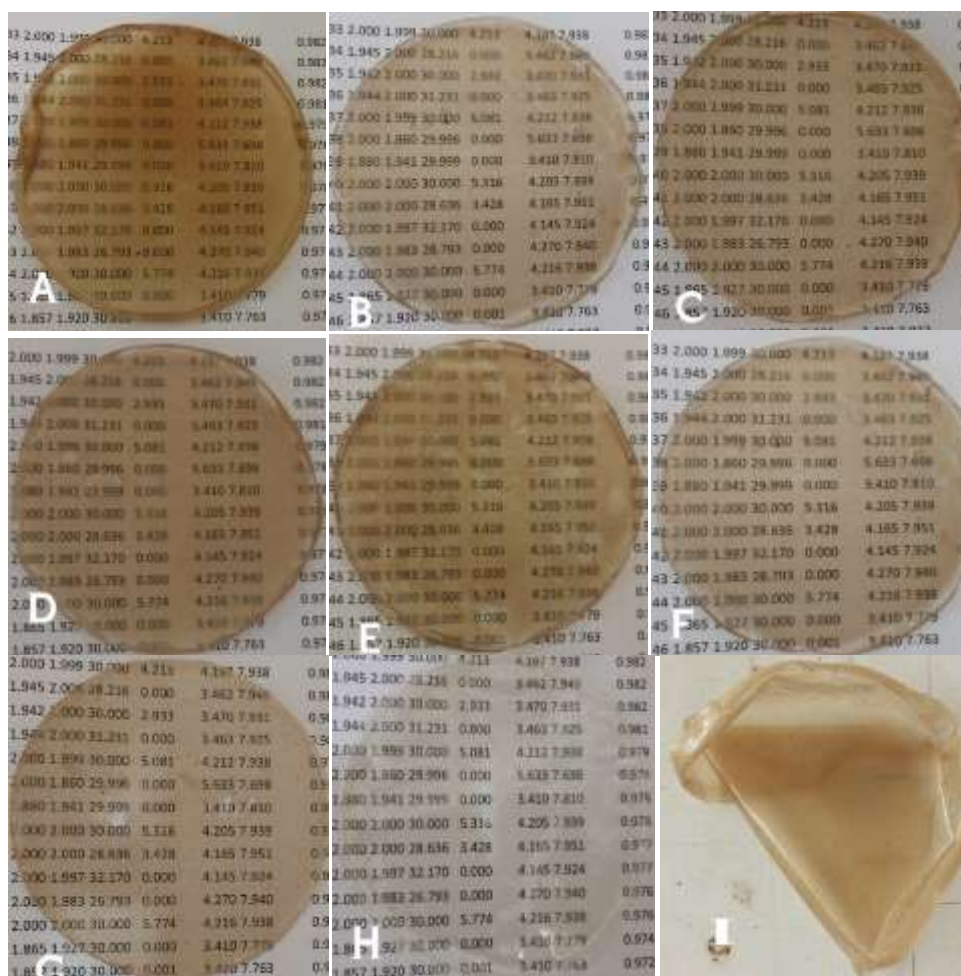
The form and transparency of glycerol and shea butter plasticized films from blends of chitosan and *Borassus aethiopum* shoot starch are shown in Plate II and III.

The representation labeled A in Plate II shows the nature of film from chitosan/*Borassus aethiopum* shoot starch plasticized with glycerol. Nature of shea butter plasticized film is represented in Plate II, labeled B and the C shows the flexible nature of the combined effect of glycerol and shea butter on the film.



**Plate II: Nature of *Borassusaethiopum*starch/Chitosan Films (BSCF) Plasticized by Glycerol and Shea Butter**

The representation labeled A in Plate III, shows the effect of 30 % w/w shea butter and 13 %w/w glycerol on the transparency of film from 2 % starch and chitosan. C, E and F label in Plate III shows the effect of low concentrations of shea butter to 30 %w/w glycerol on 2 % starch and chitosan. The label B, D and G are films from 1 % chitosan and starch. The less opaque film labeled H in Plate II, is formed from 2 % of chitosan plasticized with glycerol and shea butter. The film labeled I shows the degree of flexibility when properly plasticized film with glycerol and shea butter.



**Plate III: Degree of Transparency and Flexibility of Films Prepared from Optimized *Burassus aethiopicum* Shoot Starch and Chitosan Blends**

Significant decrease in the transparency of BSCF is observed with the increase in starch concentration from 1 % to 2 % as shown in Table 4.6. The films transmittance increased from 0.389 to 1.461 irrespective of the plasticizer type.



**Table 4.6: Transparency Index of Optimized BSCF**

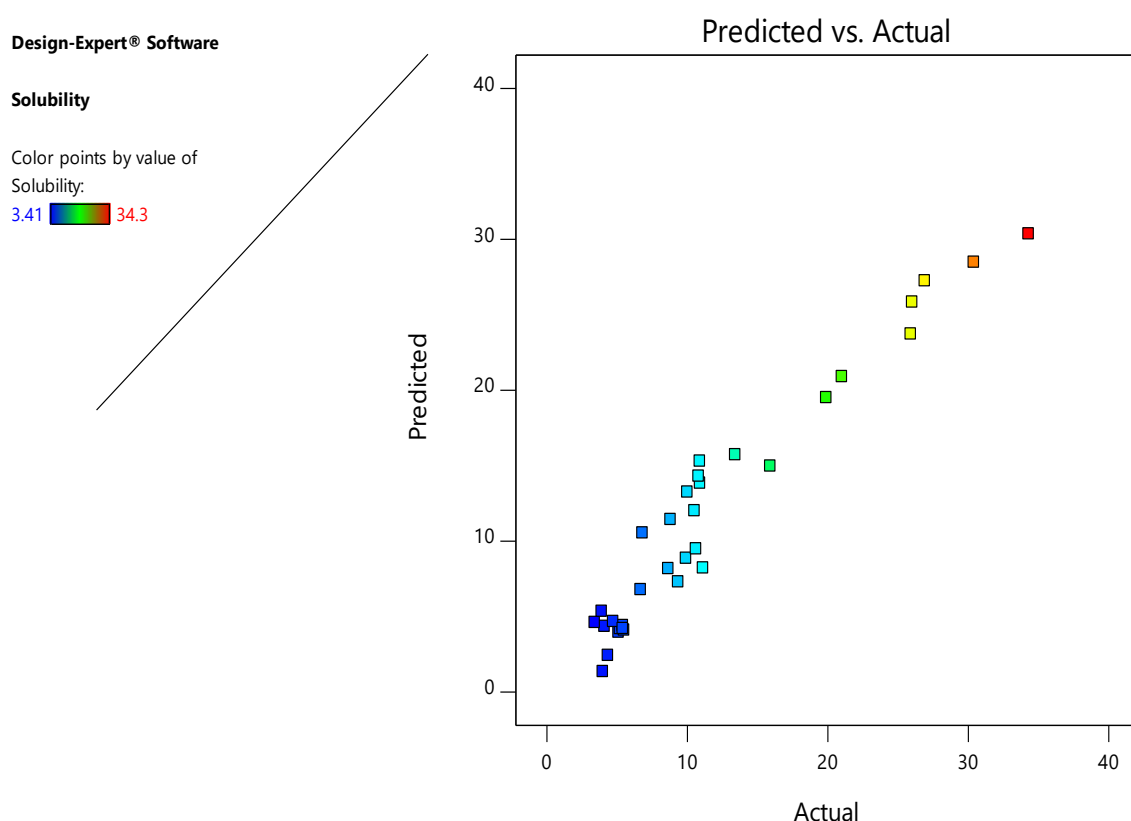
B.S(g)	CHI(g)	GLY(%w/w)	SB(%w/w)	Transparency(%)
1	1	30	1	30.1
1	1	1	30	25.2
1	1	30	30	21.5
2	1	30	10	9.7
2	0	0	10	10.7
2	2	30	1	9.1
2	2	30	13	6.8
0	2	30	10	40.4

#### 4.1.4 Optimization of production compositions of films from blends of *Borassus aethiopum* shoot starch and chitosan

The optimization of film from *Borassus aethiopum* shoot starch/chitosan blends was targeted toward two critical properties of the starch films (water resistivity and tensile strength). Degree of water solubility of the modified starch film presents the response. Prior to the selection of the minimum and the maximum values of the independent variables (B.S, CHI, GLY and S.B), preliminary experiment was carried out. The codes, ranges, levels and the type of factor studied are presented in Table 4, appendix D.

