FABRICATION AND CHARACTERIZATION OF CROSSLINKED CELLULOSE NANOCRYSTALS RE-ENFORCED BIPOLYMERIC FILMS FOR PRESERVATION OF LEAFY VEGETABLES

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THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF FOOD PROCESS ENGINEERING OF THE SCHOOL OF ENGINEERING SCIENCES, UNIVERSITY OF GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN FOOD PROCESS ENGINEERING

JULY, 2021

DECLARATIONS

Candidate's Declaration

I hereby declare that this MPhil thesis which has results of my own original research was prepared in accordance with the University of Ghana academic regulations and that no part of it has been presented for another degree in this university or elsewhere

Candidate's name: **CYRIL SAMUEL NII LARTE LARTEY**

Signature……  …… Date: 14/01/2022

Supervisors Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Ghana.

Supervisor's name: DR. VITUS APALANGYA

Signature…  Date: 14/01/2022

Co-supervisor's name: PROF. ABU YAYA

Signature……  Date: 14/01/2022

ABSTRACT

Biodegradable polymer films are increasingly being used as alternate packaging material due to the less environmental and health burden compared to synthetic plastics. Biobased films are also attractive candidates for packaging fresh food such as vegetables and fruits as they possess high moisture retention capacity and maintain desirable sufficient moist surface around the fresh food. Due to the high hydrophilicity of biobased or biodegradable polymers, the practical application of biobased polymer films in food packaging is challenging as their large hydrophilic groups on surface and in between polymer chains can absorb excess moisture which may lead to polymer chain disentanglement and dissolution as excess water can disrupt the weak physical forces (intermolecular hydrogen bonding) holding the individual polymer chains together in the film. The objective of the work is to perform film post treatment techniques in reducing the number of free hydrophilic groups on the surface of the film as well as dehydrate the film using chemical crosslinking and thermal treatment respectively. In this work, a simple packaging film was fabricated by solution casting method using polyvinyl alcohol (PVA), chitosan, citric acid as cross linker and cellulose nanocrystals (CNC) in varied amounts, as fillers to enhance mechanical strength. The films were characterized using tensile machine, Fourier Transformed Infra-red spectroscopy, thermal analysis, moisture absorption studies, film dissolution, film permeability studies and X-ray diffraction analysis. Varying the amount of CNC affected the thermal stability of the films, wherein increased amounts improved thermal properties. TGA studies showed that T max (temperature where highest mass loss occurs) in E3 (398°C) increased to 404 °C in E12 with increased amounts of CNC.

Acylation with acetic anhydride, heat treatment of films at 100°C and a combination of both treatments improved film stability in water. Swelling degree observed after 15 minutes of

immersion of films in distilled water were at their barest minimum in all films subjected to any of the treatments.

The films significantly decreased the rate of browning of fresh lettuce, keeping its fresh green colour for approximately eleven days before signs of browning set in. This was in contrast to fresh lettuce packaged with poly ethylene films which turn brown after three to four days. The fabricated films may serve as active packaging system for extending the shelf life of fresh lettuce, and potentially other vegetables and fruits in the food distribution chain.

DEDICATION

This work is dedicated to my mother

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LIST OF ABBREVIATIONS

AC	Acylated
ACHT	Acylated Heat Treated
CNC	Cellulose nanocrystals
CS	Chitosan
CA	Citric acid
FTIR	Fourier Transform and Infrared Spectroscopy
GRAS	Generally recognized as safe
HT	Heat Treated
MAP	Modified Atmosphere Packaging
MC	Moisture Content
PVA	Polyvinyl alcohol
SD	Swelling Degree
TS	Tensile strength
TGA	Thermogravimetric Analysis
WS	Water Solubility
WVP	Water Vapour Permeability
XRD	X-Ray Diffraction
LDPE	Low Density Poly Ethylene
LCA	Life Cycle Assessment

CHAPTER 1

1.0 INTRODUCTION

1.1 Study Background

Food preservation remains a relevant field of study due to the annual reported high losses of food and wastage across the globe. The FAO in 2011 estimated through a simple food mass flow model, that about a third of the overall raw mass of food from both natural and processed sources is wasted or lost globally. Sub Saharan Africa records a remarkable loss rate of 37% representing approximately 120-170 kg a year per capita (Gustavsson, Jenny; Cederberg, Christel; Sonesson, 2011). The severity and causes of food spoilage may vary globally. In underdeveloped sub-Saharan African countries, about 20 to 30% of cereals and legumes and approximately 20 to 50% of roots, tubers, fruits and vegetables, are lost in storage, during transport, and at retail (Rutten & Verma, 2014). Biological spoilage may be attributable to food losses globally, however, the high water activity coupled with microbial growth enabling humid climate, weak cold chain and poor packaging technologies make biological spoilage a major cause of food spoilage in sub-Sahara Africa countries (Connolly-Boutin & Smit, 2016; Duchenne-Moutien & Neetoo, 2021; Hodges et al., 2011; Lenné & Ward, 2010). There are therefore efforts aimed at minimizing these food losses using different handling, storage and packaging technologies from farm, during distribution and to the final consumer.

In food packaging, different strategies are adopted in protecting, regulating moisture activity, stalling microbial proliferation, and reducing oxidation of packaged food products. Food packaging is therefore considered an important tool in reducing food loss by extending the

freshness and quality of food product. Different classes of food may require different packaging strategies depending on their physiological composition, convenience and susceptibility to spoilage.

Vegetables and fruits are packaged, distributed, and retailed to consumers in convenient, lightweight and transparent petroleum based plastic films such as polyethylene, Polyethylene terephthalate films or containers etc. (Opara & Mditshwa, 2013). Besides the ecological burden emanating from the persistence of these plastics on the environment, these fossil-based plastics are not breathable. This results in moisture accumulating in the packaged food product causing wilting and other physiological injuries (Barlow & Morgan, 2013; Kader et al., 1989; Sorrentino et al., 2007)(Agüero et al., 2011).

However, there is growing interest in adopting biodegradable, breathable and biobased polymeric packaging systems with less health and environmental risks. (Kanmani & Rhim, 2014; Malathi et al., 2016; H. Wang et al., 2018). Examples of frequently utilized biopolymers include cellulose, chitosan, collagen carrageenan, corn zein, alginate, soy and various starches (Trache et al., 2017). As packaging material, bio polymeric films are to function as containers for food, provide protection from surrounding factors and adequately maintain food quality (Arvanitoyannis, 1999). However, a major setback in the use of these biopolymers is that they absorb so much moisture when in contact with water. The high sensitivity to water is due to their hydrophilic hydroxyl groups (-OH), and as a result deteriorate and dissolve in water. With an increase in relative humidity, their water sorption increases, making them unsuitable for packaging (Bourtoom, 2009; Fabra et al., 2014).

An effective way to improve general film properties is the formulation of polymer composites. A polymer composite is a material with multiple phases, where either two or more polymers are

blended or reinforcing fillers are integrated with a polymer matrix such that the end product is unique with desirable thermal, mechanical and reduced moisture sensitivity properties, which would be absent if a single constituent polymer was used instead (G. Wang et al., 2017).

Polyvinyl alcohol is a synthetic polymer produced by partial or full hydrolysis of the aliphatic polymer polyvinyl acetate. It is biodegradable, water soluble and possesses good adhesive, emulsifying and film forming properties (Kanatt et al., 2012).

Chitosan is a natural, biocompatible, biodegradable, nontoxic, antimicrobial polysaccharide obtained from chitin. Chitin is referred to as Chitosan when it is more than 60% deacetylated (Khouri, 2019). After cellulose, chitosan is the next most abundant polysaccharide occurring in nature, making it easily accessible (Leceta et al., 2013). As a result of these positive characteristics, it has been extensively applied in areas such as medicine, agriculture, food processing, cosmetics, waste and water treatment (Ibrahim & El-Zairy, 2015).

The above enumerated properties of chitosan make it an ideal film forming polymer for packaging in the food industry. There are however several limitations that do not make solely chitosan films ideal for packaging. First, singly chitosan films tend to be rigid and often require the inclusion of plasticizers to lessen the forces of friction due to hydrogen and ionic bonds within the polymer chains (Olabarrieta et al., 2001). Also, chitosan possesses poor water barrier characteristics as a result of its hydrophilic property, making it unsuitable as a packaging material (J. R. Ye et al., 2014).

The above stated drawback with respect to rigidity can be curbed with the inclusion of polyols during film formation (Srinivasa et al., 2007). Chitosan possesses a number of hydroxyl and amine groups within its structure and is therefore capable of being mixed with the polyol PVA, resulting

in the formation of hydrogen bonds which improve the mechanical properties of films produced (Abraham et al., 2016). Particulate polyols such as cellulose nanocrystals have been used as reinforcing fillers in enhancing the mechanical properties of biobased polymers.

Cellulose nanocrystals (CNC) are rod-like crystalline structures formed as a result of the acid hydrolysis of native cellulose fibers. They are readily available, easily affordable, light weight, biodegradable, nontoxic, easily modified (chemically), among many features which have resulted in their widespread industrial application (Habibi et al., 2010). As fillers for polymer composites, they form bonds between themselves and the corresponding polymer matrix such that any incident load will result in an easy dissipation of the stress energy by a large extent along the junctions of these bonds, causing stretching instead of breaking. The presence of reversible cross-links also make it possible for polymers to recover from the strain (J. Yang et al., 2014). Citric acid has been identified as a good crosslinking reagent in the formation of some polymer films (Awadhiya et al., 2016; Reddy & Yang, 2010; Stone et al., 2013).

Citric acid (CA) is a nontoxic, polycarboxylic acid. A unique property of citric acid is its ability to act as a sanitizer, it has been used as the basis of many sanitizers in the food industry to reduce microbial contaminations (libin Zhu, 2017).

The combination of CA and CNC has been found to result in a solution with good plastic formation properties and improved hydrophobicity through crosslinking (Nuruddin et al., 2020).

The polymer blend of polyvinyl alcohol (PVA), chitosan (CS), Citric Acid (CA) as cross linker and cellulose Nanocrystals (CNC) serving as fillers, may result in a filmogenic solution that produces packaging films that may exhibit good mechanical properties and less sensitive to moisture.

However, whereas the hydrophilic groups (hydroxyl (-OH) and amino (-NH_2)) within the composite polymer film are either covalently crosslinked or physically interacting, the numerous –OH and –NH_2 groups on the surface of the film are free and can absorb water molecules in the surroundings (Klein & Poverenov, 2020). The absorbed water molecules can diffuse into the film thereby reversing the physical interactions within the film. This can lead to disentanglement of the polymer chains, leading to dissolution. Moreover, since most biobased films are fabricated by solution casting, it is entirely possible that some pockets of water can be trapped between polymer chains in the film. This can significantly reduce the degree of crosslinking between polymer chains thereby weakening film. It is therefore imperative that the number of free hydroxyl groups on the surface of the film should be reduced to ensure film stability in water. This can be achieved through chemical crosslinking of the –OH and –NH_2 groups on the surface of the film. The pockets of trapped water molecules can be reduced through post heat treatment of the film which can enhance the crosslinking of polymer chains within the film (Rubentheren et al., 2016).

1.2 Problem Statement

About 20-60 % of vegetables are lost annually from the farmgate to distribution and storage in Ghana (Kitinoja et al., 2018; Paper et al., 2015). Existing methods of packaging using fossil-based packaging systems are ineffective as they are not breathable and accumulate moisture on packaged food thereby causing wilting and other physiological injuries on the fresh produce. Biobased or biodegradable films are promising alternatives to fossil-based packaging systems as their films are porous, and possess moisture absorbing hydrophilic groups (-OH, NH_2). However, single polymer biobased films exhibit weak mechanical properties and easily dissolve in fresh food packaged environment where the water activity is high. A careful choice of multiple polymers and fillers

with suitable functional groups can promote physical and covalent (through chemical crosslinking) interaction among the constituting polymers. This interaction may strengthen the film while maintaining sufficient hydrophilic groups and ions which can maintain the moisture retaining capacity through intermolecular hydrogen bonding and hydration respectively. To gain understanding on how the interaction of multiple polymers and fillers can strengthen and maintain the moisture retention of biodegradable polymer blends, the interaction between chitosan, poly (vinyl) alcohol, citric acid and varying levels of cellulose nanocrystals was investigated. Moreover, to appreciate the effect of film post acylation and temperature treatment on the moisture retention and mechanical strength of the films, effects of acylation on the films will be investigate at room and elevated temperature. Physically enforcing films with CNCs and post-treating them by acylation and heat treatment can help to mechanically strengthen films and provide good moisture retaining properties. They will serve as better alternatives to existing fossil-based packaging films with less burden on the environment.

1.3. Approach

Pursuant to the above stated need, a simple approach involving the fabrication of composite polymer poly (vinyl) alcohol (PVA) and chitosan crosslinked with citric acid and physically reinforced with cellulose nanocrystals films are designed and fabricated. A polymer composite with reinforcing fillers could exists in multiple phases, with an end product or film unique with desirable properties which would be absent in the individual constituting polymer films (G. Wang et al., 2017). Moreover, it is expected that the blending of PVA and chitosan to form composite films unlike the single polymer films, containing different biodegradable polymers (PVA, chitosan and citric acid) with different functional groups such as hydroxyl (-OH), amino ($-NH_2$), carboxylic

($-CO_3^-$), hydroxyl could react with the numerous -OH groups on the film thereby minimizing the film dissolution in water. This reaction could also lead to crosslinking of the individual polymer chains through the formation of amides (-CONHR), and esters (-COOR) in the film thereby strengthening the structural integrity of the film (Y. Park et al., 2005). It is also expected that the formed -CONHR, and -COOR bonds will absorb moisture and maintain a sufficient moisture on the surface of packaged fresh vegetables which leads to shelf-life extension of the vegetables as a formation and acylation of composite polymer films produced from chitosan and PVA is designed. The procedures involved include the formation of a composite polymer. In this mix, chitosan and PVA are firstly prepared separately and blended. To confer mechanical strength as well as antimicrobial features, CNC and citric acid are introduced into the polymeric mix. CNC is varied to determine the best formulation. Glycerol is added in minute quantities to introduce a plastic feel to the films formed. After the successful formation of films, acetic anhydride is used as solvent for the acylation of the films to confer hydrophobicity in them.

1.4 Hypothesis

- Incorporation of CNCs into chitosan and PVA films would improve the mechanical, and thermal properties as well as reduce the moisture sensitivity of the films (El Miri et al., 2015, Yadav et al., 2019. Y. Xu et al., 2018).
- Acylation and Heat treatment of chitosan and PVA films, cross-linked with citric acid and CNCs would improve their mechanical and thermal properties as well as moisture stability.

- Films produced from Chitosan and PVA, crosslinked with citric acid and CNC would have the ability to significantly increase the shelf life of leafy vegetables by reducing their rate of browning.

1.5 Aims

The aim of this project is to formulate a composite biodegradable film from Chitosan and PVA, crosslinked with Citric acid and CNCs with the overarching aim of obtaining composite films with improved structural and mechanical properties. It is expected that the CNCs would serve as reinforcing fillers with the main objective of strengthening as well as serving as moisture barriers.

Moreover, this project is aimed at reducing the high moisture sensitivity of biobased films by subjecting them to acylation and thermal treatments as well as to study the ability of the films to reduce the rate of browning of lettuce as a model vegetable.

1.6 Objectives

- To determine the effect of CNC on the physicochemical properties of films consisting of PVA/Chitosan/Citric acid and CNC.
- To determine the effect of Acylation, Heat treatment and a combination of both, on the physicochemical properties of the films.
- To study the ability of the films to reduce the rate of browning of lettuce as a model vegetable.

1.7 Rationale

Current research into packaging is geared towards the use of more biodegradable packaging systems (A. Das & Lackner, n.d.; Dilkes-Hoffman et al., 2018; Zeljko, 2017). The sense of urgency to produce effective biodegradable packaging is stemmed from the fact that as the global population increases, the demand for food supply would likewise increase. It is already estimated that the global demand for food will surge by 50% in the year 2050 due to population growth (Guillard et al., 2018). Petroleum based packaging, which is more dominant, is projected to cause adverse environmental challenges in the foreseeable future. A life cycle assessment (LCA) of petroleum based packaging, which considers their origin in terms of production, right down to their disposal, clearly expounds the environmental impact of these traditional plastics considering the high energy required to produce them as well as their considerable contribution to global warming (Taylor et al., 2011).

Considering biodegradable packaging, films produced from a combination of PVA and chitosan, crosslinked with citric acid have had successful results. Citric acid enhances the mechanical features and confers antimicrobial property to PVA/chitosan films (Wen et al., 2021). However, there is a possibility of achieving even further enhanced mechanical properties via the inclusion of CNCs which are noted for improving mechanical properties of polymer films (W. Yang et al., 2020).

In section 1.1, the weak performance of biobased and biodegradable films in water is clearly stated, it is therefore imperative to subject such films to some form of treatment to improve their water resistant property. Some works have involved bioconjugation with hydrophobic acids (Q. Ye et al., 2019), crosslinking (Zheng et al., 2017), and the application of ultrasonic treatment (Vrabič Brodnjak, 2017), all in the effort to improve moisture sensitivity. Such methods have their pros

and cons, and some are easier to accomplish than others. The need for simple, cost effective yet efficient treatment methods such as acylation (Uranga et al., 2018) and heat treatment (S. Li et al., 2020) makes these procedures worth investigating.

Inclusive of the advantages that biobased, biodegradable films have over petroleum based ones is their antimicrobial property. Many bio based, biodegradable polymers can be used in their state or modified in some form to confer some antimicrobial properties upon them. Whereas chitosan has inherent antimicrobial property, cellulose can be modified by functionalization or grafting, and blending with cationic molecules, essential oils and other antimicrobial polymers. Starch is modified by etherification, graft copolymerization and flocculation for the introduction of antimicrobial properties (Muñoz-Bonilla et al., 2019).The production of a biodegradable film with antimicrobial properties and improved sensitivity to water can therefore be utilized in the packaging of a myriad of foodstuff, especially those with short shelf life, the tendency to easily brown and require pores for respiration such as lettuce and possibly many others.

CHAPTER 2

2.0. LITERATURE REVIEW

2.1. Shelf Life of Leafy Vegetables

The mechanism of spoilage of minimally processed leafy vegetables is multifaceted. The myriad of factors that influence the deterioration process can be grouped into four major classifications namely biological changes (which include softening, development of off-flavors, loss of texture, enzymatic spoilage and surface browning) mechanical damage (general decay and dehydration), microbial activity (Microbial spoilage and pathogen infestation) and physiological spoilage (caused by increased respiration, increased ethylene production and brown spots) (Artés & Allende, 2005).

The transportation and retail of these processed fresh cuts tend to be the main activities which introduce the spoilage factors (Piagentini et al., 2005). During transportation, there is a need to keep the fresh cuts under the appropriate temperature and relative humidity conditions to the end that microbial contamination is prevented and the growth of inherent microbes delayed (Bolin & Huxsoll, 1989). Temperature and humidity values over the duration of transportation are imperative to shelf life estimation and as such must be monitored closely to ensure that the products are in an acceptable state (Sapers et al., 2009).

Retail activities tend to result in the introduction of spoilage organisms due to many permutations of events that may occur. Primarily, the fresh cuts may end up in the hands of food services, where food workers easily introduce microbes if they do not practice good hygiene (De Roever, 1998). On the other hand, consumers themselves may be the source of microbial contamination. A common sight during retail of fresh cuts is when consumers touch the products in order to make

an assessment as to whether they should purchase or decline. At this stage consumers may contaminate the product if they held it with microbe infested hands. Other factors relating to the consumer include handling fresh cuts in the home without first washing the hands, leaving the vegetables in or around a contaminated sink, refrigeration under unsuitable temperature and many others (James, 2005). Amidst the many challenges with shelf life, there are some measures that have been put in place to ensure preservation.

2.2. Existing Methods of Preservation

Many techniques have been adopted for the preservation and subsequent packaging of leafy vegetables. Commonly utilized among them are modified atmosphere packaging (MAP), chilling, and the use of chemical preservatives such as antioxidants, antimicrobial solutions and many others (Allende et al., 2003).

The efforts for shelf life extension, including pre-cooling and chilling, commence right from the point the raw materials arrive in the processing factory (Gross et al., 2016). It is imperative to keep them at the appropriate temperature at all times to greatly slow down the rate of deterioration. The mechanism of cooling involves the reduction of temperature and a corresponding increase in humidity which in turn reduces moisture loss considerably (Wills & Golding, 2016).

Chemical disinfectants are also highly utilized in the preservative process for leafy vegetables. Spinach, Parsley and Dill for instance are dipped in sodium hypochlorite to rid them of spoilage microbes (Zenoozian, 2011), chlorine bleach, hydroxide, peroxyacetic acid and sodium bicarbonate are known sanitizers in the preservation of cabbage and lettuce (Allwood et al., 2004),

citric acid is also used in the disinfection of lettuce, tomatoes and carrots (Bermúdez-Aguirre & Barbosa-Cánovas, 2013).

When it comes to packaging, food processing companies and retailers tend to make use of synthetic polymer films such as polyethylene, polypropylene and polystyrene films for the packaging of leafy vegetables. This is due to advantages such as cost effectiveness and good structural and mechanical properties (Galgano et al., 2015; Siracusa et al., 2008). Synthetic packaging is however well associated with unfavorable ecological implications, therefore biodegradable plastic films are being utilized more in recent times as alternate packaging to synthetic films.

2.3 Lettuce and Shelf life

Among leafy vegetables, lettuce (*Lactuca sativa*) is globally considered as the highest in rank in terms of prominence (Křístková et al., 2008). The lettuce plant provides resources that are utilized in a wide range of areas. The leaves are consumed as food, whether cooked or added to sandwiches or salads, the stems are also consumed in some parts of the world, the seeds are raw material for oil extraction, and the latex is used in the production of a well-known sedative (Mou, 2008).

Lettuce is a cool season crop and as such it can thrive in both tropical and temperate zones, however, the time of planting is key with respect to temperate zones.

Horticulturally, lettuce is classified according to the shape of the leaves. There are six classes namely, leaf lettuce, stem lettuce, Latin lettuce also known as 'little Gem', butterhead also referred to as 'cabbage lettuce', romaine or 'cos lettuce' and crisphead which has other names such as 'iceberg' or 'head lettuce'. (Ryder, 2002).

Quality indicators for lettuce include the colour of the leaves, texture and taste. Depending on the class of lettuce, leaf colour ranges from yellow, yellow green, light green and dark green. With respect to texture, some leaves are crunchy and crisp, as is the case of crisphead, and others are oily and tender, as is the case with butterhead.

