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Development of Enhanced Food Packaging Systems based on Blends of Bio-Based and Biodegradable Polymers Exhibiting Antimicrobial Properties

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Development of Enhanced Food Packaging Systems based on Blends of Bio-Based and Biodegradable Polymers Exhibiting Antimicrobial Properties

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Declaration of Authorship

I, Aishat Aderayo Agboluaje, declare that this thesis titled, "Development of Enhanced Food Packaging Systems from Blends of Bio-Based and Biodegradable Polymer Composites Exhibiting Antimicrobial Properties" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at thisUniversity.
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- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clearexactly what was done by others and what I have contributed myself.

Signed:

_____

Date:

_____

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Abstract

Food packaging is one of the important aspects of food processing, which contributes greatly to decreasing post-harvest food losses. While ensuring that foods are adequately packaged, it is also important to consider the impact of these food packaging materials on the environment. Accordingly, this research work was aimed at investigating the potential of bio-based and biodegradable blends of polyvinyl alcohol (PVA) and chitosan loaded with antimicrobial agents such as turmeric, clove, and cinnamon for use in food packaging applications. The methodology involved creating PVA/chitosan films with varying compositions via solvent casting method and selecting the best composition exhibiting the highest tensile strength, followed by its incorporation with antimicrobial agents. The morphology of the film was investigated via scanning electron microscopy (SEM), which showed mostly homogeneous and compact texture. Interaction between film components was analyzed via Fourier transform infrared spectroscopy (FTIR), which indicated that the incorporation of antimicrobial agents did not alter the structure of the PVA/chitosan films. Thermogravimetric analysis showed that there was no significant effect on the thermal degradation profile of the antimicrobial films when compared with plain film. The mechanical properties of PVA/chitosan films were significantly altered by the addition of antimicrobial agents while antioxidant and antimicrobial activity of the films were enhanced. The shelf life of chicken breast meat and white cheese packaged within these films were investigated via pH and microbiological assay and the results showed effective inhibition against coliform and *S. aureus* in cheese and chicken breast meat. These results suggest that PVA/chitosan films have a great potential in the food packaging industry.

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List of Abbreviations

CFU	Colony forming unit
CIN	PVA/chitosan + cinnamon film
CLO	PVA/chitosan + clove film
CS	Chitosan
DPPH	1,1-diphenyl-2-picrylhydrazyl
DTG	First derivative thermogram
FT-IR	Fourier transform infrared spectroscopy
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
SEM	Scanning electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidin-1-yl-oxidanyl
TGA	Thermogravimetric Analysis
T_{final}	Final degradation temperature
T_{max}	Degradation temperature at 50% weight loss
T_{onset}	Initial temperature of degradation
TUR	PVA/chitosan + turmeric film

Chapter 1

1. Introduction

The World Summit on Food Security estimated that approximately one billion people across the globe are living in hunger (FAO, 2014). A possible solution to this would be to increase investment in food production, however this would be more effective when complemented with more sustainable strategies to reduce food loss altogether as this ultimately improves food security (UNEP/GRID-Arendal, 2010). One of the ways to reduce these food losses and achieve food security is by using suitable packaging.

Food packaging is an integral aspect of the food processing chain, it is considered an avenue to improve economic development by decreasing rate of food spoilage, ensuring safety of food, and improving food trade on a global scale (Olsmats and Wallteg, 2009; FAO, 2014). There are opinions that increasing investments in the development of improved and advanced food packaging can be instrumental in the reduction of food wastage and ultimately improve food safety and quality especially in developing countries. These will eventually improve the livelihood of small-scale food producers by enhancing integration of food products into sustainable value chains and improving market access (FAO, 2014).

Sustainable and biodegradable food packaging systems are largely gaining recognition due to the environmental and probable health hazards associated with the use of nonbiodegradable plastics and the wastes generated (Cesur et al., 2018). In 2018, about 359 million tonnes (MT) of plastics were produced and 385 MT were consumed within the year; this depicts that the number of plastics consumed surpasses the quantity produced because of the high value placed on the use of plastics (Rai et al., 2021). The tons of wastes generated from poor disposal find their way into oceans and cause hazards to aquatic animals when they break down to microplastics via ultraviolet radiation and ocean waves (Lebreton et al., 2019). Upon ingestion, micro-plastics accumulate in the digestive tract of the animals and eventually gets into the food chain when consumed by humans, hence raising health

concerns (Ciriminna and Pagliaro, 2020). In addition, recycling of plastics consumes a lot of energy and incineration of these wastes leads to the emission of toxic chemicals such as dioxin (Averous and Pollet, 2012).

These environmental and health concerns have given rise to use of alternative materials such as biodegradable polymers which will have the potential to eliminate problems related to the use of petrochemical resources (Averous and Pollet, 2012). Biodegradable polymers pride themselves in their excellent properties such as good sealing ability, air permeability, availability and they are lower in cost compared to petroleum-based plastics (Pawar & Purwar, 2013). They are environmentally advantageous because they can be degraded by microorganisms to produce beneficial compounds like carbon dioxide, biomass, water, and methane, all of which have soil-enriching abilities (Wojnowska-Baryła et al., 2020; RameshKumar et al., 2020). Biodegradable polymers can be obtained naturally or artificially. Some examples of natural biopolymers include proteins, polynucleotides, and polysaccharides while synthetic biopolymers consist of polyethers, polyvinyl esters, aliphatic polyesters, and polyvinyl alcohols (Zhou, 2008).

The growing awareness of consumers towards a green environment and a healthy life has created an increase in demand for high quality and shelf-stable foods packaged in sustainable materials with unique properties like having antimicrobial activity (Uz, 2009; Appendini and Hotchkiss, 2002). Antimicrobial packages offer protection and enhanced shelf-life to packaged food by retarding and inhibiting growth of pathogenic and spoilage microorganisms (Wang et al., 2022). Natural extracts from herbs and spices are safer options to be used in food packaging to reduce the use of chemical and/or synthetic food preservatives which are raising health and environmental concerns (Atarés & Chiralt, 2016; Rather et al., 2021). These plant extracts, also known as phytochemicals, naturally serve as a form of defense mechanism against attacks from insects, animals, and microorganisms. Phytochemicals have been found to have therapeutic benefits due to their antimicrobial and

antioxidant properties (Rather et al., 2021). Direct addition of essential oils to preserve foods is expensive, coupled with the possibility of toxicity and the strong aroma they confer hence affecting the sensory properties of foods. A more probable solution of incorporating essential oils in small amounts while assuring their effectiveness will be to add them during the formulation process of packaging films (Sanchez-Gonzalez et al., 2011).

The work presented in this thesis aims at investigating the effectiveness of active, antimicrobial, biodegradable packaging films in the preservation of food. First part of the research involves the preparation of varying proportions of chitosan and polyvinyl alcohol-based polymers, followed by selecting the best sample based on its mechanical, thermal, and structural properties. The membranes are then modified by the addition of antimicrobial agents and eventually serve as food packaging materials to test their antimicrobial efficacy.

Chapter 2

2. Literature Review

This chapter discusses topics such as biodegradable polymers (biopolymers), their classifications, and the specific polymers used in this study will be highlighted. This chapter will also touch on the role of antimicrobial packaging, the different methods of obtaining antimicrobial food packages, the specific antimicrobial agents used in this study, and lastly a brief about microbial food spoilage.

2.1 Biopolymers

Biopolymers are large molecules made from monomers joined together by covalent bonds. Since inception, biodegradable polymers have been widely used in several fields such as medicine, construction, food, and agriculture; and this is possible because of their non-toxicity, ability to break down into beneficial products, high bioactivity, biocompatibility, and appreciable mechanical properties (Tan et al., 2015). Their characteristics as biodegradable polymers are majorly determined by: (a) intrinsic properties of the polymer; (b) forming properties of the polymer during processing; and (c) properties of the product formed because of the intrinsic and processing properties (Sampath et al., 2016).

2.1.1 Classification of Biodegradable Polymers

Biopolymers are generally classified according to their sources and method of production (Figure 1). They may be obtained from natural sources such as agricultural products from plants (starch, alginate, etc.) or animals (chitosan, gelatin etc.). On the other hand, they may also be produced via microbial action (bacterial cellulose, polyhydroxybutyrate, etc.) and from synthetic routes from biomass (polylactic acid) or petrochemicals (polyvinyl alcohol) as shown in the figure 1 below. Major biopolymers used in food packaging are the bio-based polymers obtained from lipids, proteins, and polysaccharides (Jeevahan & Chandrasekaran, 2019).

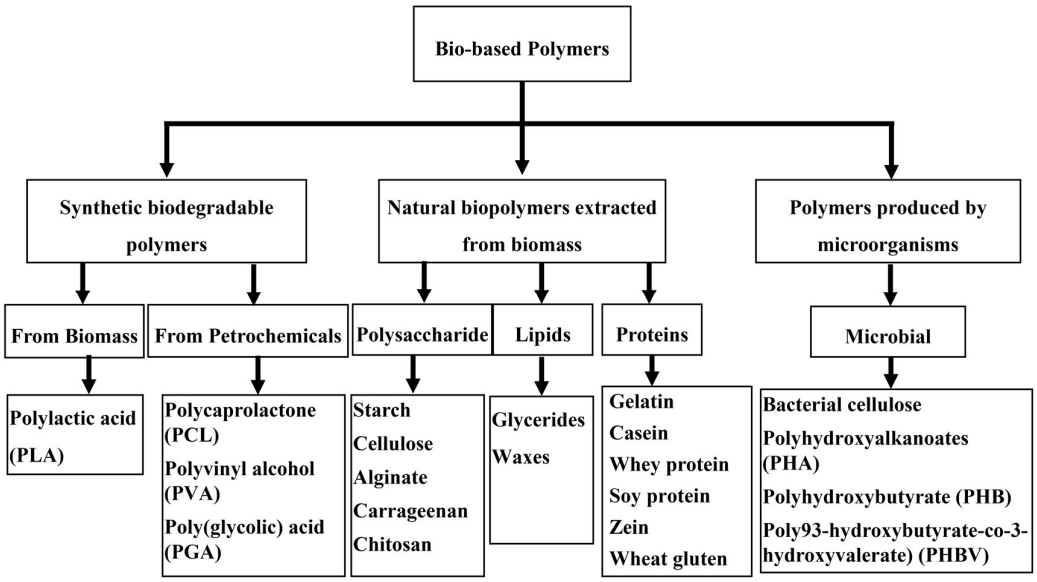


Figure 1: Classification of biopolymers (Baghi et al., 2022)

The classification of packaging polymers (natural and petroleum-based) according to their biodegradability is shown in Figure 2. Conventional plastics such as polystyrene, polyvinyl chloride, and polyethylene are classified as fossil-based and non-biodegradable. Polyvinyl alcohol (PVA), polycaprolactone (PCL) etc. are fossil based and biodegradable while polymers like cellulose acetate, biopolyethylene (bio-PE) are biobased and non-biodegradable and finally bioplastics such as polylactic acid (PLA), starch etc. are bio-based and biodegradable. This last group has been researched extensively and found to have potential in food packaging (Lindström & Österberg, 2020).

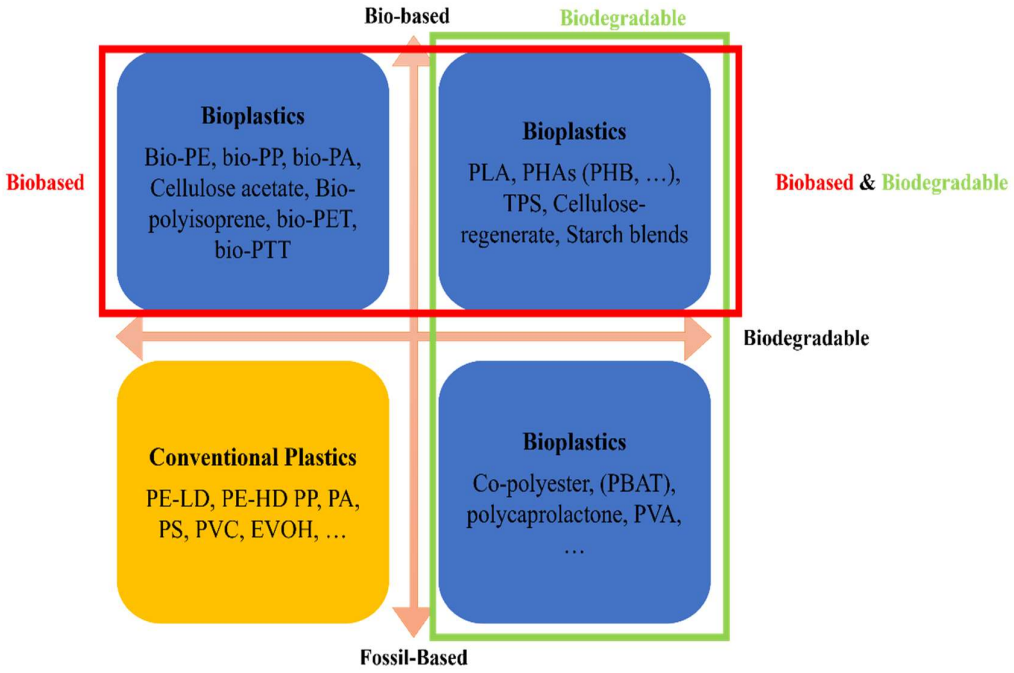


Figure 2: Classification of packaging polymers according to their biodegradability (Lindström & Österberg, 2020; Baghi et al., 2022).

2.1.2 Benefits of Biopolymers

Firstly, they are obtained from renewable sources; this makes them contribute positively to a sustainable, green environment and is one of the reasons they are gaining more importance. It also increases the availability of raw materials to be directed towards the manufacture of other petroleum-based products. Secondly, they are biocompatible and degrade into non-toxic materials such as water and carbon dioxide which are beneficial to the environment. In addition, biopolymers require less energy during processing which is important in reducing the amount of CO_2 emitted and can also lead to reduced cost of processing (Averous and Pollet, 2012). Also, synthetic biopolymers such as polyvinyl alcohol possess improved mechanical properties; for example, they have enhanced clarity, flexibility, gloss, tensile strength, and durability (Shankar and Rhim, 2018).