Thirty three experimental runs in Table 4, appendix D were carried out in order to establish the relationship that exists between the independent variables and the

dependent variable of the modified starch. The variables were analyzed using Historical Data Design in order to have the predicted and the residual values which is a measure of closeness between the actual and predicted solubility and tensile strength. Zero residual sum show a perfect closeness, the 9E-06 residual value in this study shows that the predicted is adequately closed to the actual values. The plot in Figure 4.2 and 4.3 shows closeness between the predicted against the actual solubility and tensile strength of BSCF as most of the points falls along the lines.



**Figure 4.2: Plot of Predicted Against the Actual Solubility of BSCF**

From the three models tested for model significance, the two factors interaction (2FI) model has the highest  $R^2$  (0.9392), Adjusted  $R^2$  (0.9115), and Predicted  $R^2$  (0.8301), as well as significant P-value of  $<0.0001$ . The Predicted  $R^2$  of 0.8301 is in reasonable agreement with the Adjusted  $R^2$  of 0.9115; i.e. the difference is less than 0.2. The linear

model was rejected due to lower value of  $R^2$  (0.7032), Adjusted  $R^2$  (0.6608), and Predicted  $R^2$  (0.5819), while the quadratic model was rejected due to insignificant P-value of 0.8899 and it is aliased.

**Table 4.7: Model Summary Statistics for Solubility of BSCF**

Source	Std. Dev.	P-Value	$R^2$	Adjusted $R^2$	Predicted $R^2$	
Linear	4.95	<0.0001	0.7032	0.6608	0.5819	
<b>2FI</b>	<b>2.53</b>	<b>&lt;0.0001</b>	<b>0.9392</b>	<b>0.9115</b>	<b>0.8301</b>	<b>Suggested</b>
Quadratic	2.68	0.8899	0.9411	0.9008	0.8078	Aliased

#### **4.1.4.1 Final Equation in Terms of Coded Factors**

$$\text{Solubility} = +3.75 + 6.44A - 13.72B + 0.8658C - 7.53D + 8.06AB + 0.2001AC + 2.94AD - 0.8201BC + 5.04BD + 0.1235CD$$

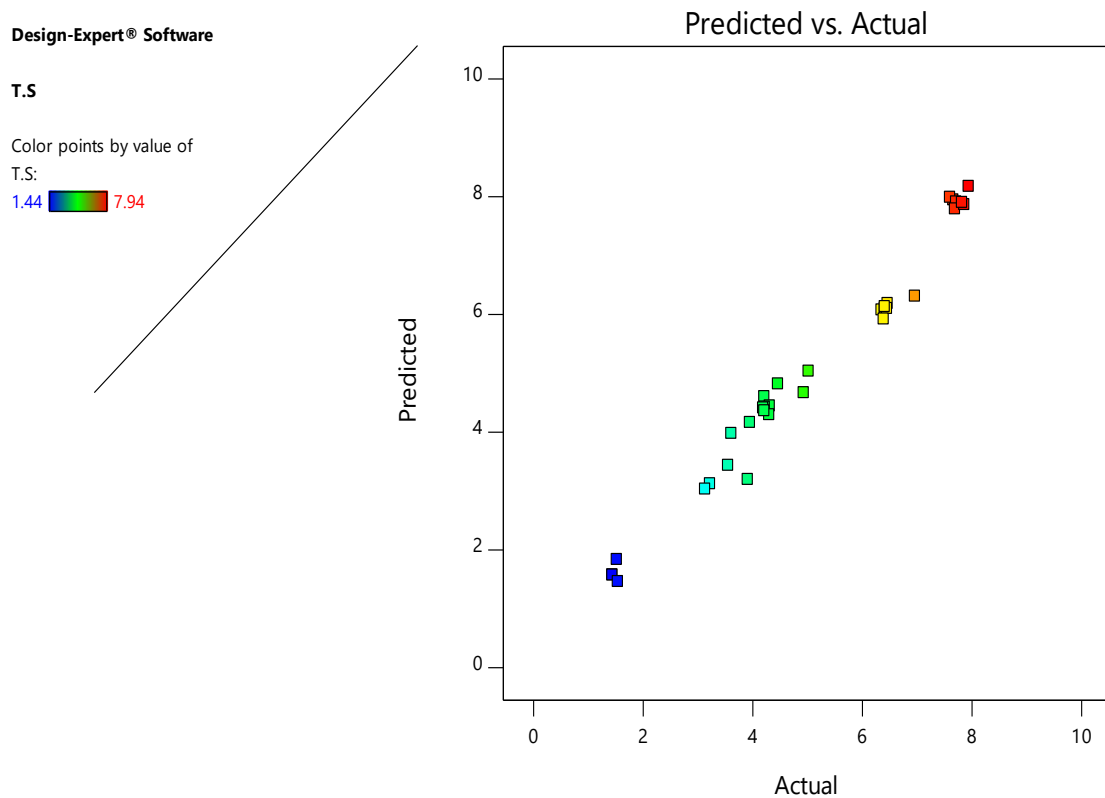
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

#### **4.1.4.2 Final Equation in Terms of Actual Factors**

$$\text{Solubility} = +33.24392 - 4.75820B.S - 26.00460CHI + 0.054487GLY - 0.625314S.B + 8.06044B.S*CHI + 0.008006B.S*GLY + 0.117419B.S*S.B - 0.032804CHI * GLY + 0.201748CHI* S.B + 0.000198GLY* S.B$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative

impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.



**Figure 4.3: Plot of Predicted Against the Actual Tensile Strength of BSCF**

Out of the three models tested for model significance, the two factors interaction (2FI) model has the highest  $R^2$  (0.9794), Adjusted  $R^2$  (0.9701), and Predicted  $R^2$  (0.9554), as well as significant P-value of 0.0031. The Predicted  $R^2$  of 0.9554 is in reasonable agreement with the Adjusted  $R^2$  of 0.9701; i.e. the difference is less than 0.0147. The linear model was rejected due to lower value of  $R^2$  (0.9529), Adjusted  $R^2$  (0.9461), and Predicted  $R^2$  (0.9357), while the quadratic model was rejected because it is aliased.

