SYNTHESIS AND CHARACTERIZATION OF SILVER, ZINC OXIDE AND TITANIUM DIOXIDE STARCH BASED NANOCOMPOSITES FILM FOR MAIZE PACKAGING

BY

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ABSTRACT

Maize is a major cereal and one of the most important food crops in Nigeria. The entire production process (sowing, harvesting, postharvest drying, and storage) of grains are possible sources of fungi contamination and possible way through which they find their way to the human body by way of contaminated food. The aim of this research was to produce starch films embedded Silver (Ag), Zinc Oxide (ZnO) and Titanium dioxide (TiO_2) nanoparticles for post-harvest preservation of maize. *Hyptis suaveolens* leaf extract was used to reduce Ag, ZnO and TiO_2 salts to nanoparticles and Characterized using UV-visible spectrophotometer and Nano zetasizer. Fresh Maize samples was collected and the fungal profile was done using a standard method. The starch film was embedded with the nanoparticles to form a composite which was characterized for thickness, moisture content, swelling index, solubility and biodegradability. The morphology of the film was confirmed using scanning electron microscopy. While the shelf life and fungal growth evaluation was done over a period of more than two months. The results of the synthesized nanoparticles shows an absorption spectral of 407 nm for Ag, 350 nm for Zinc Oxide and 318nm for TiO_2 nanoparticles. While the particle size of 61.71nm was confirmed for Ag, 61.30 nm for ZnO and 71.40 nm for TiO_2 *Aspergillus terreus* and *Mucor pusillius* were the fungi identified in the maize samples. The nano based starch film preserved the physical morphology and inhibit the growth of contaminated fungi when compared to packaged film without nanoparticles embedded. Also there was seed deterioration in samples packaged with film without nanoparticles and convectional polymer. In conclusion, the starch based nanofilm could be suggested for post-harvest packaging and storage.

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LIST OF ABBREVIATIONS

NPs	Nanoparticles
Ag-NPs	Silver Nanoparticles
ZnO-NPs	Zinc Oxides Nanoaparticles
TiO_2-NPs	Titanium Dioxide Nanoparticles
FDA	Food and Drug Administration
IITA	Internal Institute of Tropical Agriculture
AFB1	Aflatoxin B1
ZON	Zearalenone
DON	Deoxynivalenol
FB1	Fumonisin B1
PHL	Post-harvest losses
SSA	Sub-Saharan Africa
FAO	Food and Agricultural Organization
MC	Moisture Content
SI	Swelling Index
Aw	Water Activity
PP	Polypropylene
PET	Polyethylene Terephthalate
HDPE	High-density Polyethylene
LDPE	Low-density Polyethylene
PVC	Polyvinyl chloride
PS	Polystyrene
PLA	Polylactide
OECD	Organization for Economic Cooperation and Development
SEM	Scanning Electron Microscope
EDX	Energy Dispersive X-ray Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Crop production in Nigeria is dominated by small-scale farmers who are engaged in the production of the bulk of food requirements of the country. These groups make up about 80 % of Nigeria's farming population and are responsible for 80 % – 90 % of food production (Badu-Apraku *et al*., 2021). Food packaging is essential in maintaining the safety and quality of products, from processing and manufacturing to handling and storage, until the products reach the consumers (Tunma, 2018). Maize is one of the most important staple foods in the world today and its combination with rice and wheat supply more than 50 % of global caloric intake (Nag, 2017).

Maize consumption is wide spread across the country and among households. According to International Institute of Tropical Agriculture (2021), maize consumption in Nigeria as at 2021 stood at 10.9 million metric tons and the post-harvest losses of maize in SubSaharan Africa (SSA) have significantly increased in the past decade (Food and Agricultural Organization, 2018). This is mainly due to climatic conditions, bio deterioration brought about by pest organisms such as insects, molds, and fungi, rodent and birds, as well as poor post-harvesting practices (Oyeka *et al*., 2019). This has resulted in food shortage and labor losses as well as decline in the economic value.

Mycotoxin contamination of food and feed is a major concern in sub-Sahara African countries, particularly Nigeria. It represents a significant limit to health of human, livestock as well as the international trade. Among the grains that can be contaminated by mycotoxins are maize, rice, wheat, sorghum, barley, etc. For a long time, petroleumbased

plastic materials have been used for food packaging to protect the contents from insects. However, these synthetic non-biodegradable plastics have led to serious ecological problems due to environmental pollution, triggering the development of new biodegradable and less toxic biomaterials as alternatives.

Starch is considered as a widely accessible polysaccharide, which is obtained through different types of crops. In addition, it is considered as a promising alternative material for the fabrication of biodegradable materials instead of the currently used synthetic polymers (Wang *et al*., 2018). Active packaging in which antimicrobial agent is embedded or coated on a packaging material ensures the quality and safety of the food product through the interaction between the packaging film, food stuffs, and internal and external environments (Realini and Marcos, 2017). This technology refers to the incorporation of active compounds into packaging film.

The unique properties of nanomaterials, such as their small size, high surface-to-volume ratio, and quantum effects, make them suitable for diverse applications in the food industry (Omerovic *et al*., 2021). In particular, metal and metal oxide nanoparticles (such as Silver, zinc oxide, copper/copper oxide, titanium dioxide and magnesium oxide nanoparticles), mesoporous particles, graphene, and carbon dots have attracted remarkable interest in the food industry for their intrinsic antibacterial activity. The antimicrobial nanomaterials appalied in active packaging keep the food safe from harmful and spoilage microbes thus enabling long term freshness during storage and higher convenience for consumers (Hoseinnejad *et al*., 2018, Sharma *et al*., 2020).

Green synthesis of nanoparticles provides an environmentally benign, low-toxic, costeffective and efficient protocol to synthesize and fabricate nanoparticles. This method employ biological systems like bacteria, fungi, viruses, yeast, actinomycetes, plant

extracts, etc. Kumar and Guatam (2019) biochemically synthesis of zinc oxides nanoparticles through green synthesis approach using fresh leaf extract of *Azadiarachta indica*, in a similar manner Raja *et al*., (2019) synthesized zinc oxiden nanoparticles using *hyptis* leaf extract and Akinola *et al*., (2020) synthesized titanium dioxide nanoparticles biofabricated via phytosynthetic route using extracts of *Cola nitida.*

The plant *Hyptis suaveolens* (L.) Poit belongs to the family Lamiaceae is native of Tropical America. The different parts of *Hyptis suaveolens* plant, like the bark, seed, and leaves, are used to Cure swellings, abscesses, haemorrhoids, and improve memory (Raja *et al*., 2019). Sharma *et al.*, (2013) reported that the *Hyptis suaveolens* contains phytochemicals such as sterols, phenols, Saponins, terpenes, alkaloids, and flavonoids as secondary metabolites. Therefore, *Hyptis suaveolens* leaf extract was used as a reducing agent in the phytosynthesis of nanoparticles and make the synthesis ecofriedly (Raja *et al*., 2019). The blending of starch with any synthetic or natural polymer (Peighambardoust *et al*., 2019) or the incorporation of nanoparticles into starch are promising pathways to enhance novel biodegradable materials with amended features.

1.2 Aim of the Study:

To phytosynthesized and Characterized Silver, Zinc Oxides and Titanium dioxide nanoparticles using *Hyptis suaveolens* leaf extract and their inclusion in the starch film production for postharvest maize packaging.

1.3 Objectives of the Study

The objectives of this research work are to determine

i. Phytosynthesis and characterization of Ag, ZnO and TiO_2 nanoparticles

ii. Proximate composition of corn flour iii. Production and characterization of starch nanocomposite films

iv. Postharvest and post packaged maize fungi profile test

v. Evaluation of shelf life of the Maize packaged with silver, zinc oxide and titanium dioxide starch nanocomposite films

1.4 Statement of the Research Problem

Maize is one of the most important sources of food for human/animal nutrition as well as raw materials for industrial processes. Microbial contamination of maize results into a decrease in its nutritional quality and reduce shelf life. However, eating food prepared from contaminated maize has been linked to malnutrition due to insufficient nutrients in the grains or food poisoning from fungi which can cause hepatocellular carcinoma (Oyeka *et al*., 2019). The ability of fungi to infect maize grains easily upon contact with it makes conventional methods insufficient to deal with the menace. However, the presence of these mycotoxins affects the safety, quality, and functional properties of grains. Moreover, the organoleptic properties of products made from these grains could also be altered because some fungi strains produce potent odors.

1.5 Justification for the Research Study

Maize is a crucial food in Nigeria and it is vulnerable to microbial contamination due to different geographical climatic conditions and poor handling (Yilma *et al*., 2019). Food industry (FI) is one of the most important parts of any country's industry when it comes to food safety and security, one of the important aspects of food safety is the packaging (Neme *et al*., 2021). The use of nano-packaging is to improve the food's durability and shelf life. Nanotechnology can help to improve the efficiency and quality of packaging materials thereby ensuring food safety. The urgency of preventing foodborne diseases requires acceleration in the development of antimicrobial food packaging, (a special packaging that releases active biocide substances) in order to improve food quality, extend shelf life and prevent or delay the spoilage.

So far, in all kinds of nanoparticles developed and characterized, silver-based nanoparticles (NPs) have taken an important place due to their inherent feature of antimicrobial activity even in solid-state samples, and have therefore been used as bacteriostatic agents from ancient times. Silver-salts materials also have an inhibition effect on the growth of diverse pathogens affecting human health to protect them from infection. ZnO-Nanoparticles are recognized as inexpensive with potential antimicrobial properties. So the applications of ZnO-Nanoparticles packaging in food systems concentrate on its antimicrobial effect, and they are used to prolong the fresh food products shelf-life. Considerable studies indicated that addition of TiO_2-Nanopartiles had promoted the suitability of developed films applied in food packaging. TiO_2Nanoparticles and their applications in the food packaging area have attracted extensive attentions attributed to their antimicrobial activity.

Metal oxide nanoparticles such as zinc oxide and titanium dioxide are often used as photocatalysis agent to degrade organic molecules and microorganisms. The photocatalytic reaction of nano-ZnO and nano-TiO_2 attributes to generation of reactive oxygen species (ROS), resulting in the oxidation of cell cytoplasm and leading to cell death (Monish *et al*., 2018). Several research works documented that both silver, ZnO, and TiO_2 nanoparticles have antimicrobial activity against bacterial, molds and yeast. (Elegbede and Lateef, 2019). The bionanocomposites materials are designed to improve the functional characteristics of food packaging and can incorporate antimicrobial agents, antioxidants, plant extracts and enzymes to lengthen shelf-life of food products. The innovation of nanomaterials in the food packaging science has brought many changes in food preservation, storage, distribution and consumption. The preventing of microbial growth in foods by antimicrobial activity of nanomaterial has enabled extending the shelflife of foods to certain degrees with better management of spoilage in food products.

Furthermore, the nanotechnology provides numerous choices for cost-effective, ecofriendly, degradable and renewable packaging materials, which have been gaining more attention and acceptance to solve the ecological environment pollutions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Production and Importance of Maize in Nigeria

Maize is a major cereal and one of the most important food crops in Nigeria. About 50 species exist and consist of different colors, textures, and grain shapes and sizes. White, Yellow, and red are the most common cultivated maize types (International Institute of Tropical Agriculture, 2021). The white and yellow varieties are preferred by most people depending on the region. Maize production in Africa was around 75 million tons in 2018, representing 7.5 % of world maize production. Maize occupies approximately 24 % of farmland in Africa and the average yield stagnates at around 2 tons/hectare/year. The largest African producer is Nigeria with over 33 million tons, followed by South Africa, Egypt, and Ethiopia (International Institute of Tropical Agriculture, 2021).