2.1.3 Limitations of Biopolymers

Despite their remarkable advantages that make them attractive, biopolymers have a few disadvantages and concerns that limit the commercialization and industrial use of biobased products. Biopolymers (especially natural variants) have poor mechanical properties, they degrade rapidly under certain conditions, susceptibility to microbial growth and contamination of foods when packaged. Some biopolymers are highly hydrophilic, causing them to have reduced barriers against water vapor (Shankar and Rhim, 2018).

Several researchers have studied many of these biopolymers and they have been successfully modified to give products with improved and desirable properties. The polymers of interest in this study are discussed below.

2.2 Polyvinyl Alcohol (PVA)

PVA can occur as a creamy to whitish granular or powdered semi-crystals which has no taste or odor (Aslam et al., 2018). It is a biodegradable and synthetic polymer which has been used in a vast majority of ways such as in cosmetics, pharmaceutical, textile, construction, and food industries. The Food and Drug Administration of the United States has classified PVA as Generally Recognized As Safe (GRAS), proving its non-toxicity and eco-friendly nature (Abdullah & Dong, 2019; Griffiths, 2007). This polymer is known and widely used for its oxygen resistance, transparency, excellent thermal and mechanical properties and so on (Abdullah & Dong, 2019). Conversely, PVA also has certain characteristics that pose as drawbacks to its use; it is somewhat expensive, poorly resistant to water, and undergoes a long degradation time in compost and soil (Abdullah & Dong, 2019; Kopčilová et al., 2013). Structurally, PVA comprises mainly of carbon chains and several repeating units of hydroxyl groups (Figure 3) hence the reason for its poor water resistance (Liu et al., 2022). The presence of abundant hydroxyl groups also influences biodegradability and gives PVA its ability to combine easily with other polymers via hydrogen bonding, making it particularly useful in packaging (Gaaz et al., 2015; Abdullah

et al., 2017). Manipulating the molecular structure of PVA during manufacturing gives it the ability to be produced in different ranges to give different levels of strength and other properties for a wide variety of applications (Griffiths, 2007).

Methods employed in preparing PVA solutions include thermoforming, extrusion, and solution casting. The most common being solution casting, even though it has been considered as inefficient when compared with others (Zou et al., 2008). Solution casting is most preferred because it is easily dissolved in water and takes relatively short time and temperature (30 minutes at 90℃) whereas extrusion requires much higher temperatures (Tang & Alavi, 2011; Gaaz et al., 2015). Partially and fully hydrolyzed PVA melts at 180–190℃ and 230℃ respectively, meanwhile it begins to degrade at around 150℃ which suggests that PVA might undergo changes in its structure and other characteristics during the extrusion process (Singha et al., 2015).

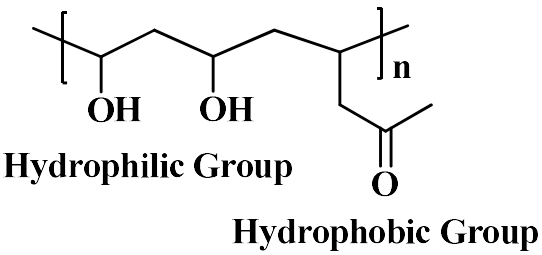


Figure 3: Chemical structure of polyvinyl alcohol (Lamminmäki et al., 2011)

2.2.1 PVA Synthesis

The most common route to obtain PVA is via the hydrolysis of vinyl acetate (VAc) in a process called free radical polymerization as illustrated in Figure 4 below. The process is carried out under strong alkaline conditions and in the presence of methanol during which an intermediate product is formed (polyvinyl acetate or PVAc). Polyvinyl alcohol (PVA) is finally formed by partially or totally replacing the ester group on the vinyl acetate via the hydroxyl groups on the methanol (Alvarado et al., 2019).

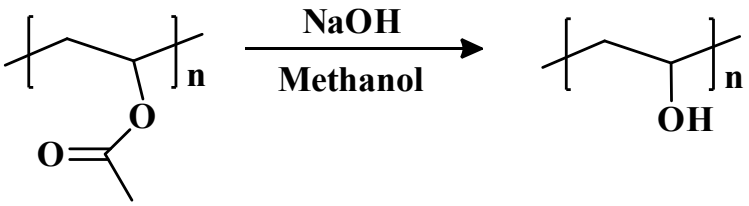


Figure 4: Hydrolysis of PVAc to obtain PVA (Alvarado et al., 2019).

Characteristics of PVA (e.g., viscosity, melting point, tensile strength, solubility, refractive index, pH etc.) are influenced by the level of hydrolysis (total or partial), the molecular weight as well as the conditions of the reaction (DeMerlis & Schoneker, 2003; Tang & Alavi, 2011; Alvarado et al., 2019). For example, PVA polymers with lower degree of hydrolysis and low molecular weight will dissolve faster, be more flexible and more sensitive to water. On the other hand, polymers with high molecular weight and percentage of hydrolysis will be more viscous, exhibit higher tensile strength and water resistance as summarized in Figure 5.

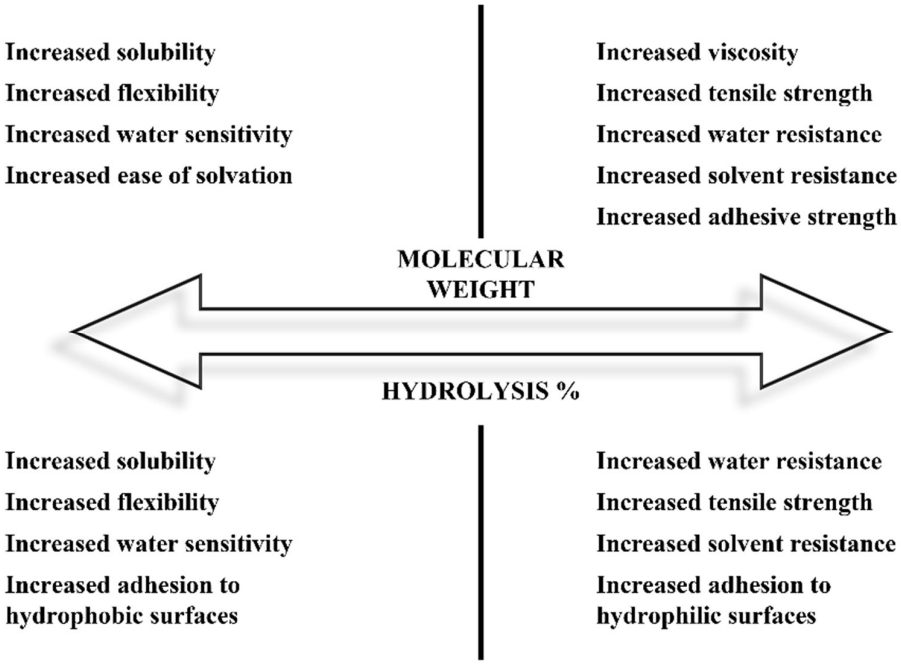


Figure 5: Effect of molecular weight and degree of hydrolysis on PVA properties (Tang & Alavi, 2011).

2.2.2 PVA in Food Packaging

PVA has been used especially in developing biodegradable packaging materials in combination with other polymers. A blend of PVA/carboxymethyl chitosan and citric acid was successfully used to obtain films with antibacterial and anti-fog properties, improved mechanical properties and increased soil degradation rate (Wen et al., 2021). Recently, water resistant PVA based packages have been developed, and they have compared favorably with petroleum-based plastic such as polypropylene and polyethylene. In addition to these, they have also showed higher barrier properties while also being biodegradable, non-hazardous, and non-toxic (de Oliveira et al., 2020). Wang et al. (2022) developed a film composed of PVA, silk protein, TEMPO-oxidized bacterial cellulose, and silver nanoparticles. These films had excellent water vapor barrier and mechanical properties, in addition to efficient UV protective properties, which increased their potential as active food packages. Biodegradability of PVA/starch films was investigated by Tanase et al. (2016) and the result confirmed an improvement in biodegradation rate by 32.45% as the amount of starch in the blend increased up to 20%. This research helps to prove that blending PVA with natural, biobased polymers could improve the biodegradability of PVA. Suganthi et al. (2020) successfully prepared antibacterial films by incorporating organic acids (malic, lactic, and taric acids) into PVA films.

2.3 Chitosan

Chitosan, a polysaccharide derived from shells of crustaceans, is a natural, straight chain and partially acetylated (1–4)-2-amino-2-deoxy-β-D-glucan. It is known for its biodegradable, biocompatible and antimicrobial nature and widely used in the food packaging industry because of its excellent barrier against unwanted aroma and gas as well as its good film-forming ability (Narasagoudr et al., 2020; Cui et al., 2017; Tripathi et al., 2009). Apart from its ability to resist the growth of a wide array of food spoilage and pathogenic organisms such as fungi, and bacteria; chitosan is also readily soluble in a range

of dilute acidic solvents such as acetic, citric, lactic, malic, formic acids etc. (Tripathi et al., 2009; Mohammadkhani et al., 2021). The ability of chitosan to solubilize in these readily available, cheap, and eco-friendly solvents is because of the non-bonding electron pairs located within the amino group of chitosan structure (Mohammadkhani et al., 2021). However, its high permeability to water poses as a disadvantage so it needs to be blended with other polymers to improve its properties (Narasagoudr et al., 2020).

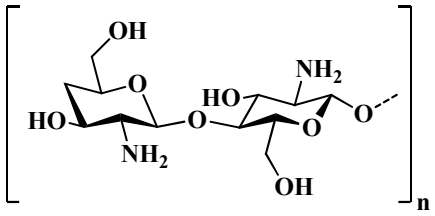


Figure 6: Chemical structure of chitosan (Younes & Rinaudo, 2015)

2.3.1 Synthesis of Chitosan

Chitosan can be obtained either from the cell wall of some microorganisms or from exoskeletons of insects, crustaceans (such as crabs, lobsters etc.) and molluscs (such as squids, oysters etc.). According to Pokhrel et al. (2016), chitosan was extracted from prawn shells via three major chemical processes which are demineralization, deproteinization and decolorization. Demineralization process involves acid treatment using hydrochloric acid for two hours, followed by washing until a neutral pH was obtained; deproteinization involves treating with an alkali solution such as sodium hydroxide while heating at about 65°C for three hours. The solution was washed to obtain neutral solids which were then oven dried at 40°C. The dried chitin was then decolorized using 2% sodium hypochlorite solution while stirring for 30 minutes at 45°C, followed by washing until neutrality was achieved. Finally, chitosan was isolated from the chitin by deacetylation, Figure 7. The process was carried out by treating the chitin with a 50% sodium hydroxide solution at a 1:10 weight/volume ratio and a temperature of 100°C under nitrogen atmosphere, after which the obtained residue was cooled and washed until a neutral pH was obtained.

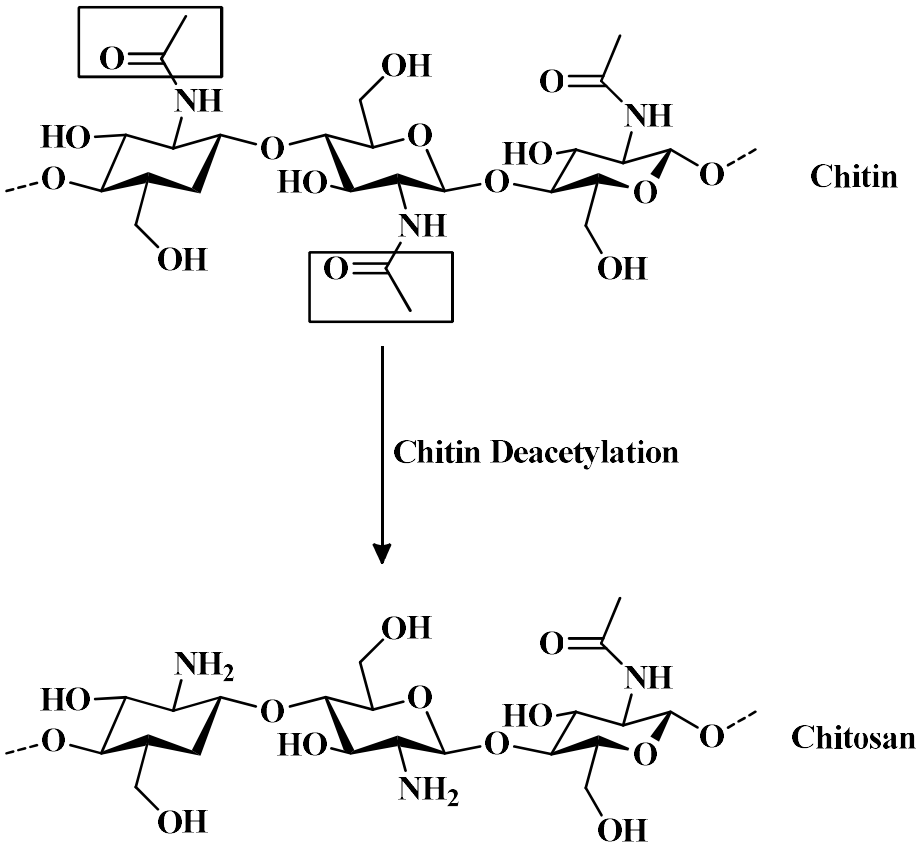


Figure 7: Chemical deacetylation of chitin to obtain chitosan (Dima et al., 2017).

2.3.2 Chitosan in Food Packaging

Numerous studies have been carried out to evaluate the effect of chitosan-based polymers in the food industry, especially in attempts to create eco-friendly packaging alternatives and as an antimicrobial food packaging material. Foods such as mango, carrot, tomato, pomegranate, banana, fish etc. have been packaged with pure chitosan films and confirmed to be effective in improving stability during storage, prevent microbial growth, retain antioxidant activity, and lengthen shelf life of the foods. Addition of plasticizers such as glycerol have also proven to improve the flexibility, chain mobility and strength of chitosan polymers (Wang et al., 2018). Chitosan has also been combined with other biopolymers such as proteins, organic acids, polysaccharides and so on. Several researchers have studied chitosan and cellulose-derived composite films and they have been reported to have improved mechanical, barrier, and optical properties (Bansal et al., 2016; Khan et al., 2014; Li et al., 2013; Sundaram et al., 2016). Films made from a combination of chitosan and alginate have also given positive results in terms of their thermal properties, water vapor permeability and gas-exchange properties (Acevedo-Fani et al., 2015; Poverenov et al., 2014a). Edible films made from gelatin and chitosan composites was used to preserve red bell peppers, the treatment reduced the rate of microbial spoilage and increased the shelf life of the foods (Poverenov et al., 2014b). Addition of extracts from plants (e.g. citrus, thyme, clove, cinnamon) and animals (e.g. bees) have also produced films which improved food quality due to their excellent antimicrobial, antioxidant, barrier, and mechanical properties among others (Wang et al., 2018).