**Table 4.8: Model Summary Statistics for Tensile Strength of BSCF**

Source	Std. Dev.	Sequential p-value	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	
Linear	0.4768	< 0.0001	0.9529	0.9461	0.9357	
<b>2FI</b>	<b>0.3555</b>	<b>0.0031</b>	<b>0.9794</b>	<b>0.9701</b>	<b>0.9554</b>	<b>Suggested</b>
Quadratic	0.2390	0.0004	0.9920	0.9865	0.9784	Aliased

**4.1.4.3: Final equation in terms of coded factors**

$$\text{T.S.} = +4.66 + 1.46\text{A} + 3.15\text{B} - 0.2004\text{C} - 0.0759\text{D} - 1.33\text{AB} + 0.2013\text{AC} - 0.0132\text{AD} - 0.0873\text{BC} + 0.0394\text{BD} + 0.1047\text{CD}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

**4.1.4.4: Final equation in terms of actual factors**

$$\begin{aligned} \text{T.S.} = & -0.770290 + 2.60779\text{B.S} + 4.53296\text{CHI} - 0.016762\text{GLY} - 0.008275\text{S.B} - 1.33425 \\ & \text{B.S} * \text{CHI} + 0.008050\text{B.S} * \text{GLY} - 0.000527\text{B.S} * \text{S.B} - 0.003491\text{CHI} * \\ & \text{GLY} + 0.001578\text{CHI} * \text{S.B} + 0.000167\text{GLY} * \text{S.B} \end{aligned}$$

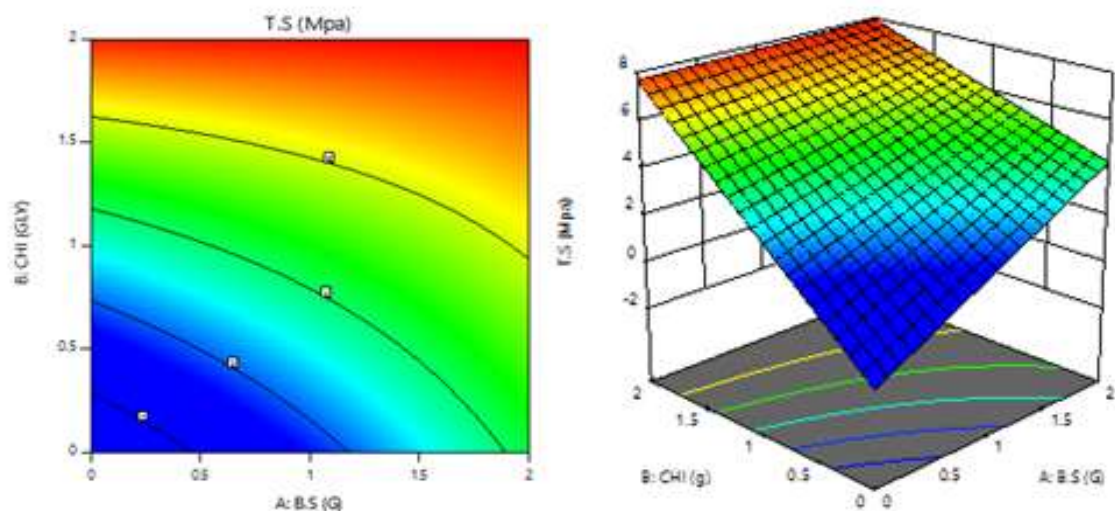
The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative

impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

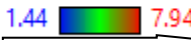
#### 4.1.5.3 Interactive effect of the process variables on the tensile strength of BSCF

The contour plot and 3D representation in Figure 4.6 shows the interactive effect of chitosan and *Borassus aethiopum* starch. The area with blue coloration is the solubility zone while the red zone is the desirability of the film.

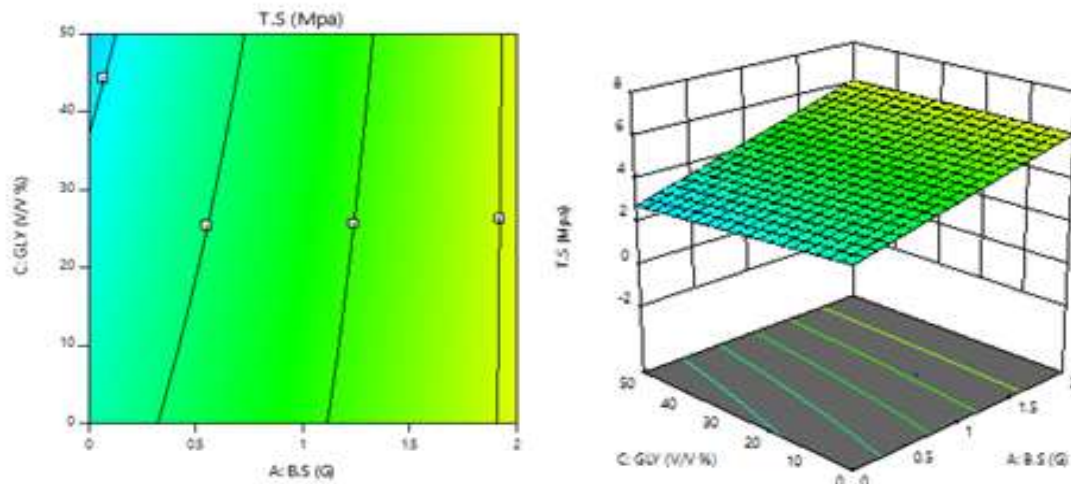
The interactive effect of CHI and B.S is shown in Figure 4.4. As both the CHI and B.S increases from 0 to 2 %, the tensile strength increases from 0 to 6 MPa. The highest tensile strength is represented with the green area while the lowest tensile strength falls within the blue area.




**Figure 4.4: 3D Representation and Contour Plot of the Interactive Effect of CHI and B.S on Tensile Strength of BSCF**

Key  T.S (Mpa) 1.44 7.94 Strength increases as colour graduate from to green to yellow to red.

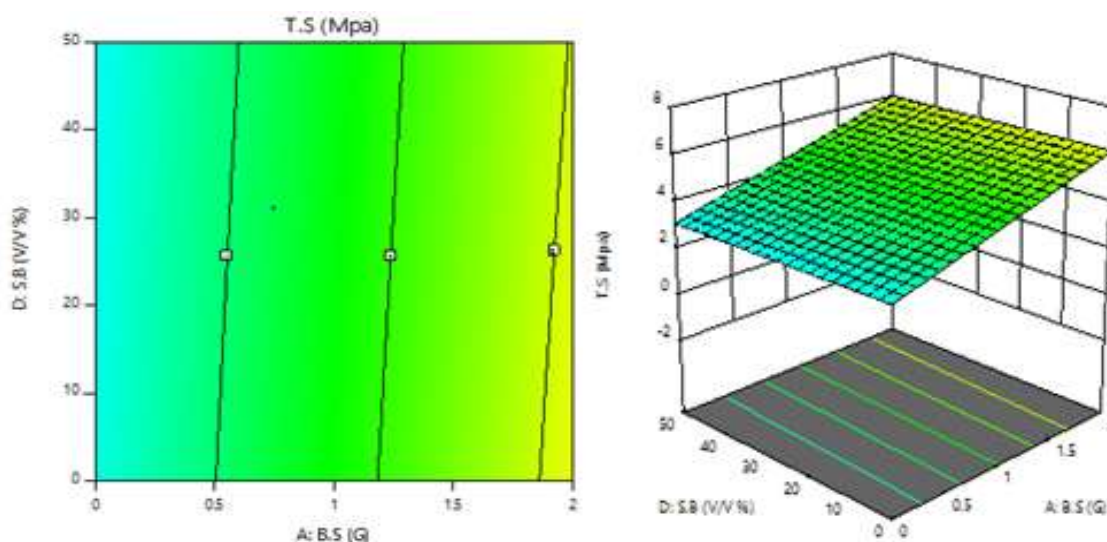
The interactive effects between B.S and GLY are presented in Figure 4.5. As B.S increases from 0 to 2 % and GLY increases from 0 to 50 %, the tensile strength also increases from 3 to 6 MPa.