Africa imports 28% of its required maize grain from countries outside the continent as most of the maize production in Africa is done under rain-fed conditions. Its genetic plasticity has made it the most widely cultivated crop in the country from the wet evergreen climate of the forest zone to the dry ecology of the Sudan savanna (Shee *et al*., 2019). Being photoperiod insensitive, it can be grown any time of the year, giving greater flexibility to fitting into different cropping patterns. It is one of the dominant cereal crops in the Guinea and Sudan savannas in northern Nigeria.

Over the years, maize has become an important crop, taking over acre ages from traditional crops such as millet and sorghum. In 2018, about 10.2 million tons of maize was produced from 4.8 million hectares, making Nigeria the highest producer in Africa (Food and Agricultural Organization, 2018). Research efforts by breeders and agronomists have led to the production of many technologies including the breeding of high yielding varieties that are tolerant to drought, diseases, low nitrogen, and Striga

infestation. Despite the availability of these varieties, yields are still low in the Nigerian savannas.

2. 1.1 Maize harvesting methods

Maize may be harvested manually or using mechanical means. Depending on the handling, each method predisposes maize to fungal infection. Manual harvesting involves de-husking the cob and throwing it to an identified site within the farm. The site may be bare or covered by the farm weeds. The de-husked cobs are normally thrown to the sites where on landing, can come in contact with soil or brush against weeds. The soil is an environment that is very rich in fungi, some of which may be capable of infecting the maize grains. Likewise, the weeds may host fungi which may also infect the maize. Alternatively, mechanical harvesting may create damage sites on the maize, which become the foci of infection by fungi (Birgin *et al*., 2020) The conditions at harvest may also predispose the maize to infection by fungi. Maize harvested during wet conditions is prone to fungal infection because moisture promotes their growth on the maize (Birgin *et al.*, 2020).

The maturity stage at which maize is harvested is also important with respect to predisposition of maize to infection by fungi. Maize harvested when the grains are still soft; contains high moisture content; and hence easily infected by the fungi. Similarly, maize harvested late may be exposed to damage by birds and small mammals, which damage the produce by de-husking of the top part of the cob followed by feeding on the grains. When it rains, water seeps in, moistens the damaged grains, which become sites of prolific growth of fungi. The infection of the grain thus spread from the de-husked, top part of the cob, downwards. In some varieties the cob opens at the top, thus de-husking in a similar manner as the ones damaged by the animals thus getting infected by to fungi as previously described (Birgin *et al*., 2020).

2.1.2 Maize storage structure

The storage facilities are important in preserving the quality of the produce. Stores that are spacious, well aerated and without leaking roofs ensures post-harvest longevity of the produce. On the other hand, poorly constructed and unmaintained stores allow access of small mammals that nibble and pests that hole the maize grains. Storage facilities used by farmers depend on the scale of farming. Large scale farmers use storage structures such as sophisticated silos. On the other hand, small scale farmers use all kinds of containers or bags.

In Kenya, the commonly used maize storage structures by farmers include traditional wooden granaries, grass line granaries and gunny bags. Maize storage in these structures is vulnerable to entry of water and rodents. The rodents mechanically damage the grains by nibbling on them as they feed thus creates ports of entry by fungi. The growth of fungi is promoted by the moisture arising from leaking roofs and walls as well as from urine and droppings from the rodents (Birgin *et al*., 2020).

2.2 Diversity of Post-Harvest Fungi in Maize.

The conditions at which the maize grains arrive at the store determine the fungi that predominate and the subsequent infection by the storage fungi. If the grains were harvested immaturely or under wet conditions, they are infected by the field fungi such as *Rhizopus, Mucor, Rhizoctonia, Cladosporium*, *Trichothecium, Fusarium* and *Altenaria* (Birgin *et al*., 2020). Post-harvest fungi infections usually occur after harvest either during transit or during storage of grains. Fungi that develop on grains during storage usually survive at low moisture contents. Major examples of these storage fungi include *Aspergillus sp.* and *Penicillium sp.* (Omotayo *et al*., 2019). Studies done by Lamboni and

Hell (2019) of Togo on traditional African granaries identified species of *Aspergillus, Rhizoctonia, Penicillium* and *Fusarium oxysporum* as post-harvest fungi commonly infecting maize in the graneries. Similar reports by Julian *et al*., (2016) in Honduras indicated that *Fusarium moniliforme, Fusarium moniliforme, Penicillium spp, Stenocarpella maydis, Sordaria macrospora* and *Acremonium* spp were the predominant species detected in stored maize.

2.2.1 Mycotoxin production by storage fungi in maize.

Fungi that grow on maize grains in the store not only damage and spoil the quality of maize, but they also contaminate them with toxic metabolites called mycotoxins. These mycotoxins are toxic to both animals and humans upon consumption. The mycotoxins released by fungi are the most potent metabolites known to man because they are capable of causing diseases at low concentration (Birgin *et al*., 2020). Two reasons have been brought forth explaining the potency of mycotoxin sreleased by fungi. Firstly, these mycotoxins are heat resistant and cannot be destroyed by conventional heat treatments even when the infected seeds are cooked. Secondly, their release from the fungus colony to food is rapid and affects variety of processed and unprocessed food products. More interestingly, when dairy cattle are fed with mouldy grains another metabolite of the mycotoxin that is found in the grains is secreted in the milk and is as potent as the original metabolite.

Studies have shown that the most important species of fungi and mycotoxins that could contaminate maize grains include *Aspergillus flavus* releasing aflatoxins, *Fusarium verticillioides, Fusarium proliferatum* releasing fumonisins and *Fusarium graminearum* releasing trichothecenes and zearalenone (Maina *et al*., 2017). Detected aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON) and fumonisin B1 (FB1) as mycotoxins on stored maize grain samples in the institute of animal husbandry, in

Belgrade. Adetunji, *et al*., (2018) also identified Aflatoxin B1 and Fumonisins B1 asmycotoxins in stored maize in five major agro ecological zones in Nigeria released by fungal species and *Aspergillus parasiticus,* and *Fusarium oxysporium* respectively. Some of the health impacts following the consumption of these mycotoxins may include liver cancer caused by consuming aflatoxins, abortion in humans following intake of zearalenone and oesophageal cancer caused by consumption of fumonisins (Omotayo *et al*., 2019).

On the other hand, reports from stored maize contamination in Kenya spanning a period of over 15 years show that Aflatoxins and fumonisins are the major mycotoxins found in stored maize (Mahuku *et al*., 2019). This is probably due to the fact that most research on mycotoxin contamination non stored maize in Kenya is focused mostly on the presence of Aflatoxins and fumonisins only. Thus, more research work has to be carried out to determine the presence of other mycotoxins in stored maize in Kenya.

2.2.2 Mycotoxins contamination of food and feeds in nigeria

Mycotoxins are toxic secondary fungal metabolites frequently found as contaminants in food and feed with attendant negative effects on humans and animal's health when ingested. They are fungal secondary metabolites that may develop in almost any food or feedstuff during the growing season, at harvest time, or during processing or storage, depending on the environment and method of handling (Abass *et al*., 2017). The occurrence of mycotoxin in food and feed chains is ubiquitous, affecting both human and animal health as well as economically affecting developing and developed countries via exposure to variable concentrations.

Recently, contamination of food and feed by mycotoxin is a global concern day by day and have caused serious out breaks worldwide affecting human and animal health, as well

as losses in nations economy (Cinar and Onbaşi, 2019). It is estimated that 25 % of world's food crop is contaminated by fungi-producing mycotoxins, causing huge losses in billions of dollars in domestic and international trade involving agricultural products (Cinar and Onbaşi, 2019). In 2017, it was reported that high level of mycotoxins content in food are the cause for the loss of billions of naira which Nigeria could have realised from non-oil export in nine years if the level of contamination were below safe limit (Imade *et al*., 2021).

In sub-Saharan Africa (SSA), the prevalent mycotoxins of concerned affecting the health of human, animals and economy are aflatoxins (43.75 %) followed by fumonisins (FUM, 21.87 %), ochratoxin (12.5 %), zearalenone (ZEN, 9.38 %), deoxynivalenol (DON, 6.25 %), beauvericin (BEA, 6.25 %) (Darwish *et al.*, 2014), while others constitute 3.13 %. Globally in 2019, data from January to December showed the most prevalent mycotoxins were FUM (70 %) and DON (68 %) (Sokefun *et al*., 2018). A 10-year survey by GruberDorninger *et al*., (2019) in Sub-Saharan Africa, reviewed an increased shift in prevalence in Fumonisins (72.6 %); ZEN (52.2 %) and DON (49.5 %) amidst other emerging mycotoxins (Kebede *et al*., 2020).

2.3 Postharvest Losses of Maize

Post-harvest losses (PHL) is defined "as grain loss which occurs after separation from the site of growth or production to the point where the grain is prepared for consumption (Food and Agricultural Organization, 2018). Other authors describe PHL as measurable quantitative, qualitative and economics of grain loss across the supply chain or the postharvest system, from the time of harvest till its consumption (World Bank, 2020).

The Food and Agriculture Organization (Food and Agricultural Organization, 2018) of the United Nations and World Bank data revealed that PHL of cereal in SSA ranged

between 5-40 %, worth around $4 billion (Zorya *et al.*, 2017). Which was stated in a recent report of a joint FAO/World Bank report (Zorya *et al*., 2017). In addition, the report shows PHL of cereal in Eastern and Southern Africa account for over 40 % of the total PHL in SSA countries. This represents losses of about $1.6 billion in value each year. Such losses are equivalent to the annual caloric requirement for at least 20 million people (Food and Agricultural Organization, 2018) or more than half of the value of total food aid received by SSA in a decade. Furthermore, it has been reported by Global Strategy to Improve Agricultural and Rural Statistics (2017) that postharvest losses of maize in various storage facilities in undeveloped tropical countries ranged from 15-25 %.

2.3.1 Types of Losses

Post-harvest losses can be classified into three main categories: quantitative loss, qualitative loss, and economic or commercial loss. Also, they can be classified as direct and indirect losses.

Quantitative loss indicates the reduction in physical weight, and can be readily quantified and valued. For example, a portion of grain damage by pests or lost during transportation.

Qualitative loss is contamination of grain by molds and includes loss in nutritional quality, edibility, consumer acceptability of the products and the caloric value (Zorya *et al*., 2017).

Economic loss is the reduction in monetary value of the product due to a reduction in quality and or/ quantity of food (Tefera *et al*., 2017).

2.4 Factors Promoting Microbial Growth and Mycotoxin Production in Grains.

When deciding whether moisture, temperature, etc., affects the safety of grains, other factors should be considered to settle on a scientifically proven conclusion. Extrinsic

factors (temperature, relative humidity, mechanical injury on seeds during harvest or processing, insects, and rodents infestation) are environmental and physical factors surrounding the grains. Whereas, those attributed to the characteristics of the grains are intrinsic factors (pH, acidity, nutrient composition, biological structure, moisture content/water activity, redox potential, naturally occurring and added antimicrobial factors). Details on how these factors contribute to or promote microbial contamination of grains are examined below.

2.4.1 Nutrient content

Every organism requires essential nutrients for growth and maintenance of metabolic functions. Hence, the type and concentration of nutrients needed depends on the class of microorganism. A source of energy, water, nitrogen, vitamins, minerals, and other compounds provide these nutrients. The growth of *Aspergillus flavus* on grains was significantly affected by the concentration of soluble sugars. Low sugar levels retarded its growth, whereas concentrations between 3.0 and 6.0 % resulted in rapid growth, and the subsequent production of aflatoxin B1. Nevertheless, aflatoxin B1 production was significantly promoted due to the bioavailability of amino acids (arginine, glutamic acid, aspartic acid) and zinc in the grains (Vidal *et al*., 2018).