Synthetic polymers have also been combined with chitosan; a study on chitosan/PLA films exhibited improved mechanical, tensile and thermal properties (Bie et al., 2013). Similarly, chitosan/salicylic acid and chitosan/fumaric acid films conferred protective properties on foods, for example, eliminating chilling injury in cucumbers (Zhang et al., 2015). Adding poly (ethylene) oxide influenced thermal properties and hydrophobicity of the resulting

films (Kohsari et al., 2016). Incorporating polycaprolactone and isothiocyanate into chitosan films increased their antibacterial properties (Alix et al., 2013; Guo et al., 2015).

2.4 Polyvinyl Alcohol/Chitosan Composite Films

PVA and chitosan composites have been reported to give good films due to the intermolecular hydrogen bonds between both polymers (Wang et al., 2015). Increase in the amount of PVA in the blends improves tensile strength, elasticity, elongation, plasticity, and barrier against oxygen and water. This gives chitosan/PVA film the ability to be used as antimicrobial packages (Wang et al., 2015; Giannakas et al., 2016). Blending chitosan and PVA has the potential to improve the low biodegradability of PVA because chitosan is a natural and biobased polymer (Broek et al, 2015). Nanofiller have also been widely researched to improve different properties of PVA/chitosan blends. Khoo et al. (2013) reduced water absorption and thermal degradation of PVA/chitosan films by adding halloysite nanotubes while Butnaru et al. (2016) used cloisite 30B nanoclays to reinforce PVA/chitosan blend which improved their mechanical and thermal properties. Al-Tayyar et al. (2020) developed films composed of chitosan, PVA, and silicon dioxide nanoparticles doped with zinc oxide nanoparticles ($ZnO\text{-}SiO_2$). These films offered antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*, reduced the level of food-borne pathogens, and improved the appearance of bread. Crosslinking of these polymer blends have also been explored and reported to improve its mechanical strength when compared to unloaded samples (Jahan et al., 2016). Researchers have also reported that incorporating bioactive compounds into PVA/chitosan results in films with unique characteristics such as excellent mechanical properties and resistance to fire (Pal & Katiyar, 2016). Anthocyanin extracted from red cabbage (*Brassica oleraceae*) has been incorporated into chitosan/PVA blends as time-temperature signals for intelligent packaging (Pereira et al., 2015). Annu et al. (2021) added extracts from *Ocimum tenuiflorium* to the chitosan/PVA matrix to obtain films with good antioxidant properties and improved barrier against light

and water. A common challenge experienced when blending polymers is obtaining homogeneous mixtures, although PVA and chitosan mixtures have exhibited good solution homogeneity because of the functional hydroxyl and amino groups in both polymers (DeMerlis & Schoneker, 2003).

2.5 Active Films in Food Packaging

Food packaging undoubtedly plays important roles in food production, from containing raw materials from the farm or fields down to transporting, storage, processing, marketing until the food product gets to the table of the final consumer. Throughout these processes, food packaging materials are expected to slow down or completely prevent all forms of chemical, physical, or microbiological degradation (Anis et al., 2021; Lee, 2010). It then becomes a challenge to develop packaging materials, which do not only offer preservative function but also enhance shelf life of food products (Anis et al., 2021). While developing packaging materials, it is important to consider factors such as design of the package, cost of material, and reactions that may occur during food storage such as oxidation (Alamri et al., 2021; Bhargava et al., 2020). Reactions in foods tend to reduce their acceptability and overall quality (Bhargava et al., 2020). Even though active films are not yet widespread in the market, materials such as leaves have been used for many years to prepare and package foods because they have excellent barrier against gas and microbes in addition to conferring color, flavor, aroma, enzymes as well as keeping food fresh (Dainelli et al., 2008). Materials used in active films are expected to be safe and compatible with the food matrix because they migrate from the films and interact with food (Ahmed et al., 2017).

2.6 Antimicrobial Food Packaging

There is a consensus amongst several researchers that antimicrobial food packages preserve food by creating a barrier between food, spoilage organisms and/or other pathogens while keeping the food safe without any change in its composition. This is achieved by a controlled release of the incorporated antimicrobials (Valencia-Chamorro et al., 2011; Barros-Velazquez, 2016; Malhotra et al., 2015).

2.6.1 Preparation Methods for Antimicrobial Food Packages

These methods have been categorized as follows:

a) Direct incorporation of antimicrobial agents into polymers during solution mixing
b) Ionic or covalent immobilization of antimicrobials onto polymer membranes
c) Addition of antimicrobial agents in form of absorbent pads or sachets into food packages
d) Coating of antimicrobials on membrane surfaces (surface coating)
e) Utilization of film-forming antimicrobial polymers
f) Combination methods involving any of the above mentioned

Source: (Appendini & Hotchkiss, 2002; Khaneghah et al., 2018; Coma, 2008).

Among these techniques, the combination methods have proven to be very effective in obtaining antimicrobial packages with multiple inhibitory functions against microbes. For instance, addition of photocatalytic antimicrobial titanium oxide nanoparticles to chitosan biopolymer with film-forming capacity gave rise to antimicrobial films with the ability to destroy microorganisms under visible light (Zhang et al., 2017). Some of these methods are most effective at laboratory scale while some have potential as industrial methods. Although industrial processes require high temperature and pressure which makes the application unsuitable for heat-sensitive antimicrobials which are the most abundant and most studied variants (Fu & Dudley, 2021).

2.7 Types of Antimicrobial Substances

Antimicrobials can be broadly divided into natural and synthetic, based on their origin.

2.7.1 Synthetic Antimicrobials

Synthetic antimicrobials are basically preservatives that have been approved for use in food (GRAS). They are not from animal, plant, or microbial sources and most of them are nanoparticles made from metals or metal oxides (Kuplennik et al., 2015). Some examples of these substances are nanoparticles obtained from zinc oxide (ZnO), silver (Ag), copper (Cu), titanium dioxide (TiO2) etc. These synthetic nanoparticles (NPs) are produced by physical and/or chemical modifications and are usually directly incorporated into packaging films because they have great antimicrobial activity and are stable in harsh conditions required in film manufacturing (Fu & Dudley, 2021; Garcia et al., 2018). However, coating of nanoparticles on the surfaces of films have shown better efficacy as antimicrobial agents as against direct incorporation (Azlin-Hasim et al., 2018). Zinc oxide nanoparticles have been reported to have the ability to penetrate bacterial cells, producing reactive oxygen substances such as hydrogen peroxide that can interact with DNA hence causing cell death (Garcia et al., 2018). TiO_2 nanoparticle is a substance which is photocatalytic, and its antimicrobial activity is initiated in the presence of light and water which produces radicals and inactivates polyunsaturated phospholipid in the cell membrane by oxidation hence keeping food stable (Alrousan et al., 2009).

2.7.2 Natural Antimicrobials

Natural antimicrobials are those obtained from plants, animals or microorganisms and have been receiving more attention because of their importance on the environment and growing awareness in green technology (Fu et al., 2016). Nisin, a common type of bacteriocin synthesized in the ribosomes of *Lactococcus lactis* is an FDA-approved natural antimicrobial and has excellent inhibition against gram-positive bacteria (e.g. Clostridium botulinum) because it produces pores in the cytoplasm. However, it has no inhibition power

against gram-negative forms because they have barriers on their outer membrane (de Arauz et al., 2009; Breukink & de Kruijiff, 2006). Lysozyme is another type of natural antimicrobial. It is an enzyme which can inhibit bacteria, especially gram-positive variants. The inhibition power of lysozyme stems from its ability to break glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan, which leads to leaching of cell wall content and death of bacteria (Muriel-Galet et al., 2013; Irkin & Esmer, 2015). Organic acids include lactic acid, tartaric acid, propionic acid, sorbic acid, etc. They have been traditionally used as food additives and proven to inhibit fungal and bacterial cells (Cha & Chinnan, 2004). Under normal conditions, cells of microorganisms are optimal around a neutral pH, but when the pH is reduced, cellular activities are disrupted which eventually ends in cell death (Coban, 2020). Organic acids can be gotten via chemical or biological processes. Those produced biologically by microorganisms in a process of fermentation have more advantages that those produced chemically because of the sustainability, eco-friendliness, and reliability (Hermann et al., 2007). Using organic acids as antimicrobial agents in foods depends on factors such as the minimum inhibition concentration, molecular weight, nature of microorganism, chemical formula, and dissociation constant. Most organic acids have their dissociation constant within a pH of 3 to 5 and these are suggested to be the most suitable for use with foods. Similarly, organic acids with carbon atoms less than seven have also been stated to be more effective as antimicrobials (Coban, 2020). Another group of natural antimicrobials are plant-derived substances such as essential oils and powdered extracts. These groups are not only FDA-approved but effective against all kinds of microorganisms (yeasts, molds, gram-positive and gram-negative bacteria) and they do this by disrupting the outer membranes of microbial cells thereby increasing cell permeability and leading to loss of cell contents and eventually cell death (Falleh et al., 2020; Burt, 2004; Hyldgaard et al., 2012). These compounds contain phenolic substances, and their antimicrobial efficacy is linked with the proportion of

phenolic compounds in them (the higher the level of phenols, the more effective against microbial inhibition). Some of the antimicrobial agents with high phenol contents and the active compounds in them includes oregano (carvacrol), thyme (thymol), clove (eugenol), turmeric (curcumin), cinnamon (cinnamaldehyde), ginger (gingerol), etc. (Dhifi et al., 2016; Pancholi et al., 2021; Ojagh et al., 2010).

2.7.2.1 Clove (Eugenol)

Clove is the scented dried flower obtained from the tree of Syzgium aromaticum (also referred to as Eugenia cariophylata). This spice is well known for its age-long use as a food preservative and medicine due to its potency as an antimicrobial, antioxidant, analgesic, cytotoxic, antiviral, anesthetic, and hepatoprotective properties (Hussain et al., 2017). Clove is rich in phenolic compounds, some of which are responsible for its pleasant aroma. Compounds like hydroxybenzoic acids, hydroxycinnamic acid, flavonoids, salicylic, and ferulic acids. The most prominent among these bioactive compounds is eugenol (Figure 8), present in as much as 9,381 to 14,650 mg/100 g in fresh plants (Shan et al., 2005; Mittal et al., 2014). The most used form of clove is in the form of oil rather than its powdered extract. Essential oils extracted from the aerial parts of the plant contains over 70% eugenol, 15% eugenyl acetate, and about 12% beta- caryophyllene which are the main compounds responsible for its antimicrobial activity (Mittal et al., 2014; Rather et al., 2021). However, despite its potency, clove is not effective against bacteria inhibition for long periods of time because it is volatile and unstable, hence it is usually incorporated with other polymers to enhance its stability (Cui et al., 2015; Mulla et al., 2017). Chitosan nanoparticles containing clove oil have been studied to evaluate the stability of clove oil. It was confirmed to be effective against *E. coli* and preserved the color and flavor of cucumbers for over five days (Cui et al., 2018). Clove oil has also been proven to enhance wound healing when added to polycaprolactone-gelatin nanofibers (Unalan et al., 2019). Pectin-clove edible coating have also been developed and used to preserve bream fillets and the result showed that lipid

oxidation was reduced, and the coatings extended the shelf life for up to 15 days with effective inhibition against gram-negative bacteria (Nisar et al., 2019).

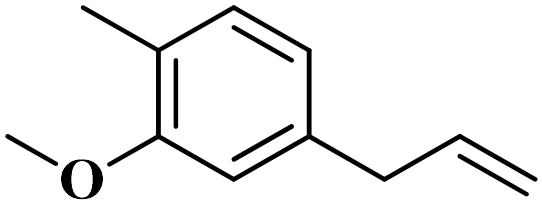


Figure 8: Structure of eugenol (Hussain et al., 2017)

2.7.2.2 Turmeric (Curcumin)

Turmeric is botanically referred to as Curcuma longa, it is a popular rhizome belonging to the ginger family (Zingberaceae) (Pawar et al., 2014). Turmeric is popular as a therapeutic herb and a culinary spice added to foods, it also has religious significance in some regions of India (Prasad & Aggarwal, 2011). The medicinal importance of turmeric is because of the presence of yellow pigmented curcuminoids, bioactive compounds which comprises of curcumin, bisdemethoxycurcumin (BDMC), and demethoxycurcumin (DMC), all of which can be isolated from turmeric (Rathore et al., 2020; Bhūtyā et al., 2011; Salehi et al., 2018). Curcumin is the principal, most studied among these compounds, and it has given turmeric even more popularity and importance. Curcumin (Figure 9) is a fat-soluble polyphenol with a molecular formula of C21H20O6 and a low molecular weight of 368.38 g/mol (Adamczak et al., 2020). It has been used traditionally as a relief from gastrointestinal disorders like indigestion, diarrhea, and gastric ulcers (Kwiecien et al., 2019). It also functions as an antibacterial, anti-inflammatory, and used in treatment of several ailments such as liver disorders, cough, asthma, rheumatism, anorexia, and for healing burns and wounds etc. (Prasad & Aggarwal, 2011; Nair, 2019).

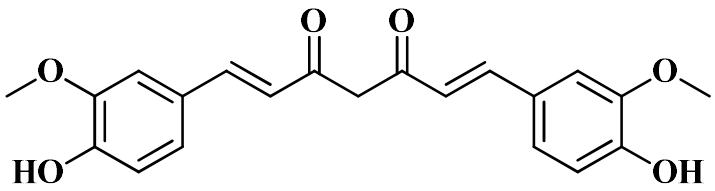


Figure 9: Chemical structure of curcumin (Adamczak et al., 2020)

Turmeric and curcumin are now widely used as food additives due to their flavoring, coloring, and preservative properties (Basnet & Skalko-Basnet, 2011; Prasad & Aggarwal, 2011). Despite its poor solubility in water, curcumin has still been confirmed to have strong antibacterial activity (Kotha & Luthria, 2019). This compound has also found importance in food packaging especially in the development of active, smart, and multifunctional biodegradable packages. Curcumin has the potential to act as a biosensor for detecting pH changes in smart packaging of foods. This is because the compound is highly sensitive to acid-base reactions and responds via color changes that are visible to consumers without opening the package (Chen et al., 2020; Liu et al., 2018). In acidic conditions (pH: 3.0–7.0), curcumin exhibits a yellow color but with an increase in the pH to about 8, the color changes to red. This color change results from the reaction between phenolic hydroxyl and hydroxide ion to form a phenoxide ion (Chen et al., 2020). In active packaging, curcumin has been studied for its antimicrobial and antioxidant properties and has shown to be effective against foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimurium*, etc. (Oliveira Filho et al., 2021).