**Figure 4.5: Contour plot and 3D Representation of the Interactive Effect of GLY and B.S on Tensile Strength of BSCF**

Key  T.S (Mpa) 1.44 7.94 Strength increases as colour graduate from to green to yellow to red.

The interactive effect of S.B and B.S on the tensile strength of BSCF is presented in Figure 4.6. It can be clearly seen that as S.B increases from 0 to 50 % and B.S increases from 0 to 2 %, the tensile strength increases from 4 to 6 MPa. This shows that the combined effect of both S.B and B.S has little contribution to the tensile strength of the BSCF.

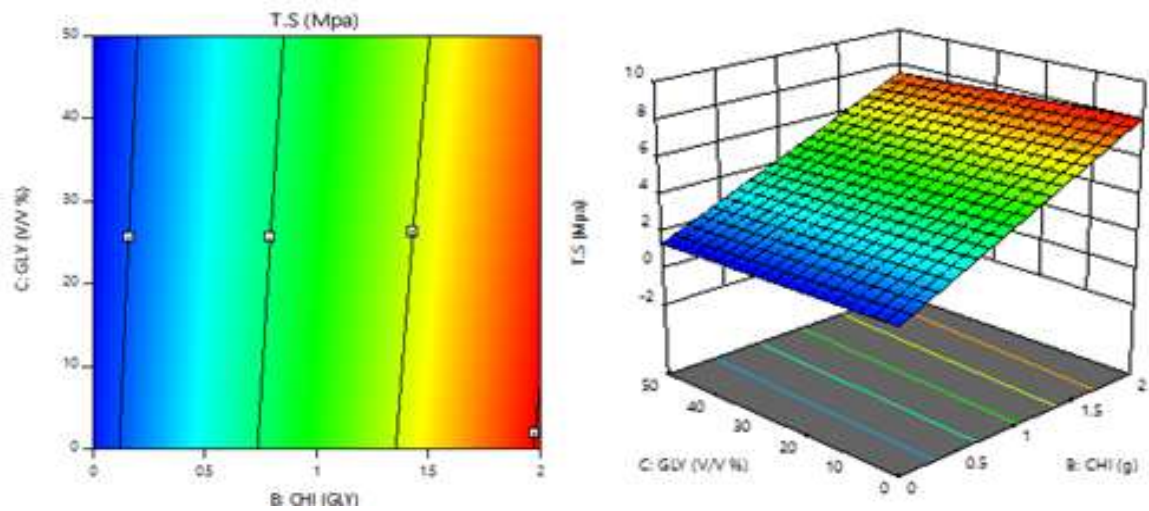


**Figure 4.6: Contour Plot and 3D Representation of the Interactive Effect of S.B And B.S on Tensile Strength**

**T.S (Mpa)**  
 Key 1.44 7.94 Strength increases as colour graduate from to green to yellow to red

The contour plot of the interactive effect is presented in Figure 4.7. As the amount of the glycerol was increased from 0 to 50 % and the CHI was also increased from 0 to 2 %, the tensile strength also experiences an increase from 2 to 8 MPa. However, the tensile strength of 8 MPa can only be obtained at reduced GLY and increased CHI content.

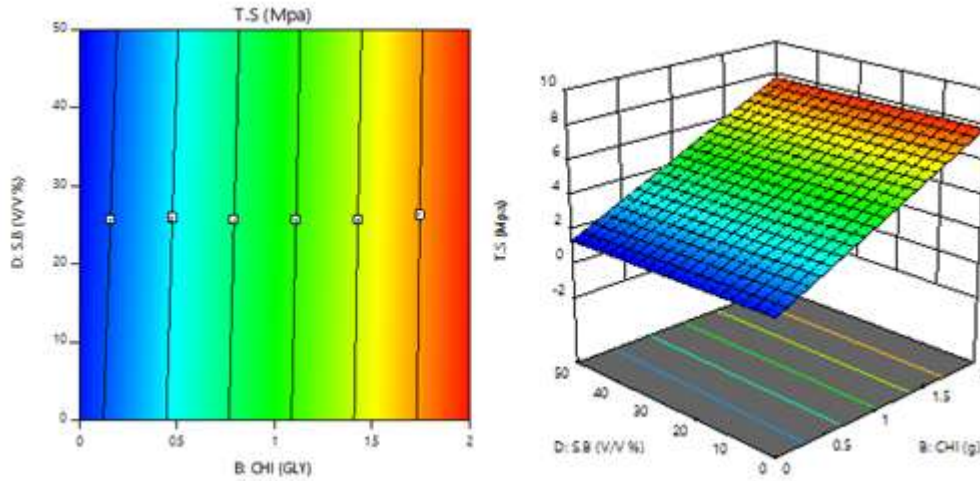




**Figure 4.7: Contour Plot and 3D Representation of the Interactive Effect of GLY and CHI on Tensile Strength of BSCF**

Key **T.S (Mpa)** 1.44 7.94 Strength increases as colour graduate from to green to yellow to red

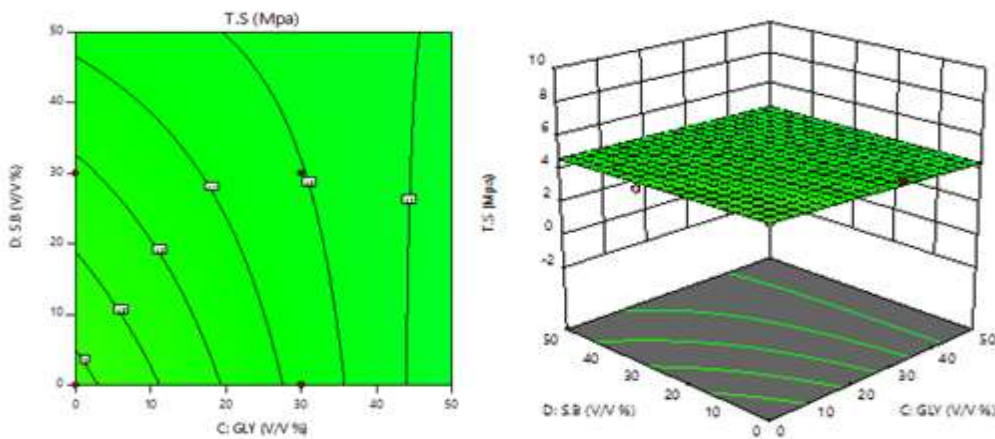
The contour plot of the interactive effect is presented in Figure 4.8. As the amount of the S.B was increased from 0 to 50 % and the CHI was also increased from 0 to 2 %, the tensile strength also experiences an increase from 2 to 7 MPa.



**Figure 4.8: Contour Plot and 3D Representation of the Interactive Effect of S.B and CHI on Tensile Strength of BSCF**

**T.S (Mpa)**  
 Key 1.44 7.94 Strength increases as colour graduate from to green to yellow to red

The combined effect of S.B and GLY is presented in Figure 4.9. As both S.B and GLY increases from 0 to 50 %, the tensile strength decreases from 5 to 4.5 MPa.