In a similar study, Li *et al*., (2016), reported different concentrations of mycotoxins (aflatoxinB1 (AFB), deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA) on numerous swine feeds. These outcomes could be attributed to the nutritional composition of the feeds. The nutritional requirement of pigs depends on the state (gestating, finisher, grower, starter, etc.) hence varied feed rations are given which contain different nutrient concentration; as a result influence fungi growth and subsequent mycotoxins production. The bioavailability of nutrients in most grains would support the growth of a wide range of microorganisms. Although each strain of mold has the genetic

potential to produce a particular mycotoxin, nutrient bioavailability could influence their levels significantly (Breidbach and Greener, 2017).

2.4.2 Biological structure

Grains have biological structures which prevent the penetration and growth of microorganisms. The testa of seeds and shell of nuts are examples of such structures. Some physical structures/barriers may exert antimicrobial potential. Intact biological structures prevent the entry of microbes, subsequent growth and production of mycotoxins in grains. However, these structures are destroyed during harvesting, transporting, or processing of the grains. Insect infestation could pave way for microbial proliferation of grains (Cao *et al*., 2018). Extract of Peanut testa was reported to exhibit pronounced antifungal activities against *Penicillium sp., A. niger*, and *Actinomucor sp*.

The cardinal and purple peanut testa produceda significant zone of inhibition at concentrations of 0.8 and 2.0 g/L, respectively. It was concluded that the fungicidal potentials of the testa depend on the type of peanut (Sun *et al*., 2016). Nevertheless, the environment, variety, type of farming system adopted, duration of storage, etc., may affect the fungicidal potency of these peanut testae. biocidal activities of *Dacryodes edulis* and *Garcinia kola* testae have been reported (Sabillon *et al*., 2016). The antimicrobial activities of these testae are associated with the presence of phytochemicals (alkaloids, saponins, etc.), and was confirmed in experimental studies by Friesen and Huminicki (2019). Zhu *et al*., (2017) reported that pathogens lack the enzyme necessary to break down the protective layers covering grains.

2.4.3 Moisture content (MC)

The oldest method of preserving food is controlling the MC. It is applicable during grain storage since the moisture influences the growth of microorganisms and subsequent

production of mycotoxins. The water requirement of microbes is known as the water activity (a_w) of the food or environment and is defined as the ratio of the water vapor pressure of the food substrate to the vapor pressure of pure water at a constant temperature (Luckstadt, 2019). The a_w of grains describes the degree to which water is bound in the grains, its availability to participate in chemical/biochemical reactions, and its accessibility to facilitate the growth of microorganisms (Los *et al*., 2018) which leads to the synthesis of metabolites. Cereals have a a_w between 0.10 and 0.20 when adequately dried, making it difficult for microbes to reproduce. Although the optimum MC for growth and subsequent toxin production for the various aflatoxigenic fungi varies, many achieve the best growth and toxin synthesis at an MC of 17.5 % (Stratton *et al*., 2017). *Aspergillus* requires about 13 % moisture or a relative humidity of 65 % (aw, of 0.65) for growth and toxin synthesis (Kumar, 2018).

Also, when molds are allowed to flourish, they could predispose the stored grain to mite and insect infestation because mites feeds on molds (Sun *et al*., 2019). A low MC could curb problems like molds infestations, discoloration, respiration loss, insect damage, and moisture absorption. Adequate drying of grains (produce) to lower moisture levels is critical to create unfavorable conditions to inhibit microbial and insect proliferation. It is recommended to dry harvested produce to safer moisture levels of 10–13 %. Low moisture help keep grains longer without losing nutrients and other vital bioactive compounds (Ziuzina *et al*., 2017). Water activity in stored grains could increase depending on climatic conditions, cellular respiration of microorganisms, or urine from rodents. Improper drying, especially during winter or autumn, could also elevate water activity levels.

2.4.4 pH, acidity and redox potential

For centuries, people have learned to increase the acidity of food either through fermentation, or by adding weak acids in the form of preservatives. These techniques have proven successful. Organic acids are effective preservatives in their undissociated state. pKa is the term used to illustrate the dissociation of an acid. Therefore, lowering the pH of grains increases the effectiveness of organic acids as preservatives (Baldwin *et al*., 2017). Naturally, grains in the field are undried and possess high pH; however, drying decreases the MC and subsequently the water activity thereby reducing the pH (Adadi and Obeng, 2018). Bartolucci (2017) reported that the lower the pH value the higher the total acidity (TA), which inhibits the growth of microorganisms.

The pH of grains could interact with other parameters (aw, salt, temperature, redox potential) in the food to inhibit microbial growth. The general rule of food microbiology states that pathogens do not grow, or grow slowly, at pH below 4.6, but there are exceptions. For instance, at pH 4.2, an organism was able to survive and synthesize a mycotoxin (Barakova *et al*., 2018). Rice and maize have pH about 6.02 ± 0.01 and 6.53 ± 0.01 during the rainy season and 6.20 ± 0.20 and 6.42 ± 0.12, in the dry season (Kasithevar *et al*., 2017). The season seems to influence the water activity, thus altering the pH of the grains.

The rainy season is defined by continuous rain, resulting in the elevation of the MC of the grains, which affects the pH. According to Musa *et al*., (2017), fungi can secrete butyrate, oxalate, maleate, citrate, gluconate, and succinate into their environment, thereby changing the acidity of the ecological niche. *Sclerotinia sclerotiorum and Botrytis sp*. secrete oxalic acid while *Penicillium sp*., and *Aspergillus sp*., synthesize mainly gluconic and citric acids (Kasithevar *et al*., 2017). Fungi can grow comfortably in pH above 8.5; however, below pH 2.2, their growth was inhibited. Microorganisms can modify the pH

of the environment in which they reside, making it challenging for farmers to control the pH of stored grain. A phenomenon like this could lead to significant economic loss due to microbial proliferation. Therefore, controlling the pH of grains is necessary to manipulate fungi growth during storage (Nsengumuremyi e*t al*., 2021)

2.4.5 Temperature

All microorganisms have a defined temperature range within which they can grow and synthesize toxins which cause food poisoning. Therefore, understanding the temperatures range, coupled with other intrinsic and extrinsic factors, are crucial to selecting the proper storage conditions for grain storage. Temperature has a dramatic impact on the growth and lag period of an organism. The growth rates of most microorganisms are favored at low temperatures, though there are exceptions. Reaction rates for specific enzymes in an organism become slower at lower temperatures. Also, low temperatures minimize the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms in the cell Bartolucci (2017). The expression of proteins are temperature regulated. A slight change in temperature can influence bacterial and archaeal community structure.

A wide range of temperatures play a vital role in the growth and synthesis of toxins in fungi. For instance, *Penicillium* and *Cladosporium* were able to grow below 20° C whereas the growth of *Aspergillus species* were inhibited. However, at a temperature above 20° C, the growth was maximized (Boehm *et al*., 2017). Virulent *A. niger* has optimal growth between 30–35° C, thus, rendering stored produce susceptible to a toxin secreted by these fungi. Warmer (33° C) and more humid conditions may increase aflatoxin prevalence. However, the opposite scenario is expected in tropics, since most aflatoxigenic fungi will not survive the expected 40° C (Periakaruppan *et al*., 2017). The knowledge of optimal temperature for microbial growth and mycotoxin synthesis gives more accurate assessment of the potential risk to human health (Yeat *et al*., 2018).

2.5 Effects of Mycotoxins on Human Health

Mycotoxins are considered a significant health and economic problem. Mycotoxins can find their way to the human body by way of contaminated food, skin contact, or inhalation (Khalil *et al*., 2018). The most common form of exposure is through oral ingestion of contaminated food (Deabes *et al*., 2018). The level of exposure and the type of mycotoxins which one is exposed to determine the nature of adverse effects on the human, either in the form of an allergic reaction, infections, or a toxic disease.

The seriousness of mycotoxins depends on the toxicity of the mycotoxin involved, the age, wellbeing of the exposed individual, and the length of exposure (Xu *et al*., 2017). Mycotoxins such as aflatoxins have been documented causing liver cancer (Kim *et al*., 2019). Other serious conditions, such as chronic interstitial nephropathy, Balkan endemic nephropathy, and urothelial tumors, as well as testicular cancer in men, have also been linked to mycotoxins (Park *et al*., 2019). Acute diseases, namely abdominal pains, headache, dizziness, throat irritation, and nausea, have also been associated with mycotoxin exposure in humans (Majeed, 2017). It is, therefore, important to ensure that grains are free of mycotoxin contamination

2.6 Food Shelf Life and Packaging

Conventional plastic or paper packaging materials are not sufficient to fulfill the today's needs. Packaging materials required to preserve food or vegetables for long times must meet important requirements. Thanks to nanotechnology, a great improvement in the food packaging materials is attained through addition of nanoparticles that add new properties to the packaging. Properties such as oxygen and water vapor barrier is essential since water and oxygen facilitate the environment for pathogenic microorganisms. So that addition of oxygen scavenger such as TiO_2 as a photocatalyst reduces amount of oxygen

in the oxygen-sensitive food staff (Zhang *et al*., 2017). On the other hand, addition of the antibacterial agent to the food might cause instant inhibition in the food microorganisms. However, the survive population will grow again upon depletion of the antibacterial material causing reduction of food shelf life (Rehim and Alhamidi, 2018). Since the main goals of a packaging material are extending shelf life, safety assurance of the food and maintaining food quality, the novel antibacterial packaging should be designed to fulfill these goals.

2.6.1 Food safety

Food safety and security has been a major issue in many frontier countries due to unregulated nature of food and feed products. Abass *et al*., (2017) reported that that dried cassava products are potential sources of mycotoxin contamination in human diet in Nigeria. The production of various indigenous African food such as injera, banku, amasi, fufu, garri produced in home under spontaneous conditions with little or no hygienic control could also contribute to high mycotoxin contamination (Adekoya *et al*., 2017). One of the most significant aspects of food safety is the packaging. Nanotechnology can help to improve the efficiency and quality of packaging materials, ensuring food safety.

Packages of nanoparticles can intelligently respond to environmental conditions or warn consumers about air pollution or toxic substances. In the FP industry, plastics are widely used. The primary goal of using nano-packaging is to improve the food's durability and shelf life. Gas, light, and moisture exchange between the outside and inside of the package must all be controlled for this purpose. Antibacterial substances, enzymes, nutrients, and flavors can all be released through nano-packaging (Othman, 2017). The food in the nanopackaging has a longer shelf life as a result of this. Some nano-packages are designed to release substances that inhibit microbial growth. It is supposed that multiple mycotoxins in food and feed are direct consequence of poor and none compliant with

quality control measures such as regulation of moisture content in grains (Adekoya *et al*., 2017).

2.6.2 Food packaging

Food packaging was initially used as the means of containers in which food stuffs were transported from production place to retail or consumption places. With many practical functions, food packaging has grown as a crucial section in the food industry. Packaging materials act as a barrier between the food, heat, moisture, and gases, and the movement of microorganism or insects. Packaging provides protection of tampering resistance and special physical, chemical, or biological needs (Mizielinska *et al*., 2018). With packaging, the cost of food could be reduced through economies of scale in mass production and efficiency in bulk distribution.

During the past decades, the increasing demands on product safety, shelf life extension, cost efficiency, environmental issues, and consumer convenience catalyzed the development of new packaging materials. In order to meet such varied demands from consumers, manufacturers, and society, various innovative packaging systems, such as active, intelligent and smart packaging systems, are emerging factors of all these backgrounds (Mizielinska *et al*., 2018).