2.7.2.3 Cinnamon (Cinnamaldehyde)

Cinnamon is a GRAS spice with a peculiar flavor and aroma and widely used as a food additive. Like all herbs and spices, cinnamon also contains bioactive compounds which accounts for its characteristics. The compound with the most percentage is cinnamaldehyde, although other compounds such as linalool and β-caryophyllene are present (Tzortzakis, 2009). Trans-cinnamaldehyde is another important constituent majorly present in cinnamon essential oil (Yeh et al., 2013). Traditionally, cinnamon has been identified as an antioxidant, anti-inflammatory agent, antifungal, nematicidal, antidiabetic, as well as a coagulant to prevent excess bleeding and a treatment for dental problems like toothaches and bad breath (Tzortzakis, 2009; Tung et al., 2010; Rao & Gan, 2014). Cinnamaldehyde,

also known as 3-phenyl-2-propenal or cinnamic aldehyde (Figure 10) gives cinnamon its antimicrobial and antioxidant properties; its inhibitory effect occurs when the lipophilic group penetrates the cells of microorganisms and causes cell death (Qin et al., 2015). Therefore, cinnamon has been employed as an antimicrobial agent in the development of food packages. Petrou et al. (2012) incorporated cinnamon oil into edible film made from apple puree and it inhibited the growth of spoilage organisms in chicken breasts. Chitosan-cinnamon edible coating was applied in the preservation of rainbow trout and the result showed that quality of the fish was retained and shelf life extended (Ojagh et al., 2010). In the same vein, Valizadeh et al. (2019) incorporated cinnamon essential oil into film containing chitosan, carboxymethyl cellulose, and oleic acid. This film provided antioxidant and antimicrobial activity against Pseudomonas aeruginosa and Listeria monocytogenes, it also improved the flexibility and water vapor permeability of the films.

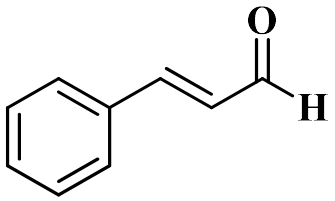


Figure 10: Chemical structure of cinnamaldehyde (Rao & Gan, 2014)

2.8 Microbial spoilage of food

Food spoilage involves a myriad of reactions occurring between the organisms, food additives, preservatives, and the food matrix under appropriate pH, temperature, water activity, oxygen, and carbon dioxide levels (Doyle, 2007). The most common organisms associated with spoilage of foods are yeasts, molds, and a range of bacteria including Staphylococcus, Listeria monocytogenes, *Escherichia coli*, Campylobacter, Shigella, Salmonella, Bacillus, and Clostridium perfringens (Dhama et al., 2013). Protein-rich foods such as dairy (milk, cheese etc.), and poultry (chicken, turkey) are excellent medium for microbial growth due to their nutritional composition and high moisture content and water activity (Boor & Fromm, 2006; Doyle, 2007). It is important to understand the factors that influence growth of foodborne pathogens so that necessary modifications and/or precautions will be taken to avoid their proliferation in foods. Water activity is the most important factor when considering the growth of microorganisms in food, a low water activity is important for longer shelf life and reduced activity of microbes (Doyle, 2007; Lopez-Malo & Alzamora, 2015). pH and temperature are also very important factors that influence the growth and reproduction rates of microorganisms. It is also important to note that different types of microbes and their strains respond differently to pH and temperature changes (Kim et al., 2018), hence the reason why all microorganisms have a minimum, optimum and maximum pH and temperature growth range. The optimum is the best pH or temperature in which rapid growth of microbes occur, while minimum and maximum pH or temperature signifies slow or no growth regions. With respect to pH, most bacteria grow best around pH 5 to 8, hence are referred to as neutrophiles. While organisms that grow best within acid pH (1–3) are acidophiles while those that have their optimum pH between 8–10 are alkaliphiles (Jin & Kirk, 2018).

Chapter 3

3. Materials and Methods

3.1 Materials

Polyvinyl alcohol (PVA) (white crystalline powder, 4% viscosity, 98–99% hydrolysis, and molecular weight of 85,000 to 1,24,000), chitosan powder (95% deacetylation), acetic acid, clove oil, and turmeric (curcumin) were purchased from international company for scientific and medical supplies, Cairo. The PVA and chitosan purchased were under the brand name Techno Pharmachem, India. Polyethylene glycol and glutaraldehyde were obtained from the chemistry laboratory at the American university in Cairo. Cinnamon powder, white cheese and fresh chicken breasts were purchased at a nearby supermarket. Distilled water was used throughout the experiments.

3.2 Methods

The methodology involved first developing different compositions of PVA and chitosan polymer films and then analyzing their mechanical properties. The different compositions were achieved by varying the concentrations of PVA and chitosan at different percentages. The polymer with the most acceptable mechanical property was selected and used to develop the antimicrobial films using clove oil, turmeric, and cinnamon antimicrobial agents each at 0.4%, 0.8%, and 1.2% concentrations. Antimicrobial and antioxidant analysis were then carried out on all films to select the most acceptable concentration. The antimicrobial films containing 1.2% concentration of the antimicrobial agents emerged as the optimized sample and was evaluated using different characterization methods and its effectiveness in shelf stability studies.

3.2.1 Film Preparation

Solvent casting method was used to prepare the biodegradable packaging films. For the plain films, 2% chitosan (by weight) was dissolved in 2% acetic acid and 8% of PVA was dissolved in distilled water (60°C) in a laboratory bottle while stirring until a homogenized

mixture was obtained. Both solutions were mixed, PEG was added as a plasticizer to improve flexibility of films glutaraldehyde was added to improve thermal stability and tensile strength. The solution was sieved using a muslin cloth and left for a few minutes for degassing to occur. The solution was then casted over glass plates of internal dimensions 16cm by 16cm and left at room temperature until the solvent completely evaporated, Figure 11. For the antimicrobial films, the same method as above was adopted with the addition of the antimicrobial agents (cinnamon, turmeric, and clove) at 1.2% concentration. The dried films were peeled off and stored for further analyses. The films were labeled as follows. PVA/chitosan films without antimicrobial agent = PLAIN, PVA/chitosan films with cinnamon = CIN, PVA/chitosan films with turmeric = TUR, PVA/chitosan films with clove = CLO.

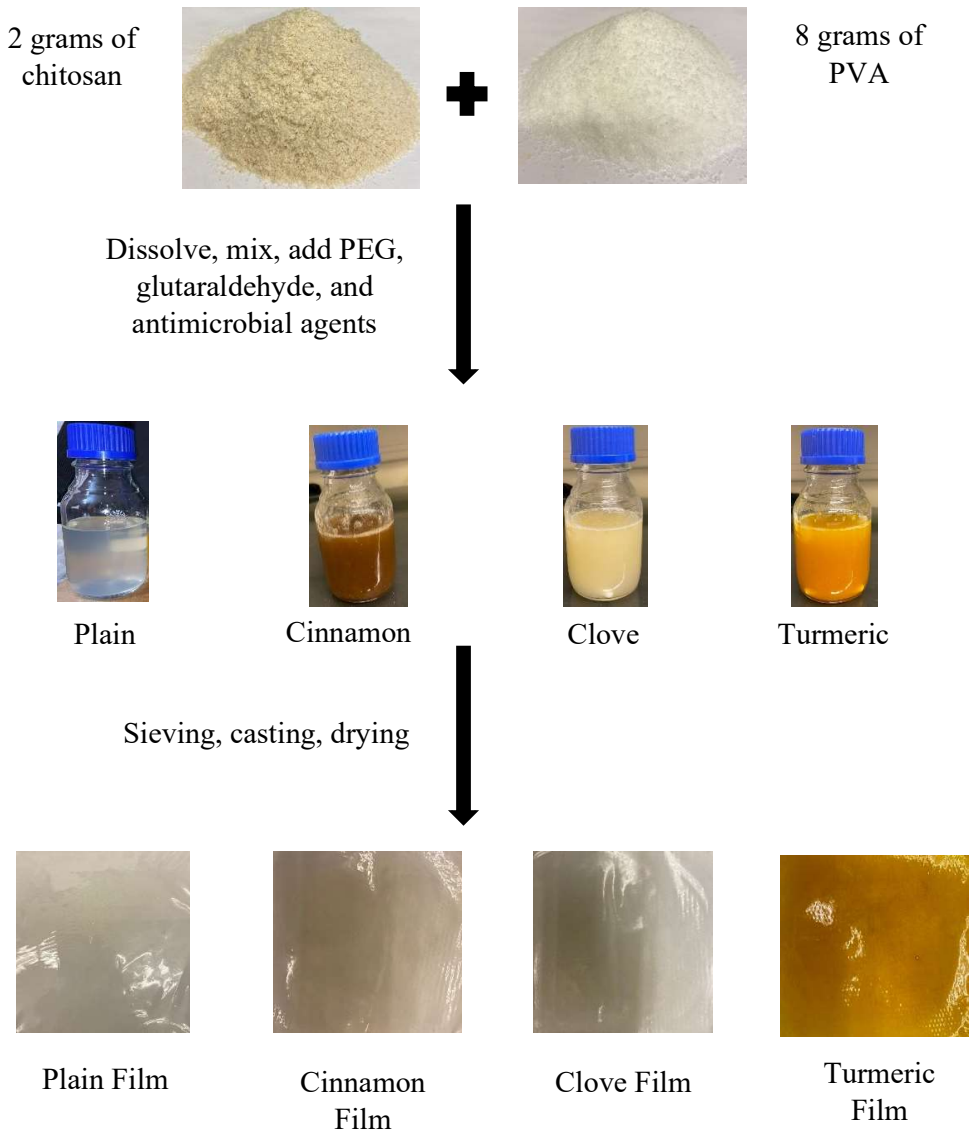


Figure 11: A scheme showing PVA/chitosan film preparation

3.3 Characterization Methods

3.3.1 Scanning Electron Microscopy (SEM)

Morphology of the membranes were investigated using a scanning electron microscope (Supra 5S LEQ, Zeiss). The experiment was carried out at the science and technology research center (STRC) at The American University in Cairo. SEM works by employing high energy scanning or imaging of the samples.

3.3.2 Thermogravimetric Analysis (TGA)

Thermal stability of the films was evaluated using a thermogravimetric analyzer model Q50, Lukens Drive, New Castle, USA. Small pieces of samples were cut, placed in a platinum pan, and heated from 20 to 600 ℃ at a rate of 10 ℃/minute under nitrogen atmosphere with a flow rate of 50 ml/min. The change in weight was plotted against the corresponding temperature and displayed on TGA curves.

3.3.3 Fourier-Transform Infrared Spectroscopy (FTIR)

FT-IR spectra of plain and antimicrobial films were investigated to determine their chemical structures and possible interactions between their components. The spectra were obtained by Thermo Scientific Nicolet 380 FT-IR, Waltham, MA, USA in The American University in Cairo. The samples were cut into 2 cm × 2 cm and the spectra was measured under a wavelength range between 500 and 4000 cm^{-1} at room temperature.

3.3.4 Mechanical Properties

Stress-strain isotherms were obtained according to the ASTM D822. The dried films were cut into dimensions 6 cm × 1.5 cm. The thickness of the films was measured using a manual micrometer screw gauge at three random parts of each sample and the mean values were 0.18 mm for plain PVA/chitosan, 0.14 mm for PVA/chitosan plus clove, 0.12 mm for PVA/chitosan plus cinnamon and 0.15 mm for PVA/chitosan plus turmeric. Two distinct lines were drawn at 3 cm apart before hanging the sample, to determine the change in length for each stress applied. The films were secured between two clamps, the lower clamp is

immobile while the upper is connected to strain gauge. Tensile force was applied by constant straining of the upper clamp which caused stretching of the film. The force was recorded via a digital oscilloscope connected to a transducer supplying the data through constant voltage DC supply. The readings were taken after 15 minutes to allow for equilibrium. The elastic force (f) was determined versus elongation and the equilibrium elastic force (f*) was calculated according to the following equation:

$$f^* = {}^{f}/_{A^*}$$

Where f is the elastic force in Newton and A^* is the cross-sectional area (cm^2). The stress-strain graph was plotted.

3.3.5 Antibacterial activity of active films

Agar-plating colony forming unit (CFU) counting test was used to evaluate the antibacterial activity of the polymer sheets against gram-positive *Staphylococcus aureus* (*S. aureus*) and gram-negative *Escherichia coli* (*E. coli*). Bacterial suspension (McFarland standard 0.5) was prepared and incubated in Mueller-Hinton broth medium. Then, 200 µL of the prepared bacterial suspension was added into 96-well plate containing the test samples and control sample, followed by incubation of the well plate at 37 °C for 24 hours. 20 µL bacterial solution was then cultured on the surface of dried nutrient agar plates, followed by incubation at 37 °C for 24 hrs. The bacterial colony on the plates was observed by a digital camera, and the number of colonies was counted. The experiment was done in triplicate to ensure the accuracy. Antibacterial efficacy was calculated as follows:

$$\text{Antibacterial ratio (\%)} = \frac{(\text{CFUs in control group} - \text{CFUs in experimental group})}{\text{CFUs in control group}} \times 100$$

3.3.6 Antioxidant activity

The antioxidant activity of the films was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. DPPH is a stable free radical at room temperature, it produces a violet solution in ethanol and when an antioxidant molecule is added to the solution, DPPH loses electron, and the solution goes from violet to colorless after a specified time. This occurs because The absorbance is then measured by spectrophotometry. In literature, % inhibition in DPPH absorbance is also free radical scavenging activity (FRSA) or radical scavenging activity (RSA). The films were cut into small pieces (1 cm × 1 cm), immersed in ethanol, and left for a while for the active compounds to be extracted. The resulting solution was measured at different concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 μg/ml) and mixed with 1 ml of DPPH by vigorous shaking, then the mixture was allowed to stand at room temperature for 30 minutes. The absorbance was measured via spectrophotometer (UV-VIS Milton Roy) at 517 nm. The percent DPPH scavenging activity was calculated by using following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of control (DPPH solution alone) and A_1 is the absorbance of DPPH in the presence of test sample.