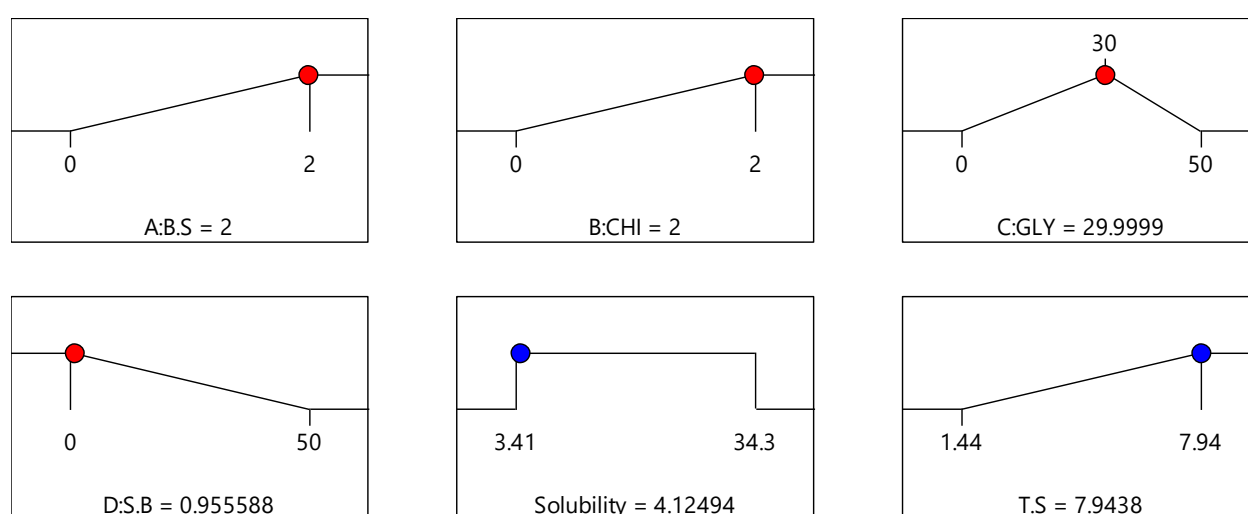


**Figure 4.9: Contour Plot and 3D Representation of the Interactive Effect of S.B and GLY on Tensile Strength of BSCF**

**T.S (Mpa)**  
 Key 1.44 7.94 Strength increases as colour graduate from to green to yellow to red

**Table 4.9: Optimization Study Constraints on the Tensile Strength of BSCF**

Name	Goal	Lower Limit	Upper Limit
A:B.S	Maximize	0	2
B:CHI	Maximize	0	2
C:GLY	is target = 30	0	50
D:S.B	Minimize	0	50
Solubility	is in range	3.41	34.3
T.S	Maximize	1.44	7.94



Desirability = 0.996

**Figure 4.10: Optimum Point Prediction of Tensile strength on BSCF**

#### 4.1.6 Effect of glycerol and shea butter on the degradability of films from blends of *Borassus aethiopum* shoot starch and chitosan

The tests for the time taken for the various compositions of the films to decompose were carried out placing equal size of the films in a loamy soil. Films from 1% w/v of *Borassus aethiopum* shoot starch were observed to have decomposed first. Table 4.10-

4.12, shows the time taken for the tested samples and their various concentrations to have completely decomposed.

**Table 4.10: Degradation Time for Films Produced from 1 %w/v *Borassus aethiopum* Shoot Starch and 0.5-1 %w/w Chitosan Plasticized with Glycerol and Shea Butter**

<b>B.Starch (BS) (g)</b>	<b>Chitosan (CHI) (g)</b>	<b>Glycerol (GLY) (w/v%)</b>	<b>Shea Butter (SB) (w/v%)</b>	<b>Time (days)</b>
1	0	0	0	6
1	0	30	0	6
1	0	0	30	8
1	0	30	30	7
1	0.5	0	0	10
1	0.5	30	0	9
1	0.5	0	30	11
1	0.5	30	30	10
1	1	0	0	11
1	1	30	0	11
1	1	0	30	13
1	1	30	30	12

It took six days for a quarter of 100 mm film produced from 1 %w/v *Borassus aethiopum* shoot starch to decompose by the action of soil microbes. Time taken was not altered by the presence of glycerol but shea butter plasticized film decomposed after eight days in the soil. The decomposition was faster in film plasticized with both glycerol and shea butter (7 days). The incorporation of 0.5 % w/w of chitosan resulted

to slower decomposition rate even though glycerol introduction terms to increase the rate of decomposition. Shea butter introduction decreases the decomposition time as shown in Table 4.10. Films from equal ratio of starch to chitosan term to have a lower decomposition rate. The introduction of glycerol did not affect the decomposition time of films from 1 %w/v of both *Borassus aethiopum* shoot starch and chitosan. Shea butter presence further lower the decomposition rate (13 days), but the combinatory effect of glycerol and shea butter on 1 %w/v of the film increased the decomposition by aday.

**Table 4.11: Degradation Time for Films From 2 %w/v *Borassus aethiopum* Shoot Starch Plasticized with Glycerol and Shea Butter**

<b>B.Starch (BS) (g)</b>	<b>Chitosan (CHI) (g)</b>	<b>Glycerol (GLY) (w/v%)</b>	<b>Shea Butter (SB) (w/v%)</b>	<b>Time</b>
2	0	0	0	9
2	0	30	0	9
2	0	50	0	9
2	0	0	30	10
2	0	0	50	10

Films from increased concentration of *Borassus aethiopum* shoot starch at 2 %w/v decompose on the 9<sup>th</sup> day even in the presence of glycerol at high and low concentration. On the other hand, the presence of shea butter reduces the decomposition rate by a day both at high and low concentration as recorded in table 4.11.

The introduction of 1 % chitosan to 2 %w/v *Borassus aethiopum* shoot starch further elongates the degradation time of the films. The presence of glycerol and shea butter tends to reduced to decomposition time. Film plasticized by shea butter demonstrated a longer decomposition time.

**Table 4.12: Degradation Time for 2 %w/vB.Starch and 1-2 %w/w Chitosan Films Plasticized with 30/50 %w/w GLY and SB**

<b>B.Starch (BS) (g)</b>	<b>Chitosan (CHI) (g)</b>	<b>Glycerol (GLY) (w/v%)</b>	<b>Shea Butter (SB) (w/v%)</b>	<b>Time</b>
2	1	0	0	14
2	1	30	0	13
2	1	0	30	19
2	1	30	30	20
2	2	0	0	18
2	2	30	0	17
2	2	0	30	30
2	2	30	30	29
2	2	50	0	17
2	2	0	50	33
2	2	50	50	31

Increase solute concentration of chitosan to starch composite affects the degradation time. Equal ratio of starch to chitosan at 2 %w/v in the absent of plasticizers increases the decomposition time (18 days). Glycerol incorporation lowered the degradation time by a day but the presence of shea butter elongates the degradation time (30 days).

Increase in glycerol concentration does not affect the degradation time but films from increased concentration of shea butter have a longer degradation time up to 33 days, as recorded in Table 4.12.