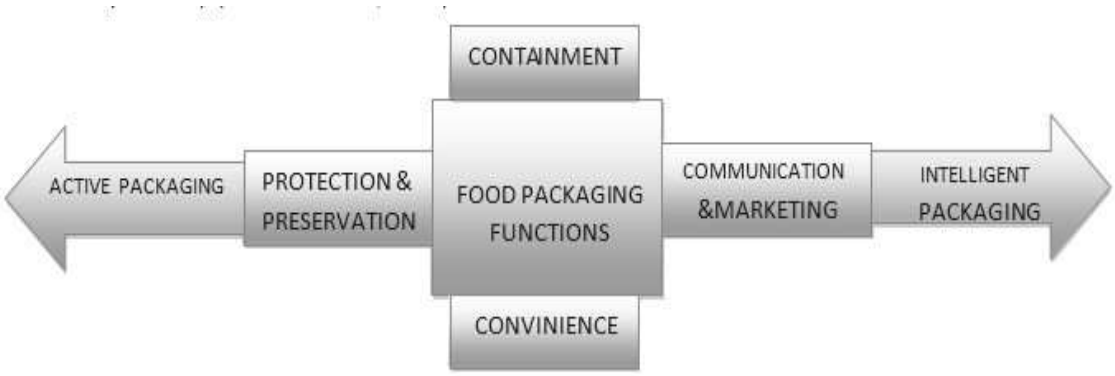

Figure 2.1. Food Packaging Functions: (Azeredo, 2013)

2.6.3 Plastic packaging

Parkesine, a cellulose derivative invented by Alexander Parkes, is the first manmade plastic and was publically presented at the 1862 Great International Exhibition in London (Guern, 2018). Since then, plastics have been developed and diversified. The plastics that are used today are predominantly made from crude oil or natural gas and are also known as petroleum-based plastics (Hutson, 2019). Plastics are good choices for food packaging given their cheapness, easy process ability, light weight, good resistance to oil and chemicals, excellent gas and water vapor barrier properties, and easily reusability and recyclability in terms of sustainability (Sedlacekova, 2017).

Some conventional petroleum or fossil-based plastics used in packaging include polypropylene (PP), polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyvinyl chloride (PVC), polystyrene (PS), and other plastics, such as bioplastic polylactide (PLA). Polyethylene (PE) and PP are of the most common and generally used materials in food packaging because they possess excellent chemical and moisture resistance; moreover, they are easy to process and cheap (Khanam and Almadeed, 2015).

Poly ethylene consists of two basic categories, namely HDPE and LDPE, wherein HDPE plastics have numerous short side branches and a tightly packed structure, whereas LDPE have numerous long branches (Shin, 2020). Although they are good chemical and moisture barriers, they are relatively permeable to oxygen and are thus poor odor barriers. PE also has relatively lower heat resistance than other plastics, and PE films melt at relatively low temperatures (Cort *et al*., 2017). PET is an emerging material that has been used in food packaging for the last several years because it has higher heat resistance and mechanical strength than many plastics (Cort *et al*., 2017). Moreover, it is an inert material that possesses good gas and moisture barrier properties and can be modified to

present specific properties that are suitable for various packaging applications. This characteristic makes PET a good option as a packaging material.

PLA is a promising biobased and biodegradable polymer that can be used as a food packaging material (Yadav *et al.,* 2018). PLA is ideal for fresh organic packaging because it has good breathing properties. However, pure PLA exhibits some limitations, such as water permeability, brittleness, and easy degradation under significant increases in temperature (Yadav *et al.,* 2018). Compositing PLA with other components can confer PLA with increased tensile strength and water resistance, antimicrobial properties, and reduced processing costs (Sufer *et al*., 2017). However, these plastics are mostly nonbiodegradable, nonrenewable, and noncompostable, therefore causing major environmental and disposal issues worldwide. They are the most challenging packaging materials to recycle. Traditional plastics are so durable that they are not readily degraded in their ambient surroundings; they persist in the environment because polymers require numerous or even hundreds of years to decompose in the normal natural environment (Singh *et al.,* 2017). According to a report from the organization for economic cooperation and development (OECD) Environment Directorate, over 60 % of plastic wastes come from packaging. Singh *et al*., (2017) stated that 90 % of plastic solid wastes are recyclable, but 80 % of them are sent to landfills, 8 % are incinerated, and only 7 % are recycled. The landfill disposal of HDPE also causes serious consequences because it produces greenhouse gases (Singh *et al.,* 2017).

2.6.4 Bioplastic packaging

Food packaging should be natural and environmentally friendly. Bioplastics or biopolymers from renewable resources have attracted growing interest from industries as a solution to the environmental problems and limited resources of petroleum-based polymers (Byun and Kim, 2021). The bioplastics used in the packaging market accounted

for approximately 65% of the global bioplastic production (Singh *et al*., 2017). Some currently produced and applied biopolymers based on renewable resources include PLA, cellulose, and starch, which are biopolymers that are directly obtained from agrowastes. However, biobased does not equal "biodegradable" or compostable (Dammer and Partanan, 2016).

Biobased products include raw materials that are renewable and can be replenished via natural processes (Niaounakis, 2019). Biodegradable products include polymers that can be degraded by microorganisms within a certain period of time in the environment (Niaounakis, 2019). Compostable bioplastics are a subset of biodegradable plastic. Therefore, all compostable bioplastics are biodegradable but not all biodegradable bioplastics are compostable (Green Dot Bioplastic, 2020). However, if used alone for packaging purposes, biopolymers or bioplastics show some limitations due to their poor water barrier properties, brittleness, high vapor permeability, and low heat deflection temperatures (Mohanty and Swain, 2017). Thus, biopolymers are strengthened with nanofillers to enhance their mechanical properties, barrier properties, and heat (Mohanty and Swain, 2017).

Starch is one of the least expensive biomaterials. It is also abundant, biodegradable, and renewable, and its possibility of blending with conventional polymers has garnered wide interest in the bioplastic market (deMoura *et al*., 2017). Blending starch with a nonbiodegradable plastic can also promote the biodegradability of the plastic (Talegaonkar *et al*., 2017). Native starch lacks thermoplastic properties (Zhang *et al*., 2019). Thermoplastic starch (TPS) can be obtained through the addition of plasticizers under heating after starch destruction (Talegaonkar *et al*,.2017). Starch is often used as a filler for other bioplastics to reduce production cost (Byun and Kim, 2021). Figure 3 below shows the general structure of starch.

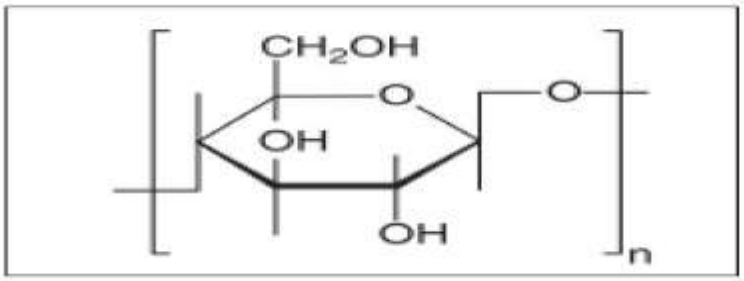

Figure 2.2**.** General Structure of Starch.

2.7 Application of Nanotechnology in Food Packaging

Nanotechnology is the science of very small materials that has a big impact in food industry including packaging. A variety of nanomaterials such as silver nanoparticle, titanium nitride nanoparticle, and nano-titanium dioxide, nano-zinc oxide, and nanoclay are introduced as functional additives to food packaging (Tager, 2018).

Nanotechnology enabled food packaging can be divided into three main categories **Improved packaging**:

Nanoparticles are mixed with polymer chain to improve the gas barrier properties, as well as, temperature, humidity resistance of packaging. The use of nanocomposite in contact with food is approved by United States Food and Drug Administration.

Active Packaging:

The use of nanomaterials is helpful to interact directly with food or environment to allow better protection of the product. Several nanomaterils like nanocopper oxide, nanosilver, nanotitanium dioxide, nanomagnesium oxide and carbon nanotubes can provide antimicrobial properties. Presently, the use of silver nanoparticles as antibacterial agents in food packaging is increasing.

Intelligent/Smart Packaging:

It is designed for sensing biochemical or microbial changes in the food. It can detect specific pathogen developing in the food or specific gases from food spoiling. Some smart packaging has been developed to use as tracing device for food safety.

2.7.1 Metal and metal oxide nanoparticles as antibacterial nanofillers

Metal and metal-oxide NPs exhibit different antibacterial efficiencies toward Grampositive and Gram-negative bacteria, and may even have different effects on the bacterial strains within the same Gram-positive or Gram-negative group (Zanet *et al*., 2019). This effect originates probably from the presence of various membrane components in

different bacteria that are the first barrier and the first target for NPs.

2.7.2 Silver nanoparticles (AgNPs) biopolymers

Silver are the most commonly used nanofiller to endow the composite films with antimicrobial properties (Shao *et al*., 2018). The US Food and Drug Administration (2022) authorized direct addition of Ag salts up to 17 μg/kg as a disinfectant in bottled water. The antibacterial effect of Ag nanoparticles is achieved through the adhesion of Ag NPs to the bacterial membrane and/or penetration inside bacterial cells. Ag nanoparticles may interact with membrane lipids or with DNA and proteins inside the cells (Qian *et al*., 2018). Ag NPs were also shown to down regulate the expression of antioxidant enzymes such as glutathione, superoxide dismutase, and catalase depriving the bacterial cell of defense against oxidative stress (Yuan *et al*., 2017). Another mode of action involves the release of highly toxic silver ions that bind protein sulfhydryl groups or intercalate within the DNA chain. Moreover, Ag -ions released by Ag NPs can generate a high concentration of ROS and interact with bacterial proteins from the respiratory chain causing cell death (Long *et al*., 2017).

In active packaging, Ag NPs are immobilized into polymeric materials, which allows controlled release of Ag-ions suppressing in this way their cytotoxicity. The migration of silver from the packaging film to food may occur through the detachment of AgNPs from the composites or the oxidative dissolution of silver ions. It was shown that the highest level of silver migrating from various nanocomposites into food simulants occurred with acidic food (Echegoyen *et al.*, 2016). The addition of AgNPs may also improve the mechanical properties of packaging films. Abreu *et al*., (2015) applied a solution casting method to prepare nanostructured starch films containing organically modified nanoclay and Ag NPs. They demonstrated that Ag NPs improved the clay dispersion, contributing to higher homogeneity of films and improving their mechanical properties.

The presence of Ag NPs reduced water vapor and oxygen permeability, providing excellent barrier properties. Moreover, the nanocomposite showed antimicrobial activity against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*. A composite film prepared of cellulose nanofibrils impregnated with Ag NPs through casting and evaporating at room temperature showed strong inhibition effectiveness against *E. coli* and *Listeria monocytogenes* and no significant cytotoxicity to human epithelial cell or colon cell lines (Yu *et al*., 2019).

A green synthesis method is generally used for preparing Ag NPs for food packaging applications. Wu *et al*., (2018) synthesized laponite-immobilized Ag NPs by reactive template grain growth method using quaternized chitosan as a green reducing agent and stabilizer. Prepared Ag NPs and laponite improved chitosan films for the wrapping of litchi fruits, by increasing the film density, improving its mechanical properties and making it more uniform and homogenous. The tripartite interactions between NPs, chitosan, and laponite were proposed as a crucial factor in reducing the water vapor permeability, oxygen transmission rate, solubility, swelling of the films, and in increasing

the film's antimicrobial activity against *E.coli*, *S. aureus*, *Aspergillus niger*, and *Penicillium citrinum*. The film enabled to extend the shelf life of litchi fruit from 4 to 7 days. Melanin, as a reducing agent, enables green synthesis and capping of Ag NPs within the carrageenan film (Roy *et al*., 2019).