3.4 Shelf Stability Studies

3.4.1 Sample Preparation

Chicken breast meat and white cheese were chosen to test the efficacy of the PVA/chitosan active films. Fresh boneless and skinless chicken breast was cleaned, cut into small cubes, and formed with a burger press after which the meat was sandwiched between two films of dimension 12 cm × 12 cm and the ends of the films were closed with scotch tape to prevent entry of air and other foreign substances, Figure 12. As for the white cheese, they were cut and packaged by wrapping the films around them and securing with a scotch tape as shown

in Figure 13. All samples were stored at 5 ℃ for 14 days with pH and microbiological analysis carried out at days 0, 7 and 14. These analyses were carried out at NAWAH scientific laboratory, Al-Mokattam, Cairo, Egypt.

3.4.2 pH Analysis

pH of the was measured with a pH meter. 10 grams of the food samples was added to 90 mL of distilled water and homogenized with a blender. The resulting mixture was used for the pH determination.

3.4.3 Antimicrobial Analysis of Packaged Foods

25 grams of the food samples were weighed and added to 225 ml of buffered peptone water (initial suspension representing dilution 10^{-1} of sample). The mixture was homogenized with a stomacher for 2 minutes and serial dilution was performed using 9 ml of buffered peptone water up to 10^{-8}.

For aerobic plate count, 1 ml of the initial suspension was transferred into an empty petri dish and mixed with 20 ml of plate count agar using pour plate method. Plating was performed in duplicate and incubated at 30 ℃ for 72 hours.

For total coliform, 1 ml of the initial suspension was mixed with 20 ml Violet Red Bile Lactose agar (VRBL) medium using pour plate method. After solidification, a second layer of VRBL was poured onto the plates. Incubation was carried out at 37 ℃ for 42 hours after which characteristic colony types were counted.

For coagulase-positive *Staphylococcus aureus*, plates of Baird-Parker medium supplemented with egg yolk tellurite emulsion was prepared and dried for 30 minutes in a biosafety cabinet. 250 µL of the initial suspended was inoculated onto the dried plate using spread plate method. Four plates were inoculated for each dilution up to 1 ml of sample. Plates were also prepared using dilution factor of 10^{-2} and all plates were incubated at 37 ℃ and examined at 24–48 hours. The number of typical colonies were identified and confirmed by coagulase positive test. Number of colonies were counted from plates with less than 150

colonies. All colonies were reported in colony forming unit per gram (cfu/g) and plotted as logarithm of the colony forming unit per gram (log cfu/g).

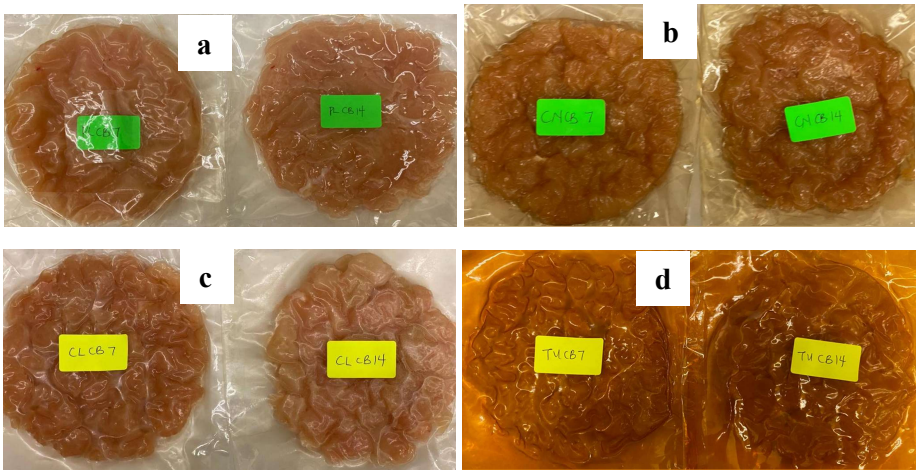


Figure 12: Chicken breast meat packaged with plain and active PVA/chitosan film

(a) = plain PVA/chitosan film (b) = PVA/chitosan film with cinnamon
(c) = PVA/chitosan film with clove (d) = PVA/chitosan film with turmeric

Figure 13: White cheese packaged with plain and active PVA/chitosan film

(a) = plain PVA/chitosan film (b) = PVA/chitosan film with cinnamon
(d) = PVA/chitosan film with clove (d) = PVA/chitosan film with turmeric

Chapter 4

4. Results and Discussion

4.1 SEM of Antimicrobial Films

The SEM snapshots of plain and active antimicrobial PVA/chitosan films are presented in Figure 14. All samples except the clove-containing film (CLO) showed homogeneity and compactness, suggesting significant interfacial adhesion between PVA, chitosan and the other additives. The SEM snapshots show that plain films as well as those loaded with turmeric (TUR) and cinnamon (CIN) showed smooth surfaces, indicating no phase separation between the blends' components or between the polymeric materials and the added antimicrobial substances. However, the SEM snapshot of the polymeric film loaded with clove oil showed clear phase separation due to the hydrophobic nature of the clove oil and the hydrophilic nature of the PVA/chitosan polymeric film, which caused the droplets to coalesce and separate out. Nevertheless, on a macroscopic scale, the film itself showed homogeneity between the various components of the polymeric blend, which suggest that the phase separation between the clove oil droplets and the polymeric blend was, in fact, a microphase separation on the nanoscale region. The coalescence and flocculation developed during the film drying have also been attributed to the roughness observed in these films (Peng & Li, 2014). It has been suggested that the volatile nature of the essential oils could also create cavities on the film surfaces (Ahmed et al., 2016; Ahmad et al., 2012).

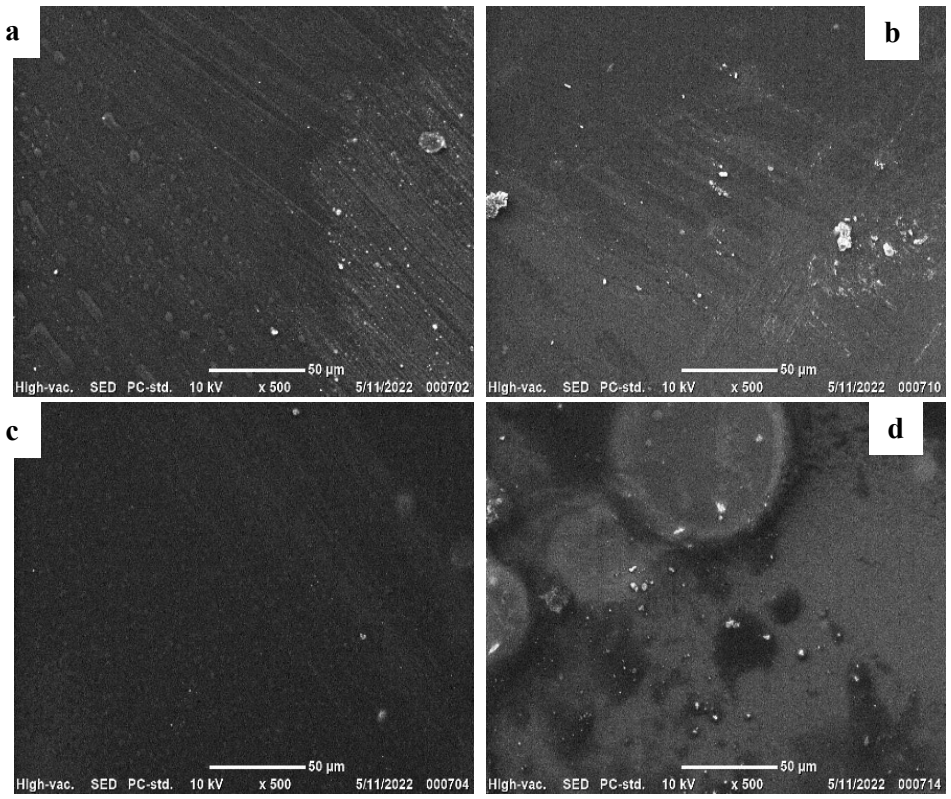


Figure 14: SEM snapshots of plain (a), PVA/chitosan with turmeric (b), PVA/chitosan with cinnamon (c), PVA/chitosan with clove (d)

4.2 TGA

Thermogravimetric analysis was carried out to investigate the influence of the antimicrobial agents on the thermal stability of PVA/chitosan films, Figure 15. The first derivative thermogravimetric analysis (DTG) curves are also shown in Figure 16. It is obvious from the figures that adding the different antimicrobial agents did not significantly cause a change in the thermal stability of the films. All samples showed three distinct stages of degradation at different temperature ranges. The degradation temperatures at the beginning of the thermal profile, at 50% weight loss and at the final stages of the thermal treatment had close values to each other when compared to the plain PVA/chitosan film as seen in Table 1. Therefore, it can be concluded that adding the antimicrobial agents to the polymeric blends did not have a major influence on the thermal degradation profile of the samples. TUR exhibited a low degradation profile among antimicrobial-loaded films, while CLO showed the highest thermal stability. Generally, the first mass loss is associated with evaporation of adsorbed water within the polymer molecules. The second mass loss accounts for most of the degradation that occurs; this includes decomposition of PVA, depolymerization of chitosan, evaporation of the antimicrobial agents, degradation of the glutaraldehyde cross-linking agent and the polyethylene glycol plasticizer. The film with the highest mass loss at the second phase was CIN, losing 69.57% between temperatures of 170 to 315 ℃. This stage has also been correlated with dehydroxylation of PVA according to Sambudi et al. (2016). Liu et al. (2022) reported that PVA is not highly thermally stable and confirmed that incorporation of chitosan into PVA films had improved the thermal stability.

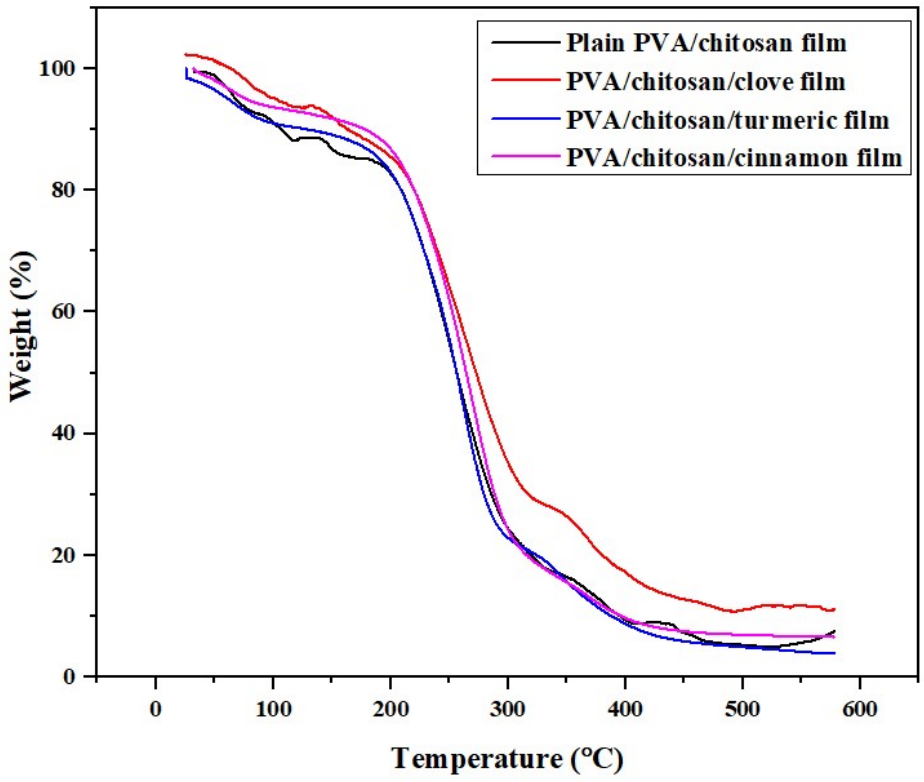


Figure 15: TGA thermogram of plain and antimicrobial-loaded PVA/chitosan films

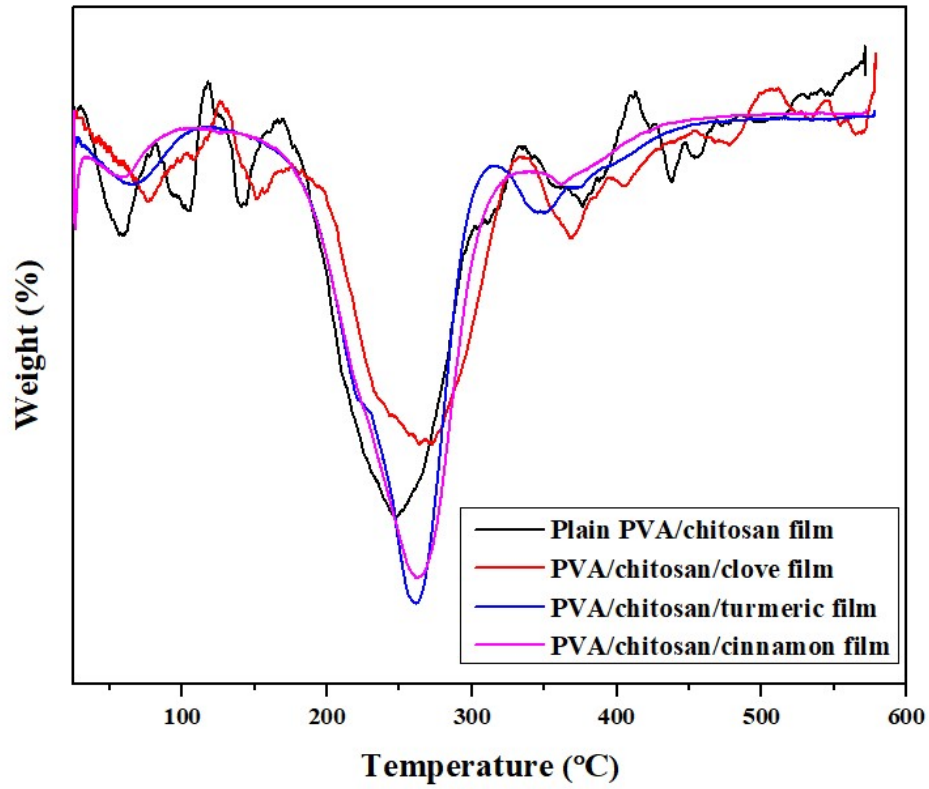


Figure 16: DTG thermogram of plain and antimicrobial-loaded PVA/chitosan films

Table 1: Characteristic temperatures for plain and active PVA/chitosan films

Sample	T_{onset} (°C)	$T_{50\%}$ (°C)	T_{final} (°C)
PLAIN	153.56	256.20	389.78
TUR	162.75	257.30	399.30
CLO	182.49	273.00	437.50
CIN	170.62	265.27	401.77