## **4.2 Discussion**

### **4.2.1 Starch percentage composition**

In this study, the dry weight of *Burassus aethiopum* shoot starch was 20 %, which cannot be considered as a high starch source by dry weight because other starch sources like potatoe has dry weight from 13 to 23 %, cassava starch has 32 to 35 % and corn starch has 50 to 70 % as reported by Haase (2003) and Montagnac *et al.* (2009). The yield of starch is related to dry matter content (Nwachukwu *et al.*, 1997), which is important in film processing because, the higher the dry matter content, less starch content is needed for film production. Thus minimizing the quantity of starch needed for the production of biodegradable films. Starch from potatoes with dry weight of 13 to 23 % is deployed in many industrial applications (General thickener, binder, texturizer, anti-caking and gelling agent) and since the starch from *Burassus aethiopum* has a dry weight of 20 % which is in range with potatoe starch, it can therefore be utilized in many industrial applications such as gelling agent, thickner, texturizer and binders. *Burassus aethiopum* shoot starch has 75.4 % amylose content, which is considered as high amylose starch when compared with other starch sources like corn starch which have 28 to 70 % (Shogren, 1998), wheat has 35 % (Hung *et al.*, 2007), potato has 20 % (Haase, 2003) and cassava has 23.7 % (Yuan *et al.*, 2007). Starch amylose content is an important factor for biodegradable film production as it is responsible for gelatinization and retrogradation, which are required during film formation. Normal starch consists of about 75 % amylopectin and 25 % amylose, waxy starches consist of mainly amylopectin and 0–8 % amylose, and high amylose starches consist of 40–70 %

amylose and more (Thawien, 2012). Pure amylose structure is very stable, with strong molecular orientations, which are denser and stronger (Lourdin *et al.*, 1995). The ability of amylose to produce self supporting films has been known for a long time and this is ascribed to the ability of its linear chains to interact by hydrogen bonds to a higher degree than the branched amylopectin chains (Rindlav *et al.*, 2002). The studies of Alves *et al.* (2007) on the effect of amylose enrichment on cassava starch films properties also pointed out the mechanical and barrier properties of cassava films were influenced by the amylose contents. The amylose enrichment produced stronger films as a result of starch network formation during drying of film-forming solutions when water evaporates. During this stage the interactions of starch chains induced by higher amylose contents could facilitate the formation of matrix with more polymer content per area. Muscat *et al.* (2012) further studied the effect of low and high amylose starches on film forming behavior. They discovered that, films with high amylose content showed higher tensile strength and modulus of elasticity values and lower elongation values than low amylose starch films. During this stage, the proximity of starch chains induced by higher amylose contents could facilitate the formation of a matrix with more polymer content per area.

The excellent film forming ability of *Borassus aethiopum* shoot starch under low temperature depict the characteristics of films from other starch sources such as corn starch with high amylose ratio ( $\leq 70$  %). This implies that films from *Borassus eathiopum* shoot starch could be deployed for several domestic and industrial applications.



#### **4.2.2 Properties of films produced from *Borassus aethiopum* shoot starch and chitosan blends**

Results from Table 4.1 shows the various ratios of starch to chitosan blends in the absence of plasticizers. The thickness of the films ranges from 0.043 to 0.205 mm which is observed to be dependent on the films solute compositions. The variations of thickness within the different formulations, is associated to the film solute concentrations. From Table 4.1, 1 %w/v of *Borassus eathiopum* shoot starch film has lower thickness because of the low concentration of the base material in forming the film, but as the concentration of both the starch and chitosan increases, the thickness increased significantly which confirms the relationship between solute concentration and size. This result is in accordance with the study of Ghasemlou *et al.* (2013) on corn starch. The thickness of the corn starch increased from 0.151 to 0.266 mm as the solute concentration increases. Thickness is a crucial parameter for mechanical and barrier properties of the films. It depends on the film preparation method, the flatness of the dish surface during the drying process. Despite the same volume of film forming solutions were used for the film casting, the drying time for complete solvent evaporation increased with increased solute concentration. The films thickness is dependent on both film composition and processing methods (Garcia *et al.*, 2009). Several authors reported the effects of thickness on the permeability of edible matrix (Galus and Lenart, 2013). Thus, the basic parameter used in determining the feasibility of films as packaging materials for consumable products.

In the absence of any plasticizers, the solubility of films from *Burassus aethiopum* shoot starch decreased from 30.4 to 25.91 % as solute concentration of starch increases and further decreased to 5.11 % in the presence of 2 % chitosan as reported in Table 4.1. The reduction in films solubility in the presence of chitosan may be due to the

nature of chitosan. Uneven acetyl group distribution in chitosan is often associated to its hydrophobic nature which is the reason why films with chitosan incorporation has low solubility in water but the degree of deacetylation often affects these properties.

This property of starch is very important to ascertain films reaction with water, so that when ingested it can be digested properly, or when discarded into the environment, it can decompose naturally. Soluble-film packaging is convenient to use in ready to eat food products due to its ability to melts in boiled water or in the consumer's mouth (Pitak and Rakshit, 2011). In another way round, there is a need for a biodegradable film to have a low solubility for the enhancement of product integrity and water resistance (Perez and Krochta, 1999). Therefore, the films solubility is tailored to the needs of the user since high solubility cannot protect the product from humidity and water loss.

Elongation at break (EAB) is the extendibility of film length from initial length to the point of break. That is, the ability of films to deform before finally breaking. Films from *Burassus aethiopum* shoot starch have range of elongation at break from 9.34 to 29.15 % as reported in Table 4.1. As the concentration of the starch increases, the EAB decreased. This result is similar to that of cassava starch which has 11 to 27 % elongation at break as reported by Parra *et al.* (2004). This reduction in films elongation at break with increased starch solute concentration may be due the formation of strong intermolecular bonds within the starch molecules. Chitosan incorporation did not ameliorate this property (low EAB) as seen in Table 4.1. This effect is basically due to the formation of stronger bonds within the chitosan chain. The strong intermolecular bonds between the hydrogen molecules of chitosan  $\text{NH}_4^+$  backbone and  $\text{OH}^-$  of the *Burassus aethiopum* shoot starch did not permit proper stretching thereby increasing the films tensile strength which is may be the reason for the lower elongation at break.

The tensile strength values of the blended films with chitosan increased as chitosan to *Burassus aethiopum* shoot starch ratio increases, as shown in Table 4.1. This effect is attributed to the formation of intermolecular hydrogen bonds between  $\text{NH}_4^+$  of the chitosan backbone and  $\text{OH}^-$  of the *Burassus aethiopum* shoot starch. Increase in the concentration of both starch and chitosan gave rise to a significant increase in the films tensile strength (TS) from 1.52 to 7.94 MPa in the absence of plasticizers. These results may be due to the fact that starch films obtained from high amylose content starch usually have bigger crystalline domains as reported by Garcia *et al.* (2000), leading to greater mechanical resistance compared to films made from starch with a lower amylose content Phetwarotai *et al.* (2012). TS is one of the major properties of starch films required for packaging.

#### **4.2.3 Effects of glycerol and shea butter as plasticizers on some properties of films produced from the composites of *Borassus aethiopum* shoot starch and chitosan blends**

The results in table 4.2-4.5 indicated that glycerol and shea butter interaction significantly affected films thickness, solubility elongation at break and tensile strength. The introduction of glycerol was observed to have increased the thickness of the films both at 1 % and 2 % of the starch films. This effect of increasing concentrations of the base material on the increase of the films thickness observed in this study relates with the results of previous studies reported by Kokoszka *et al.* (2010), on soy protein isolate-based edible films with glycerol as plasticizer, and Song *et al.* (2012), on barley bran protein-gelatin composite films with plasticizers. The observed increase in thickness due to the addition of glycerol is because the glycerol molecules tend to occupy the voids in the starch matrix and interact with the film forming polymer, which causes the distance between the polymers to increase thus enlarging the film. Arham *et*

*al.* (2016), reported that the plasticizer added to starch film can form starch-plasticizer polymer, so that the inter starch bond is replaced by the starch-glycerol-starch bond which led to the increase of the film thickness. The reported of Nemet *et al.* (2010), also shows that the film-forming solution with higher concentrations of glycerol has a higher dry matter content resulting in a thicker film. Shea butter on the other hand, tends to reduce the thickness of the films, probably due to its ability to reduce the hygroscopic effect of glycerol as observed in Table 4.2-4.5. This decrease may be due to the hydrophobic nature of the oil leading to significant reduction of the films moisture contents thereby lowering the thickness. This effect could have a positive value in the area of storage where particular moisture could be maintain using shea butter plasticized films.