2.7.3 Titanium dioxide nanoparticles (TiO_2 Nps)

TiO_2 particles are commonly used as a food additive to provide a whitening effect that makes the foodstuffs look brighter and more appealing (Ropers *et al*., 2017). In food packaging, TiO_2 NPs are used for their wide range of antimicrobial properties (Makvandi *et al*., 2020). Until recently, TiO_2 NPs were considered as safe. However, the European Food Safety Agency (EFSA) revealed some concerns due to the ability of TiO_2 NPs to alter the intestinal barrier, which pushed some Europian countries to ban TiO_2 NPs in food (Boutillier *et al*., 2020). Numerous reports confirmed the toxic effects of TiO_2 NPs on human cells. In vivo tests revealed that upon inhalation or oral exposure, TiO_2 NPs accumulate in lungs, digestive tract, liver, heart, spleen, kidneys, and heart (BaranowskaWojcik *et al*., 2020).

However, nanosized TiO_2 is still among the few approved nanomaterials used by the food industry.The main mechanism of antimicrobial activity of TiO_2 NPs involves ROS production under exposure to UV light (Wang *et al*., 2018). This photocatalytic mechanism involves: (i) electron–hole pairs production and migration to the TiO_2 NP surface, (ii) interaction of photogenerated holes with adsorbed HO/OH leading to the generation of hydroxyl radicals, (iii) electron binding to empty oxygen portions and superoxide formation. TiO_2 NPs incorporated into the wheat gluten-based biopolymer ensured antimicrobial properties of the food packaging (El-Wakil *et al*., 2019). Wheat gluten was chosen as a plant protein because it is inexpensive and shows good filmforming properties. Advanced nanocomposite film was fabricated by incorporation

of glycerol, cellulose nanocrystals, and TiO_2 NPs to the wheat gluten by casting/evaporation. Obtained films showed enhanced mechanical properties in terms of increased tensile strength and decrease of elongation at break, and low water vapor permeability and water vapor uptake due to the increased surface hydrophobicity.

The addition of TiO_2 NPs was shown to further prevent water absorption. Fresh white cheese prepared from buffalo milk is characterized by rapid spoilage under aerobic conditions. It deteriorates usually in less than 7 days when packed using traditional petrochemical plastics. To provide prolonged storage and shelf life of buffalo milk cheese a chitosan/PVA/TiO_2 NPs nanocomposite was elaborated (Youssef *et al*., 2018). Mechanical properties, such as tensile strength and elongation at break, were significantly improved by the addition of TiO_2. This was associated with a uniform dispersion of NPs providing stable stress distribution and diminishing the formation of stress concentration centers. In addition, the TiO_2 NPs incorporation increased the hydrophobicity of the film, improving its protective effect against moisture during the storage period. The chitosan/PVA/TiO_2 films exhibited good antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa,* and *E. coli,* as well as good antifungal properties against *C. albicans*, thus enabled prolonged cheese shelf life.

2.7.4 Zinc oxide nanoparticles (ZnO NPs)

ZnO is classified as an essential micronutrient for human and animal health and considered as GRAS (generally recognized as safe) by the FDA. ZnO is used by food industries as a nutritional supplement and as a food additive due to its white appearance (Auger *et al*., 2019; Jeon *et al*., 2020). In nanosized form, ZnO is used for food packaging because its presence in the polymeric matrix provides improved mechanical strength, barrier properties, and material stability together with high antibacterial and antifungal activities (Auger *et al*., 2019).

The dynamic role of ZnO NPs in food preservation entails the production of ROS, especially hydrogen peroxide in the presence of light (Auger *et al*., 2019). Their antibacterial efficiency was shown for a broad range of microorganisms such as *B. subtilis, S. aureus, E. coli, Salmonella, P. aeruginosa, C. jejuni* (Auger *et al*., 2019). Seray *et al*., (2020) described poly(butylene adipate-co-terephthalate)/ZnO NPs films. The nanoparticles embedded into the polymer assured the controlled release of Zn -ions, resulting in the safe application of the film.

Several novel nano-biocomposites combining ZnO NPs and essential oils have been developed for food packaging in the last few years. Wu *et al*. (2019) used protein isolatebased biocomposite films, synthesized through a liquid precipitation method that incorporated both plant-sourced cinnamaldehyde and hexagonal wurtzite ZnO NPs. The aim was to obtain an antibacterial and antifungal material harnessing the synergistic effect of ZnO NPs and cinnamaldehyde. ZnO NPs improved mechanical characteristics and oxygen and water vapor barrier properties of the biopolymer. The composite films exhibited homogeneous, uniform, compact, and nonporous surfaces. Interestingly, both cinnamaldehyde and ZnO NPs prevent light transmission, providing a beneficial feature of these films because the oxidative deterioration of food was diminished along with an extension of the food shelf life. The antifungal activity of the film against *Aspergillus niger* was increased in comparison to the pristine film. In another study, a polylactide/polyethylene glycol/polycaprolactone blend with incorporated ZnO NPs and clove essential oil in the form of a film was developed using the solution casting technique (Ahmed *et al.*, 2019).

2.8 **Plant Description**

Hyptis suaveolens is a rigid annual herb of aggressive nature (Mudgal *et al.*, 1997). It starts its vegetative phase either from perrenating root stock or viable seeds either from

persistent seed bank or from fresh stock with the onset of monsoon rains. It can attain height of approximately 2.5 meters within a growing season. It stem is quadrate and bears hair. Leaves are either ovate or obovate with 3-5 cm long and 2-4 cm wide with serrulate margins and a long petiole. Lower surface of the leaves bears hairs; petioles up to 3 cm long. Flowering starts in it at an early age of two to three months.

Figure 2.3. Hyptis Plant (Mishra, 2021)

2.8.1 Traditional values of hyptis plant

Hyptis suaveolens has both medicinal individuality as well as insecticidal properties. *Hyptis* literature indicates that leaf extracts cure swellings, abscesses and haemorrhoids. Infusion is used in infections of the uterus; leaf juice is taken in cases of colic and stomach ache (Sharma *et al*., 2013). The shoot tops of the plant are edible and also used for flavouring purpose. Leaves are used in the preparation of mint flavoured beverages. Roots are chewed with betel nuts as a stomachic and its decoction is used as an appetizer (Sharma *et al*., 2013) while some parts of the plant are used for the treatment of headache. In Indonesia, the plant infusion is used to treat catarrhal (inflammation of mucous membranes, especially of the nose and throat) conditions, affections of the uterus, parasitic cutaneous diseases while the leaves are used as stomachic.

In West Africa the leaves of *H. suaveolens* are employed as antifertility agent (Sharma *et al*., 2013). In case of a burning sensation when passing urine (Dysuria) and other urinary

complains, dry seeds of *H. suaveolens* are soaked overnight in a glass of water and taken in the morning on an empty stomach along with small amounts of sugar for about a week (Sharma *et al*., 2013. The plant has been reported to possess antifertility, antiinflammatory and antiplasmodial properties (Housin News, 2023). This medicinal plant therefore, has great potential for benefitting people in countries suffering from poverty and malnutrition. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents. Among the various plants, *Hyptis suaveolens* leaves extract is known for its medicinal properties. Phytochemical screening indicated the presence of chemicals such as alkaloids, flavonoids, phenols, saponins, terpenes, and sterols (Mishra *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Corn starch, glycerol, sodium hydroxide, Acetic acid, dextrose agar and petridish were purchased locally at the market, while Zinc nitrate hexahydrate, titanium dioxide and silver nitrate are products of Sigma Aldrich. All the chemicals purchased were of analytical grade.

3.1.1 Plant sample

Fresh leaves of *Hyptis suaveolens* was obtained in Minna, Niger State Nigeria. The collected fresh leaf was taken to Plant Biology Department for identification (2021) with voucher number with voucher number FUT/PLB/LM/003 at Federal University of Technology. Minna, Niger State.

3.2 Methods

3.2.1 Preparation of *Hyptis suaveolens* leave extract

The leaf was washed and dried for 15 days at room temperature in the laboratory of the Department of Biochemistry. The fine powder of dried plant leaves was crushed/grind with the help of mortar pester. After that about 5 g of the *Hyptis suaveolens* leaves powder was weighed into 100 ml of deionized water and Boiling was done 100° C for 10 minutes to get the pure plant extract. Then the aqueous plant extract was doubled filter to get a pure extract for further analysis.

3.2.2 Media preparation

The preparation of dextrose culture growth media was done according to the manufacturers' instructions using deionised water. The media was autoclaved at 121° C for 15 minutes and chloramphenicol (Fisher Bioreagents, Pasley, UK) was used as an anti-bacterial agent prior to autoclaving the media. The molten media were poured into 9 cm Petri dishes (approx. 17.5 mL per dish). **3.2.3 Fungal identification and isolation**

After the media was prepared, the maize samples inoculation was done using the serial dilution technique based on the method of Yilma *et al*., (2019). One gram of each maize sample was soaked for 3hrs in a sterile test tube containing 5mL of sterile distilled water and shaken vigorously. Exactly 1mL of the water was transferred to a dilution tube containing 9 mL of sterile water. Subsequently sterile 1mL pipette tips were used for serial dilution to 10^{-4} z. Exactly 100 µL from each dilution were taken using a sterile 200 µL pipette tip placed centrally on the surface of the duplicate Petri dishes for each dilution.

This was spread with a surface-sterilised L-shaped glass rod on the SDS Agar. The Petri dishes were incubated for 48 hours at 25° C. Fungal isolates were grouped to genus level based on fungal identification manual (Barnett and Hunter, 1998; Sarah *et al*., 2016).

Isolates were observed based on colony growth rates, texture, degree of sporulation. The fungal species were identified using colonies and morphological identification based on fungal atlas literature at the Department of Microbiology, Federal University of Technology, Minna.

3.2.4 Biochemical synthesis of zinc oxide nanoparticles

Zinc Oxides nanoparticles were synthesized according to the method described by Kumar and Gautam (2019). Exactly 200 ml of deionized water was added to 20 g of zinc nitrate hexahydrate $Zn(NO_3)_2.6H_20$. The solution was stirred on a magnetic stirrer at 50 rpm for 30 minutes. After which 50 ml of the aqueous extract was added with continuous stirring until a homogenous mixture was obtained. Then few drops of 0.5 M NaOH was added drop wise to attain pH of 8 with continuous stirring until a thick paste was formed. The mixture was allowed to age for 24 hours which resulted in the yield of a bulky sol. The sol was then washed with ethanol and water copiously until a clear solution was obtained. The sol was oven dried at 105^o C for 12 hours and later calcined in a furnace at 450^o C for 3 hours.

3.2.5 Biosynthesis of silver nanoparticles

The nanoparticles was biosynthesized by adding 100 ml of silver nitrate solution (1 mM) with 25 ml of leaf extract and heated with stirrer at 70^o C for 60 minutes at pH 7 as described by Ahmed *et al.,* (2016). The reaction mixture was observed for color change. The resultant reddish brown-colored reaction mixture was then centrifuged at 12,000 rpm for 10 minutes. The pellet obtained was washed three times with deionized water and finally with acetone. The resultant pellet was dried and stored for further characterizations.

3.2.6 Biosynthesis of titanium dioxide nanoparticles

Titanium dioxide nanoparticles was synthesize according to method described by Tunma (2018). Exactly 20 ml aqueous leaf extract was added to 100 ml of 1 M TiO_2 solution. The mixture was then incubated for 12 hours at room temperature. The solution was then centrifuge at 8000 rpm for 10 minutes. The supernatant was discarded and settled pellets was dried at 100^{o} C for 4 hours.