PLAIN = Plain PVA/chitosan film
TUR= PVA/chitosan film with turmeric
CLO= PVA/chitosan film with clove oil
CIN= PVA/chitosan film with cinnamon

4.3 FTIR Analysis

The molecular interaction between PVA/chitosan films containing natural antimicrobial extracts were studied via FTIR analysis. The spectra observed in all samples showed that all characteristic peaks were represented in both the plain and active films and confirmed the successful incorporation of natural extracts as well as the compatibility between PVA and chitosan. Plain PVA/CS films showed a broad transmittance at 3293.9 cm^{-1}, which may be characteristic for hydrogen bonding and stretching connections between the hydroxyl (-OH) group of PVA and the amine ($-NH_2$) group of chitosan as similar bands were seen in all the samples (between the range of 3277.9 and 3311.7 cm^{-1}). Similar peak was observed by Zheng et al. (2014) for PVA/cellulose nanofibril; this confirms that transmittance between 2000 to 4000 cm^{-1} is largely due to PVA H-bonding with polysaccharides. Peaks observed around 1500 to 2000 cm^{-1}, although with different transmittance percentages were seen in all samples. This could indicate an efficient treatment of acetic acid with chitosan as reported by Essel et al. (2018). Bands seen around 2900 cm^{-1} represent the presence and stretching of C-H alkyl groups (Reddy et al., 2019). There were also characteristic sharp peaks around 1000 to about 1700 cm-1 in the active films, which can be correlated to C=C stretching, C=O stretching, C-O stretching, C-H bending, and O-H bending, which are all peculiar to the aromatic active agents in the natural extracts added to the films (eugenol in clove, cinnamaldehyde in cinnamon and curcumin in turmeric) and confirming their presence (Talari et al., 2016). Peaks observed around 800 cm^{-1} have been attributed to the presence of saccharin structure in chitosan (Zakaria et al., 2012).

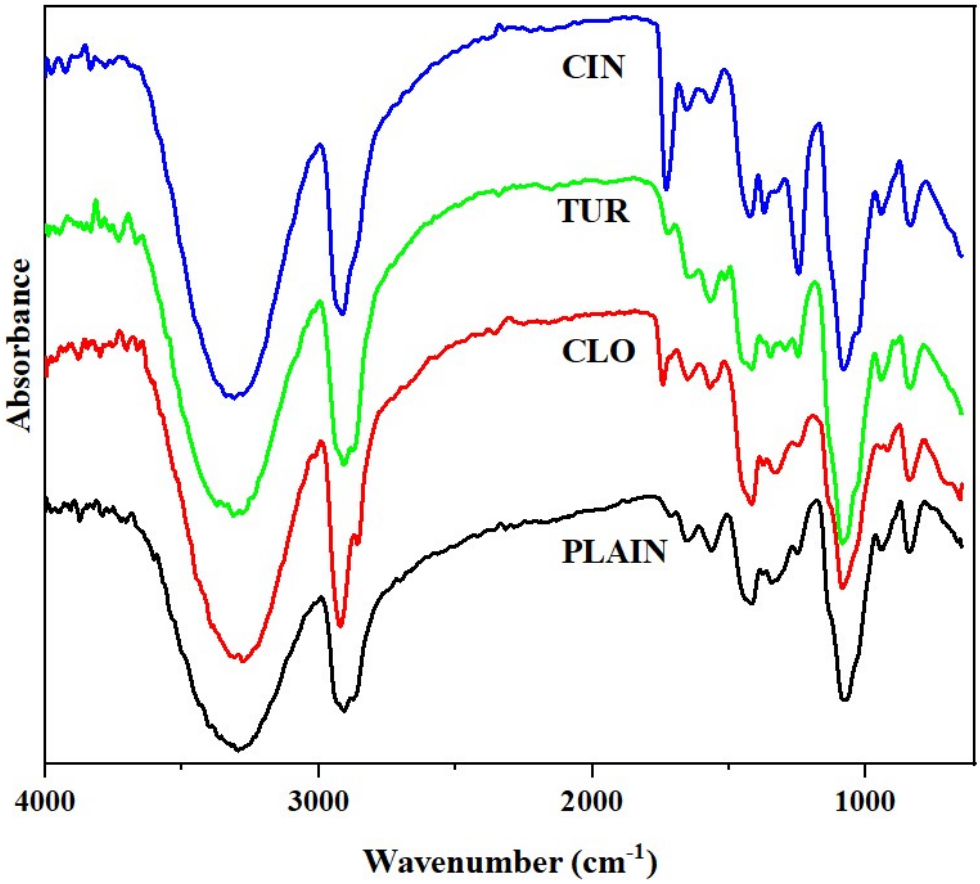


Figure 17: FTIR spectra of plain and antimicrobial-loaded PVA/chitosan films

PLAIN = Plain PVA/chitosan film
TUR= PVA/chitosan film with turmeric
CLO= PVA/chitosan film with clove oil
CIN= PVA/chitosan film with cinnamon

4.4 Mechanical Properties

Mechanical properties are important in determining the suitability of films in food packaging application. For this reason, different compositions of PVA/chitosan polymers were prepared, and their mechanical properties were investigated so that the sample with the most acceptable property is selected, Figure 18. Naturally, chitosan produces brittle and stiff films (Jahan et al., 2016); blending with polyvinyl alcohol helps to reduce this phenomenon and imparts some flexibility on the films by increasing the free volume (Kanatt et al., 2012). In Figure 18, it can be observed that the blend composition containing 20% chitosan and 80% PVA had the highest maximum elongation (α) while maintaining its high modulus values, hence this blend composition was chosen as the basis for blends used throughout this research work. Films containing 70, 80 and 90% chitosan were also prepared but they were extremely stiff and highly brittle for food packaging application as shown in Figure 18 for samples containing 60% chitosan.

The stress-strain graphs of PVA/chitosan films blended with different types of natural antimicrobial agents namely, turmeric, clove, and cinnamon are shown in Figure 19. It was observed that incorporation of these agents has influenced the mechanical properties of the films. It is obvious from this figure that the use of clove antimicrobial agent diminished the flexibility of the films while maintaining high modulus. This is possibly due to a composite effect resulting from the presence of clove oil droplets acting as a filler and rendering the blend as a composite. This can also be confirmed by observing the SEM snapshots where, unlike other films with cinnamon and turmeric, the clove oil droplets formed a separate phase from the polymeric blend, Figure 14.

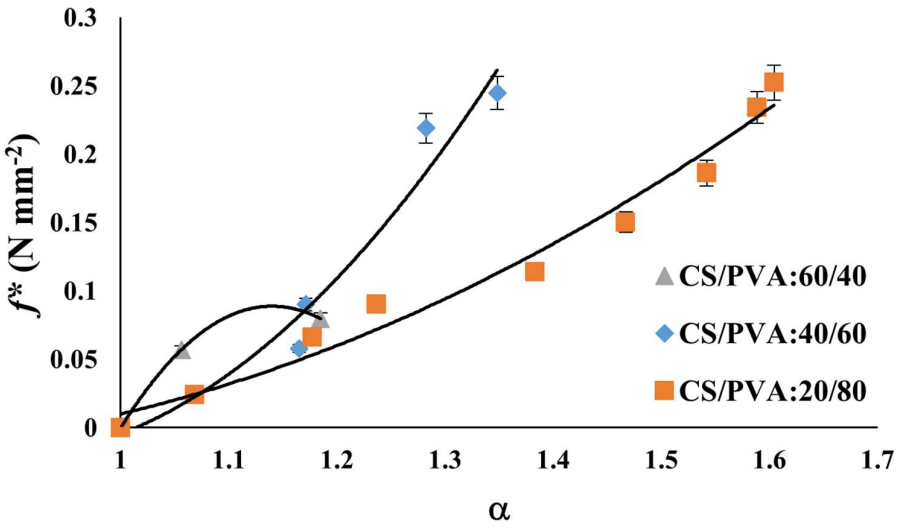


Figure 18: Stress-Strain curve for different compositions of PVA/chitosan films

CS = Chitosan PVA = Polyvinyl alcohol

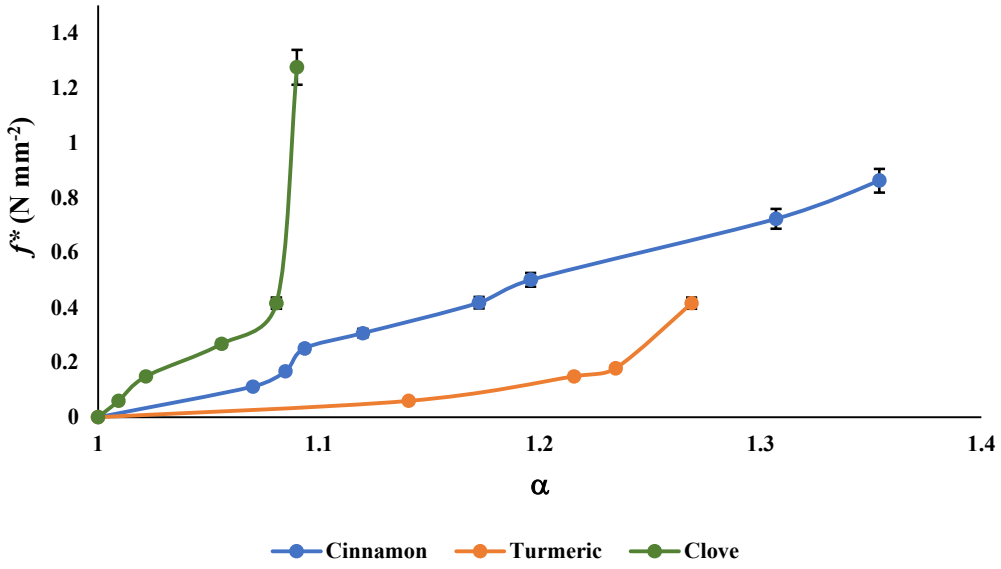


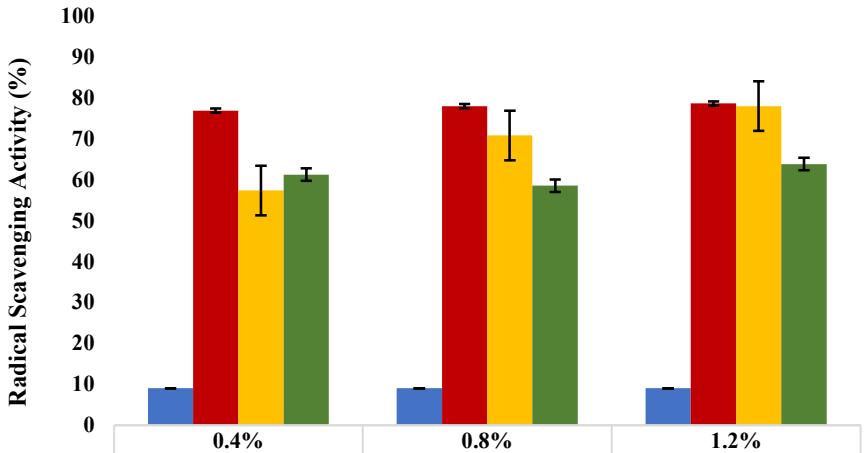
Figure 19: Stress-Strain curve for PVA/chitosan films containing natural antimicrobial agents

4.5 Antioxidant Activity

The antioxidant properties of plain and antimicrobial-loaded films were analyzed as a measure of the %DPPH radical scavenging activity (RSA) at different concentrations. Films (1 cm by 1 cm) containing 0.4, 0.8, and 1.2% of the antimicrobial agents were prepared and tested for their antioxidant activity, Figure 20. TUR showed highest scavenging activity even at low concentrations, followed by CIN, while CLO exhibited varying RSA. This may be due to agglomeration of clove oil at high concentration because of its hydrophobicity within a hydrophilic polymer matrix. However, the films loaded with 1.2% of the antimicrobial agents had the highest scavenging percentage thus they were selected and used in shelf-life studies.

Free radicals are known to be associated with oxidation leading to development of off odors, off-flavors, and eventually spoilage in foods. Therefore, there is a need to investigate the antioxidant activities of natural compounds to improve physiological condition of foods such as delayed spoilage (de Torre et al., 2019). The antioxidant activities of the antimicrobial-loaded films were significantly enhanced when compared to the plain film. Plain PVA/chitosan film recorded no antioxidant activity (9%) while sample TUR with 1.2% showed the highest radical scavenging activity at 78.7%, although this was closely followed by sample CIN (78.1%), however CLO had the least antioxidant activity (63.9%). Yashaswini and Iyer (2019) similarly reported a figure of about 64% for chitosan-based films with clove oil. Although, same authors created films with turmeric essential oil but reported a scavenging activity of about 56% which is contrary to the report obtained in this research. This might support the claim that reduced scavenging activity could be due to evaporation of essential oils during solvent drying stage of film preparation. Absence of scavenging activity in the control films might be due to the presence of PVA because Yang et al. (2016) reported that pure chitosan film exhibits some level of antioxidant activity. This is because the chitosan structure contains free amino groups (NH2) in chitosan which react

with free radicals to give stable radicals. This free NH_2 group becomes unavailable in the PVA/chitosan matrix because it has been used as a reactive site in the intermolecular bonding (hydrogen bond) with PVA (Annu et al., 2021). Low composition of chitosan in the blend could also indicate the reason for absence of antioxidant activity in the control film. Enhanced antioxidant activity observed in the loaded films may be due to hydrophilicity of the composite films because solubility is crucial in determining the release of bioactive compounds on food surfaces (Peng et al., 2013). Researchers have also correlated high antioxidant activity to high phenolic contents in natural extracts because this leads to increased ability to donate hydrogen hence acting as a radical scavenger (Sankhalkar & Vernekar, 2016; Annu et al., 2021; Hiremani et al., 2021).