Solubility is a chemical property referring to the ability for a given substance (solute) to dissolve in a solvent. In the case of films from starch base composites, solubility is the measure of dissolution of films in water at a given time. The results in Table 4.2-4.5 indicated that the addition of glycerol significantly affected the film solubility. Increase in film's solubility was observed in the presence of glycerol. This development was also observed by Farahnaky *et al.* (2013) in the manufacture of wheat starch edible film, Shekarabi *et al.* (2014) in the production of plums gum/ carboxyl methyl cellulose films and Wittaya (2013), in the manufacture of edible film from mung bean. Increment of the film solubility due to the influence of glycerol is caused by hygroscopic effect of glycerol. Bourtoom (2008), reported that the increase of the starch films solubility with plasticizer incorporation can be explained by the hydrophilic properties of plasticizer (Glycerol), where the dry matter solubilized in water is likely formed by the plasticizer. Therefore, the increase in the glycerol content of the film will increase the content of dry matter solubilized in water, thereby increasing the films solubility. Shea butter on

the other hand significantly reduces the solubility of the film in water as shown in Table 4.2-4.5. Shea butter is highly hydrophobic in nature due to the higher concentration of unsaturated fatty acids which carries one or more double bond atoms within its chain which limit the capacity to hold additional hydrogen atom from water. High water resistivity films in the presence of chitosan and shea butter implies that the films could be deployed for use in packaging without fear of dissolving in water.

Films for packaging function to protect goods during handling, transportation and marketing. This requires high tensile strength (TS). The results shown in Table 4.2-4.5 indicated that the incorporation of plasticizers significantly affected the TS. Increase in glycerol concentration resulted to a decrease in tensile strength of the films. This phenomenon is also reported by Zhong and Xia (2008), when studying the effect of glycerol on chitosan/cassava starch/gelatin blends. They observed that glycerol introduction reduces the films TS from 49.40 to 16.71 MPa. On the other hand, Shea butter addition did not significantly increase the tensile strength of the film. This effect is due to the reduction of strong cohesive bond caused by the presence of fatty acids in shea butter. The decrease of TS with increasing of glycerol concentration observed in this study was also observed by Arhma *et al.* (2016), that the addition of glycerol in the manufacture of biodegradable film can reduced TS due to a decrease in the interaction between water molecules and agar. In addition, the increase in plasticizer concentrations increased the moisture content of the film because of its high hygroscopic nature, which also contributes to the reduction of the forces between the adjacent macromolecules (Sobral *et al.*, 2008). Similarly, Liu *et al.* (2009) reported that increasing the glycerol concentration in the manufactured of starch-chitosan films tend to lower the TS value. The phenomenon of a decrease in TS with increase in plasticizer concentration has been reported by several other researchers (Saremnezhad *et al.*

(2011), Arrieta *et al.* (2014), Wiset *et al.* (2014). The introduction of chitosan with and without plasticizers significantly increased the films TS as shown in Table 4.1-4.5. This improvement in TS by chitosan is due to its strong inter-molecular bond formation and several investigations have reported similar results. Al-Hassan and Norziah (2012), Jongjareonrak *et al.* (2006), Park *et al.* (2002), Polnaya *et al.* (2012), Song *et al.* (2012), Zhai *et al.* (2004), investigated the effect of chitosan on corn starch film properties. The tensile strength and the flexibility of starch film were improved largely after incorporation of chitosan into starch film. In accordance with conventional standard, packing films should have a tensile strength of more than 3.5 MPa. This means, the TS of films from plasticized chitosan/*Borassus aethiopum* shoot starch with 7.8 MPa is qualify to be deployed for packing.

Elongation at break (EAB) is the ability of the films to extend before breaking. It describes the nature of the film plasticity. The result in Table 4.2-4.5 shows that glycerol incorporation significantly increased the EAB, whereas the concentration and interactions between shea butter and glycerol tend to significantly lower the EAB. This result is in accordance with the work of Parra *et al.* (2004) on cassava starch films plasticized by glycerol. The EAB of their films increased from 11 to 27 % in the presence of glycerol. Concentration, type of base material, and solvent used in the manufacture of film affect the elongation and tensile strength of the film (Herliany *et al.*, 2013). Increasing glycerol concentrations tends to increase the EAB due to reduction of the intermolecular interactions between the polymer chains which have an impact on increasing the elongation and flexibility of the film, as reported by Kokoszka *et al.* (2010), Zhong and Xia (2008). Increase in the amount of glycerol will reduce the strength of intermolecular forces that enhance mobility between molecular chains and lead to an increase in the elongation (Katili *et al.*, 2013). (Oses *et al.*, 2009) also

reported that the increased content of plasticizer up to a certain concentration will increase the elongation of the film with glycerol plasticizer, increasing the elongation occurred only at concentration of  $\leq 40$  %. Similarly Liu *et al.* (2009) have also reported that the addition of glycerol at 5 %w/w and higher concentrations for starch-chitosan film resulted to a higher EAB values due to plasticization. The addition of glycerol promoted the interactions among chitosan, starch and glycerol through hydrogen bonding in films. The increasing of the EAB at the incorporation of chitosan at lower concentration may be attributed to the formation of intermolecular hydrogen bonding between  $\text{NH}_3^+$  of the chitosan backbone and  $\text{OH}^-$  of the starch. The amino groups ( $\text{NH}_2$ ) of chitosan were protonated to  $\text{NH}_3^+$  in the acetic acid solution, whereas the ordered crystalline structures of starch molecules were destroyed with the gelatinization process, resulting in the  $\text{OH}^-$  groups being exposed to readily formed hydrogen bonds with  $\text{NH}_3^+$  of the chitosan. The EAB decreased with increasing the concentration of chitosan, this may be as a result of too high chitosan content providing intra-molecular hydrogen bonds rather than inter-molecular hydrogen bonds between polymers as stated by Bangyekan *et al.* (2006). This result implies that the interaction of glycerol and shea butter oil helps maintain an acceptable EAB for packaging.

#### **4.2.4 Effects of plasticizers on the transparency of films produced from the composites of *Borassus aethiopum* shoot starch and chitosan blends**

The transparencies of *Borassus aethiopum* shoot starch/chitosan films (BSCF) are shown in Table 4.5.1. The film transmittance values were used to assess the transparency of the films. Transparency is proportional to absorbance. The 1 % BSCF had the highest transmittance of 30.1 %. Film transparency decreased significantly with an increase in the concentration of *Borassus aethiopum* shoot starch formulations in the presence of glycerol and shear butter oil as plasticizer. A similar tendency was reported

by Mbey *et al.* (2012) for cassava starch-kaolinite composite films; they reported that when talc was added to the plasticized cassava starch matrix, there was a reduction of transmittance. The possible reason for higher opacity value in films is due to the fact that *Borassus aethiopum* shoot starch is not a white starch and the oil increases this characteristic. When the light passes through these films, a much lower extent of light is transmitted through the film which results in higher opacity value. Sun *et al.* (2014) also reported that the introduction of CaCO<sub>3</sub> nanoparticles in corn starch films which led to a significant increase in films opacity from 1.19 to 2.23. In general, higher light absorbance of films related to desirable properties of food packaging since it was an excellent barrier to prevent light induced lipid oxidation. Therefore films from *Borassus aethiopum* shoot starch could be deployed in the area of food packing.