Lettuce, like other precut green vegetables does not maintain wholesome quality for more than 120 minutes when kept at room temperature. It will also wilt rapidly when in direct contact with the sun. When subject to refrigeration however, lettuce can be preserved for up to approximately 14 days (Chua et al., 2008). Other current preservative methods include Modified Atmosphere Packaging (Jacxsens et al., 2001; McManamon et al., 2019; McMillin, 2020; Soltani Firouz et al., 2021). , the use of essential oils (Ponce et al., 2011). , antioxidants (Martín-Diana et al., 2008) among others.

There have been promising results in the use of bio based packaging films for lettuce preservation. Das et al., 2021 combined Alginate and vanillin to prepare a packaging film that considerably extended the shelf life of lettuce. Combination of starch, PVA, alginate and small amounts of essential oils such as lemongrass or copaiba results in the formation of biodegradable films that have successfully extended the shelf life of lettuce significantly (Brandelero et al., 2016).

2.4 Biodegradable and Biopolymer Plastic Films

Biodegradable plastics are ideally defined as materials which possess the unique property of being able to breakdown completely into carbon dioxide and water, otherwise known as biomass, via the activities of microorganisms present in nature such as bacteria, fungi and algae (Sawada, 1998). Biodegradability does not necessarily take into account if the source of resources used are non-

renewable fossil or renewable biomass, the property in focus is their tendency to breakdown into carbon dioxide and water (Iwata, 2015). These are not to be confused with bio-based plastics which strictly refer to materials produced from renewable carbon resources.

Naturally occurring polymers are simply referred to as Biopolymers. All biopolymers fall under one of three sources from which they are classified. The first class includes polymers formed by a chemical procedure which makes use of renewable monomers as precursors, such as in the case of polylactic acid. The second includes polymers synthesized from microorganisms, an example being polyhydroxyalkanoates. Finally, there are the polymers extracted from plant or animal sources such as proteins and polysaccharides (Galgano et al., 2015).

The plenitude of biopolymers in nature and their ease of degradation should have made them the most ideal source of packaging material, but their inherent poor mechanical and barrier properties relative to synthetic polymers currently in use, make them unsuitable to a great degree (Abdul Khalil et al., 2018).

Measures to improve the mechanical and barrier properties of these biopolymers over the years include gamma irradiation (Jo et al., 2005), addition of plasticizers (Audic & Chaufer, 2005), chemical modification (Demirgöz et al., 2000), the addition of additives such as nanoclay (Almasi et al., 2010), CNC's (Kassab et al., 2019), and many others (Saurabh et al., 2015).

The second most abundant biopolymer occurring in nature is chitin, which is found in the exoskeleton of crustaceans, and from which the polymer chitosan is derived (Q. Li et al., 1992).

2.5 Chitosan and its Properties

Chitosan is the second most abundant polymer sourced from nature after cellulose (Zaku et al., 2011). When the polymer chitin partially deacetylated, it is known as chitosan (Weska et al., 2007). From observation, the main structural difference between chitin and chitosan is the degree of deacetylation. The exact percent degree of deacetylation which classifies chitosan clearly is not certain. Previous works classify the acceptable degree of deacetylation to be 50% (El Knidri et al., 2018) and 60% (Khouri, 2019).

The conversion can be achieved either via the utilization of enzymes or alkali deacetylation. Either way, the process of chitin conversion into chitosan involves the breakdown of part of the inherent N-acetyl-D-glucosamine links present in chitin, resulting in the formation of D-glucosamine units. These units possess a free amine group which gives chitosan many of its key properties. The amine groups make the polymer capable of being soluble in aqueous media (Chen & Tsaih, 1998). They also have the ability to accept positive charges enabling them to bond well with substances like dyes and metal ions and are applied in such industries (Shajahan et al., 2017). The antimicrobial property of chitosan has also been attributed to the presence of the free amine groups (Al-Manhel et al., 2018).

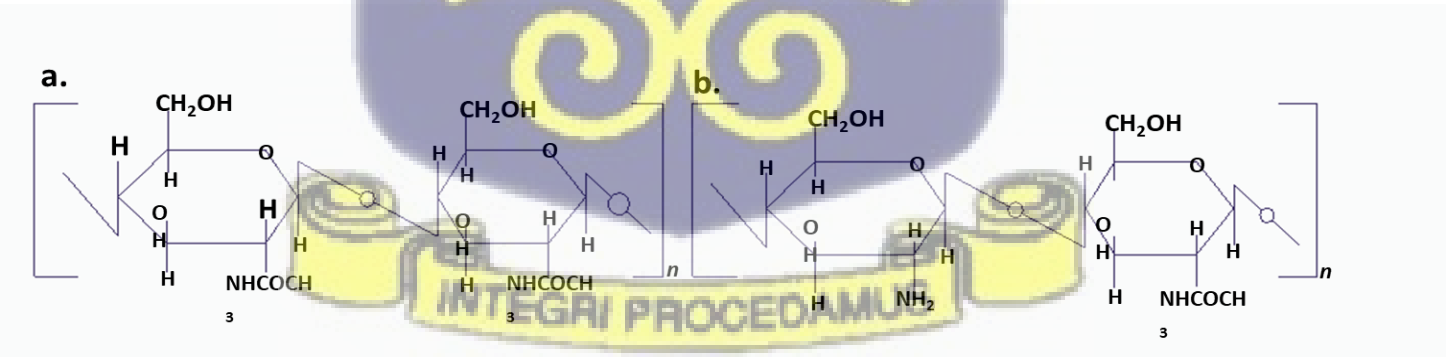


Fig 2.1 Structure of Chitin possessing N-Acetyl-D-Glucosamine chains with β (1→4) glycosidic bonds. b) Structure of Chitosan possessing D-Glucosamine chains with free Amine groups

Chitosan is insoluble in many solutions. Water, common organic and basic solvents are all incapable of dissolving chitosan due to the hydrogen bonding within its structure, crystalline nature and high molecular weight (Zong et al., 2000). The polymer however easily dissolves in dilute mineral and organic acid solutions like formic acid, acetic acid, hydrochloric acid, citric and phosphoric acid to name a few(Chen, P., Lai, Y., Kuo, T., Liu, 2007).

The film casting method is therefore an ideal way to produce Chitosan films. The challenge however is that chitosan based films are unable to resist structural damage under high heat conditions, have very high hydrophilicity, and generally possess poor mechanical properties, being brittle and inelastic (Lacroix, 2009). Various procedures such as incorporation of organic and inorganic Nano fillers, crosslinking and blending with other polymers have been adopted in the attempt to improve barrier and mechanical features (Pires et al., 2021).

Chitosan based packaging films can be prepared as pure films, chitosan/biopolymer films, combined with synthetic polymers among many permutations (Kuorwel et al., 2015; Romanazzi et al., 2017; Verlee et al., 2017).

Chitosan has been combined with many polymers with commendable results; Starch (Y. X. Xu et al., 2005), keratin (Tanabe et al., 2002), carboxymethyl cellulose, sodium alginate (Lan et al., 2018), gelatin to produce edible films (H. Wang et al., 2021) and PVA (Liu et al., 2017) just to name a few.

PVA and chitosan have been blended to produce films suitable for the extension of minimally processed tomatoes (Tripathi et al., 2009), both polymers have also been further blended with guar gum and crosslinked with hydroxyl citric acid to produce films that inhibit the activities of *S. aureus* and *E. coli* bacteria (Bhat et al., 2021). In the field of active packaging, chitosan/PVA films have been combined with anthocyanins from red cabbage to produce intelligent packages with time temperature indicators (V. A. Pereira et al., 2015).

2.6 PVA and its Properties

Polyvinyl Alcohol is a synthetic polymer commonly known as PVA, and sometimes referred to as ethanol homopolymer (Morrison, 1990). It is formed from the saponification of vinyl acetate at a controlled rate and usually consists of acetate groups that are not hydrolyzed. The process involves the partial replacement of the ester group of vinyl acetate with a hydroxyl group in the presence of aqueous sodium hydroxide (Aslam et al., 2018). The resulting number of hydroxyl groups present in the molecular structure of PVA is what influences its physical, chemical and mechanical properties (Tubbs, 1966). Features like solubility, adhesiveness, plasticity and tensile strength can also be varied by changing the length of vinyl acetate and the degree of hydrolysis in different alkaline or acidic solutions (Ben Halima, 2016).

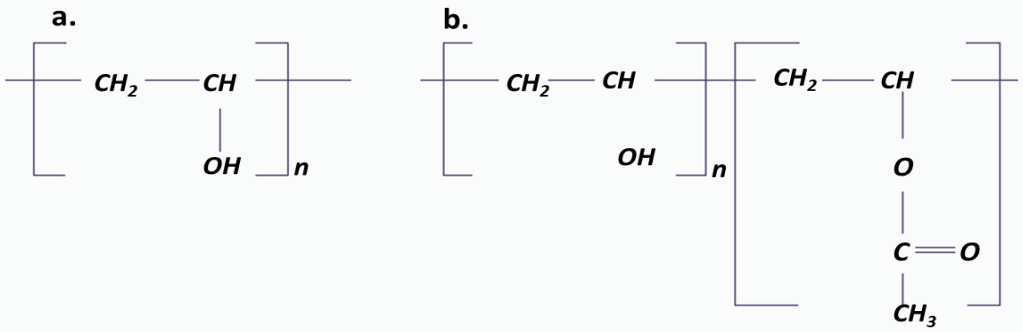


Fig 2.2 Structural formula for a) fully hydrolyzed PVA b) Partially hydrolyzed PVA

PVA is a white or cream coloured powder which has no particular taste or odor. It is a non-toxic, fairly thermostable and biocompatible semi crystalline polymer with many remarkable properties (Abdullah et al., 2017).

The melting and flash points of PVA are 22℃ and 78.4℃ respectively and the polymer is known degrade at a slow rate when the temperature is greater than 100℃ but less than 200℃. At temperatures higher than 200℃ PVA is known to degrade very quickly (Nair, 1989).

Over the years, a lot of research into the various applications of the polymer have resulted in different morphological representations. PVA is therefore present in hydrogel form, as scaffolds, fibers, membranes, beads and films (Chiu & Lin, 2016; Goumri et al., 2016; Hemalatha et al., 2015; Lin & Chen, 1995; Peppas & Hassan, 2000; M. Ye et al., 2014).

With respect to film production, PVA is usually combined with other polymers to form composite films which possess improved features. Researchers combined gellan and PVA via the solution casting method to determine thermal properties and the molecular interactions present in the composite film (Sudhamani et al., 2003). The combined polymers were found to possess a

homogeneous composition and the mechanical attributes indicated that the films could be used as edible food packages. PVA has repeatedly been combined with starch to improve the mechanical properties of starch based materials (Noshirvani et al., 2016; H. R. Park et al., 2005; Priya et al., 2014). In the medical sector, PVA has been combined with silk to produce silk spheres with well-tailored micro and nanosphere sizes that prove to be useful in loading and releasing drugs, overcoming the challenge of silk's natural tendency to self-assemble under conditions of heat, pH change, presence of salt and high shear (X. Wang et al., 2010). A combination of soy protein isolate and PVA has been shown to result in a composite film with improved moisture barrier properties (Su et al., 2010). The rate of biodegradation of PVA blend films PVA/PVC (polyvinyl chloride) and PVA/PCL (poly capro-lactone) in soils and soils with landfill leachate were studied into detail. It was proven that as standalone films, PVA had the best rate of degradation and also Improved the degradation rate of the other polymers (de Campos et al., 2011). PVA has also been successfully blended with β-chitin obtained from squid pens. The solvent used was formic acid and the resultant films were of higher tensile strength and lower swelling degree in distilled water (Peesan et al., 2003).

Substantial amount of work has been carried out on the formation of composite films from a blend of PVA and chitosan. Biocompatibility studies on PVA/Chitosan blends show that they are non-toxic, biocompatible and tolerant of each other (De Souza Costa et al., 2009). The antimicrobial properties of this blend have also been studied with respect to the shelf life extension of tomatoes when used as coatings (Tripathi et al., 2008), strawberries when used as a bilayer films (Liu et al., 2017) and mangoes when modified into an active package via the inclusion of D-limonene (Lan et al., 2020). Aside alternate polymers, PVA and chitosan may be blended with crosslinking agents to enhance chemical bonding. Citric acid is one of such cross-linking agents.

2.7 Mechanism of Crosslinking

Crosslinking is the term used to describe the forming of a link or in a sense a bridge between at least two distinct biological structures. These links may either be physically or chemically induced and result in a modification of the mechanical, thermal and biological properties of the composite formed (Oryan et al., 2018). Considering polymer chain interactions, there are three types of crosslinking possible namely covalent (which is considered most stable), ionic and physical (typically hydrogen bonds or Van der Waals forces) crosslinking (Tillet et al., 2011).

With respect to chemical crosslinking, the use of crosslinking agents is considered. These agents are responsible for aiding in the formation of chemical bonds within a structure of linear molecules. They do this by causing the linear molecules to interact with each other by virtue of their inherent functional groups (Nielsen, 2008).

Crosslinking results in a myriad of benefits depending on the polymers involved and the expected improvement in the end product. Some of these benefits include improvement of elasticity which is seen in the case of vulcanization of rubber (Kato et al., 2015), decrease in viscosity, insolubility in water, increased glass transition temperature, strength and toughness, lowered melting points, transformation of thermoplasts into thermosets, among many others (Maitra & Shukla, 2014).

2.8 Citric Acid as Film Modifier

Citric acid is very prominent in the food industry since it is considered as GRAS (generally recognized as safe) by the FAO/WHO joint committee on food additives (Soccol et al., 2006).

The bulk of Citric acid is produced through the biological process of submerged fermentation of sources of carbohydrates such as molasses, sucrose and other starch based media. The fermentation is done solely by the *Aspergillus niger* fungus (Jianlong, 2000).

Citric acid may either exist as anhydrous (water-free) citric acid or citric acid monohydrate (containing one water molecule) (Lafontaine et al., 2013). Different transition temperatures for the conversion of citric acid monohydrate to anhydrous citric acid have been proposed by several authors, between 36.15 ℃ to 36.45 ℃ (Bennet & Yuill, 1934), 36.0 ± 0.5 ℃ (de Kruif et al., 1982), 35.8 ℃ (Dalman, 1937) among many others.

The presence of multiple carboxylic groups in the structure of citric acid has been the basis of its use as a crosslinking agent in several research projects (Gawish et al., 2009). Citric acid has been used as a crosslinking agent in the production of starch films, conferring unto them improved tensile strength, thermal stability, and resistance to degradation in the presence of water (Reddy & Yang, 2010). In the production of cellulose based hydrogels, citric acid functions as a non-toxic and cost effective crosslinking agent (Z. Yang et al., 2010). When used in the crosslinking of chitosan films, citric acid gives the resultant films a smoother surface, an amorphous structure and increased resistance to mechanical stress. The structure of PVA is modified when crosslinked with citric acid and the chemical surface is improved. Parameters such as roughness and adhesion are generally improved (Nascimento et al., 2021). Citric acid has also further been applied as crosslinking agent for PVA/chitosan membranes (Lusiana et al., 2013).

In another study, the introduction of citric acid via surface grafting unto prepared chitosan films was observed to improve hydrophilic property due to the presence of carboxyl groups from the organic acid which confer high molecular polarity and as such, increased hydrophilicity (Liu et al., 2016). Another crosslinker of great importance and potential is CNC.

2.9 The Role of Cellulose Nanocrystals (CNC) in Film Formation

The predominant method of producing CNC is the acid hydrolysis of cellulose based material, which results in reduced amorphousness and prominent crystallinity (Lim et al., 2019). During the acid hydrolysis, CNCs become isolated from the amorphous intercrystalline regions of the cellulose source they stem from (Trache et al., 2017).

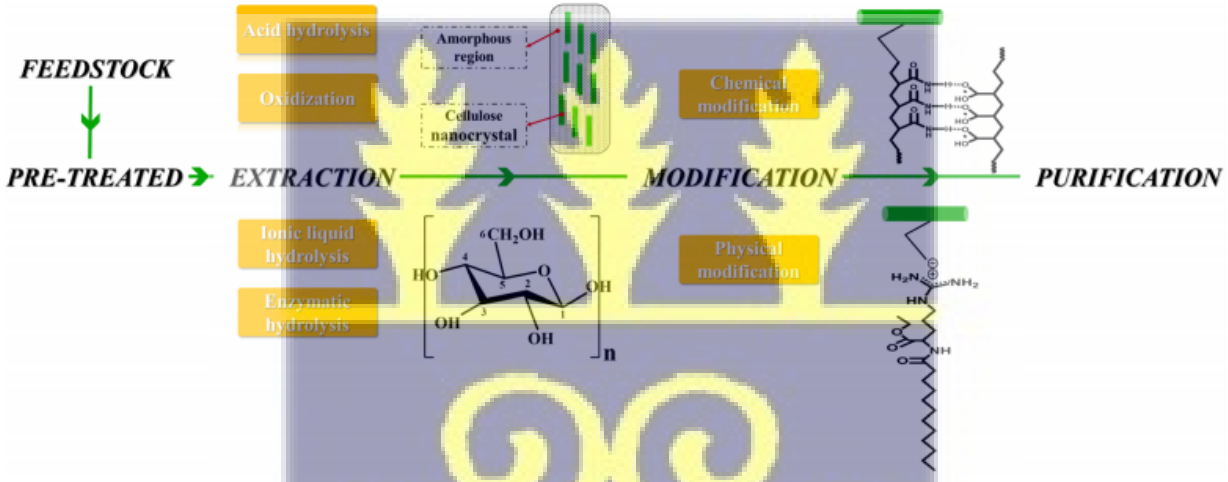


Fig 2.3 Schematic diagram for the production of CNCs ((Huang et al., 2020)

Examples of these cellulose based material include wood pulp, phloem fibers, cotton linters, tunicin, algal cellulose and bacterial cellulose (Domingues et al., 2014; Mariano et al., 2014; Moon et al., 2011).

Advantages of incorporating CNC into systems are numerous, it possesses remarkable thermal properties (Lu et al., 2014) mechanical features (Geng et al., 2016; Khoshkava & Kamal, 2014), high tensile strength (Gray et al., 2018), low density, optical transparency and has a large surface area (Trache et al., 2017).

CNCs have been discovered to possess notable reinforcing abilities when applied to some polymer nanocomposites. An early discovery of the ability of a little percentage of cellulose nanowhiskers, in a composite mix with latex, considerably improved mechanical properties, opened a door of research into this field (Favier et al., 1995). Since then, a myriad of polymers have been studied as the focal points of possible reinforcements with CNCs. Polylactic acid (PLA) was reinforced with CNC and the end result was a CNC-PLA nanocomposite film with good transparency and enhanced barrier properties, where for instance 1 wt. % CNC resulted in an 81% increase in Water Vapour Permeability (WVP) as compared to pure PLA films (Miao & Hamad, 2016). In another paper, Low density Poly (ethylene) (LDPE) was reinforced with CNCs and resultant composite film was the best reinforcement of LDPE because the storage capacity was discovered to have increased by 236% and the overall strength by 314% (Sapkota et al., 2014). In order to improve the properties of PVA, CNCs were incorporated into the polymer mix to produce composite films via the film casting method. It was discovered that the tensile strength of the film initially increased with increasing CNC, but at a threshold, the trend shifted and there was a decrease in tensile strength with increasing CNC amounts (A. L. S. Pereira et al., 2014). Further research identified a similar trend with respect to the maximum stress when investigating cellulose nanowhiskers reinforced polyvinyl alcohol copolymers. In the results, the curves for the stress and strain presented a peak value after sometime and dwindled with increasing amounts of cellulose nanocrystals (Roohani et al., 2008).

Mujtataba & Salaberria, (2017) prepared CNCs from flax and determined the optimum percentage CNC loading required to efficiently chitosan films. CNC was varied by 5, 10, 20 and 30% (w/w) and it was discovered that values for Tensile strength (TS) preliminarily increased with increased loading of CNCs till it reached a peak load of 20% CNC and afterwards begun to reduce in value. A similar trend was noticed in the values for the Young's Modulus.

The ability of CNCs to increase tensile strength and Young's Modulus is attributed to the interaction between anionic CNCs with cationic polymers and chemicals (Limousin et al., 2020).

Although CNC's in their optimum amounts confer considerable tensile strength to bio based films, the inherent weakness of these films is noticed when they come into contact with moisture, where they breakdown almost immediately through random chain scission (Kalita et al., 2020). Many methods have been investigated to find a solution to the poor water barrier of biopolymer films, and among these are the post acylation and heat treatment of prepared biopolymer films.

2.10 Acylation and Heat Treatment of Prepared Films

The degree of acetylation of chitosan when varied, serves as an efficient tool in tuning the properties of chitosan based films such as mechanical strength, biodegradation and water sorption percentage (Mima et al., 1983; Tomihata & Ikada, 1997). Similarly in the case of PVA, the degree of substitution influences properties such as the degree of swelling, which is essential in biopolymer film formation, wherein the higher the degree of substitution, the lower the swelling degree (Nametkin et al., 1981). Both degree of acetylation and degree of substitution are affected by acylation reactions.

Acylation generally involves the addition of an acyl group (R-C=O) to an organic compound which results in the formation of a ketone (R2C=O) group (Gooch, 2007). The resulting compound could also be an ester or aldehyde. It is stable and less likely to undergo rearrangement.

The process takes places in the presence of acylation reagents, which usually fall into either one of three classes namely: perfluoro acid anhydrides, fluoracylimidazoles, and nonhalogenated groups (Parkinson, 2012).

Acetic anhydride is a perfluoro acid anhydride capable of triggering acylation reactions easily at room temperature (Zaikin & Halket, 2003). It is popularly used in the conversion of glycerol into triacetin which is used in cryogenics, plastics and fuel additives (Silva et al., 2010).

J. Xu et al., (1996). Proved that the acylation of chitosan films with acetic anhydride greatly improved the property of biodegradability. The mechanism for acylation involved immersing chitosan films within acetic anhydride solution with stirring at different time intervals.

Microfibillated cellulose films have also been acylated, with the resulting films displaying excellent water resistant properties due to increased hydrophobicity (Deng et al., 2016).

Heat treatment involves subjecting the film to high temperature over a period of time. The effects of application of heat result in the reduction of free hydroxyl groups present in composite polymers making them unavailable for interaction with surrounding moisture. Heat treatment has been proven to increase tensile strength with a tradeoff in elongation values which are greatly reduced (Rubentheren et al., 2016).