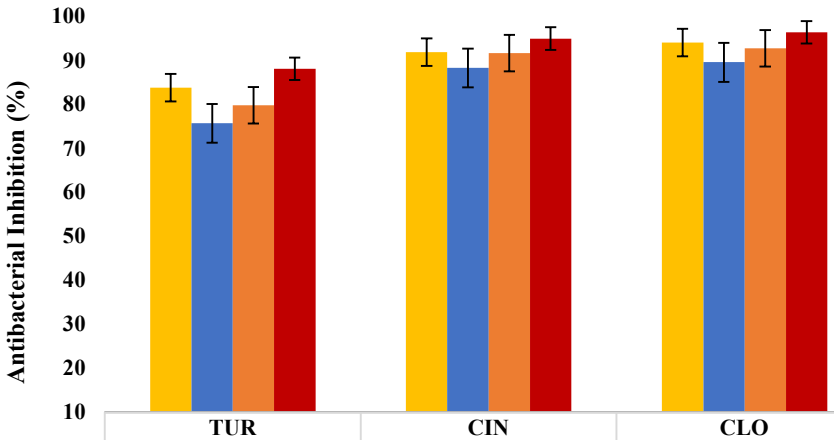
	0.4%	0.8%	1.2%
Plain PVA/CS	9.0	9.0	9.0
PVA/CS+Turmeric	77.0	78.1	78.7
PVA/CS+Cinnamon	57.4	70.9	78.1
PVA/CS+Clove	61.3	58.6	63.9

Figure 20: Effect of antimicrobial agents at different concentrations on radical scavenging activity PVA/chitosan films.

4.6 Antibacterial Activity

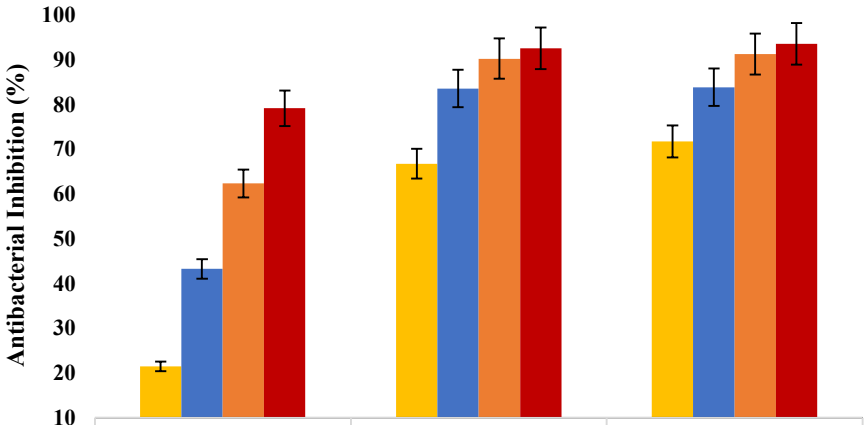
The antibacterial properties of turmeric, cinnamon, and clove-containing films at different concentrations (0.4, 0.8 & 1.2%) are shown below. These films were tested against two common food pathogens, gram-positive *Staphylococcus aureus* (Figure 21) and gram-negative *Escherichia coli* (Figure 22). The highest microbial inhibition was observed in the films containing 1.2% of the antimicrobial agents.

After 24 hours of incubation, plain PVA/chitosan film did not have any inhibitory effect against *S. aureus* and *E. coli*. However, the composite films containing natural antimicrobial agents recorded high antimicrobial activity with a similar trend observed for both organisms. Film loaded with clove oil effectively inhibited the growth of *S. aureus* and *E. coli* and recorded the highest value in both cases (93.6% and 96.4% respectively). This was closely followed by CLO film (92.6% and 95%), while TUR film had the least inhibition against the organisms (79.2% and 88.1% respectively). The bacterial population (log cfu/ml) of the different concentrations of active PVA/chitosan films and free samples are indicated in Table 2. The values show that bacterial population and inhibition efficiency have an inverse relationship. This means that the bacterial population in log cfu/ml decreases as inhibition percentage increases. Plate count of free samples were higher those of the samples in the PVA/chitosan films, indicating a lower inhibition effect on the microorganisms. The bacterial count (log cfu/ml) of active films obtained in this study was lower than the values for PVA/chitosan films containing ZnO-SiO_2 nanocomposites reported by Al-Tayyar et al. (2020). The findings obtained in this research gives an idea of the antibacterial potency of each of the natural agents used. The CFU values show that *E. coli* was slightly more sensitive to the antimicrobial agents than *S. aureus*. This occurrence may be due to differences in cell structure and metabolism. This finding was similar to those of Lan et al. (2020). These results indicate that the developed films may have good potential as active food packages, especially for fresh and perishable foods.



	TUR	CIN	CLO
1.2% pure sample	83.8	91.9	94.1
0.4% concn. film	75.7	88.3	89.6
0.8% concn. film	79.8	91.7	92.8
1.2% concn. film	88.1	95	96.4

Figure 21: Antibacterial activity of PVA/chitosan films with different concentrations of antimicrobial agents against *S. aureus*. TUR= PVA/chitosan film with turmeric CLO= PVA/chitosan film with clove oil CIN= PVA/chitosan film with cinnamon



	TUR	CIN	CLO
1.2% pure sample	21.5	66.8	71.8
0.4% concn. film	43.3	83.6	83.9
0.8% concn. film	62.4	90.3	91.3
1.2% concn. film	79.2	92.6	93.6

Figure 22: Antibacterial activity of PVA/chitosan films with different concentrations of antimicrobial agents against *E. coli*. TUR= PVA/chitosan film with turmeric CLO= PVA/chitosan film with clove oil CIN= PVA/chitosan film with cinnamon

Table 2: Bacterial population of *S. aureus* and *E.coli* in PVA/chitosan films and free samples at different concentrations of antimicrobial agents

Plate count (log cfu/ml)						
	S. aureus			***E. coli***		
	0.4%	**0.8%**	**1.2%**	**0.4%**	**0.8%**	**1.2%**
PLAIN	7.21	7.21	7.21	6.94	6.94	6.94
Samples in a film						
TUR/Film	6.60	6.51	6.28	6.51	6.26	6.69
CLO/Film	6.23	6.07	5.77	5.88	5.74	6.15
CIN/Film	6.28	6.13	5.91	5.93	5.81	6.16
Free Samples						
TUR	6.77	6.61	6.42	6.94	6.90	6.83
CLO	6.26	6.17	5.98	6.53	6.47	6.39
CIN	6.32	6.22	6.12	6.57	6.50	6.46

PLAIN = Plain PVA/chitosan film TUR/Film = PVA/chitosan + turmeric film
CLO/Film = PVA/chitosan + clove film CIN/Film = PVA/chitosan + cinnamon film
TUR = Free turmeric CLO = Free clove CIN = Free cinnamon

4.7 Shelf Stability Studies

4.7.1 pH of Chicken Breast samples

Table 3: Effect of active packaging on the pH of chicken breast

	Storage Period		
	Day 0	**Day 7**	**Day 14**
PLAIN	6.46±0.00[a]	6.20±0.00[d]	7.01±0.00[a]
TUR	6.46±0.00[a]	6.64±0.00[a]	6.61±0.00[b]
CLO	6.46±0.00[a]	6.27±0.00[c]	6.50±0.00[c]
CIN	6.46±0.00[a]	6.49±0.00[b]	6.61±0.00[b]

Samples are means ± standard deviation of duplicate determination
Samples with different superscript letters in a column indicate significant difference
PLAIN = Plain PVA/chitosan film
TUR = PVA/chitosan + turmeric film
CLO = PVA/chitosan + clove film
CIN = PVA/chitosan + cinnamon film

The pH of packaged chicken breast meat was measured through a period of 14 days to investigate the effect of antimicrobial agents on samples' stability (Table 3). All microorganisms have sensitivity to pH, extremely high and low pH values can inhibit the growth of most microorganisms. However, all fresh and unprocessed foods do not have sufficient pH required to inhibit microbial growth hence there is need to monitor the pH changes in foods to indicate food stability (McGlynn, n.d).

The initial pH of the fresh chicken breast was 6.46 at day 0. After 7 days of storage at 5 ℃, sample packaged in plain PVA/chitosan film recorded the most decrease in pH (6.20), followed by the sample packaged in CLO (6.27). Contrarily, TUR and CIN films recorded increase in their pH values (6.64 and 6.49 respectively). After 14 days, the pH of all samples increased significantly. The sample packaged in plain PVA/chitosan film showed the highest value (7.01) while sample packaged in CLO film recorded the least value (6.50). No significant difference ($p<0.05$) was observed in the pH of chicken packaged with TUR and CIN films (6.61). The pH values obtained in this study are in agreement with previous

reports revealing the effect of natural antimicrobial agents in meat preservation (Bojorges et al., 2020; Konuk Takma & Korel, 2019; Khezrian & Shahbazi, 2018; Souza et al., 2018). The pH of meat is closely correlated with freshness and quality parameters such as color, microbial growth, and tenderness of meat (Bojorges et al., 2020). This study showed that addition of turmeric, clove, and cinnamon as antimicrobial agents in the packaging of chicken significantly affected pH. Although the pH values increased, but the increase was gradual in the antimicrobial-loaded films, unlike what is evidenced in the plain PVA/chitosan film (a rapid increase from 6.20 to 7.01). TUR film however kept the pH value low, showing a decrease from 6.64 to 6.61 between days 7 and 14, indicating potency of the active compound in turmeric, curcumin (Chan et al., 2011). Increase in pH of perishable food is common during storage and it indicates the occurrence of biochemical reactions such as protein denaturation and increase in microbial growth which leads to the production and accumulation of volatile alkaline by-products such as amines and ammonia (Bojorges et al., 2020; Souza et al., 2018). This shows that the antimicrobial films were able to extend the shelf life of chicken due to the presence of high amounts of phenolic compounds and their high inhibition efficiency against microorganisms which has been explained in section 4.6.

4.7.2 pH of Packaged Cheese samples

Table 4: Effect of active packaging on the pH of white cheese

	Storage Period		
	Day 0	**Day 7**	**Day 14**
PLAIN	5.64±0.00[a]	6.78±0.00[a]	6.85±0.00[a]
TUR	5.64±0.00[a]	6.52±0.00[d]	6.53±0.00[d]
CLO	5.64±0.00[a]	6.63±0.00[b]	6.80±0.00[b]
CIN	5.64±0.00[a]	6.60±0.00[c]	6.62±0.00[c]

Samples are means ± standard deviation of duplicate determination
Samples with different superscript letters in a column indicate significant difference
PLAIN = Plain PVA/chitosan film CLO = PVA/chitosan + clove film
TUR = PVA/chitosan + turmeric film CIN = PVA/chitosan + cinnamon film

Effect of packaging on the pH of soft white cheese is shown in Table 4. pH values for all samples increased as the period of storage proceeded. Fresh cheese had an initial pH value of 5.64. At day 7, this value significantly increased to 6.78 in plain PVA/chitosan film but lower in the films containing antimicrobial agents. Cheese packaged with CLO film had a pH of 6.63, followed by CIN (6.60), while the least value was observed in cheese packaged with TUR (6.52). Similarly, at 14 days of storage, the pH of the cheese samples increased following the same trend. Faccia et al. (2019) also recorded increase in pH of cheese samples from 5.9 to 6.1 and inferred that absorption of moisture could be a reason for increasing pH in cheese. Marcuzzo et al. (2013) also suggested that high pH values observed could be due to decrease in lactic acid level caused by the action of yeast. pH of cheese is an important factor in cheese processing and storage. On one hand, it influences the characteristic flavor and texture of cheese. On the other hand, it is an indication of microbial activity because it controls the concentration of lactic acid available for fermentation (Marcuzzo et al., 2013).

4.7.3 Microbiological Assay

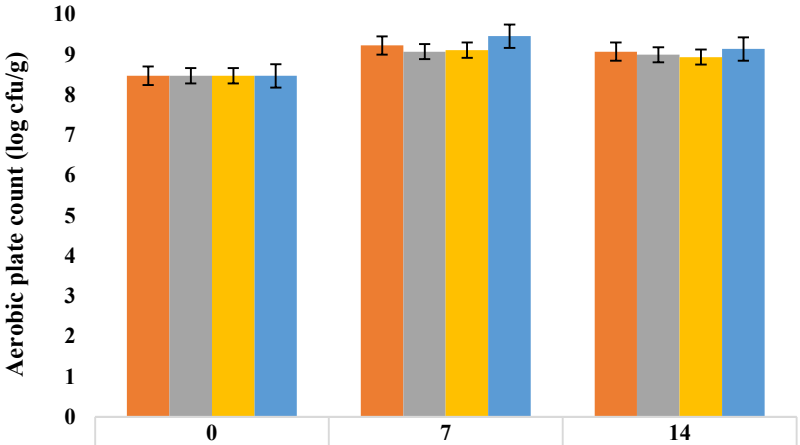
The antibacterial activity of the PVA/chitosan films loaded with turmeric, clove, and cinnamon were tested in-vitro by packaging chicken and cheese, followed by storage at 5℃ for 14 days. The microbial population of the food samples were estimated and the changes in aerobic plate count (APC), *Staphylococcus aureus*, and total coliform count are presented, Figures 23 to 28.

Chicken and cheese samples had an initial APC of 8.48 and 5.77 log cfu/g respectively, Figure 23 & 24. The initial microbial load of chicken is higher than other reports (Soysal et al., 2015; Shapi'i et al., 2020; Konuk Takma & Korel, 2019). It could be an indication that the samples may have been contaminated either during handling and/or transportation. On Day 7, these values increased significantly; the highest count for chicken was observed in the CIN films (9.46 log cfu/g) while TUR had the highest count for cheese (7.92 log cfu/g). Meanwhile, CLO recorded the least bacterial population (9.08 log cfu/g) for chicken after 7 days of storage. By the 14th day, the total bacterial load in all chicken samples decreased significantly, while cheese samples were increasing. Overall, CIN offered the most inhibition against total bacterial load in cheese, suggesting the high inhibitory activity of the active compound, cinnamaldehyde. Meanwhile CLO gave the most inhibition in chicken breast samples.