3.3 Characterization of the Biosynthesized Nanoparticles

The UV-spectroscopy measurements of the nanoparticles were carried out using a UV1800 Shimadzu spectrophotometer and the absorbance of the nanoparticles were taken at 200 – 500 nm. The particle size of the biosynthesized nanoparticles was assessed using the Malvern Zetasizer NanoZS90 (UK) to measure their particles size (Raja *et al*., 2019).

3.4 Proximate Composition of Corn Starch

The proximate analysis of the corn starch for moisture, total ash, crude fibre, fat was carried out in triplicate using methods described by Association of Official Analytical Chemists (2000). The nitrogen was determined by micro Kjeldah method described AOAC (2000) and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrate content was estimated by 'difference'. All the proximate values were reported in percentage.

3.4.1 Determination of moisture

Two grams of each sample was weighed as W1 and dried in an air oven at 105^{o} C for 3 hours. It was cooled in a dessicator and reweighed. This was repeated until a constant weight was achieved as W2. The percentage moisture content was calculated as,

Percentage moisture content = $\frac{W1-W2}{W1} \; x \; 100$ …… Eqn 3.0

3.4.2 Determination of ash

For the determination of ash, clean empty crucible was placed in a muffle furnace at 550^0 C for an hour, cooled in desiccator and then weighed as W_1. Exactly 2 g gram of corn starch was weighed into a crucible (W_2) and was inserted over a burner, until it was charred. Then the crucible was placed in muffle furnace for ashing at 550 ^{0}C for 3 hours, the appearance for gray white ash indicate complete oxidation of all organic matter in the sample (Adeniyi and Ariwoola, 2019). After ashing the crucible was cooled and weighed (W_3). Percentage ash was calculated by the following formula.

$$\text{Percentage Ash} = \frac{\text{Difference in Weight of Ash} \times 100}{\text{Weight of Sample}} \quad \ldots\ldots \text{Eqn 3.1}$$

Difference in weight of ash = $W_3 - W_1$

3.4.3 Determination of crude protein

Protein content was determined by kjeldahl method. Exactly 0.25 g of dried samples was placed in digestion flask, with 6 ml of concentrated H_2SO_4 and a speck of kjeldah1 catalyst (mixture of 10g Na_2SO_4+5g CuSO4+ 0.05g selenium). The flask was swirled in order to mix the contents thoroughly, then digested on the digestion block till the mixtures become clear (colourless or greenish in color). The digest was cooled and transferred to 100 ml volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham Distillation Apparatus and ten milliliters of digest was introduced in the distillation tube and 10 ml of 40 % NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in conical flask containing 5 ml of 4 % boric

acid solution with few drops of methyl red indicator. During distillation yellowish color appears due to NH_4OH. The distillate was then titrated against standard 0.1 N HCI solution untill the appearance of pink color. A blank was also run through all steps as above (AOAC, 2000). Percentage crude protein content of the sample was calculated by using the following formula;

% Crude Protein = 6.25 x % N (Correction factor)

% N = (S-B) x N x 0.014 x D x 100 …… Eqn 3.2

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCI

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

3.4.4 Determination of crude fat

Exactly 2.0 g of each sample was added to a pre-weighed filter paper which was dipped inside the Soxhlet extractor. It was fitted up with the reflux condenser and a flat bottom flask. The flask was filled to about 2/3 with n-hexane. This was heated using water bath and allowed to reflux for 6 hours. After the extraction was completed, the wrapped filter paper containing the sample was dried in an air-oven at a temperature of 100° C for 1 hour and cooled in a desiccator. Weight of the sample was determined after extraction, percentage crude fat was calculated as (AOAC, 2000).

$$\% \text{ Crude Fat} = \frac{W1-W2}{W1} \, x \, 100$$ …… Eqn 3.3

Where W1 = weight of sample before extraction, W2 = weight of sample after extraction.

3.4.5 Determination of crude fiber

Exactly 2.0 g of each of the defatted and dried corn starch was weighed and poured into a round bottom flask containing 200 mL of boiling 0.255 N sulphuric acid solution. The round bottom flask was connected to a condenser and brought to boil within a minute. Refluxing was done for 30 minutes with periodic swirling of the flask to remove particles adhering to the sides. This was filtered within 10 minutes using a preheated Buchner flask. The residue on the filter paper was washed with boiling water and the residue was transferred back into a clean round bottom flask containing 200 mL of boiling 0.313 N sodium hydroxide and refluxing was again carried out for 30 minutes.

The hydrolyzed mixture (after letting it rest for 1 minute) was filtered within 10 minutes in a preheated Buchner flask. The residue was washed with boiling water, with 1 % HCI solution and then again with boiling water and finally with petroleum ether. The residue was then transferred into a pre-weighed crucible and oven dried at 105^0 C till constant weight, the weight was recorded. The crucible was immediately transferred into a muffle furnace operated at 550^0 C for 3 hours, and then left to cool in a desiccator and weighed again. Percentage crude fibre was calculated as (AOAC, 2000).

Percentage crude fibre = 100 (A-B)/C ……. Eqn 3.4

Where A = weight of crucible with dry residue (g)

B = weight of crucible with ash (g)

C = weight of sample (g)

3.4.6 Carbohydrate content determination

The nitrogen free method described by A.O.A.C (2000) was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameter as Nitrogen free Extract (NFE).

Percentage carbohydrate (NFE) = 100- (m+p+F_1+A+F_2) …… Eqn 3.5

Where M = moisture, P = protein, F_1 = Fat, A = ash, F_2 = crude fiber

3.5 Production of Starch Nanocomposite Film

The principle for preparation of nanoparticles embedded starch films was based on solution casting and evaporation method as described by Li *et al.*, (2018). For this process, three grams of corn starch (CS) was mixed with 100 ml deionized water under continuous stirring at 300 rpm for 15 minutes. Then 100 mg of the prepared phytosynthesized nanoparticles (silver, zinc oxide, and titanium dioxide) was added to the solution individually, and later 1 g of Glycerol was added as plasticizer with few drops of 5 % Acetic acid solution to maintain pH (at 3-4). The final solution was mixed and heated at 90° C until the mixture becomes gelatinized for 30-45 minutes. Then the prepared solution was sonicated immediately at 90° C for 5 minutes and degassing was done using vacuum oven. The final prepared homogeneous solution was poured and spread evenly onto a flat plate and dry in an incubator at 25±1° C for 24 hours.

3.5.1 Functional characterization of starch nanocomposite films

Film Thickness

Thickness was measured using a digital micrometer with an accuracy of 0.001 mm (Mitutoyo 293-831-30 Micrometer). The thickness of each sample film was evaluated at six different positions and then averaged as described by Kumar and Gautam (2019).

Moisture content:

The moisture content (MC) of the films was measured in terms of weight loss. A 2x2 cm^2 samples film were cut from each film and then weighed. Then, the initial weight (W) of the sample was determined and then dried at 105° C for 24 hours using a hot air oven.

The final weight (W) of the film samples was measured and per cent MC of the films was calculated as follows:

$$MC\,(\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad \text{…….} \quad \text{Eqn 3.6}$$

Where W_o= initial weight of sample film before drying

W_1 = final weight of sample film after drying **Swelling**

index:

Swelling index was evaluated using method described by Cao *et al*., (2018). Film specimens with 2x2 cm^2 dimension were dried in hot air oven at 105^o C for 24 hours and weighed. Dried specimens were immersed in distilled water for 2 minutes and then removed from the distilled water. Water was drained from the swelled samples and weighed. The quantity of water absorbed by the specimens were evaluated according to equation:

$$SI(\%) = \frac{M_a - M_b}{M_a} \times 100 \quad \text{……} \quad \text{Eqn 3.7}$$

Where M_a and M_b, are the weight of pre-dried film and weight of the swelled sample, respectively.

Solubility

Water solubility of the film was measured according to the method described method by Wang *et al*., (2017). Four samples of each film with 2x2 cm^2 dimension were cut from the films. All prepared samples were dried in a hot air oven at 105^o C for 24 hours and weighed (w_o) with an accuracy of 0.0001. Dried samples were immersed in a beaker containing 15 mL of double distilled water and stored at25^o C for 24 hours. Then, swelled

samples were removed and dried again at 105^{o} C for 24 hours in a hot air oven and weighed again.

$$S(\%) = \frac{m_1 - m_2}{m_1} \times 100$$ …… Eqn 3.8

Where m_1 and m_2 are the weight of initial dried and insoluble film samples, respectively

Biodegradability of Film

Biodegradability of samples was checked using soil buried test. All specimens with dimension 1x1 cm^2 of modified starch and composite were prepared from the films. Thereafter, plastic containers filled up to the surface with compost soil was set up and the specimens were buried in the mixture below 2 cm from the mouth of the containers.

Weight loss of films were monitored after a fixed time interval of one week (Babaee *et al*., 2015).

3.6 Characterization of the Nanoparticles Embedded Starch Films

3.6.1 Scanning electron microscopy (SEM)

The surface morphology and cross sections of the nanocomposite films were observed with a JSM-5610LV scanning electron microscope (SEM;JEOL,Tokyo,Japan) according to Kumar and Gautam (2019). SEM with an accelerating voltage of 10 kV was used to study the surface morphology of nanocomposite films. Surface images with a spot size of 2 nm were captured in the magnification range (200–1000 cm).

3.6.2 Energy dispersive x-ray (EDX) spectroscopy

The EDX spectroscopy was done on S-3400N, Hitachi instrument to determine the percentage elemental compositions. In this technique, the nanoparticles were analyzed by activation using an EDS X-ray spectrophotometer, which is generally present in modern SEM. (Kim *et al*., 2018)

3.7 Packaging and Storage for Shelf Life Evaluation

The maize samples were packaged with the nanacomposite biofilms in airtight condition to prevent contamination during the observation period for over a period of three months and starch film without nanoparticle was used as control. Similarly, for comparative purpose, conventional synthetic polymer was also use to package the fresh maize sample. **3.8**

Data Analysis

Proximate analysis of the corn flour was carried out in three triplicates. The data collected were subjected to one way Analysis of Variance (ANOVA) ($p < 0.05$) using SPSS version

11.0 statistic software package. Means with significant differences were separated by Turkey multiple comparism test**.**

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Morphological Characterization of Fungi Isolated from two Maize Samples collected from Jiya Stores in Minna Metropolis

The cultural characteristics of the fungi isolates from maize samples are presented in table 4.1. The two fungi isolates are *Aspergillus terreus* and *Mucor pussillus*, while the dominant fungi is *Aspergillus terreus.*

Table 4.1: Morphological Features of Fungi Isolate from Maize Comb Collected from Jiya Store in Minna Metropolis

Maize Sample	Morphological characteristics of isolates	Microscopic features	Suspected organism
S_1	Whitish and wooly with various black dots on top	Non-apophysate sporangia and by zygospores on suspensors.	*Mucor pussilus*
S_1 and S_2	Gray brown colony and fluffy	Long conidiophores, scattered brown conidia. Hyphae has no septate	*Aspergillus terreus*

Key:

S1 = store 1

S2 = store 2

4.1.2 Characterization of Biosynthesized Silver, Zinc Oxide and Titanium Dioxide Nanoparticle

4.1.2.1 *UV-spectroscopy of biosynthesized nanoparticles showing the absorption peaks*

From Figure 4.1, all the three synthesized nanoparticle (AgNPs, ZnONPs and TiO_2NPs) have optical spectral absorption range of 200-500 nm. The silver nanoparticles have a surface plasmon resonance peak of 407 nm, the zinc oxide nanoparticles have a maximum surface plasmon resonance peak of 350 nm, while the titanium dioxide nanoparticles have a maximum Surface Plasmon Resonance absorption peak of 282 nm.