#### **4.2.5 Optimization study on the solubility and tensile strength of glycerol/shear butter plasticizers on *Borassus aethiopum* shoot starch/chitosan films (BSCF)**

The name of the independent variables, the goal of the optimization study and the lower and upper limits of each of the variable studied is to maximize the potential of BSCF. Since both *Borassus aethiopum* shoot starch (B.S) and Chitosan (CHI) have positive effect by increasing the tensile strength of the BSCF. The amount of GLY was set at 30 % because it does not much impact on the tensile strength and above this point the BSCF becomes difficult to dry. The effect of shear butter (S.B) was minimized since it has no significant contribution to the improvement on the tensile strength of the BSCF. The solubility was set in range in order to get the best process conditions and maximum tensile strength of the BSCF. The outcome of the optimization study shown in Figure 4.4 that solubility of 4.12 % and tensile strength of 7.94 MPa was predicted at B.S of 2 %, CHI of 2 %, and GLY of 30 % and S.B of 0.96 %. The tensile strength in this study is within the one reported for both rice and potato starch film of 4.48–8.14 MPa by



Domene-López *et al.* (2019). Noting that the water resistivity and mechanical strength were optimized, That is, the responses achieved for these properties indicated that, under the optimized process conditions, BCSF had better mechanical and barrier properties. This level of efficiency could lead to the utilization of these films for some packing. The examination of the tensile strength of bio-film goes a long way to determine its possible area of application (Domene-López *et al.*, 2019). The aim of this test is get the best process conditions that can give high tensile strength. It can be seen in Table 4.7, that increase in B.S from 0 to 2 % leads to increase in the tensile strength of BSCF from 3.2 to 6.1 MPa, this could be attributed to formation of stronger BSCF as more of the B.S is being converted. Increase in CHI from 0 to 2 % leads to increase in tensile strength from 1.5 to 7.9 MPa, this shows that CHI has a very strong influence on the tensile strength of BSCF and it also helps to improve on the tensile strength of the film, hence increase in CHI leads to significant improvement on the tensile strength of the starch film. Increase in GLY from 0 to 50 % decreases the tensile strength of the BSCF slightly from 4.9 to 4.6 MPa. This shows that increase in glycerol content does not have positive contribution to the tensile properties of the starch film. It can be clearly seen that increase in S.B from 0 to 50 % leads to insignificant change in tensile strength from 4.7 to 4.7 MPa. This shows that S.B does not contribute to the improvement of the tensile strength of BSCF in this study.

The contour plots of the interactive effect show that as the amount of the S.B was increased from 0 to 50 % and the CHI was also increased from 0 to 2 %, the tensile strength also experiences an increase from 2 to 7 MPa. CHI is primarily responsible for the improvement in the tensile strength of the BSCF. It can be inferred that as the CHI increases the S.B was able to hold the CHI firmly bond to the molecules of the starch thereby enhancing the significant increase of the tensile strength. It is important to note

that the interactive effect between GLY and CHI is similar to the interactive effect of S.B and CHI on the tensile strength of BSCF, because, as the amount of the glycerol was increased from 0 to 50 % and the CHI was also increased from 0 to 2 %, the tensile strength also experiences an increase from 2 to 8 MPa. However, the tensile strength of 8MPa can only be obtained at reduced GLY and increased CHI content. Since B.S has a negative effect by increasing the solubility of the BSCF, its effect was minimized as presented in Table 4.7. The effect of CHI shows positive influence on the reduction of solubility of BSCF, hence its effect was maximized. The amount of GLY was set at 30 % because above this point the BSCF becomes difficult to dry. The effect of S.B was maximized since it has positive contribution to the reduction of the solubility of the BSCF. The solubility was set in range in order to get the best process conditions and maximum tensile strength of the BSCF. The outcome of the optimization study shown in Figure 4.4, that solubility of 3.41 % and tensile strength of 5.67 MPa can be obtained at B.S of 1.35 %, CHI of 1.16 %, GLY of 30 % and S.B of 29.35 %. The low solubility reported in this study is better than the 29.74 % and 32 % reported for modified potato and rice starch film respectively by Domene-López *et al.* (2019). Increase in GLY from 0 to 50 % increases the solubility of the BSCF slightly from 2.9 to 4.5 %. Addition of glycerol is to increase the flexibility and reduce the brittleness of the starch film. The low solubility of the BSCF is as a result of effect of glycerol which causes a structural change in the BSCF. It can be clearly seen that increase in S.B from 0 to 50 % leads to decrease in solubility from 11.3 to -3.7 %. This could be attributed to the increase hydrophobic phase of the S.B in the BSCF.

#### **4.2.6 Biodegradability test of plasticized films from *Borassus aethiopum* shoot starch and chitosan**

Crude test on the degradability of the films from chitosan and *Borussus aethiopum* shoot starch composites was carried out in relation to the duration for the individual films to decompose. Films from starch and chitosan blends in the presence of shea butter as plasticizer took a longer time to degrade. The film showed 100 % degradation within a period of 33 days. The delay in degradation of films with higher chitosan and shear butter concentration may be due to their antimicrobial properties which inhibit the action of soil microorganisms to effectively and quickly degrade the film slide as reported by Han (2003). This can be advantageous in the field of food packing where starch film plasticized with shear butter in the presence of chitosan serve to prevent food spoilage due to the activities of microorganisms.

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

This study presents a good knowledge of the influence of glycerol and shea butter on the physical and mechanical properties of high amylose *Borassus eathiopum* shoot starch. Chitosan, glycerol and shea butter has significantly improved the functional properties of films from *Borassus eathiopum* shoot starch. The incorporation of these materials to the starch gave rise to films with higher water resistivity, tensile strength, elongation at break and reduced thickness. Incorporation of chitosan improves the toughness of the films as glycerol improved the films flexibility, while shea butter contributed to the improved water resistance. The optimization of the production processes and the maximization of material combinations led to recommendable properties of *Borassus aethiopum* shoot starch/chitosan films (BSCF), that is, high tensile strength and water resistivity which can deploy for many applications. Therefore, it can be concluded that the BSCF in the presence of glycerol and low concentration of shear butter as plasticizers, may be the best option for the desired properties of BSCF.

#### 5.2 Recommendations

- i. Further investigation should be carried out on films from *Borassus eathiopum* (muruchi) shoot starch, using other viable and compactable plasticizers since there are other materials that may further improves the films properties for commercial use.

- ii. Cross-linkers are also commendable to be used on starch materials due to their unique ability to further improve the inter and intra molecular interactions between various materials.
- iii. Relative humidity and gas permeability test are other parameters that are also vital in ascertaining the integrity of organic films. These parameters should be tested.
- iv. Starch modification by chemical and enzyme treatments in combination with other fiber reinforcement could improve the properties of organic film made from *Borassus eathiopum* (muruci) shoot starch, which should be considered in future research on these materials.

### **5.3 Contribution to Knowledge**

The results from this study demonstrated that equal concentration (2 %w/v) of *Borassus aethiopum* shoot starch and chitosan plasticized with 30 %w/w glycerol and 1 %w/w shea butter, produced films which are flexible with high tensile strength (7.9 MPa) and low solubility in water (4.1 %). Also, these films had a complete decomposition within 33 days in the soil. This study established that these composite films could be commercialized and deploy for packaging due to their excellent mechanical properties and biodegradability.

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