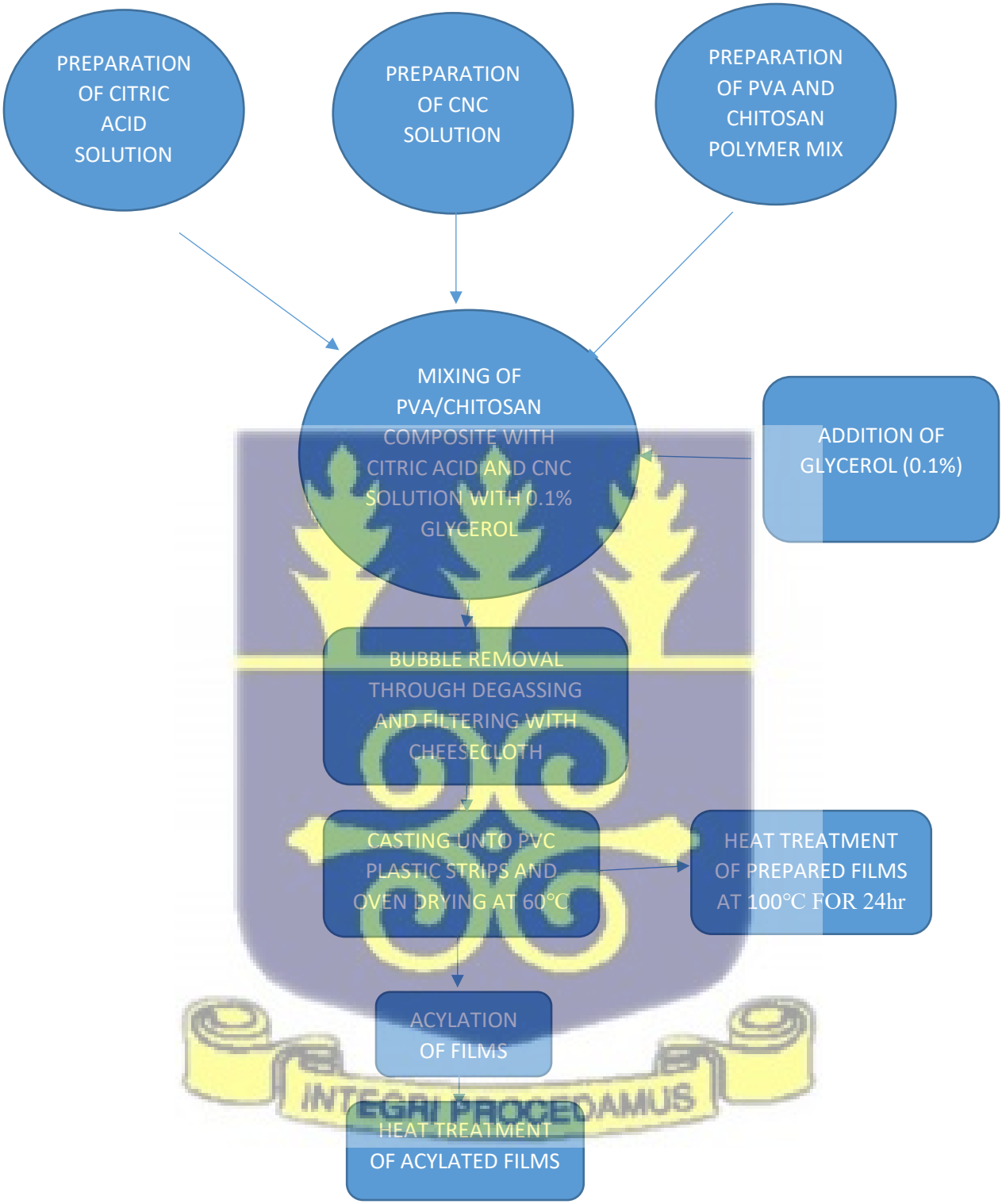
CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

Low molecular weight chitosan (deacetylation ≥ 75) was purchased from Sigma Aldrich, PVA was obtained from Daejung chemicals and metals Co. Ltd and used as received. Glacial acetic acid (>99.7%), also from Daejung chemicals was used to dissolve chitosan to form solution. Citric acid monohydrate (>99.5%, density 155 kg/l, Molecular weight 210.14) was obtained from VWR BHD chemicals. CNC (12.2 wt% batch no-2015-FLP-71) that contains 1% sulphur and sodium counter ion was obtained from University of Maine and manufactured by UDSA-US (U.S. Department of Agriculture) forest service products laboratory in Madison, USA. Glycerol obtained from Daejung chemicals and metals Co. Ltd served as a plasticizing agent in film preparation.

3.2 Flow Diagram of Method



3.3 Preparation of Citric Acid and CNC Solutions

Citric acid solution was prepared by first weighing 5g of citric acid monohydride. The 5g was then dissolved in about 30ml of deionized water and subsequently filled to 100ml to obtain Citric acid solution (5wt %).

CNC solution was prepared by first measuring 5g of CNC in a beaker. The beaker was then filled to 100 ml with deionized water to form CNC (5% w/v). The solution was stirred for 2 hours with a magnetic stirrer at 533rpm.

3.4 Film Preparation

3.4.1 All Films Prepared

The films prepared include (i) PVA/chitosan films with 0.1% glycerol denoted C*, (ii) Composite PVA/chitosan films with the addition of citric acid (14 v/v%) and 0.1% glycerol denoted D*, (iii) Composite PVA/chitosan films with citric acid and varying amounts of CNC (3% CNC denoted E3, 6% CNC denoted E6, 9% CNC denoted E9 and 12% CNC denoted E12 (iv) E3, E6, E9, E12 films acylated with acetic anhydride denoted E3AC, E6AC, E9AC and E12AC respectively, (iv) E3, E6, E9, E12 films heat treated at 100 °C for 24 hours denoted E3HT, E6HT, E9HT and E12HT respectively and finally, (v) E3AC, E6AC, E9AC and E12AC films heat treated at 100 °C for 24 hours to form acylated heat treated films E3ACHT, E6ACHT, E9ACHT and E12ACHT respectively.

3.4.2 PVA/Chitosan Film (C*)

PVA solution was prepared by preheating deionized water at 80 ℃ for 20 minutes with subsequent dissolution of the polymer in the deionized water. The solution was stirred for 5 hours to form 5% w/v PVA solution. On the other hand, 1g of chitosan was dissolved in 100ml dilute acetic acid solution (1% v/v) for 24 hours, according to literature (El-Hefian et al., 2010; Osman et al., 2001). Glycerol (0.1%) was then included in the composite mix with additional stirring for a period of 30 minutes. The resultant solution was then degassed for 90 minutes. Cheesecloth was used as filter for the solution, in order to remove undissolved chitosan and other unwanted contaminants. To remove air bubbles, the solution was kept at room temperature for two hours. It was finally cast unto PVC plastic strips (10 X 20cm) within a tray and dried at 60 ℃ for 24 hours.

3.4.3 PVA/Chitosan Film with Citric Acid (D*)

PVA solution was prepared with the same procedure stated in section 3.4.2 above. Chitosan solution was also prepared and added to the PVA solution in a ratio of 1:2 with stirring. Citric acid (5 wt. %) was previously prepared and included to form 14% of the entire mix. Glycerol (0.1%) was then added to the solution. The resulting mix was degassed for 90 minutes, filtered with cheesecloth and left under room temperature for 2 hours to eliminate remaining bubbles. These were subsequently cast unto PVC plastic strips (10 X 20cm) and dried at 60℃ for 24 hours

3.4.4 PVA/CNC Films with Citric Acid and Varying Concentrations of CNC (E3, E6, E9, and E12)

PVA (5 wt. %) and chitosan (1 wt. %) were both prepared and mixed in the ratio 2:1 respectively. Citric acid was added to the mix, forming 14 (v/v %) of solution. Varying amounts of CNC were used in the preparation of unique films. CNC (3 v/v %) content resulted in the formation of the

film denoted E3, CNC (6 v/v %) labelled as E6, CNC (9 v/v %) denoted as E9 and CNC (12 v/v %) denoted as E12. All prepared solutions were stirred, degassed, filtered, kept still and cast according to the procedure used in section 3.4.3.

3.4.5 Acylation of Prepared films

The films (E3, E6, E9, and E12) were placed in a round bottomed flask and clamped well to a magnetic stirrer/hot plate. The acetic anhydride was then poured into the round bottomed flask containing the films till all films were fully immersed. The real time temperature within the round bottomed flask was monitored with a temperature probe till a reading of 50 ℃. When the temperature within the flask rose to 50 ℃, the temperature dial of the hot plate was set just a little above 50 ℃ to ensure that the temperature within the round bottomed flask was maintained at 50℃. The acylation reaction of the films was allowed to take place amidst stirring at 533 rpm for the next 6 hours. After this time, the acetylated films were taken out and dried in the open

3.4.6 Heat Treatment of Prepared Films

The prepared films (E3, E6, E9 and E12) were placed on trays in a pre-heated hot air oven set at 100℃ for 24 hours. The films were taken out and allowed to cool down for approximately 2 hours prior to characterization.

The same procedure mentioned in this section was followed in preparing the heat treated acylated films denoted E3ACHT, E6ACHT, E9ACHT and E12ACHT.

3.5 Characterization of Films

3.5.1 Fourier Transform and Infrared Spectroscopy (FTIR) Analysis

The FTIR was conducted with a Perkin Elmer UATR Two spectrometer possessing an attenuated total reflectance accessory. The range of measurement was 450 – 4000 cm^{-1}, with a 4 cm^{-1} resolution. The procedure involved first wiping the holding crystal with ethanol, after which films were set up for spectral analysis. The data obtained was fed into Origin Pro 2019, with which the FTIR spectra were obtained.

3.5.2 Thermogravimetric Analysis (TGA)

TGA was conducted using an SDT Q600 V20.9 Build 20 DSC-TGA equipment capably of recording both DSC-TGA simultaneously. Sixteen different film samples (C*, D*, E3, E6, E9, E12, E3AC, E6AC, E9AC, E12AC, E3HT, E9HT, E12HT, E3ACHT, E6ACHT and E12ACHT), with weights within the range of 0.6mg and 3.5mg were heated up at a rate of 10℃/min till a temperature of 800℃ was reached.

3.5.3 X-Ray Diffraction (XRD) Studies

XRD was recorded using an EMPYREAN X-Ray diffraction system using X-rays at 30A and 40kv with scanning angle of 5° - 60°. The samples E6, E6AC, E6HT and E6ACHT were analyzed and XRD graphs were plotted in Origin software.

3.5.4 Mechanical Properties

Tensile test was accomplished with the aid of an electronic tensile testing machine. Gauge length for films was set to 3mm and cross head speed was 10mm per minute. Measurements of thickness were taken with a micrometer screw gauge with reading of E3 (0.066), E6 (0.069), E9 (0.059), E12 (0.053), E3AC (0.047), E6AC (0.106), E9AC (0.067), E12AC (0.082), E3HT (0.092), E6HT (0.246), E9HT (0.084), E12HT (0.056). The measurements were done at room temperature and values of tensile strength, elongation and Young's modulus were represented in a table. Graphs indicating the tensile strength and elongation of each film were also plotted with Origin Pro 2019.

3.6 Moisture Content, Swelling Degree, Water Solubility, Water Vapour Permeability and Test of Film Stability in water

3.6.1 Moisture Content (MC)

The initial weights of the various films are measured and recorded as W1. The films are then placed in an oven and heated at 105℃ for 24 hours. After this, the films are weighed a second time to obtain W2. The second weighing is usually done almost immediately to prevent the film from absorbing moisture from the atmosphere (Cazón et al., 2020). The moisture content is determined according to the equation below

$$\text{Moisture Content} = \frac{W1-W2}{W2} X\ 100\% \qquad (1)$$

3.6.2 Swelling Degree (SD)

This is to determine the extent to which the prepared films swell by virtue of the amount of water they take up (Maria V et al., 2016). After film samples are dried at 105℃ for 24 hours and the weights

measured as W2, about 100 ml of distilled water is poured into a bowl. The dried film samples are then loaded on petri dishes and submerged in the bowl containing distilled water at different time intervals. The weights of the films after immersion are recorded as W3, from which the swelling degree can be calculated from the equation below

$$\text{Swelling degree} = \frac{W3-W2}{W2} X\ 100\% \qquad (2)$$

3.6.3 Water Solubility (WS)

Bio based packaging films have already been proven to possess high hydrophilicity. The water solubility therefore gives an idea of how much of the produced film is totally lost by virtue of complete dissolution in water. W1 represents initial weights of films, W2 represents the weights of the films after oven drying at 105℃ for 24 hours, W3 represents the weight after immersion in water for specific time periods, and W4 therefore represents the weight recorded after films previously immersed in water are dried at 105℃ till a uniform weight is realized. Water solubility is therefore calculated as

$$\text{Water Solubility} = \frac{W2-W4}{W2} X\ 100\% \qquad (3)$$

3.6.4 Water Vapour Permeability (WVP)

Water vapour permeability was carried out using the cup method. In this procedure, 6 ml of deionized water was placed in the bottom of the cups used for the test. Films were used to cover the top section of the cups. Measurements taken included the distance between the film and the

solution, thickness of films, height of distilled water within the cups and the weight loss of cups over a period of 24 hours with the weights recorded at 2 hour intervals. Water vapour permeability is calculated via the equation

$$P = \left(\frac{\Delta m}{\Delta t}\right) * \left(\frac{e}{A * \Delta P}\right) \qquad (4)$$

Where $\frac{\Delta m}{\Delta t}$ represents the sample weight loss as a function of time

And e represents film thickness

A is the permeability area and,

ΔP Is pressure difference between pressure within the cup and pressure outside the film.

3.6.5 Test of Film Stability in Water

A simple test is designed to determine the behavior of the fabricated films in water. Triplicates of each film sample are prepared and cut into squares (3x3mm). The initial film weights are measured and recorded with an analytical balance at the start of the test. The films are then immersed in 100ml of distilled water in a beaker for the first minute after which they are placed on a ball of cotton for the absorption of excess water. The weight after immersion is then recorded. The same film is then subsequently immersed again in time intervals of 3, 6, 9, 12 and 15 minutes with proper adherence to post immersion procedures like placing them on cotton and measuring the new weights. The data obtained is plotted with GraphPad Prism 8.0.2.

3.7 Shelf Life Studies with Lettuce as Model Vegetable

Shelf life studies were carried out by observing some key aspects outlined by Baur et al., 2005. Fresh lettuce was purchased from the market and kept in water to keep it fresh throughout the time the set up was made. The fresh lettuce leaves were frequently picked out of water and diced into little square pieces using a sanitized surgical blade. These pieces were packaged in the various prepared films and set on display for evaluation. Films used for this procedure were, polyethylene films for control, the regular untreated films, Acylated films and Acylated heat treated films. Over a period of 11 days, the films were observed considering their overall visual quality. Shelf life studies were conducted at room temperature.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1. To Determine the Effect of CNC on the Physicochemical Properties of Hydrogels consisting PVA, chitosan, citric acid and CNCs (Objective 1).

4.1.1 Fourier Transform and Infrared Spectroscopy (FTIR) Studies

Fig 4.1a is the spectral representation of Chitosan, PVA, and the composite blend of these two polymers denoted C* (PVA/CS). For chitosan, the transmittance peak at 3360 represents the NH group-stretching (Pawlak & Mucha, 2003).

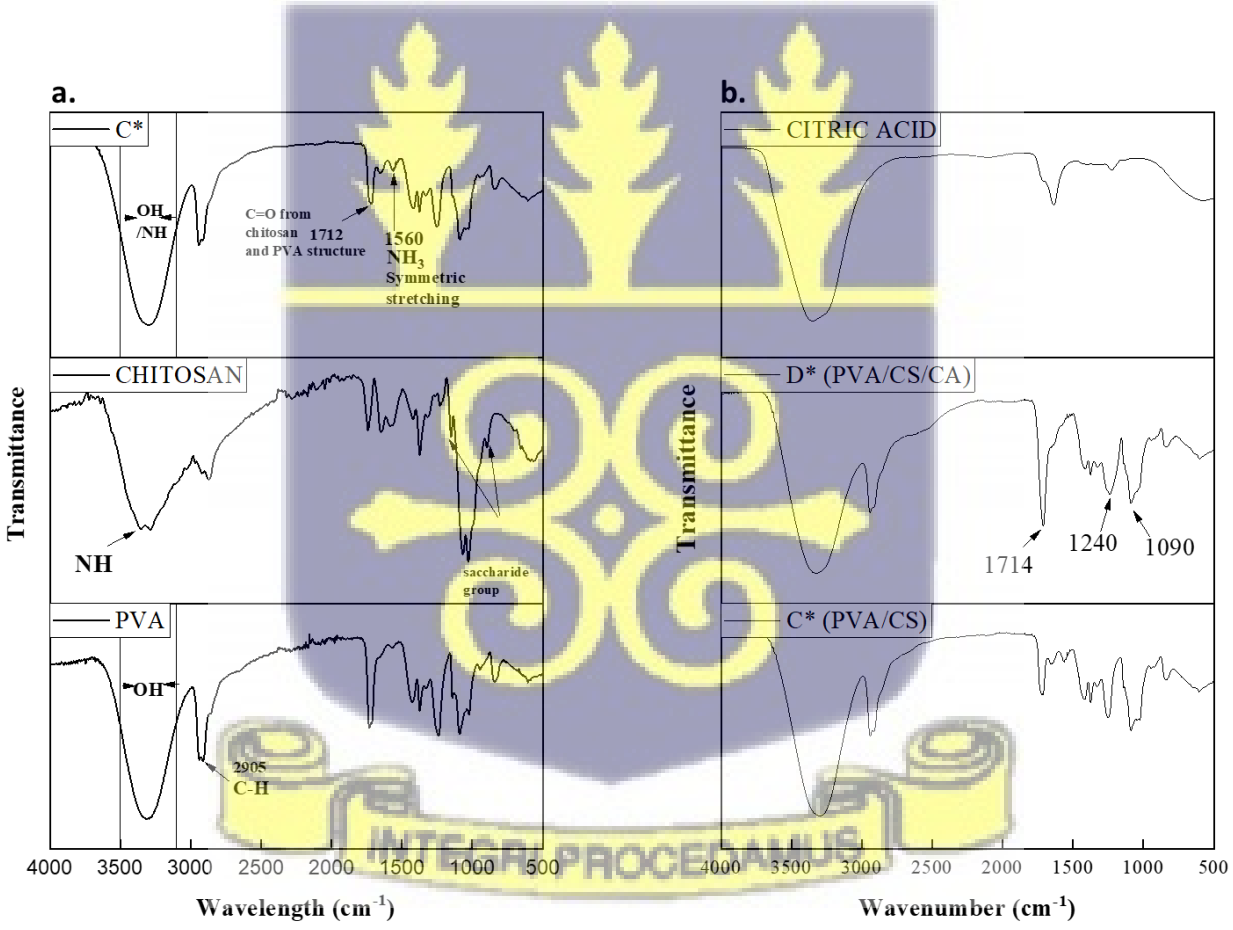


Fig 4.1 FTIR Spectra of a) pure PVA, Pure chitosan and composite blend of PVA and Chitosan denoted C*, b) C*, D* and pure citric acid (CA).

The transmission peaks 1648 and 1563 are also present, which are consistent with acetamido groups (C_2H_4NO) known to be within chitosan (Srinivasa et al., 2003). Also, the peaks 895 and 1149 are consistent with the saccharide structure of chitosan (Branca et al., 2016).

For PVA, the range 3100 and 3500 represents the O-H region, and the transmittance peak 3300 is consistent with the presence of OH within PVA. Other key transmittance peaks present at 2936, 1738, 1660, 1565 and 1327 represent C-H asymmetric stretching, C=O stretching vibrations, acetyl C=C group, bending vibrations of CH_2 and wagging vibrations of CH_2 respectively (Salman et al., 2019).

In the C* (PVA/CS) mix, the initial OH peak (3300) corresponding to pure PVA shifted to the lower wavenumber of 3284, indication some form of interaction between OH groups from PVA and the NH_2 group present in chitosan (Tripathi et al., 2009). At 1375, there is clearly N-O stretching when compared to pure chitosan and PVA films, this is indicative of ionic crosslinking of PVA and chitosan (Jahan et al., 2016). The transmission peak 1560 represents NH_3 symmetric stretching that occurs due to interaction of the NH_2 of chitosan with the OH of PVA, and the peak 1738 that represents C=O stretching in pure PVA, shifted to 1712 in the PVA/CS films confirming crosslinking occurred between the polymers.

In fig 4.1b, the addition of citric acid first in sample D* (PVA/Chitosan/Citric Acid) strengthened the peak at 1714 cm^{-1} which is typical of C=O stretching vibrations and is evidence that esterification occurred among PVA/Chitosan and citric acid (S. Wang et al., 2014). Also, the transmission peaks present at 1090 and 1240 could easily be attributed to C-O ester groups which arise when PVA and citric acid are combined (Lusiana et al., 2013).

Fig 4.2 is the FTIR spectra of polymer films infused with various concentrations cellulose nanocrystals (CNC). Spectra for CNC is also available for the purpose of comparison.