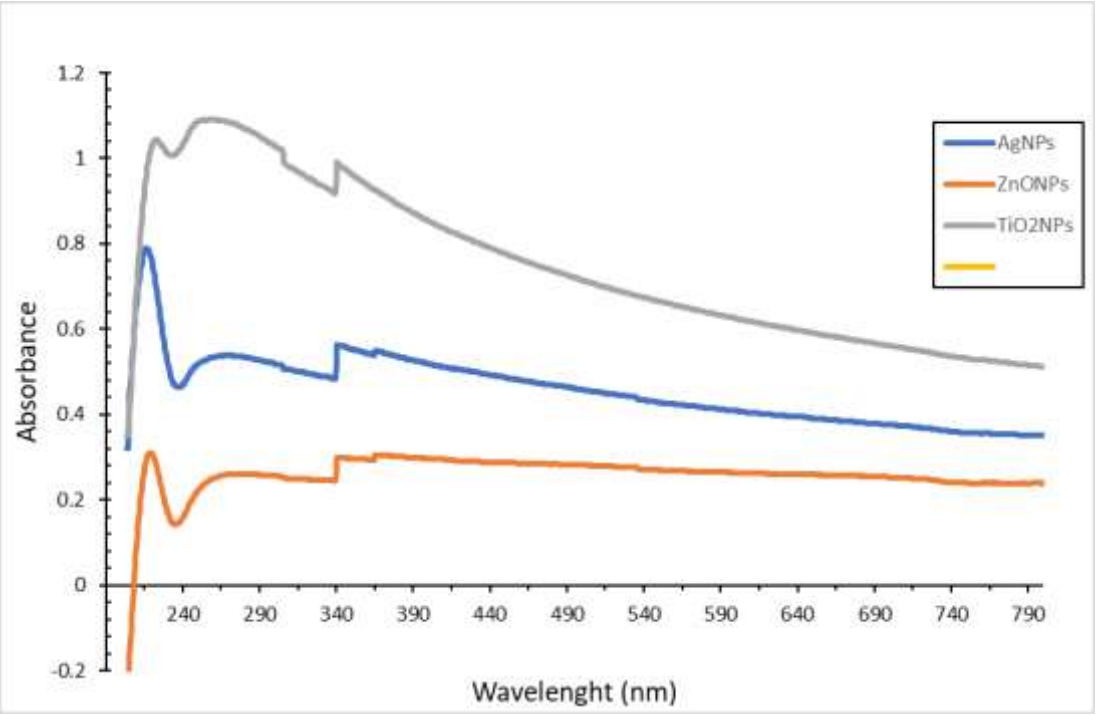

Figure 4.1. UV-Spectral of Biosynthesized Nanoparticles showing their Absorption Peaks

4.1.2.2 *Particle size distribution of biosynthesized nanoparticles*

Figure 4.2 reveals the optical zeta potentials of the biosynthesized nanoparticles, which is a function of the particles size distribution. The silver nanoparticles have an average particle size of 61.70 nm, zinc oxide nanoparticle having average particle 63.14 nm and titanium dioxide nanoparticle having the largest particle size distribution of 72.40 nm.

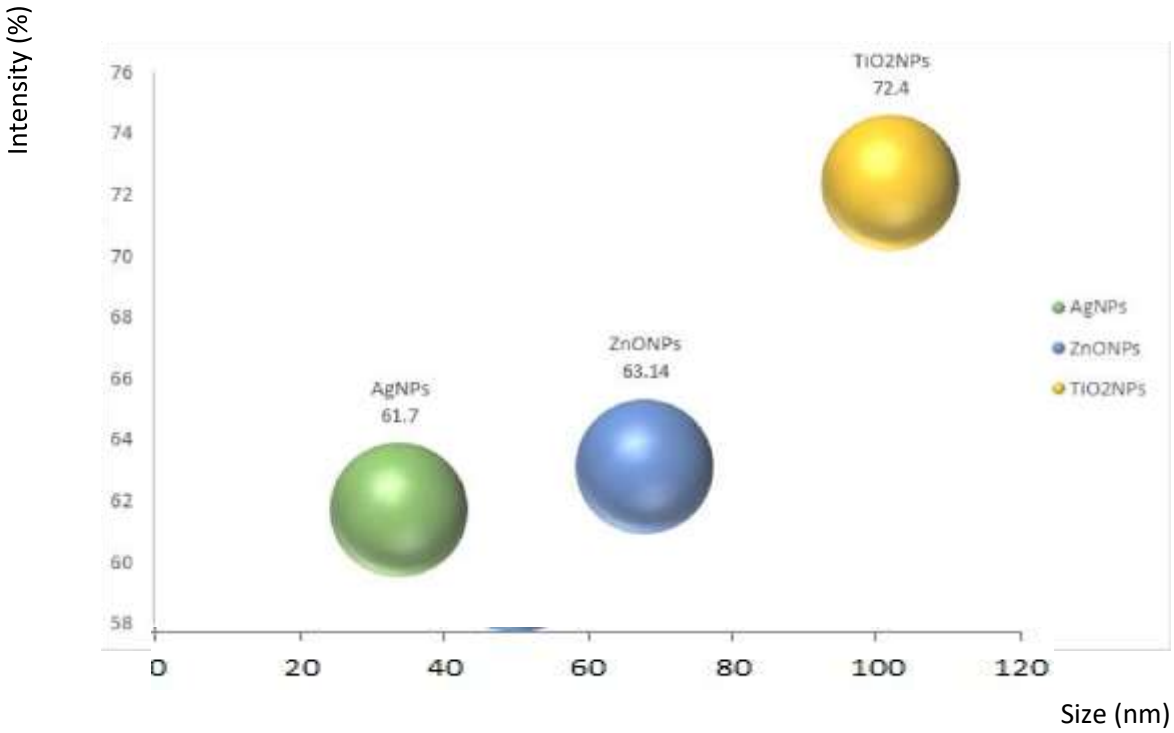

Figure 4.2. Average Particle Size Distribution of Biosynthesized Silver, Zinc Oxides and Titanium Dioxide Nanoparticles.

4.1.3 Proximate composition of corn flour used for film production

The proximate contents are presented in Table 4.2, the corn flour used for film production is a high grade refined starch with high carbohydrate content of 98.92 %, while other parameters such moisture content, ash, lipid, crude fibre and protein are present in insignificant amount.

Table 4.2: Proximate Compositions of Corn Flour

Proximate paramaters	Percentage composition (%)
Moisture content	0.38
Ash	0.30
Lipid	0.10

Crude fiber	0.10
Protein	0.2
Carbohydrate	98.92

4.1.4 Characterization of nanocomposites films

The functional properties of the developed film (Table 4.3) Indicates that the starch film without embedded nanoparticle has lower moisture content of 24.35±0.13 %, which is 0.2 % lower than that of titanium dioxide embedded film which is 25.55±0.02 %. There is no significant difference ($p>0.05$) between the moisture contents of silver 26.53±0.23 and zinc oxide, 26.08±0.21 films. There is a significant difference ($p < 0.05$) in the swelling index of all the films with starch film without embedded nanoparticle having the lowest swelling value of 52.01±0.11 %, while the titanium embedded film having the highest swelling index of 57.28±0.32 %. The films with embedded nanoparticles have thickness of 0.03±0.01 % and the film without embedded nanoparticles having lower value of 0.02±0.03 %. The starch film without embedded nanoparticles has the highest solubility rate of 39.85±0.11 %, followed by silver nanoparticles embedded film which has 37.10±0.18 %. There is no significant difference ($P>0.05$) in the solubility values of zinc oxide and titanium dioxide nanoparticles embedded films, when compared with silver embedded nanoparticles and starch film without embedded film.

The titanium nanoparticle embedded film has the lowest biodegradability of 80.12±0.05 %, while there is no significant difference in the solubility values of Starch film, silver nanoparticle embedded film and zinc oxide film (Table 4.3).

Table 4.3: Functional Properties of the Silver, Zinc Oxide and Titanium Dioxide Films

Sample	Moisture Content (%)	Swelling Index (%)	Film Thickness (%)	Solubility (%)	Biodegradability (%)
Starch film	24.35±0.13 [c]	52.01±0.11[d]	0.02±0.03[b]	39.85±0.11[a]	84.95±0.02 [a]
Ag film	26.53±0.23 [a]	53.51±0.01 [c]	0.03±0.01 [a]	37.10±0.18 [b]	84.05±0.03 [a]
ZnO film	26.08±0.21 [a]	55.14±0.16[b]	0.03±0.03 [a]	35.19±0.20 [c]	84.03±0.11 [a]
TiO_2 film	25.55±0.02 [b]	57.28±0.32 [a]	0.03±0.01 [a]	35.21±0.01 [c]	80.12±0.05 [b]

Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5% level (P<0.05).

4.1.4.1 *Energy dispersive x-ray spectroscopy (EDS) of produced nanoparticles films*

The EDS analysis of biosynthesized silver, zinc oxide and titanium dioxide nanoparticles indicate the presence of silver, zinc, titanium and other elements in the films as shown in Table 4.4. The silver film has silver, carbon, Oxygen, and iron as the constituent atom, while the zinc oxide film has zinc, carbon, oxygen and iron as the constituent atoms and the titanium dioxide film has titanium, carbon, oxygen and silicon as the constituent atoms.

Table 4.4: Energy dispersive x-ray spectroscopy of silver, zinc oxides and titanium dioxide films

FILM	ATOMIC PERCENTAGE (%)						
	Ag	**Zn**	**Ti**	**C**	**O**	**Fe**	**Si**
AgNPs	25	0	0	24	31	5	0

ZnONPs	0	35	0	25	30	7	0
TiO_2NPs	0	0	20	28	35	0	4

4.1.4.2 *Scanning electron microscopy (SEM) of produced nanocomposite films*

The SEM monograph of the biosynthesized silver, zinc oxide and titanium dioxide nanoparticles are shown in Plate I. The SEM images revealed the nanoparticles morphological of the films at 5 kv. The silver biofilm has crystalline and spherical shapes, while the zinc oxide film has oval shapes with undulating surfaces and the titanium dioxide film has spherical shapes with undulating surfaces

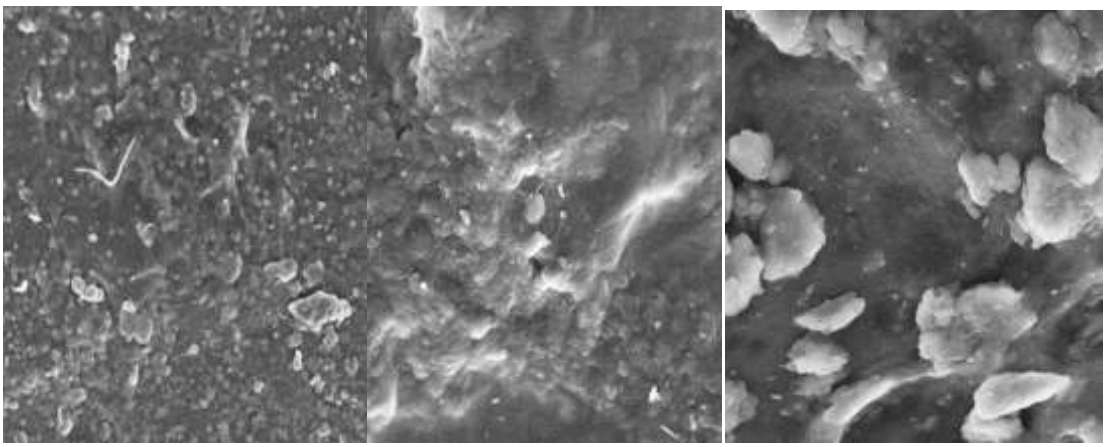

Plate I: SEM images of the various nanoparticles films (A) Silver nanoparticles film (B) Zinc oxides nanoparticles film (C) Titanium dioxide

4.1.5 Shelf life evaluation

The shelf life of the packaging film for a period of 12 weeks is shown in Plates II-V. At week one the maize in starch, conventional and nanocomposites films of silver, zinc oxide

and titanium dioxide film were all fresh with no morphological changes observed (Plate II).