Staphylococcus aureus counts in chicken breast at day 0 was 1.85 log cfu/g, Figure 25. At day 7, the population decreased in samples packaged with CLO film (0.95 log cfu/g) and the plain film (1.0 log cfu/g) while CIN and TUR films showed an increase in *S. aureus* counts (2.20 and 1.90 log cfu/g respectively). Although by day 14, the population slightly increased in CLO (1.00 log cfu/g) and reduced to 1.00 log cfu/g in TUR. Meanwhile, the *S. aureus* count in all the cheese samples was constant throughout the duration of storage, Figure 26. The data obtained shows that the films were most effective in inhibiting the growth of *S. aureus* in both chicken and cheese samples. The values obtained for cheese

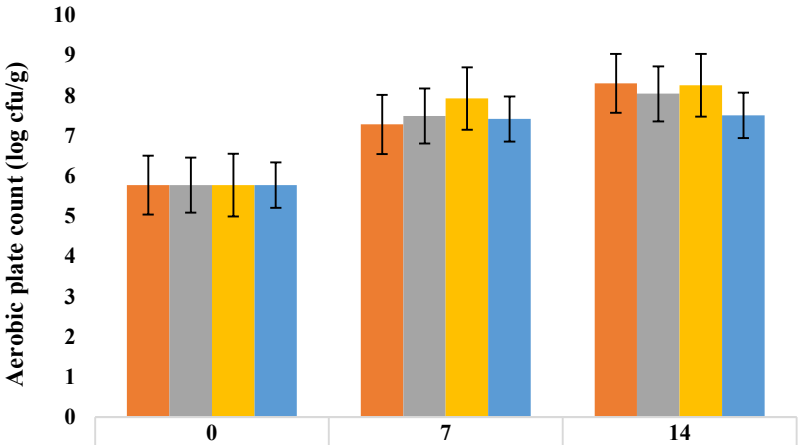
samples in this study were lower than those reported by Lin et al. (2019) who used *Moringa* oil as antimicrobial agent in chitosan-containing film.

The initial count for total coliform in chicken breast was 6.59 log cfu/g and by the 14th day, the population had decreased in plain (6.23 log cfu/g) and CLO (5.28 log cfu/g) films, Figure 27. In the antimicrobial assay discussed in Section 4.7, it was reported that the plain films do not have any antimicrobial activity. Hence, the reduction in coliform count observed may be due to the organisms approaching decline phase after undergoing rapid reproduction and producing toxic secondary metabolites which leads to cell death. Contrarily, chicken breast packaged in CIN film experienced an increasing number of coliforms at Day 7 (7.11 log cfu/g) and slight decrease in population at day 14 (7.08 log cfu/g). On the other hand, TUR experienced a decrease at day 7 (5.93 log cfu/g) and an increase in population back to the initial value of 6.59 log cfu/g. These results conformed with those reported by Souza et al. (2018). As for the cheese samples, the total coliform count remained constant from days 0 to 7, with less than 10 colonies. However, by the 14th day, CIN antimicrobial films recorded the highest coliform count (3.41 log cfu/g), followed by the plain films (1.95 log cfu/g) and TUR (1.48 log cfu/g), while CLO showed no increment in coliform load, indicating effective inhibition and bactericidal effect of the active compound (eugenol) against coliform proliferation.



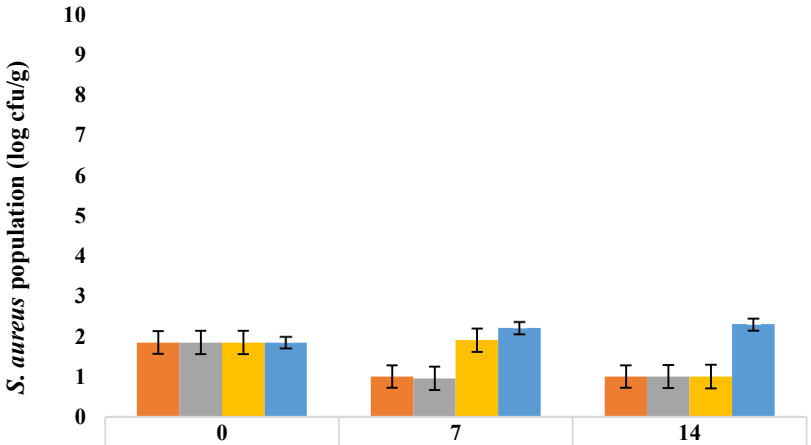
	0	7	14
Plain PVA/chitosan	8.48	9.23	9.08
PVA/chitosan+clove	8.48	9.08	9.00
PVA/chitosan+turmeric	8.48	9.11	8.94
PVA/chitosan+cinnamon	8.48	9.46	9.15

Figure 23: Effect of active packaging on aerobic plate count of chicken breast



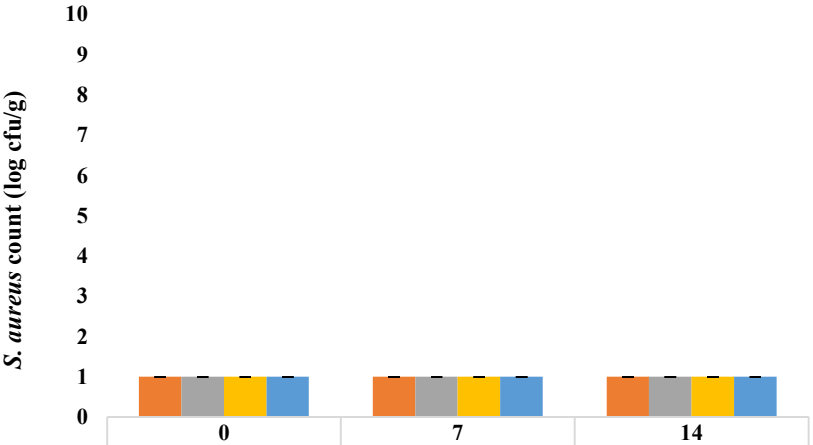
	0	7	14
Plain PVA/chitosan	5.77	7.28	8.30
PVA/chitosan+clove	5.77	7.49	8.04
PVA/chitosan+turmeric	5.77	7.92	8.26
PVA/chitosan+cinnamon	5.77	7.41	7.51

Figure 24: Effect of active packaging on aerobic plate count of white cheese



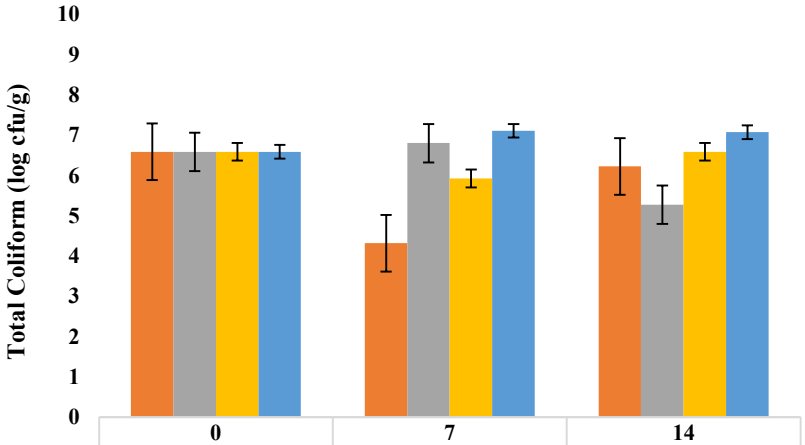
	0	7	14
Plain PVA/chitosan	1.85	1.00	1.00
PVA/chitosan+clove	1.85	0.95	1.00
PVA/chitosan+turmeric	1.85	1.90	1.00
PVA/chitosan+cinnamon	1.85	2.20	2.30

Figure 25: Effect of active packaging on *S. aureus* count in chicken breast



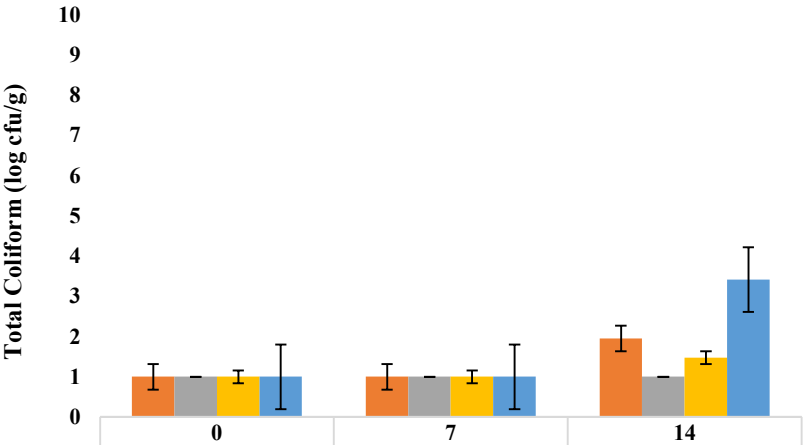
	0	7	14
Plain PVA/chitosan	1.00	1.00	1.00
PVA/chitosan+clove	1.00	1.00	1.00
PVA/chitosan+turmeric	1.00	1.00	1.00
PVA/chitosan+cinnamon	1.00	1.00	1.00

Figure 26: Effect of active packaging on *S. aureus* count in white cheese



	0	7	14
Plain PVA/chitosan	6.59	4.32	6.23
PVA/chitosan+clove	6.59	6.81	5.28
PVA/chitosan+turmeric	6.59	5.93	6.59
PVA/chitosan+cinnamon	6.59	7.11	7.08

Figure 27: Effect of active packaging on total coliform count in chicken breast.



	0	7	14
Plain PVA/chitosan	1.00	1.00	1.95
PVA/chitosan+clove	1.00	1.00	1.00
PVA/chitosan+turmeric	1.00	1.00	1.48
PVA/chitosan+cinnamon	1.00	1.00	3.41

Figure 28: Effect of active packaging on total coliform count in white cheese

Chapter 5

5. Conclusion and Future Work

The impact of petroleum-based plastics on environmental sustainability is gaining more attention nowadays. Environmentally friendly packaging films developed from bio-based polyvinyl alcohol and biodegradable chitosan are of a particular interest here and their physical, mechanical, and microbiological properties are investigated. The results of SEM revealed that incorporation of turmeric and cinnamon as antimicrobial agents gave homogenous and compact films, suggesting significant interfacial adhesion between PVA, chitosan and the additives. However, incorporation of clove oil resulted in a clear microphase separation attributed to the hydrophobic nature of the clove oil droplets and the hydrophilic nature of the PVA/chitosan polymeric film, which caused coalescence and separation of clove oil from the film matrix. Though, on a macroscopic scale, the film itself showed homogeneity and natural miscibility between the polymeric blend and the additive suggesting that the phase separation between the clove oil droplets and the polymeric blend was in fact a microphase separation on the nanoscale.

Thermogravimetric analysis revealed that adding the antimicrobial agents to the polymeric blends had no significant influence on the thermal degradation profile. The degradation temperatures of the active films at the beginning of the thermal profile, at 50% weight loss and at the final stages of the thermal treatment had close values to each other when compared to the plain PVA/chitosan film. The molecular interaction between PVA/chitosan films containing natural antimicrobial extracts were studied via FTIR analysis. The spectra observed in all samples showed that all characteristic peaks were represented in both the plain and active films and confirmed successful incorporation of natural extracts as well as compatibility between PVA and chitosan. The mechanical properties of different compositions of PVA/chitosan polymers were prepared, and the sample with the most acceptable property was the blend produced from 80% PVA and 20% chitosan. This blend

was then loaded with turmeric, cinnamon and clove at different concentrations. The mechanical properties after incorporating these antimicrobial agents were presented in a stress-strain graph, which revealed that the presence of antimicrobial agents has influenced the mechanical properties of the films through a reduction in both the force and elongation at break. However, the clove-active films maintained a high force at break on the expense of the film flexibility. This was attributed to a composite effect resulting from the presence of clove oil droplets acting as a filler and rendering the blend as a composite. This phenomenon was confirmed by observing the SEM snapshots where, unlike other films with cinnamon and turmeric, the clove oil droplets formed a separate phase from the polymeric blend.

To analyze the antioxidant activity of the films, antimicrobial agents were incorporated at 0.4%, 0.8%, and 1.2% concentrations and the antioxidant activity was estimated as a measure of the radical scavenging activity (%RSA). The results revealed that the antimicrobial-loaded films exhibited significant antioxidant activities while the plain films recorded no antioxidant activity. Films containing turmeric showed highest scavenging activity even at low concentrations, followed by cinnamon active films, while clove-containing films exhibited varying RSA which decreased at 0.8% concentration compared to 0.4%. This was attributed to agglomeration of the clove oil droplets at high concentration resulting from its hydrophobicity within a hydrophilic polymer matrix.

Likewise, the antibacterial activity of the films showed improved inhibition against two common food spoilage pathogens, *E. coli* and *S. aureus*, with the highest inhibition percentage observed in cinnamon and clove active films, especially at 1.2% concentration of the antimicrobial agents., The plain and antimicrobial PVA/chitosan films were tested in-vitro via packaging of chicken breast and white cheese, the food samples were monitored for changes in their pH and microbial count during a 14-day storage under refrigeration storage at 5℃. Results revealed that the pH of food samples was lower in the antimicrobial

loaded films, and they offered better shelf-life extension in cheese than chicken. Chicken packaged with clove-containing films showed lowest pH values while turmeric-containing films gave the least pH results in cheese samples after 14 days of storage. The microbiological assay investigated were aerobic plate count (APC), *Staphylococcus aureus*, and total coliform count. Turmeric-containing films recorded the least APC in chicken samples while cinnamon-containing films had the lowest APC for cheese after 14 days. Population of *S. aureus* in both chicken and cheese were very low throughout storage, suggesting that the antimicrobial agents were effective in hindering the growth of *S. aureus*. Similarly, incorporation of antimicrobial agents, especially clove and turmeric films gave the most inhibition against total coliform in cheese samples. Meanwhile chicken breast samples recorded higher coliform counts, though clove-containing films recorded lowest plate count.

The findings of this research indicates that PVA/chitosan films incorporated with natural bioactive compounds such as turmeric, cinnamon and clove could be of importance as active food packages.

A promising area of future work would be to study the effect of antimicrobial incorporation on the biodegradability, migration rate, and barrier properties of the PVA/chitosan films.

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