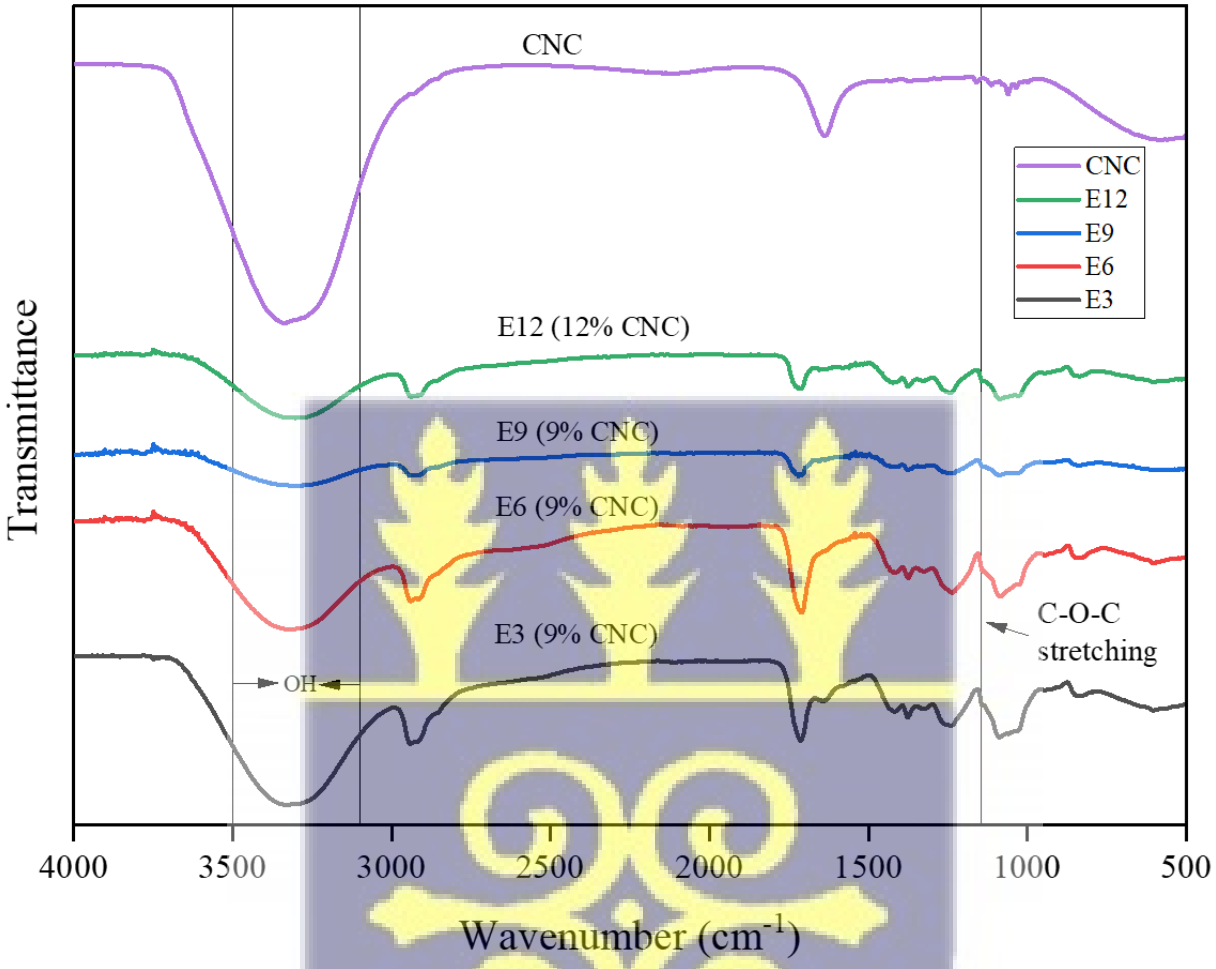


Fig. 4.2: FTIR spectra of PVA, Chitosan and citric acid infused with varying concentration CNCs

As shown in Fig 4.2, CNC was introduced in increasing amounts from sample E3 (3% CNC) to E12 (12% CNC), and with increasing concentration of CNC, the intensity of the bands around 3300 cm^{-1}, 3297 cm^{-1}, 3294 cm^{-1}, 3291 cm^{-1} corresponding to samples E3, E6, E9 and

E12 respectively, is clearly decreasing. This is due to the fact that the introduction of CNC as filler results in an increase in the available energy for the formation of hydrogen bonds (Popescu et al., 2018) and as a result, fewer O-H bonds are available. Also, in all CNC containing films, there's a peak around 1145 cm $^{-1}$ corresponding to C-O-C stretching and with increasing CNC content, the intensity of the band decreases.

4.1.2 Thermogravimetric Analysis (TGA)

The thermogram for polymer films of varying CNC amounts is also considered in Fig 4.3 below.

Films without CNC (C* and D*) are also present to serve as reference points for description.

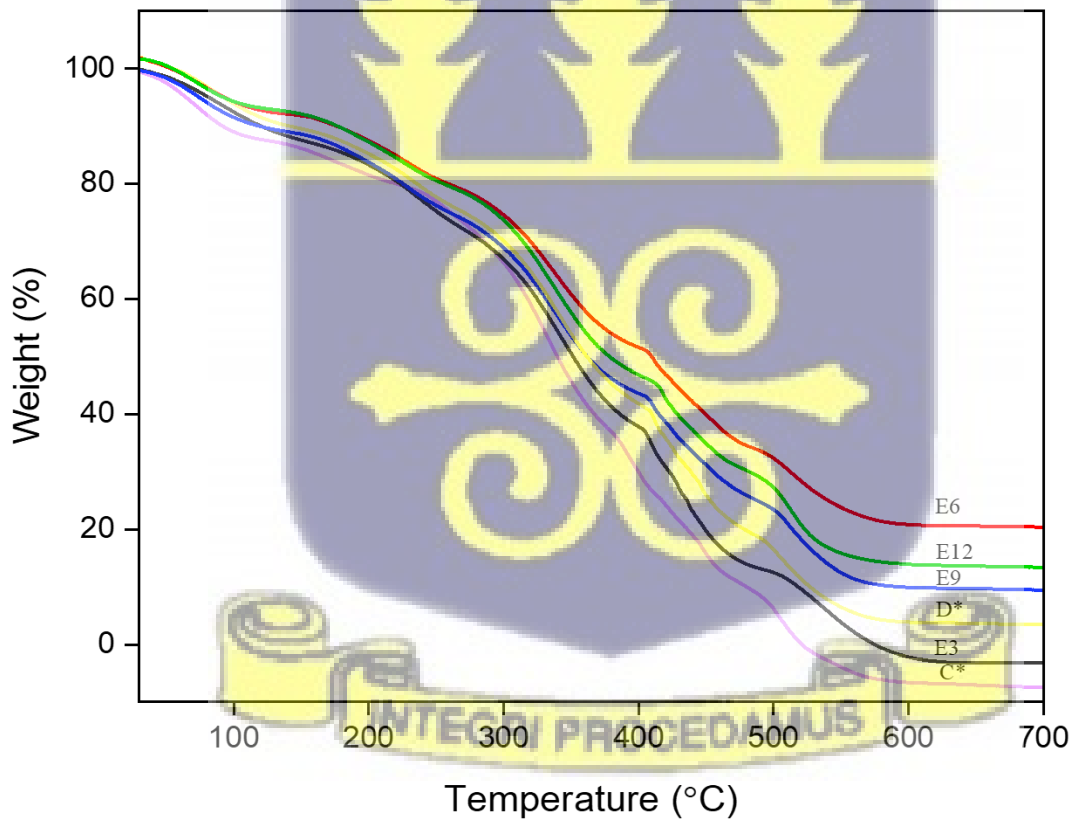


Fig. 4.3 TGA for polymer films C*, D* and films with varying amounts of CNC

The TGA analysis helped determine the dehydration and decomposition temperatures of the films created. The curves in fig 4.8 represent the weight loss pathway for 6 films (C*, D*, E3, E6, E9, E12) produced in accordance to the first objective of this project, which is to fabricate films with varying CNC content. C* from fig 4.8 represents a film formed by the combination of PVA and chitosan only. In C*, about 12.5% mass is lost from about 30 ºC to 130 ºC, most likely from the loss of surface water and residual acetic acid used in the film formation. Also, there's an observable mass loss of 41.86% from 222 ºC to 379 ºC, which corresponds to the thermal degradation of PVA and chitosan polymers (Liu et al., 2017). Film D* saw the introduction of citric acid which also serves as a cross-linking agent. The introduction of citric acid clearly improved the thermal properties of the film. Maximum weight loss in C* (41.86%) begun at 222 ºC, but in D* (42.19), the peak shifts to 268 ºC. The improvement is as a result of citric acid's ability to promote hydrogen and ionic bonding between PVA and chitosan, which reduces the mobility of the structural chains (Wilpiszewska et al., 2019).

The addition of CNC's in E3, E6, E9 and E12 films greatly improves the thermal stability of the films (Perumal et al., 2018). The maximum temperature (T max) at which the highest mass loss was recorded for film C* was 379 ºC, but this value increased to 398 ºC, 399 ºC, 399 ºC and 404 ºC in E3, E6, E9 and E12 respectively, serving as proof of improved thermal stability. In fig 4.4, the various weight loss steps are clearly defined in the derivative curves for each of the films.

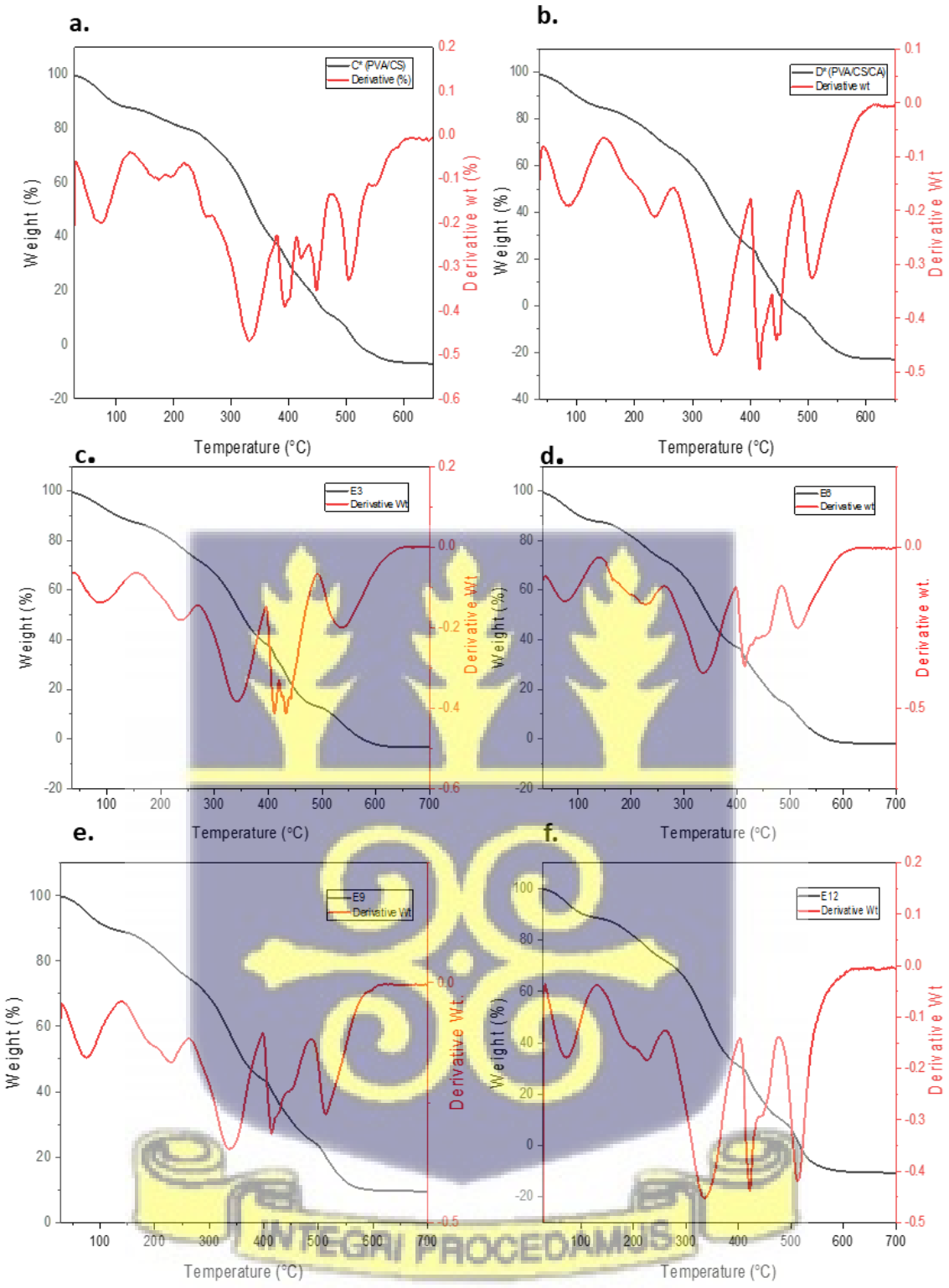


Fig 4.4 TGA showing weight and derivative weight percentage of a) C* b) D* c) E3 d) E6 e) E9 f) E12

4.1.3 Mechanical Properties

Since the fabricated films are designed with the intended purpose of food packaging, mechanical properties such as tensile strength, Young's modulus and elongation at break are essential. In fig 4.5 below, the tensile stress and strain values are plotted against each other and slope values of selected sections of the graph are calculated to determine Young's Modulus.

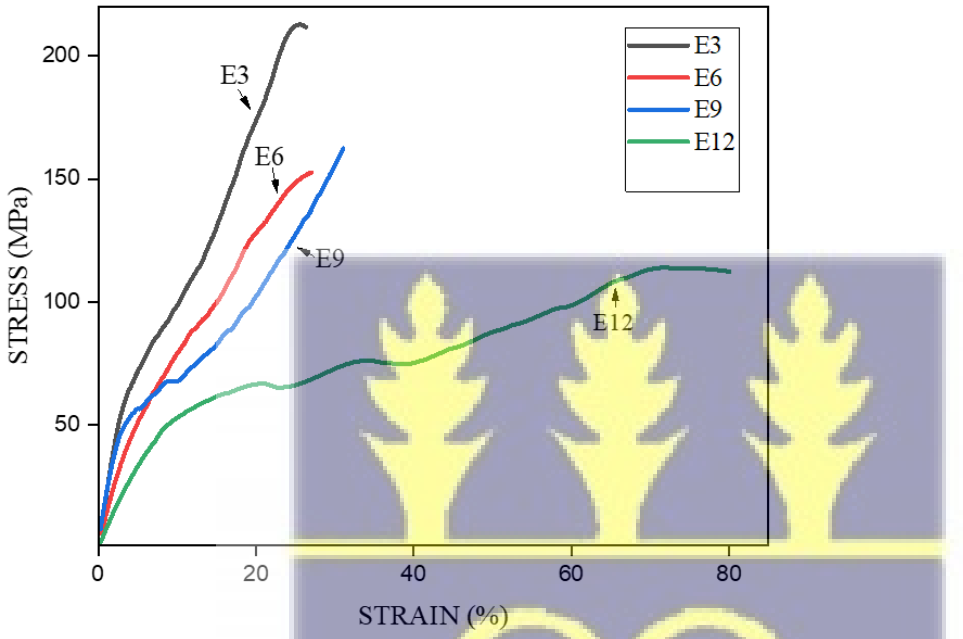


Fig. 4.5 Tensile stress and strain for E3 –E12 films

Table 4.1 consists of the specific values of tensile strength (MPa), Young's Modulus and Elongation (%) values of E3-E12 films. Unlike conventional fossil based plastic films such as polyethylene films, the values for tensile strength in the E3-E12 films are significantly higher.

Table 4.1 Values for Tensile strength, Young's modulus and Elongation of E3-E12 films

Film	Tensile Strength (MPa)	Young's Modulus	Elongation (%)
E3	212.60	7.30 ± 0.03	25.81
E6	152.67	5.16 ± 0.02	26.74
E9	162.35	3.97 ± 0.02	31.01
E12	113.75	1.06 ± 0.01	77.57

Findings on the tensile strength of low density polyethylene (LDPE) films usually indicate comparatively lower tensile strength values like 8.6 MPa (Achilias et al., 2007) and 16.9 (Suresh et al., 2011). The tensile strength values of the formulated films in table 4.1 are much higher due to the mechanism of chemical crosslinking of PVA, chitosan, CNC, and citric acid. The covalent and ionic bonds formed cause the polymer components to be locked in tightly with each other, and as a result, more force is required to break such bonds. The inclusion of CNC into polymer blends is known to increase tensile strength, the addition of 5wt. % of CNC caused the tensile strength of PVA, chitosan, CNC films to increase from 56.12MPa to 98.15MPa (Perumal et al., 2018). Citric acid is also known to increase tensile strength with increasing concentration (Shi et al., 2008). The combined effect of both CNC and citric acid is the reason for the great increase in tensile strength values. Elongation values are typically lower for plastics such as polyethylene due to the weak Van der Waals forces that hold them together (Spencer, 1984). Films E3-E12 have lower elongation values because the strong covalent and ionic bonds formed within them cannot easily be stretched.

From Fig 4.5, the graph corresponding to E3 closely resembles that of a brittle material. The presence of little amount of CNC (3%) greatly improves its tensile strength, here recorded as

212MPa (Table 4.1), but when the ultimate tensile strength is attained, the film fails immediately. With increasing CNC content, the plastic property of the film improves, and as seen in E12, the elongation at break is an impressive 77.57% (Table 4.1). The plastic behavior is because citric acid behaves both as a plasticizer and crosslinking agent (Shi et al., 2008). Fig 4.6 clearly shows the trend in increasing elongation at break with increasing CNC content.

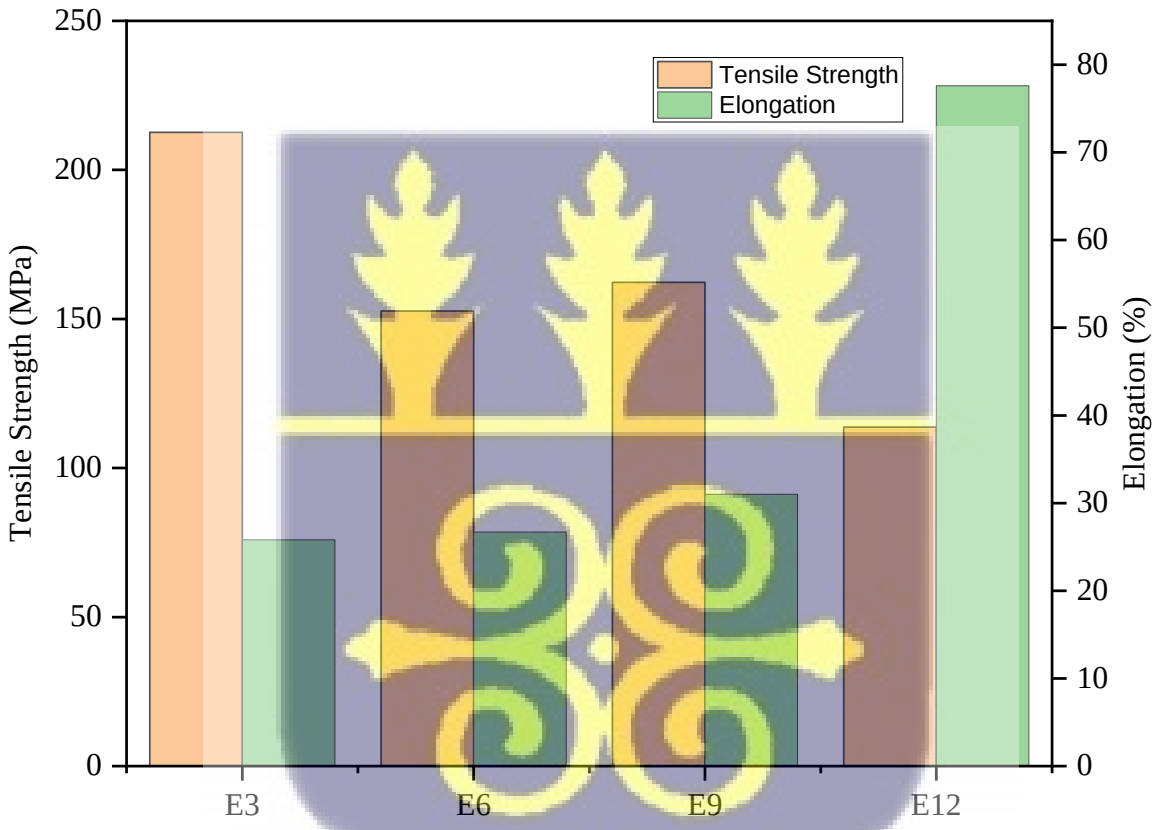


Fig 4.6 Tensile Strength and elongation of E3 –E12 films

4.1.4 Moisture Content, Swelling Degree, Water Solubility and Water Vapour Permeability

4. 1.4.1 Moisture Content

Fig 4.7a is a simple representation of the moisture content of the polymer films with data points obtained as an average of at least three measurements. From the graph, E9 is noticed to have the highest moisture content with an average of 20.74%, while C* has the least average value of 10.05%. The films had an average of 16.25% moisture content.

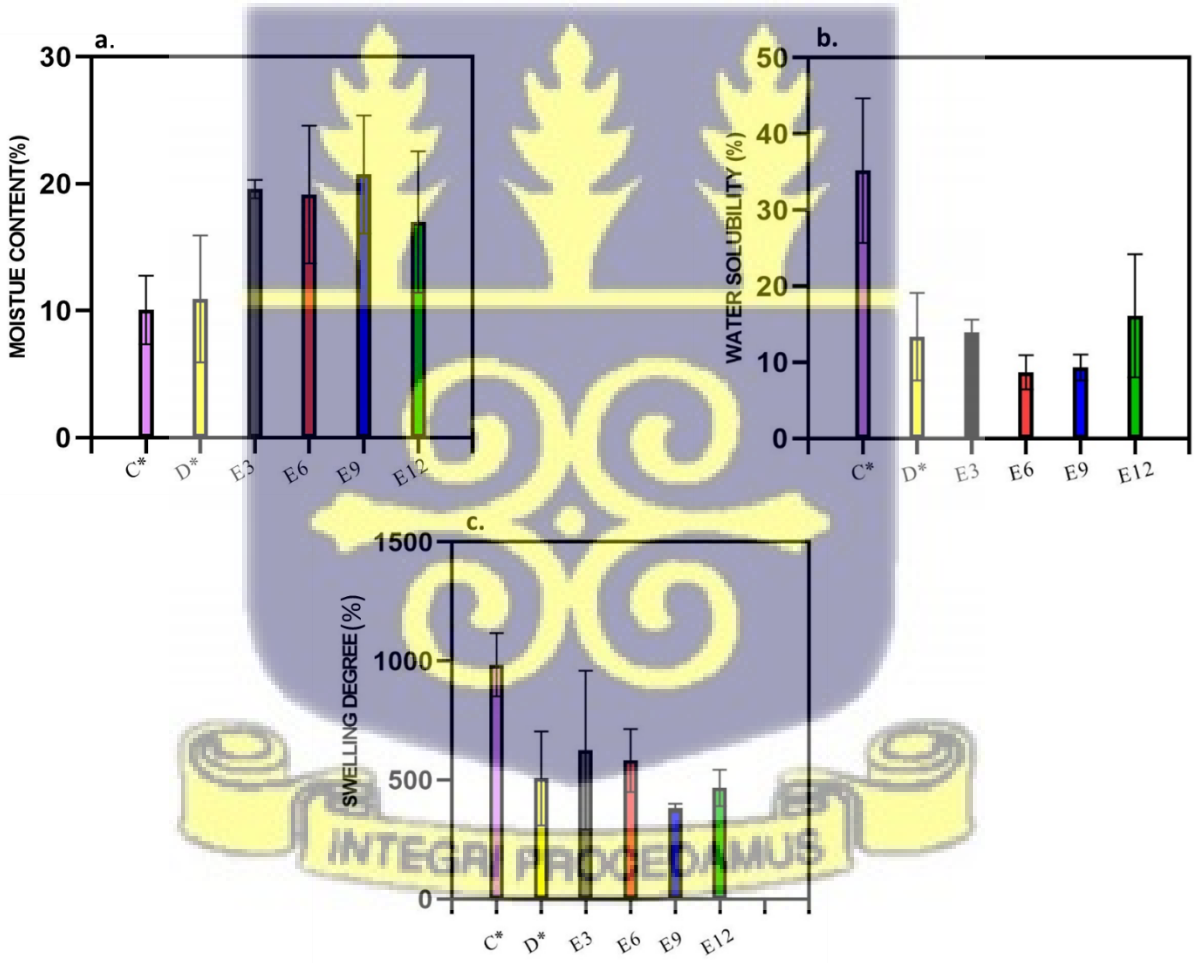


Fig 4.7 Graphs of a) Moisture Content b) Water Solubility and c) Swelling degree of films C*-E12

4. 1.4.2 Swelling Degree

Fig 4.7c clearly outlines the trend in swelling degree. It is noticed that with the increase of CNC content within the films, the swelling degree of the films is reduced. CNC's act as fillers within the polymer matrix and reduce the amount of hydroxyl groups present to interact with water molecules.

4.1.4.3 Water Solubility

The water solubility value gives an idea of the ability of the polymer films to remain in contact with water over a period of time and still maintain structural integrity, considering its naturally hydrophilic nature. In fig 4.7b, it is observed that with increasing CNC content, the formulated films are noticed to be more stable in water. The trend however differs with E12 where the CNC content is in excess and increases the tendency of films to dissolve in water.

4.1.4.4 Water Vapour Permeability (WVP)

Water vapour permeability of a packaging film plays an integral role in the ability of the package to preserve and extend the shelf life of its contents. WVP determines how much excess water will be available for deterioration by spoilage microorganisms since it determines the equilibrium moisture content of the package (Siracusa, 2012). As a result, the higher the WVP, the less effective the package. From Fig 4.8, the WVP values reduce when added to the films. The presence of CNC's introduce more twists, bends and curves which reduce the rate of water vapour diffusion (Azeredo et al., 2010). Sample D* from Fig 4.8 was also seen to have lower WVP because the

addition of citric acid to biopolymer films is known to reduce permeability (Olsson et al., 2013)

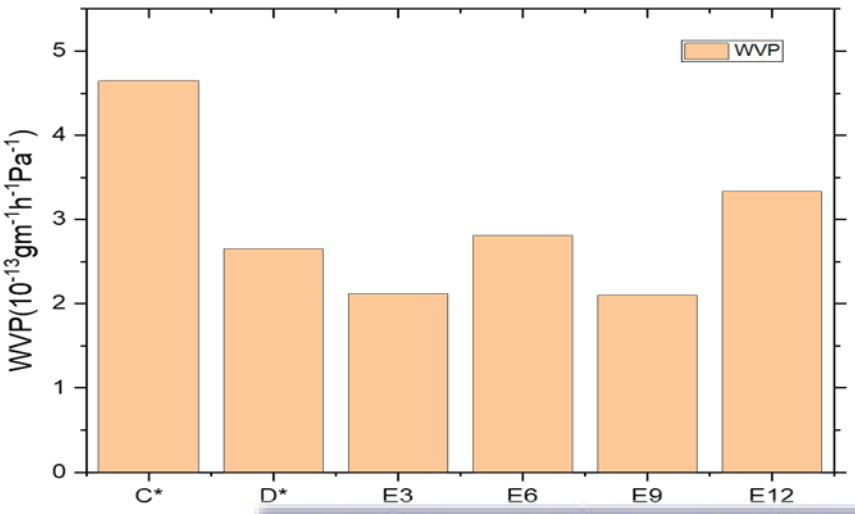


Fig 4.8 Water Vapour Permeability values C*- E12 films

In the case of E12, the water vapour permeability increased significantly compared to films where the CNC content was lower. CNC's tend to reduce film functionality when found in excess (Xiao et al., 2021). Therefore with respect to the fabrication of these hydrogel films, the addition of 12% CNC reduced the excellent barrier properties and increased water vapour permeability.

4.2 To Determine the Effect of Acylation, Heat Treatment, and Acylation Plus Heat Treatment on the Physicochemical Properties and Moisture Sensitivity of Films

4.2.1 Fourier Transform and Infrared Spectroscopy (FTIR) Studies

The second objective of this project is aimed at reducing moisture sensitivity of produced films through acylation and heat treatment. In fig 4.9a, the comparison of E3 and the acylated E3AC

spectra clearly shows a sharpening of the peaks 2850, 1560, and 1644, corresponding to C-H stretching vibrations, secondary N-H bending and C=O respectively, which is similar to N-acylation of chitosan films (Choi et al., 2007). The peak 1715 is prominent in E3 film, which is consistent with the presence of the carbonyl of an acid, indicating that carboxylic acid is preserved. There's however a gradual shift of this peak to slightly higher wavenumbers in the E3AC spectrum, indicating that the esterification process, albeit incomplete had begun. This is proven by observing the spectrum for E3ACHT, wherein heat is added to enhance acylation process, in E3ACHT, the E3 peak shifted from 1715 to 1726, indicating that an ester was formed due to successful acylation.

Comparing E3 to E3HT, considering the range 3000 to 3500, corresponding to O-H and N-H, spectrum for E3HT is relatively narrower and less intense, this is because heat treatment brings about dehydration within the sample (Rivero et al., 2012).

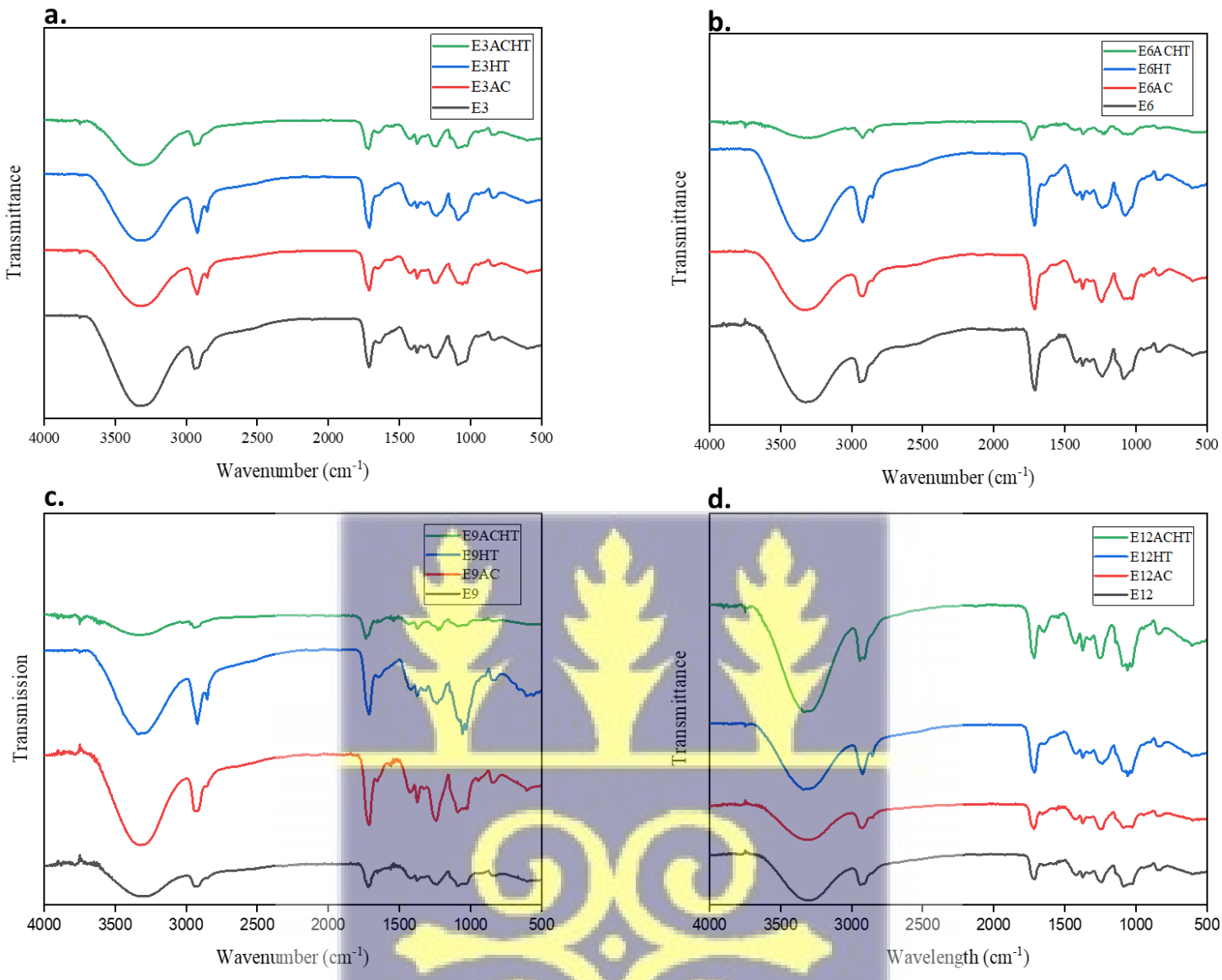


Fig 4.9 FTIR spectra of Acylated (AC), Heat Treated (HT) and Acylated Heat Treated (ACHT) versions of a) E3 b) E6 c) E9 d) E12

In fig 4.9b, a similar trend with respect to E6 and E6AC is observed with 2850, 1560 and 1644 corresponding to C-H, N-H, and C=O respectively. In E6ACHT however, it is observed that there

is a significant reduction in the intensity of the spectrum within the 3000 to 3500 range and the same phenomenon is observed in E6ACHT as well.

In fig 4.9d, films contain a higher percentage of CNC, 12% to be specific. As a result, there are excess hydroxyl groups present within the structure of films. Therefore, treatments such as acylation and heat treatment seem to have little effect on the intensity of the O-H, N-H spectra.

4.2.2 Thermogravimetric Analysis (TGA)

In this section, the TGA graphs of films that have been treated to withstand moisture better are considered. Fig 4.10 shows the various thermogram including that of acylated films (AC), heat treated films (HT) and films that have both been acylated and heat treated as well (ACHT).

In fig 4.10a), weight loss due to the egress of moisture and other volatile substances is to a maximum of about 174 ºC with respect to all E3 films under comparison. It can clearly be seen that the thermal properties of the film are improved with heat treatment and acylation. T max for the E3 film was recorded as 398 ºC as stated earlier, and rose to 413 ºC and 452 ºC for E3HT and E3AC respectively. The thermal properties were however reduced greatly in E3ACHT films, and this was a general trend in all the acylated heat treated films of the varied film formulations. The interaction of hydroxyl groups within the chemical structures of the films are responsible for their bond strength. Acylation and heat treatment as standalone measures are known to strengthen bonds within the film structure, with tolerable reduction in the number of hydroxyl groups present and as a result improve the mechanical properties of the film.

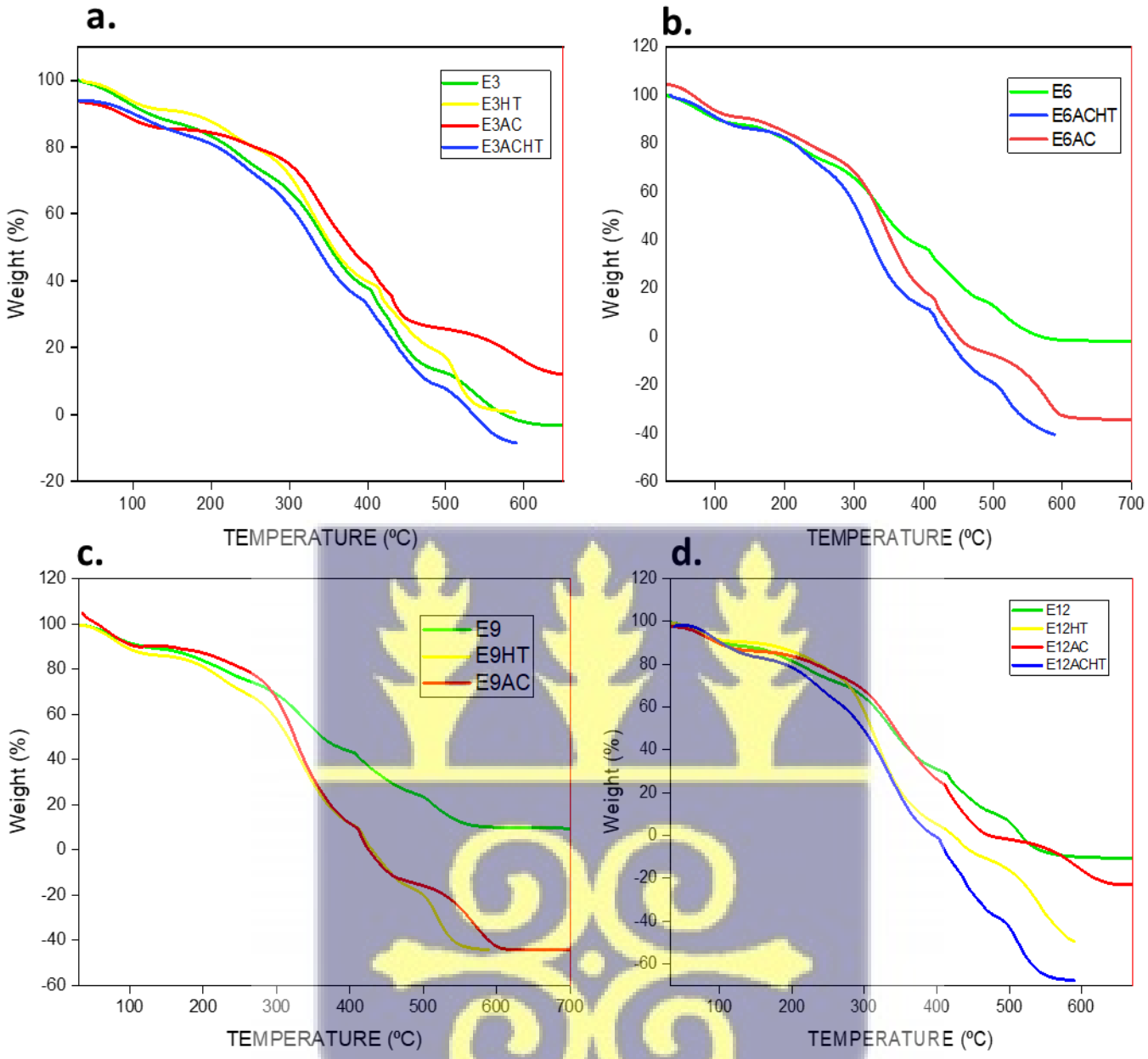


Fig 4.10 FTIR of some Acylated (AC), Heat Treated (HT) and Acylated Heat Treated (ACHT) versions of a) E3 b) E6 c) E9 d) E12

A combination of both measures however greatly reduces the available hydroxyl groups within the film matrix and therefore, the thermal properties are negatively affected.

4.2.3 X-Ray Diffraction (XRD) studies

In Fig 4.11 below, the XRD graph of one formulated film together with its variants is carefully considered. Film E6, consisting PVA, chitosan, citric acid and 6 wt% CNC was the basis to determine if acylation, heat treatment and a combination of acylation and heat treatment had any effect on the crystallinity of the fabricated film.

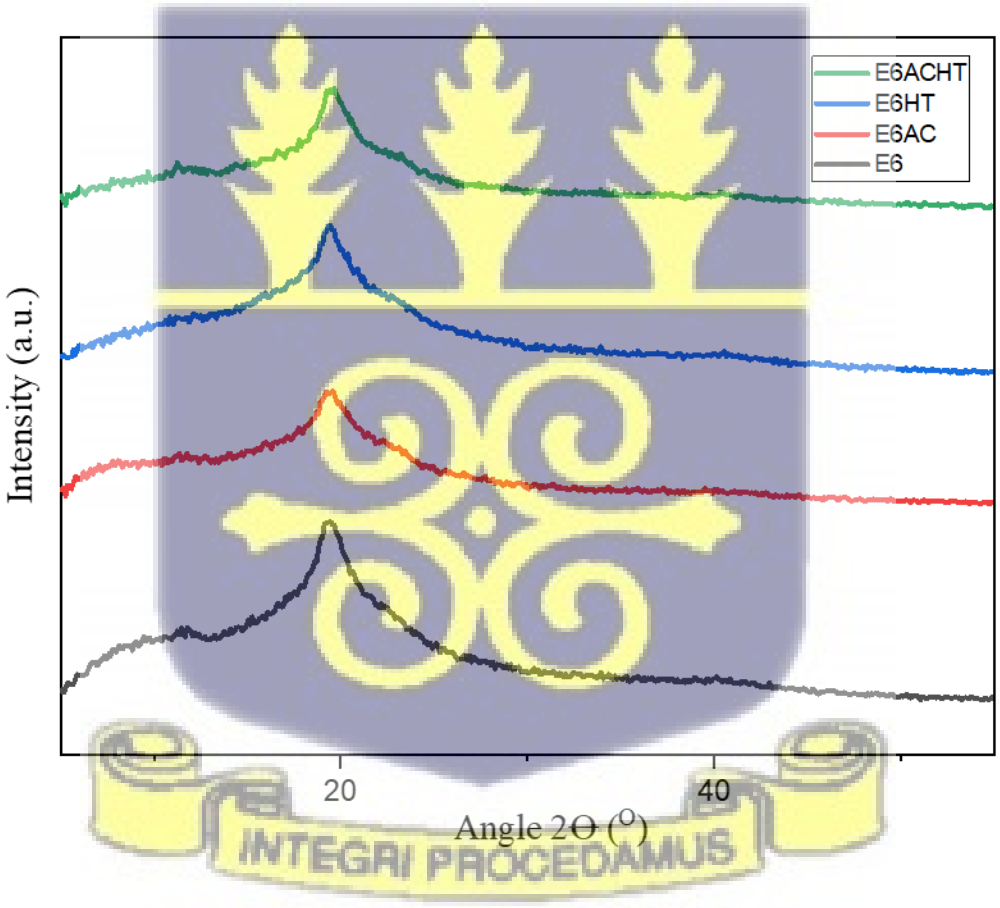


Fig 4.11 XRD graph for E6 film and its corresponding Acylated, Heat Treated and Acylated Heat Treated versions.

The peak at 19.3° is as a result of the strong presence of PVA in the composite mixture of E6, being the more abundant polymer (Shi et al., 2008). This corresponds to a semi crystalline film structure (Khodiri et al., 2020).

With acylation, heat treatment and a combination of both, it is noticed that intensity of the peak at 19.3° is reduced. This could be attributed to the presence of cross linkers, citric acid and CNC's which formed part of the constituents of the film. The presence of citric acid has been known to cause a reduction in the diffraction peak of PVA/Chitosan films (Wen et al., 2021). Acylation and heat treatment enhance the action of citric acid, giving rise to the reduction in peak intensity on the XRD graph.

4.2.4 Mechanical Properties

After treatments such as Acylation and heat treatment, tensile properties were altered. In fig 4.12a, it is noticed that the tensile properties of the non acylated films are very similar to that of the acylated. With increasing CNC content, there is a general decrease in tensile strength from 149.80 MPa in E3AC to 99.24 MPa in E12 AC. A slight increase in elongation is also observed, but not as much as is the case with non acylated films. This is because acetic anhydride is known to react strongly with citric acid, and is used to determine the amount of citric acid in milk (Marier & Boulet, 1958). As a result, the amount of citric acid available to function as a plasticizer is reduced, resulting in reduced elongation.

In fig 4.12b, values for tensile strength are higher than that of non acylated and acylated films, however the values of elongation are much further reduced. This indicates that heat treated films are stronger but fail more easily once their ultimate tensile stress is attained.

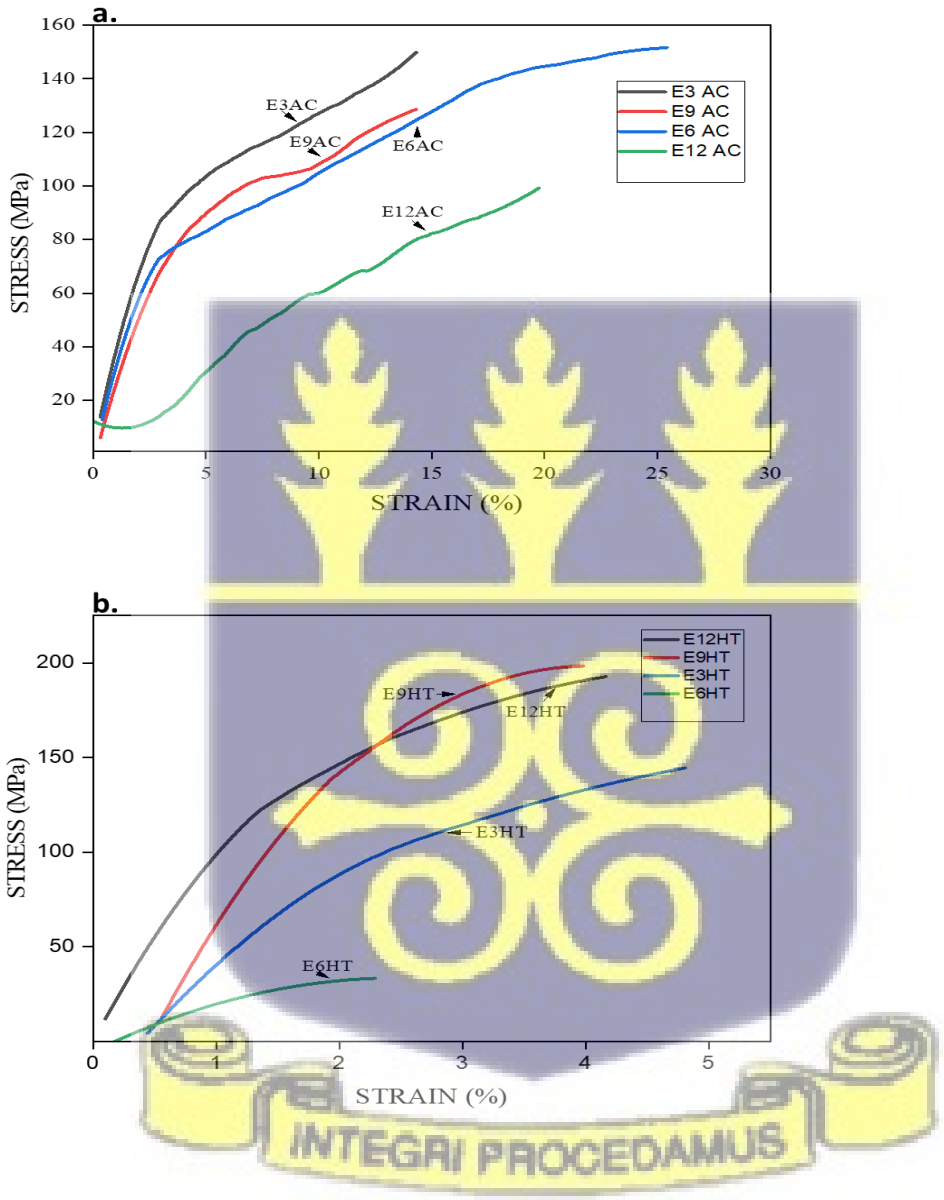


Fig 4.12 Tensile stress strain curves of a) E3 – E12 (AC) films and b) E3 – E12 (HT)

Table 4.2 shows the specific Tensile strength, Young's modulus and elongation values corresponding to Acylated and Heat Treated films. The elongation values are much lower than the untreated films discussed in table 4.1. This is due to the fact that acylation and heat treatment further strengthened the covalent and ionic bonds making it more difficult to stretch the films for long periods.

Table 4.2 Values for Tensile strength, Young's modulus and Elongation of Acylated and Heat treated films

Film	Tensile Strength (MPa)	Young's Modulus	Elongation
E3AC	149.81	7.14 ± 0.17	14.31
E6AC	151.20	4.34 ± 0.05	25.02
E9AC	128.62	6.79 ± 0.17	14.10
E12AC	99.24	4.95 ± 0.03	9.65
E3HT	144.47	29.78 ± 0.09	4.75
E6HT	33.43	15.86 ± 0.56	2.23
E9HT	198.31	52.68 ± 1.98	3.87
E12HT	192.67	37.77 ± 1.45	4.11

In fig 4.13 below, the tensile strength and elongation of all acylated and heat treated films are observed in the bar chart below.

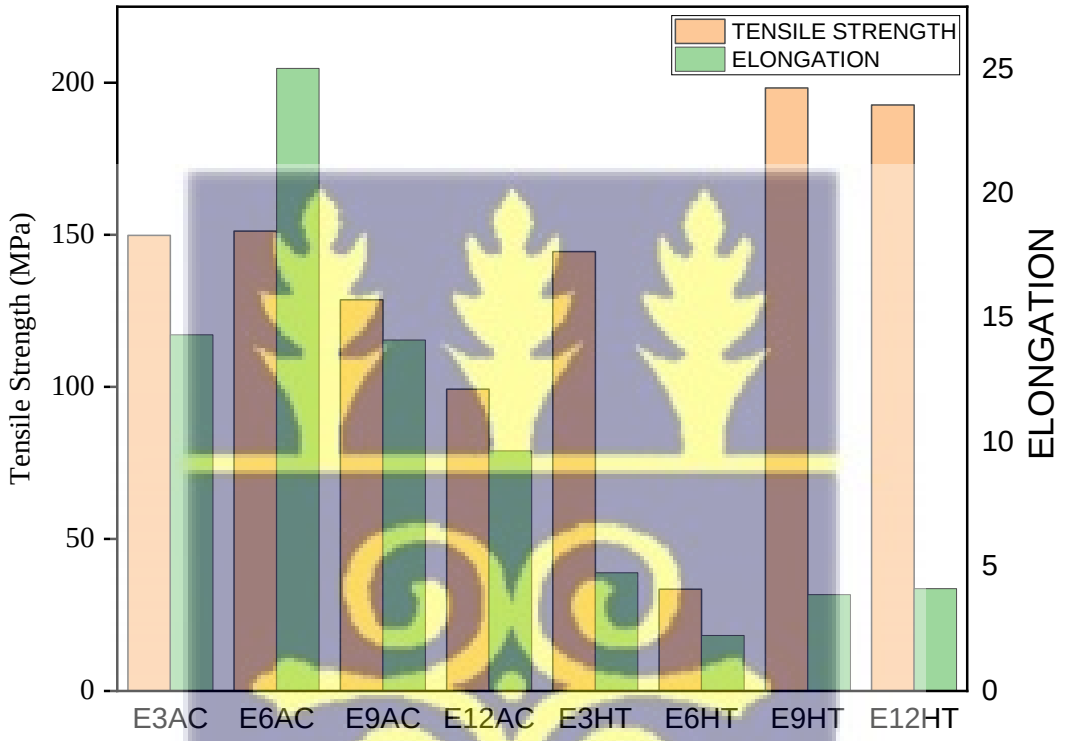


Fig 4.13 Tensile strength and elongation of acylated (AC) and Heat treated (HT) films

4.2.5 Moisture Content, Swelling Degree, Water Solubility and Water Vapour Permeability

4.2.5.1 Moisture Content

Tables 4.3, and 4.4 show the moisture content of acylated, heat treated and acylated heat treated films respectively, these values are likewise represented and described in fig 4.14.

Table 4.3 Moisture content of Acylated films

Acylated Films	Moisture Content (%)
E3AC	10.80
E6AC	13.37
E9AC	14.18
E12AC	10.57

Table 4.4 Moisture content of Heat treated films

Heat Treated films	Moisture Content (%)
E3HT	9.86
E6HT	10.28
E9HT	11.12
E12HT	11.19

Table 4.5 Moisture content of Acylated Heat treated films

Acylated Heat treated Films	Moisture Content (%)
E3ACHT	11.48
E6ACHT	12.49
E9ACHT	8.39
E12ACHT	12.44

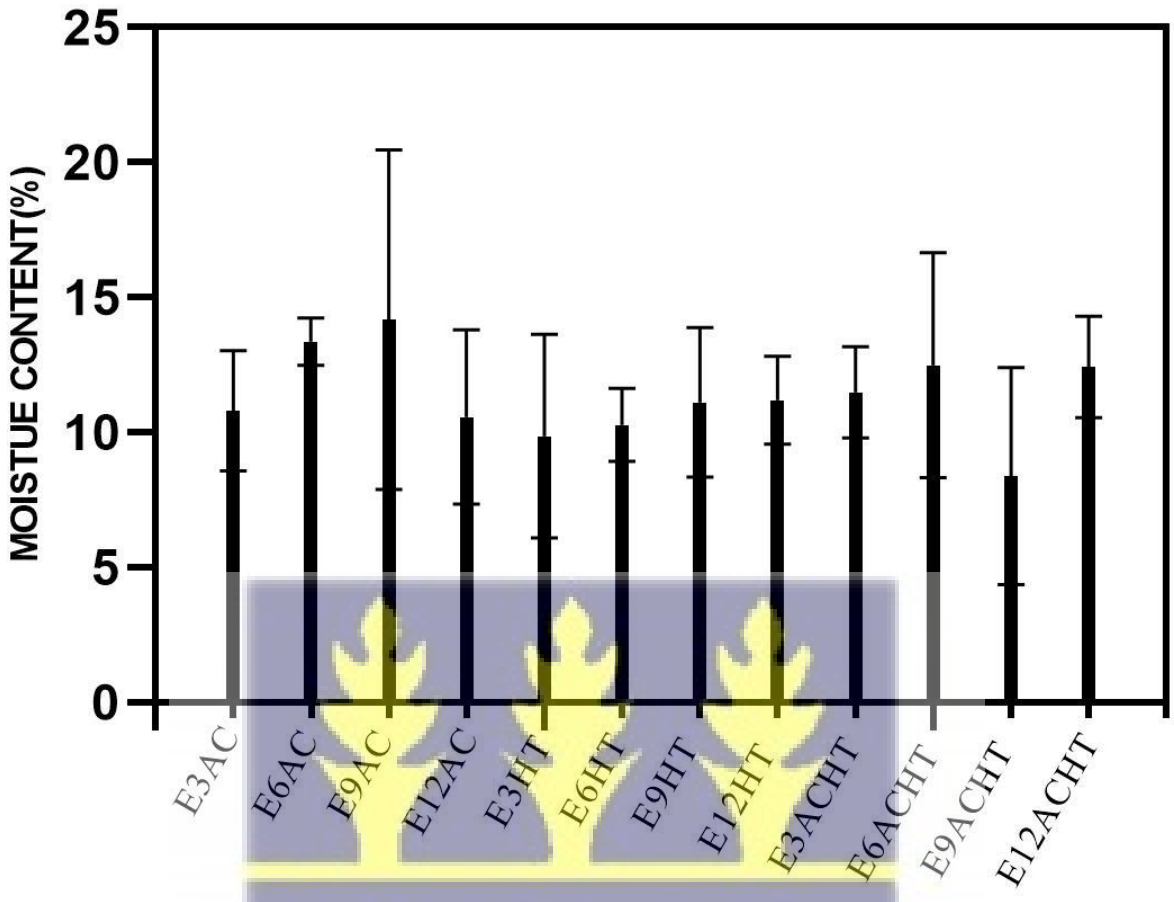


Fig 4.14 Moisture content for acylated, heat treated and acylated heat treated films

E9 is noticed to have the highest moisture content with an average of 20.74%, while E9ACHT has the least average value of 8.39%. Most films were within the 10-11% margin and an average of 12.98% moisture content.

4.2.5.1 Swelling Degree

The data for swelling degree of acylated and heat treated films is presented in fig 4.15 below.

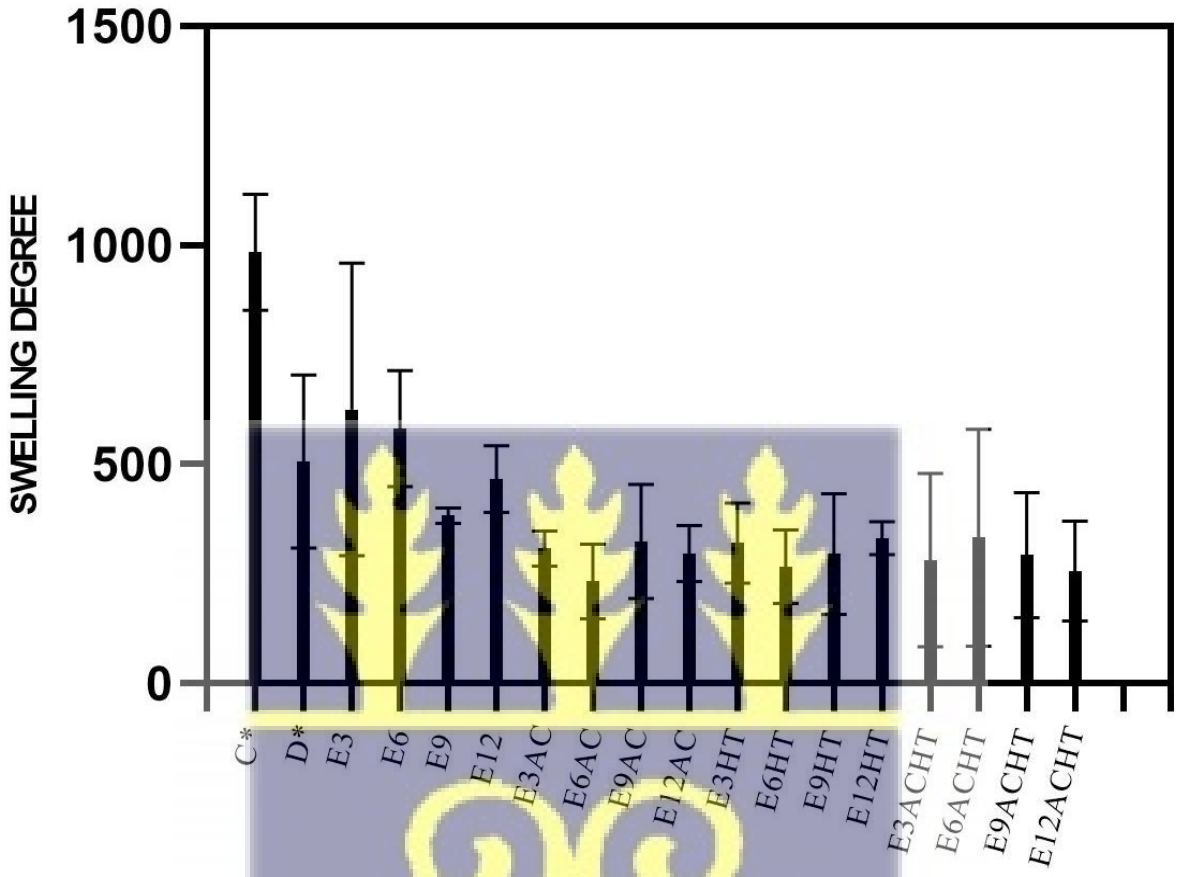


Fig 4.15 Trends in Swelling Degree for Acylated, Heat Treated Films and Acylated Heat Treated Films

In fig 4.15, the acylated, heat treated and acylated heat treated films generally have lower swelling degrees compared to the regular non treated films. The initial rise in swelling degree for all films

is expected with the presence of PVA in the polymer mix, which has the property of expanding when immersed into water (Wen et al., 2021).

Among the acylated films, E6AC was noticed to have the least swell of 233% with the average swelling degree for this group as 290.7. From the error values, there was not much significant difference in swelling degree of acylated films with increasing concentration of CNC's.

For heat treated films, swelling degree values also reduced significantly compared to regular films. Heat treatment in the presence of cross linking agents is already known to reduce swelling degree in polymer films (Rubentheren et al., 2016). The average swelling degree for all heat treated films was 285.1, indicating that acylation and heat treatment were similar in their effect on this property.

A combination of acylation and heat treatment had no significant effect in further reducing the swelling degree of the films, with the mean value for all acylated heat treated films being 291.4.

In fig 4.16 below, every regular film is compared in terms of swelling degree to its acylated, heat treated and acylated heat treated version. The graphs consistently show that treated films always have lower swelling degree compared to their corresponding regular films.

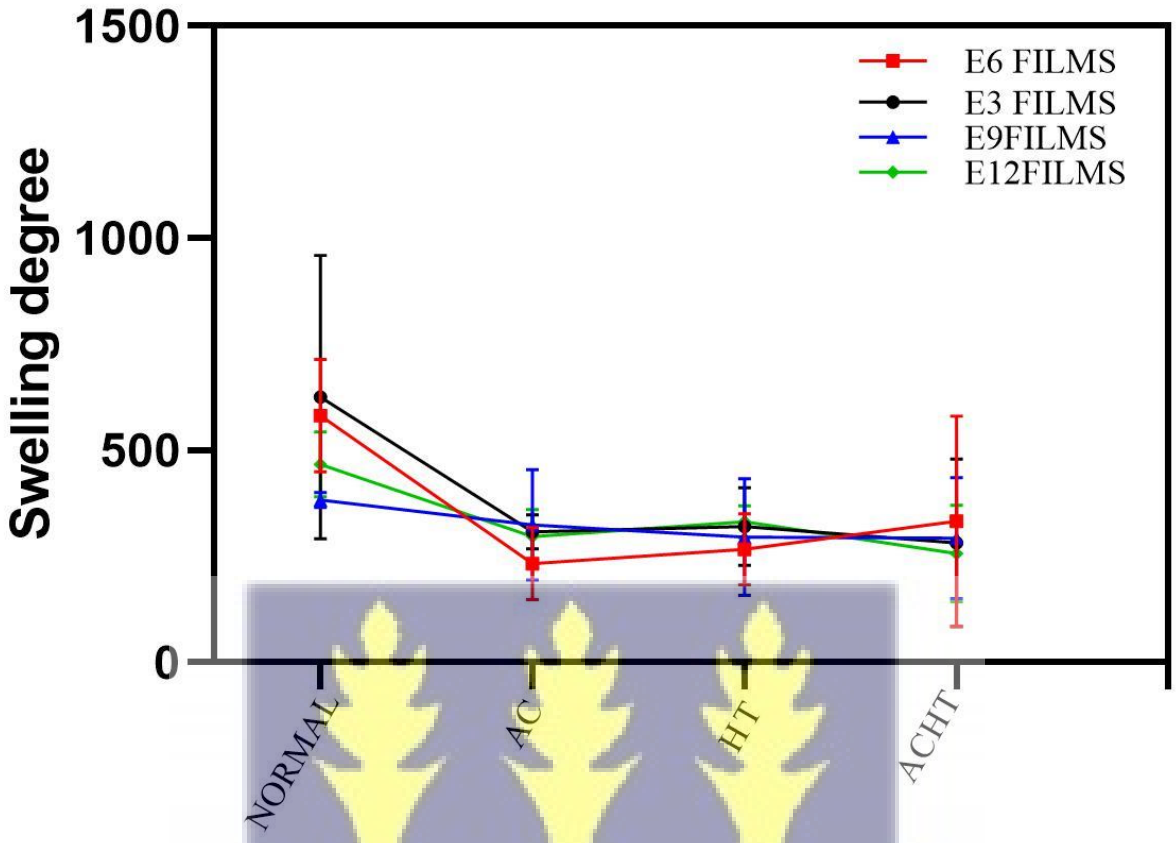


Fig 4.16 Trends in swelling degree recorded for E3-E12 regular (normal), Acylated (AC), heat treated (HT) and Acylated heat treated (ACHT) films.

4.2.5.2 Water Solubility

Water solubility values for treated films are lesser than that of regular films as shown in fig 4.17.

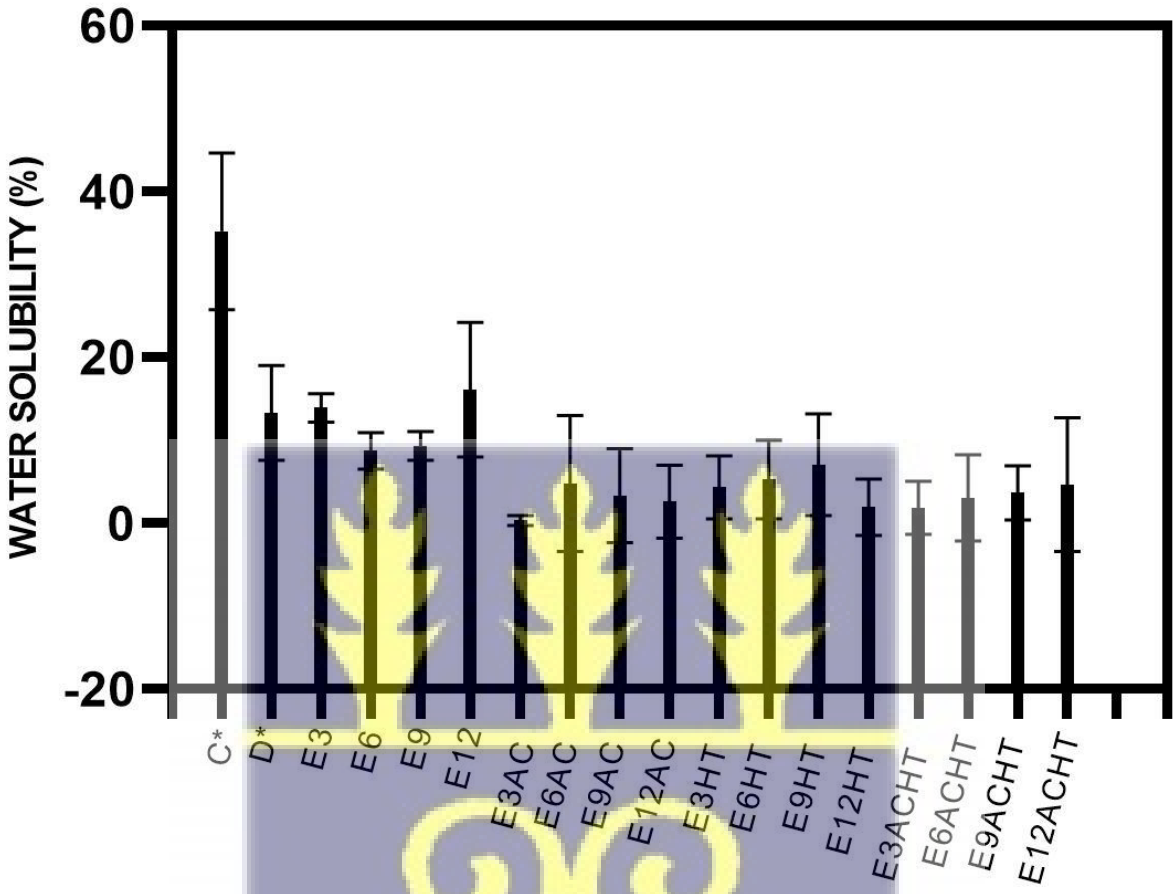


Fig 4.17 Trends in water solubility observed for C*, D*, E3-E12 regular, Acylated (AC), heat treated (HT) and Acylated heat treated (ACHT) films.

This observation indicates that treatments to improve moisture sensitivity result in the formation of films that do not dissolve easily when in contact with moisture. The degree of acylation has a strong bearing on the reduction of solubility of films in contact with moisture (Zong et al., 2000).

This is validated by the data wherein all acylated film have an average solubility of 2.74%. Heat treatment as a standalone measure also decreased the solubility of the films, having an average value of 4.64% which is slightly higher than that of acylated films. Acylation plus heat treatment resulted in films that were more stable in water than merely heat treated films and less stable compared to purely acylated films with an average solubility of 3.3%. All regular films had an average solubility of 12%.

4.2.5.3 Water Vapour Permeability

Figures 4.18a and b below show the water vapour permeability values of both acylated and heat treated films.

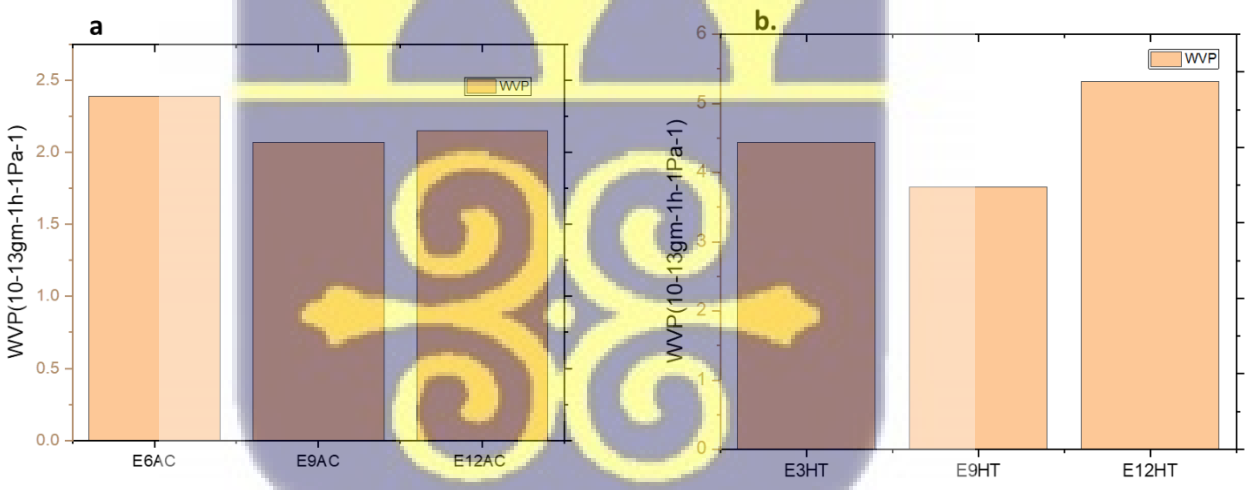


Fig 4.18 Water Vapour Permeability (WVP) of a) Acylated films b) Heat Treated films

Heat treatment from Fig 4.18b reduced the water vapour of the film slightly, from 4.6 to 4.4 x (10^{-13}gm-1h-1Pa^{-1}). The increase in temperature causes a reduction in the tendency of the film to

absorb moisture from the surroundings and this occurrence is what reduces the WVP slightly (Xianda et al., 1987). The reduction is noted in E3HT films and subsequently E9HT films, E12HT films however have increased WVP due to the presence of excess CNC's. In Fig 4.18a, the acylated films have the least values for WVP, with an over 60% reduction in value which is consistent with work done by Li et al. (2019).

4.2.5.3 Test of film stability in water

In fig 4.19 the graphs represent the behavior of the prepared films immersed in water overtime.

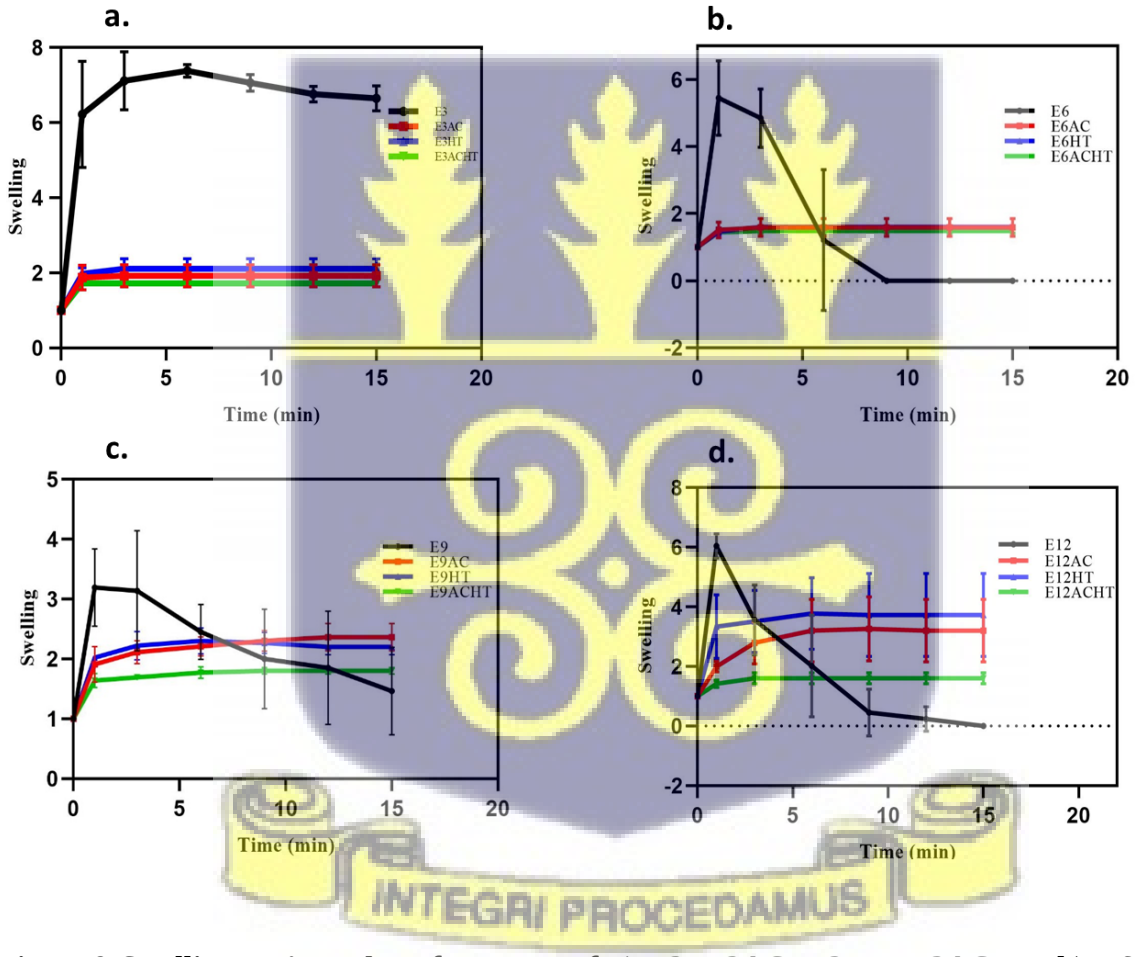


Fig 4.19 Swelling ratio and performance of a) E3, E3AC, E3HT, E3ACHT, b) E6, E6AC, E6HT, c) E9, E9AC, E9HT, E9ACHT and d) E12, E12AC, E12HT, E12ACHT films in water for 15 minutes.

Regular films with their respective acylated, heat treated and acylated heat treated versions were compared. In fig 4.19a, the regular E3 film swells over six times its normal weight after immersion for one minute and over seven times after 3 minutes. The value then reduces steadily over time. Fig 4.20 is a pictorial presentation of all films and their reaction to immersion in water over a period. There are clear films looking films as well as slightly browned films due to acylation and heat treatment procedures. Fig 4.20a shows that the E3 film completely dissolves in water after one minute but the film forming solution remains and can be weighed to determine the swelling ratio, this residue is however expected to further dissolve completely over time. As a contrast to the regular film, the E3AC, E3HT and E3ACHT films swell to approximately double their size after the first minute, with minimal swells within 3 and 6 minutes of immersion, until there is a stable weight observed over 9 to 15 minutes. Fig 4.20 (e, i & m) show that the treated films are still in shape and stable long after 15 minutes of immersion, these films will subsequently lose the excess moisture over time and return to their initial weight. It is also clear from fig 4.21 (e, f and g) that acylated, acylated heat treated and heat treated films do not dissolve in water or become overly weak like regular films.

In fig 4.19b, regular E6 films swell to about 5 times their initial weight after immersion for a minute. Due to its high solubility in water, after 3 minutes of immersion, a great portion of the initial previously swollen film is lost. After 9 minutes, the film completely dissolves in water such that weight measurements cannot be recorded, this is shown in fig 4.21a. The acylated, heat treated and acylated heat treated films had a similar trend of swelling to approximately 1.5 times their weight after the first minute after which the weight remained constant for the rest of the period.

The patterns in E9 films from fig 4.19c and E12 films in fig 4.19d were similar. The E9 film expanded 3 times its weight, and the E12 film was 5 times its weight after the first minute of

immersion. Afterwards, both films spiraled down in weight due to their high solubility property as shown in fig 4.21b and c. The acylated, heat treated and acylated heat treated films were noted to increase in weight for the 1, 3 and 6 minute time intervals of immersion, their weights however became somewhat constant after 9 minutes onwards.

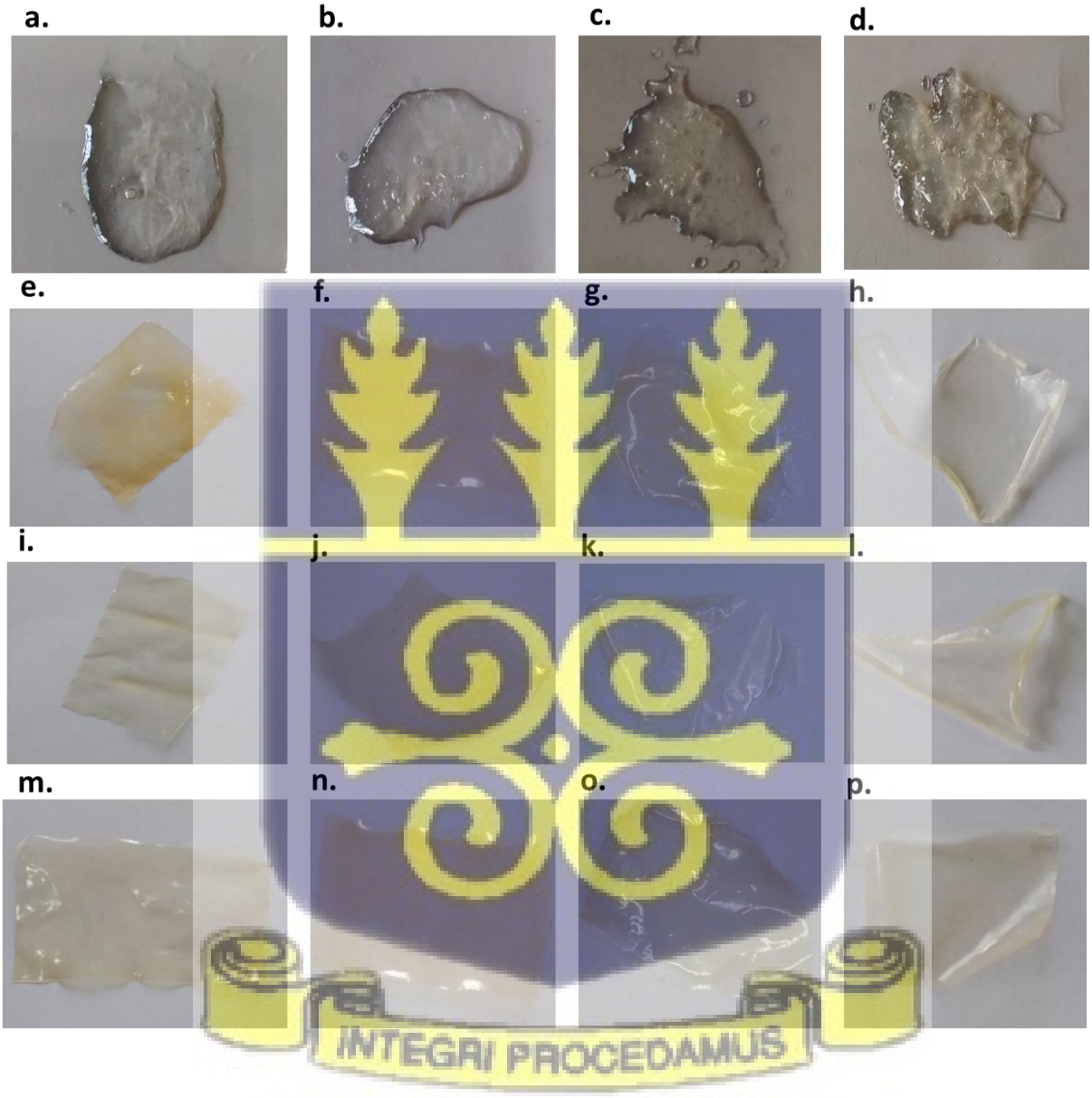


Fig 4.20 Pictorial presentation of a) E3, b) E6, c) E9 and d) E12 films after 1 minute immersion in water, e) E3AC, f) E6AC, g) E9AC, and h) E12AC films hours after 15 minutes immersion in water, i) E3HT, j) E6HT, k) E9HT, and l) E12HT films hours after 15 minutes

immersion in water and m) E3ACHT, n) E6ACHT, o) E9ACHT, and p) E12ACHT films hours after 15 minutes immersion in water.

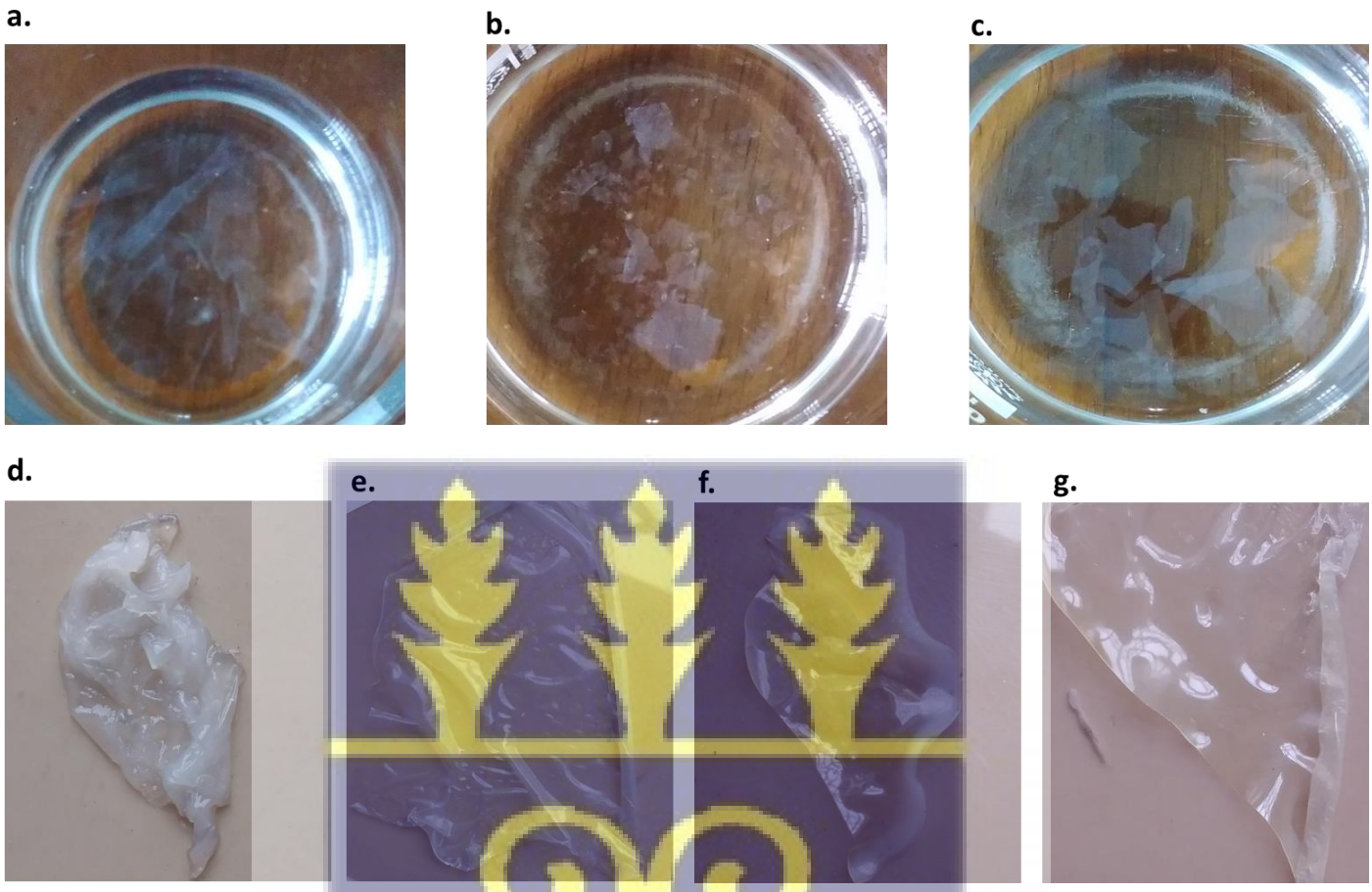


Fig 4.21 Pictorial presentation of a) E6, b) E9 and c) E12 films completely dissolved in water, d) E3, e) E6AC, f) E9ACHT, and g) E12HT films immediately after 15 minutes of immersion.

4.3 To Study the Ability of the Polymer Hydrogel Films in Extending the Shelf Life of Vegetables Using Lettuce as a Model Vegetable

4.3.1 Shelf life Studies with Lettuce as a Model Vegetable

Fig 4.21-24 show the changes in appearance of lettuce packaged in the prepared films over a period of 11 days. The control films (Fig 4.21a) begun to turn brown from the fourth day of storage most likely due to alteration of the phenol metabolism in the lettuce due to cutting (Degl'Innocenti et al., 2007).

In fig 4.21, the lettuce is packaged in the regular films E3, E6, E9 and E12 and observed over the period of 11 days. In all cases, there was little or no browning observed over the entire period of observation. PVA/chitosan composite films have been proven to halt oxygen exchange between the environment and that which they package. The presence of oxygen is key in initiating the browning process (Yu et al., 2018). Chitosan possesses antimicrobial properties and specifically inhibits the activities of Polyphenol oxidase which is the enzyme responsible for browning in lettuce (Pasquariello et al., 2015).

Fig 4.22-24 shows the behavior of lettuce when packaged in acylated, heat treated and acylated heat treated films of E3, E6, E9 and E12. It is noticed that the modification of the films had no effect on their ability to likewise sojourn browning reactions.

Acylation and heat treatment focus on ridding the films of free hydroxyl groups that confer hydrophilic properties on the film. Considering acylation, the NH_2 group present in chitosan is believed to be responsible for its antimicrobial property (Kim, 2018). The acylation reaction however prefers to bond with the OH groups. The antimicrobial property is therefore preserved.

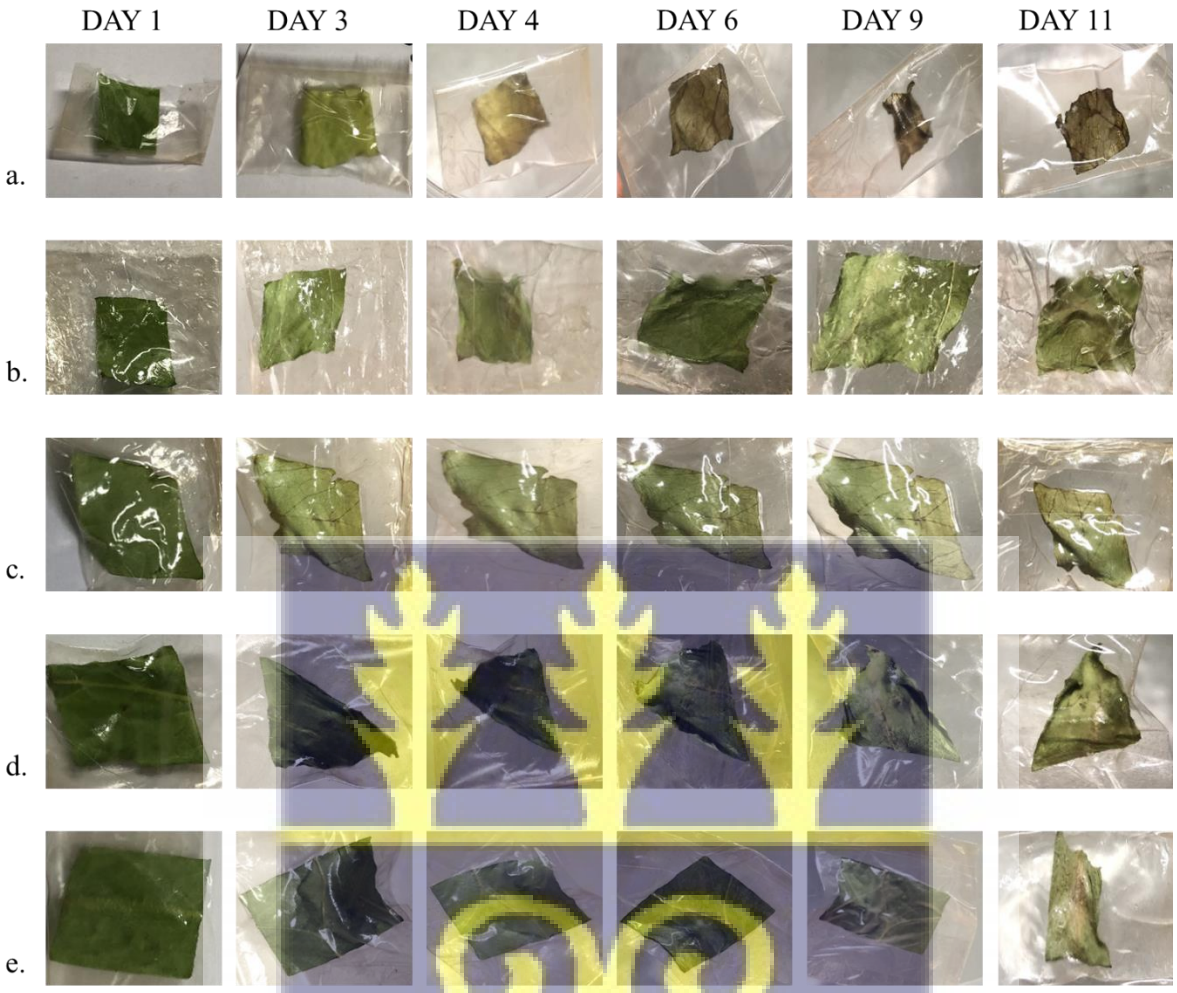


Fig 4.21 Observation of a) Control b) E3 c) E6 d) E9 e) E12 samples over a period of 11 days

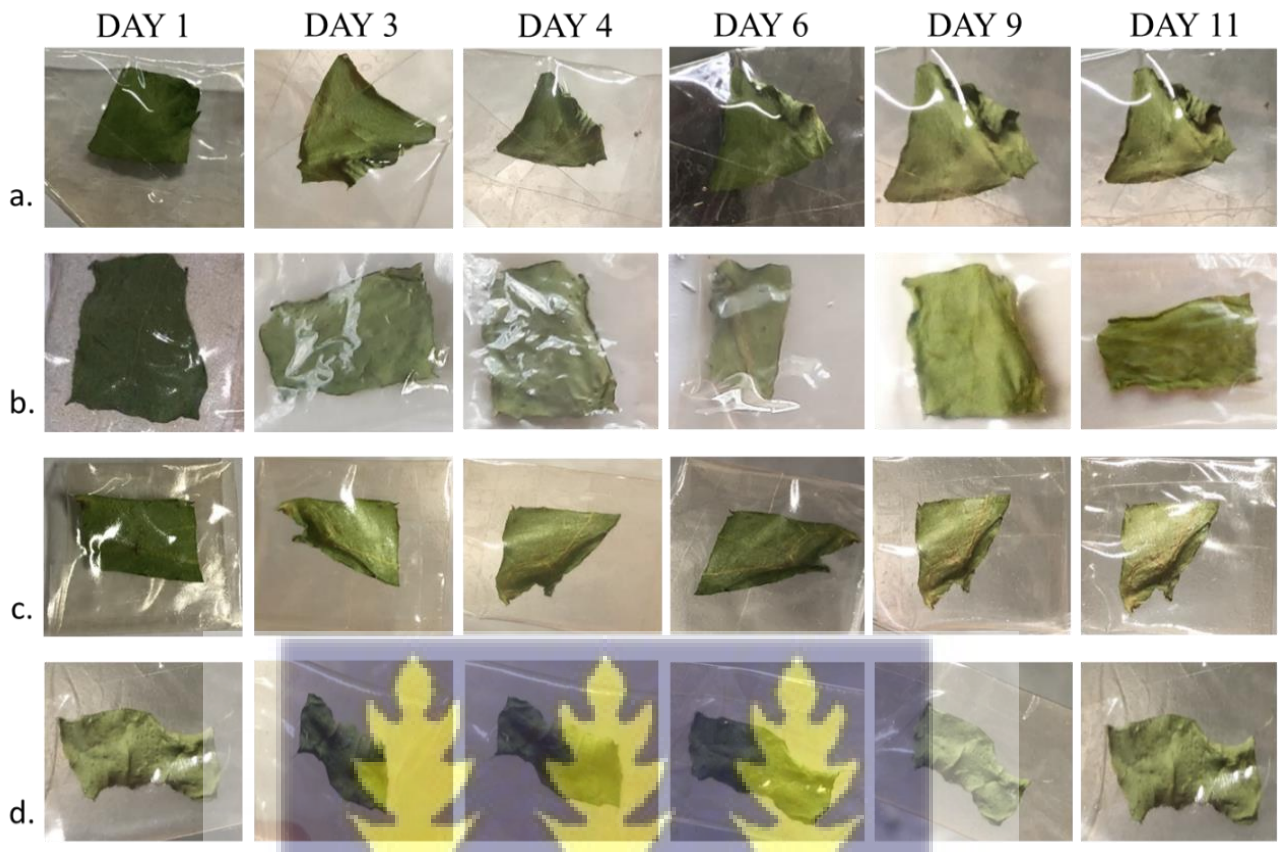


Fig 4.22 Observation of a) E3AC b) E6AC c) E9AC d) E12AC over a period of 11 days

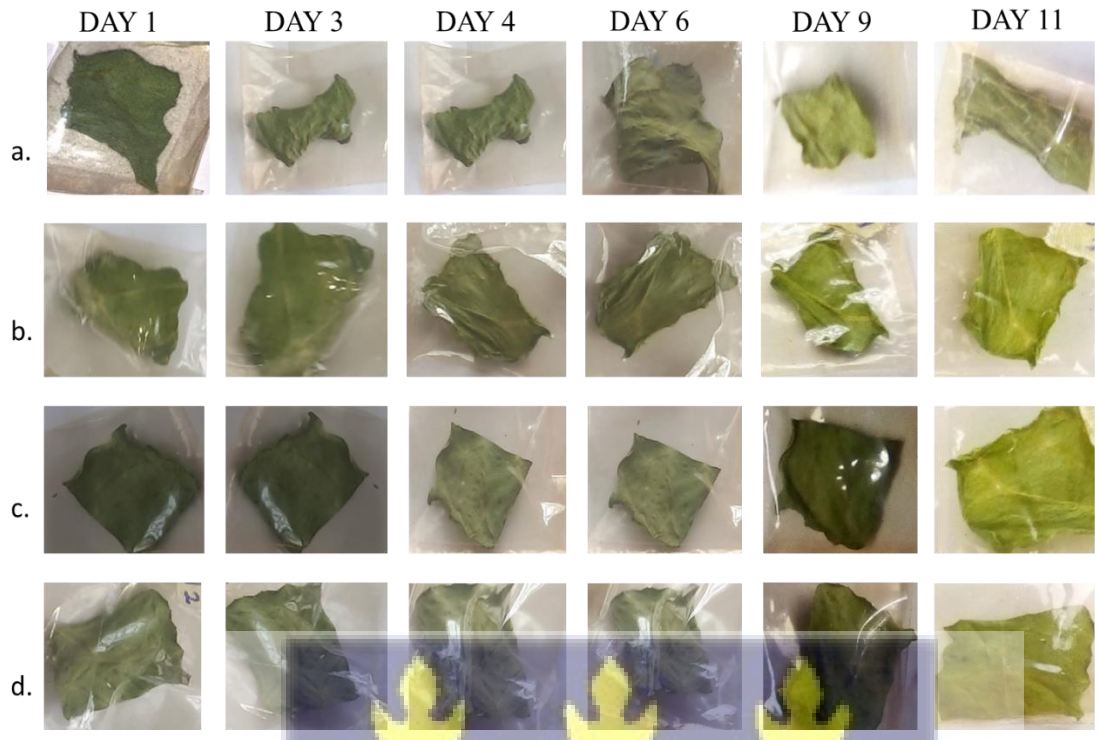


Fig 4.23 Observation of a) EHT b) E6HT c) E9HT d) E12HT over a period of 11 days

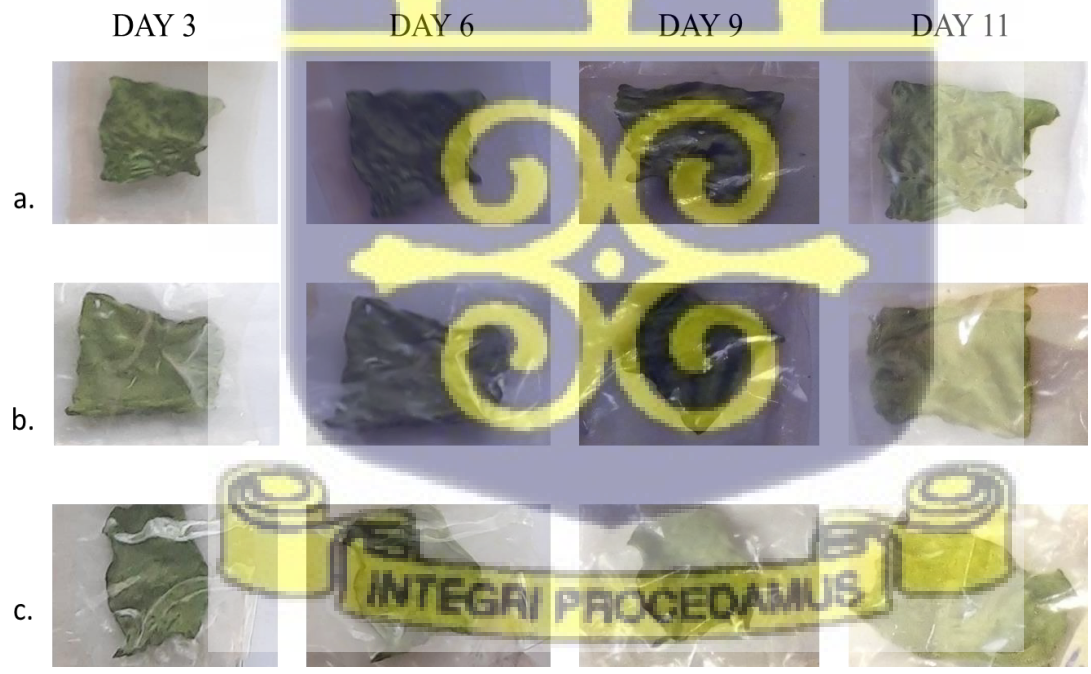


Fig 4.24 Observation of a) E6ACHT b) E9ACHT c) E12ACHT over a period of 11 days

In all prepared and treated films, another observation was the loss of moisture from the leaves. After 3 days of storage, a considerable amount of moisture was lost in the lettuce under study. This is due to the high moisture absorption ability of chitosan and PVA. Chitosan is noted for absorbing high percentages of moisture with increase in relative humidity (Aguirre-Loredo et al., 2016) and PVA has been found to have a moisture absorption rate of over 40% after 8 hours (Asrofi et al., 2019).

CHAPTER 5

5.0 CONCLUSION

5.1 Conclusion

The research aimed to fabricate and test an effective biobased/biodegradable fresh food packaging film for extension of the shelf life of fresh vegetables. The study showed that the increase of CNCs in the polymer blend strengthened the films and did not lead to reduction in moisture absorption. Generally, the inclusion of CNCs resulted in an improvement of the tensile strength and led to desirable retention of the moisture absorption capacity, but it also raises the question of what is the optimum CNC concentration require for the maximum moisture. Further work will be needed to determine the optimum levels of CNCs necessary in obtaining the maximum strength of the biobased/biodegradable films. However, the CNCs reinforced films dissolved after a prolong residence time in water, a behavior similar to the chitosan and PVA films without CNCs. The heat treatment of the films led to increase tensile strength and decrease in elongation. This is due to the reduction in pockets of water trapped in polymer chains, reducing the plasticizing effect of water in between polymer chains thereby enabling polymer chain - poly chain interaction strengthening the film while reducing the film elongation at failure. This therefore led to reduction in swelling degree of the films as less hydrophilic groups are available to interact with the water molecules. The acylation of the films strengthened the film and decreases the moisture absorption. The acylation process drained the water molecules from the film and promoted close interaction between polymer chains thereby strengthening the film. However, the highest gain in film strength and highest reduction moisture absorption were also obtained from combined heat treatment and acylation. This was probably due to combined effect of both treatments.

5.2 Recommendations for future studies

This study may serves as a good foundation upon which further research can be conducted into understanding the nature of the interaction of the biobased and biodegradable multipolymer blends with the nano fillers in improving the properties of the film. Specifically, we recommend that the following studies can be undertaken;

1. Further works should be undertaken to investigate what the optimum concentration or levels of CNCs are needed to obtain the maximum physicochemical properties of the film such as high mechanical properties and retaining the moisture absorption capacity of the films.
2. Also, it is imperative to study the mechanical properties of the films when in contact with moisture as the study in this work focused mainly on the dry weight of the films, post contact with moisture.
3. There must be further research conducted to determine the extent of the microbial action of the films due to the presence of chitosan and citric acid.
4. It is important to determine the amount of citric acid released over time in order to determine the safety of the films as packaging materials.

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APPENDICES

APPENDIX A

Moisture Content for Objective One

SAMPLE	MOISTURE CONTENT			AVERAGE	STD DEVIATION
C	12.5	10.53	7.14	10.056667	2.71116826
D	9.09	16.6	7.14	10.943333	4.994900733
E3	20	20	18.75	19.583333	0.721687836
E6	14.28	25	18.18	19.153333	5.425876273
E9	15.79	21.43	25	20.74	4.643608511
E12	17.65	22.22	11.11	16.993333	5.584033787

Water Solubility for Objective One

Sample	WATER SOLUBILITY (%)			AVERAGE	STD DEVIATION
C	29.41	46.15	30	35.18667	9.499106976
D	20	10	10	13.33333	5.773502692
E3	15.38	14.28	12	13.88667	1.723987626
E6	11.11	8.33	6.66	8.7	2.247954626
E9	11.11	9.09	7.69	9.296667	1.719340959
E12	14.28	9.09	25	16.12333	8.113595586

Swelling Degree for Objective One

Sample	SWELLING DEGREE (%)			AVERAGE	STD DEVIATION
C*	858.82	1123.07	970	983.963333	132.6772273
D*	640	600	280	506.666667	197.3153145
E3	987.5	561.53	328.57	625.866667	334.14307
E6	593.33	444.44	708.33	582.033333	132.3071957
E9	387.5	363.63	400	383.71	18.47883384
E12	378.57	512.5	509.09	466.72	76.35917692

Sample	Thickness	Diameter	Cup+Water+Film	2 hrs	4hrs	6hrs	8hrs	10hrs	12hrs	14hrs	16hrs	18hrs	20hrs	22hrs	24hrs
C*	0.02	5.7	7.112	7.0514	7.0359	7.022	7.0051	6.9858	6.9646	6.9403	6.9198	6.903	6.8824	6.8633	6.848
D*	0.025	5.6	7.1278	7.1914	7.1727	7.1596	7.1418	7.1217	7.1006	7.0765	7.0544	7.0378	7.0176	6.9967	6.977
E3	0.003	5.6	7.7839	7.8772	7.8619	7.8455	7.8297	7.8105	7.7882	7.7625	7.738	7.7198	7.6987	7.68	7.6635
E6	0.002	5.6	7.0102	7.0885	7.0639	7.044	7.0282	7.0032	6.9772	6.9548	6.9345	6.9117	6.8907	6.8691	6.8505
E9	0.0025	5.7	7.4537	7.5431	7.5303	7.5158	7.5023	7.4823	7.4574	7.4346	7.415	7.3945	7.3762	7.3553	7.334
E12	0.0025	5.7	7.3038	7.3868	7.3635	7.3472	7.3339	7.3108	7.2875	7.2658	7.2466	7.2249	7.1807	7.1404	7.1144

WATER VAPOUR PERMEABILITY FOR OBJECTIVE ONE

DATA FOR TEST OF FILM STABILITY IN WATER

E3/TIME	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	7.833333	5.1875	5.642857	6.221230159	1.414565
3	8	6.5625	6.785714	7.116071429	0.773598
6	7.333333	7.25	7.571429	7.384920635	0.166808
9	7.166667	6.8125	7.214286	7.064484127	0.21952
12	6.666667	6.625	7	6.763888889	0.205537
15	6.333333	6.625	7	6.652777778	0.3342

E6	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	6.285714	4.181818	5.875	5.447510823	1.115193
3	5.142857	5.545455	3.875	4.854437229	0.871776
6	0	3.636364	0	3.636364	2.099456
9	0	0	0	0	0
12	0	0	0	0	0
15	0	0	0	0	0

E9	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	3.827586	3.222222	2.533333	3.194381	0.647575
3	4.137931	3.148148	2.133333	3.139804	1.002325
6	2.793103	2.62963	1.933333	2.452022	0.456574
9	2.793103	2.074074	1.133333	2.00017	0.832349
12	2.758621	1.925926	0.866667	1.850404	0.948235
15	2.103448	1.62963	0.666667	1.466582	0.732137

E12	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	5.714286	6.5	5.916667	6.043650794	0.407959
3	2.928571	4.916667	2.833333	3.55952381	1.176284
6	2.142857	3.666667	0.25	2.01984127	1.711652
9	1.357143	0	0	1.35	0.783547
12	0.714286	0	0	0.714	0.412393
15	0	0	0	0	0

APPENDIX B

MOISTURE CONTENT FOR OBJECTIVE TWO

SAMPLE	MOISTURE CONTENT			AVERAGE	STD DEVIATION
E3AC	13.33	9.09	10	10.806667	2.232136495
E6AC	12.5	14.28	13.33	13.37	0.890673902
E9AC	10	21.43	11.11	14.18	6.303165871
E12AC	8.33	14.28	9.09	10.566667	3.238214529
E3HT	11.54	5.55	12.5	9.8633333	3.766169584
E6HT	9.09	11.76	10	10.283333	1.357362639
E9HT	10	14.28	9.09	11.123333	2.771359474
E12HT	13.04	10.53	10	11.19	1.623915022
E3ACHT	11.11	13.33333	10	11.481111	1.697371606
E6ACHT	8.33	12.5	16.66667	12.498889	4.168333444
E9ACHT	5.88	6.25	13.04	8.39	4.03126531
E12ACHT	10.53	14.28	12.5	12.436667	1.875802051

WATER SOLUBILITY FOR OBJECTIVE TWO

Sample	WATER SOLUBILITY (%)			AVERAGE	STD DEVIATION
E3AC	0	0	1	0.333333	0.577350269
E6AC	0	0	14.28	4.76	8.244561844
E9AC	0	0	9.9	3.3	5.715767665
E12AC	0	7.7	0	2.566667	4.445597073
E3HT	0	7.14	5.8	4.313333	3.795066973
E6HT	0	6.67	9.09	5.253333	4.707678126
E9HT	11.11	10	0	7.036667	6.119152991
E12HT	5.88	0	0	1.96	3.394819583
E3ACHT	0	0	5.56	1.853333	3.210067497
E6ACHT	9.09	0	0	3.03	5.248113947
E9ACHT	6.25	4.76	0	3.67	3.264460139
E12ACHT	0	0	14	4.666667	8.082903769

SWELLING DEGREE FOR OBJECTIVE TWO

Sample	SWELLING DEGREE (%)			AVERAGE	STD DEVIATION
E3AC	261.53	330	333.33	308.286667	40.52667804
E6AC	330	170	200	233.333333	85.04900548
E9AC	463.63	206.25	304.54	324.806667	129.8813668
E12AC	370	250	270	296.666667	64.29100507
E3HT	426.08	270.58	264.28	320.313333	91.65076832
E6HT	280	177.77	342.85	266.873333	83.31916726
E9HT	455.55	210	222.22	295.923333	138.3757082
E12HT	376.47	309.09	311.11	332.223333	38.33204578
E3ACHT	507.69	200	138.88	282.19	197.6653766
E6ACHT	618.18	180	200	332.726667	247.4120129
E9ACHT	433.33	300	147.61	293.646667	142.9659163
E12ACHT	370.58	258.33	142.85	257.253333	113.8688177

WATER VAPOUR PERMEABILITY FOR OBJECTIVE TWO

Sample	Thickness	Diameter	Cup+Water+Film	2 hrs	4hrs	6hrs	8hrs	10hrs	12hrs	14hrs	16hrs	18hrs	20hrs	22hrs	24hrs
E6AC	0.0027	5.7	7.0554	7.1411	7.1272	7.1147	7.0898	7.0669	7.0446	7.0187	6.9982	6.9791	6.9562	6.9377	6.9194
E9AC	0.0025	5.6	7.5156	7.6213	7.602	7.5839	7.5667	7.5421	7.5232	7.4989	7.4769	7.4565	7.435	7.4193	7.3981
E12AC	0.0025	5.6	7.3223	7.4023	7.3885	7.3712	7.3514	7.3313	7.3102	7.2884	7.2685	7.2547	7.233	7.2178	7.2
E3HT	0.003	5.8	9.7949	9.7736	9.7581	9.7424	9.7223	9.6964	9.6727	9.6487	9.6266	9.6064	9.5825	9.5641	9.5427
E9HT	0.0035	5.7	9.3771	9.3625	9.3725	9.3537	9.3329	9.3108	9.2878	9.2656	9.243	9.2243	9.1989	9.1817	9.1615
E12HT	0.003	5.6	9.5008	9.4854	9.4669	9.4378	9.3786	9.3562	9.3279	9.3013	9.2784	9.2587	9.2359	9.2196	9.1985

DATA FOR TEST OF FILM STABILITY IN WATER

E3AC	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.666667	1.714286	2.25	1.876984127	0.323917
3	1.666667	1.857143	2.25	1.924603175	0.29746
6	1.666667	1.857143	2.25	1.924603175	0.29746
9	1.666667	1.857143	2.25	1.924603175	0.29746
12	1.666667	1.857143	2.25	1.924603175	0.29746
15	1.666667	1.857143	2.25	1.924603175	0.29746

E6AC	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.777778	1.333333	1.428571	1.513227513	0.234004
3	1.888889	1.444444	1.428571	1.587301587	0.261303
6	1.888889	1.444444	1.428571	1.587301587	0.261303
9	1.888889	1.444444	1.428571	1.587301587	0.261303
12	1.888889	1.444444	1.428571	1.587301587	0.261303
15	1.888889	1.444444	1.428571	1.587301587	0.261303

E9AC	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	2.25	1.8	1.684211	1.911404	0.298894
3	2.25	2.2	1.894737	2.114912	0.192309
6	2.375	2.2	2.052632	2.209211	0.161381
9	2.5	2.2	2.210526	2.303509	0.170248
12	2.625	2.2	2.263158	2.362719	0.229326
15	2.625	2.2	2.263158	2.362719	0.229326

E12AC	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	2	2.166667	1.818182	1.994949495	0.174297
3	2.076923	3.5	2.818182	2.798368298	0.711745
6	2.076923	4.166667	3.363636	3.202408702	1.05416
9	2.076923	4.166667	3.545455	3.263014763	1.07312
12	2.076923	4.166667	3.363636	3.202408702	1.05416
15	2.076923	4.166667	3.363636	3.202408702	1.05416

E3HT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	2.125	2	1.8	1.975	0.163936
3	2.25	2.285714	1.8	2.111904762	0.270707
6	2.25	2.285714	1.8	2.111904762	0.270707
9	2.25	2.285714	1.8	2.111904762	0.270707
12	2.25	2.285714	1.8	2.111904762	0.270707
15	2.25	2.285714	1.8	2.111904762	0.270707

E6HT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.352941	1.5	1.555556	1.469498911	0.104694
3	1.529412	1.625	1.666667	1.607026144	0.070371
6	1.529412	1.625	1.666667	1.607026144	0.070371
9	1.529412	1.625	1.666667	1.607026144	0.070371
12	1.529412	1.625	1.666667	1.607026144	0.070371
15	1.529412	1.625	1.666667	1.607026144	0.070371

E9HT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	2.071429	2	2	2.02381	0.041239
3	2.071429	2.1	2.5	2.22381	0.239614
6	2.071429	2.5	2.333333	2.301587	0.216042
9	2.071429	2.4	2.333333	2.268254	0.173684
12	2.071429	2.2	2.333333	2.201587	0.13096
15	2.071429	2.2	2.333333	2.201587	0.13096

E12HT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	4.111111	3.777778	2.125	3.337962963	1.063596
3	4.111111	4.111111	2.3125	3.511574074	1.038429
6	4.777778	4.111111	2.4375	3.775462963	1.205703
9	4.888889	4.111111	2.1875	3.729166667	1.390607
12	4.888889	4.111111	2.1875	3.729166667	1.390607
15	4.888889	4.111111	2.1875	3.729166667	1.390607

E3ACHT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.714286	1.75	1.714286	1.726190476	0.02062
3	1.714286	1.75	1.714286	1.726190476	0.02062
6	1.714286	1.75	1.714286	1.726190476	0.02062
9	1.714286	1.75	1.714286	1.726190476	0.02062
12	1.714286	1.75	1.714286	1.726190476	0.02062
15	1.714286	1.75	1.714286	1.726190476	0.02062

E6ACHT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.444444	1.444444	1.444444	1.444444444	0
3	1.555556	1.444444	1.444444	1.481481481	0.06415
6	1.555556	1.444444	1.444444	1.481481481	0.06415
9	1.555556	1.444444	1.444444	1.481481481	0.06415
12	1.555556	1.444444	1.444444	1.481481481	0.06415
15	1.555556	1.444444	1.444444	1.481481481	0.06415

E9ACHT	SAMPLE 1	SAMPLE 2	SAMPLE 3		
0	1	1	1	1	0
1	1.5	1.7	1.714286	1.638095	0.119807
3	1.666667	1.7	1.714286	1.693651	0.024436
6	1.666667	1.8	1.857143	1.774603	0.097745
9	1.75	1.8	1.857143	1.802381	0.053611
12	1.75	1.8	1.857143	1.802381	0.053611
15	1.75	1.8	1.857143	1.802381	0.053611

E12ACHT	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE	STD DEV
0	1	1	1	1	0
1	1.5	1.272727	1.5	1.424242424	0.131216
3	1.5	1.818182	1.5	1.606060606	0.183702
6	1.5	1.818182	1.5	1.606060606	0.183702
9	1.5	1.818182	1.5	1.606060606	0.183702
12	1.5	1.818182	1.5	1.606060606	0.183702
15	1.5	1.818182	1.5	1.606060606	0.183702