At end of week two, the maize packaged with the starch film begins to show deterioration, while the maize in conventional film have started sprouting. All the samples packaged various nanoparticles films maintain their physical integrity (Plate III).

Plate IV, shows that maize sample in the starch film becomes deteriorate with cloudy film, while the sprouted maize in the conventional film has begin to dry up when compared the seeds in nanocomposites films which had their physical integrity preserved with drying of seed at a slow rate (week 5).

At week 12 (Plate V), the maize seed in the nanocomposite films were with no morphological changes when compared to week 1, 2 and 5. The nanocomposite films preserve the physical integrity of the maize, with only noticeable change is the drying of the seeds.

Starch Film Nylon Film Ag-NPs Film ZnO-NPs Film TiO_2-NPs Film

Plate II: Morphological Changes of the various Packaging Films at Week One

Starch Film Nylon Film Ag-NPs Film ZnO-NPs Film TiO_2 Film

Plate III. Morphological changes of the various packaging films at week two.

Starch Film Nylon Film Ag-NPs Film ZnO-NPs Film TiO_2-NPs Film

Plate IV: Morphological Changes of the Various Packaging Films at Week Five

Starch Film Ag-NPs Film ZnO-NPs Film TiO_2-NPs Film

Plate V: Morphological changes of the various packaging films at week twelve

4.1.6 Fungal load evaluation of post packaged maize

The microbial load of the maize samples packages with the various films are presented in Table 4.5. The film without embedded nanoparticles show deterioration and a rapid growth of *Mucor pussillus* (Table 4.5). The film with embedded nanoparticles show no growth of fungal at post packaging evaluation

Table 4.5: Post Packaged Fungal Load Evaluation

Film type	**Fungi species**	**Mean Fungal load (mfc/g)**

Control film	*Mucor pusillus and*	33.00×10^4
	Aspergillus terreus	26.00×10^4
ZnO-NPs biofilm	-	-
Ag-NPs biofilm	-	-
TiO_2-Ag NPs biofilm	-	-

Key: - = no growth

4.2 Discussion

4.2.1 Morphological characteristics of fungi identified in the maize samples

Fungi are known to contaminate food crops worldwide, thereby contributing significantly to the problem of food safety and food insecurity (Avery *et al*., 2019). In tropical countries, like Nigeria, favourable warm-to-hot climatic conditions coupled with poor pre- and post-harvest agricultural practices encourage widespread of filamentous fungal contamination of grains, such as maize and rice and cassava products (Akinyele *et al.*, 2020). From the study, it was established that *Aspergillus terreus* and *Mucor pusillus* were responsible for the spoilage of the maize grains from maize comb collected from Jiya store in Minna metropolis. *Aspergillus terreus* commonly occursin soil and is occasionally reported as pathogen in human and animals (Vassileva *et al*., 2020), it can cause aspergillosis infection with a high level of mortality particularly in immune compromised patients (Bartash *et al*., 2017).

On the other hand, *Mucor Pusillus* is a fungi that causes mucormycosis, and are present throughout the environment, particularly in soil and in association with decaying organic matter, such as leaves, compost piles, and animal dung. They are more common in soil than in air. Most people come in contact with this microscopic fungal spores every day, so it's probably impossible to completely avoid coming in contact with mucormycetes. These fungi are not harmful to most people. However, for people who have weakened immune systems, breathing in mucormycete spores can cause an infection in the lungs or sinuses which can spread to other parts of the body (Sivagnanam *et al*., 2017). The conditions at which the maize grains arrive at the store determine the fungi that predominate and the subsequent infection by the storage fungi. Post-harvest fungi infections usually occur after harvest either during transit or during storage of grains.

Fungi that develop on grains during storage usually survive at low moisture level as the grains are dry before storage (Omotayo *et al.,* 2019). It evident from the synthetic film (Plate II) that the maize was not very dried for storage, as the moisture observed in the film triggers sprouting of the maize seeds and the moistures in the seed was a suitable media for fungal growth (Plate II), while the nanoparticles embedded film inhibit the growth of the isolated fungi.

4.2.2 Characteristics of phytosynthesized nanoparticles

Green synthesis of metal and metal oxide nanoparticles has been a highly attractive research area over the last decade. Numerous kinds of natural extracts such as biocomponents like plant, bacteria, fungi, yeast, and plant extract have been employed as efficient resources for the synthesis and/or fabrication of materials (Burduniuc *et al.,* 2021). Among them, plant extract has been proven to possess high efficiency as stabilizing and reducing agents for the synthesis of nanoparticles (Elegbede and Lateef, 2019). In this study, the green synthesis of silver, zinc oxide and titanium dioxide nanoparticles was successfully achieved using leaf extract of *Hyptis suaveolens* plant. *Hyptis Suaveolens* have great economic value for small farmers as the aqueous extracts of *Hyptis Suaveolens* have considerably been used to lowered population densities of stem borers (Housing News, 2023).

This troublesome insect limits resource-poor farmer's ability to produce maize (Housing News, 2023). Bed Bugs have been reported to be repelled using the leaves of *Hyptis Suaveolens* (Housing News, 2023) and in many parts of nupe land, this plant is used as mosquito repellant. Besides this insect repellant attribute, the plant possesses hypoglycemic, anti-inflammatory, and antioxidant properties (Housing News, 2023). All these attribute of *H. suaveolens* has been linked to its phytochemical constituent.

Therefore, in this study the *hyptis suaveolens* leaf extract reduced metal salts of silver, zinc oxide and titanium dioxide to their nanoparticles forms.

The phytochemicals present in the leaf extracts have the potential to reduce metal ions in a much shorter time as compared to fungi and bacteria, which demands the longer incubation time as reported by Singh *et al*., (2018). Also, the reduction process of silver ions to silver nanoaprticles was an indication of change in color of the reaction mixture from pale brown to dark brown in line with earlier study done by Shittu and Ihebunna (2017).

The UV-spectroscopic spectra of the biosynthesized silver, zinc oxide and titanium dioxide nanoparticles from Figure 4.1 confirm the synthesis of these nanoparticles with distinctive optical properties of nanoparticles that enable intensely interact at specific wavelengths. The UV-visible light spectrum of silver nanoparticles was at maximum absorption spectral peak of 407 nm. This result agrees with the previous work of Shittu and Ihebunna (2017) where the UV-Visible spectra of various conditions indicate a strong Surface plasmon resonance within 411–415 nm. On the other hand, the zinc oxide nanoparticle shows a maximum Surface plasmon resonance spectra peak of 350 nm (Figure 4.1). This absorption peak conform to the work of Gupta and Chundawat (2020), that synthesized Zinc oxide nanoparticles using*C. roseus*and produce narrow absorption band at 366 nm. Also, Heidary *et al.,* (2019) synthesized silver and Zinc oxide nanoparticles and confirm surface plasmon resonance peak at 420 and 350 nm, respectively. In another study by Raja *et al*., (2019), UV–Vis spectrum of biosynthesized zinc oxide nanoparticles with leaf extract of *H*. *suoveolens* have Surface Plasmon Resonance band at 376 nm.

The maximum spectra peak of biosynthesized titanium dioxide nanoparticles was observed at 318 nm from Figure 4.1, which confirmed the presence of titanium dioxide

nanoparticles. Similar results was also reported earlier for synthesis of titanium dioxide nanoparticles using *Azadirachta altissima* leaf extracts by Ganesan *et al.*, (2018) where the UV spectrum of titanium dioxide nanoparticles synthesized was observed at 332 nm. The optical properties and the initial concentration of the metal and metal oxide salts play a role in the surface plasmon resonance properties of the nanoparticles in the regions of absorption.

Particle size analysis gives information on the size distribution of particles. This can be used to calculate different properties of a particle and how they will act under certain condition. This information is critical in industries to achieve different goals. The density and size of nanoparticle inversely affect the number of particles in polymer nanocomposites at filler concentration. The small nanoparticles with low density produce a larger number of nanoparticles in nanocomposites, while the big and dense nanoparticles make few nanoparticles (Ashraf *et al.*, 2021). This occurrence affects the characteristics of nanoparticles and interphase which finally changes the behavior of nanocomposites because the aggregates/agglomerates significantly decrease the number of nanoparticles.

The average particle size distribution of the synthesized silver nanoparticles (Figure 4.2) was found to be 61.7 nm which agrees with previous work done by Shittu and Ihebunna (2017) where the biosynthesized silver nanoparticles at various conditions of anions, pH and concentration gives the particle size range from 68.23–150.00 nm. The zinc oxide nanoparticles (Figure 4.2), has an average particles size distribution of 63.14nm, this conform with previous research work done by Gupta and Chundawat (2020) on Green synthesized zinc oxideo nanoparticles from *Catharanthus roseus* which gives a particles size distribution of 50.73 nm.

From Figure 4.2, the titanium dioxide nanoparticles have an average particle size of 74.20 nm that is in line with work reported by Ganesan *et al.,* (2018) where the biosynthesized TiO_2 nanoparticles using *Ageratina altissima* were found to be spherical in shape with an average diameter of 60100 nm. Nanoparticles as describe are materials with size range between 1-100 nm, and the particle sizes of biosynthesized silver, zinc oxide and titanium dioxide nanoparticles in this study are all less than 100 nm, which is a characteristic of nanoparticles.

4.2.3 Proximate parameters of corn flour used for nanocomposite films production

Starch plays a significant role in the human diet and largely used in papermaking and adhesive industry. Also, it is used to produce new products such as biodegradable plastics, drug carrier and water absorbent polymer. However, the production of starch films, glycerol are use as plasticizer to increase the elasticity. The plasticizers molecules entered in between the polymer chains which tend to increase the polymer flexibility (Tarique *et al.,* 2021). In food packaging industries, utilization of starch in food packaging and coating materials are justified due to its nontoxic, fragrance-free, colorless and excellent film forming capability (Kumar and Gautam, 2019). Therefore, it is use to produce ecofriendly food packaging materials that have capabilities to substitute petroleum-based synthetic food packaging materials is justified (Kumar and Gautam, 2019).

The proximate composition of starch flour used for starting film production in this study shows that the corn flour which is a refined starch contain about 99 % carbohydrate content, where other parameters have less significant values of moisture 0.38 %, ash 0.21 %, Lipid 0.1 %, crude fibre 0.4 % and protein 0.02 % (Table 4.1).

The insignificant values of ash, lipid, protein and crude fibre suggested that they might have been removed/reduced during the refining process. Corn starch is obtained by

extracting the starch from corn grain, specifically from the endosperm of the kernel. It is almost 100 % starch without any fibre, protein, fat or other components. This corn flour is obtained by grinding entire dried corn kernel into a fine powder, it is basically very finely ground corn meal. This is similar to Paliwal and Granados study (2002) who did a proximate composition of unrefined corn flour the following results in percemtages (%); Moisture 12.0, Ash1.1, Crude protein 9.0, Crude fat 3.4, Fibre1.0, and total carbohydrate 74.5. The unrefined starch usually have higher proportions of other proximate parameters as compared to refined corn which is almost 100 % carbohydrate.

4.2.4 Characteristics of produced nanocomposite films

The suitability of biofilms depends of their functional properties parameter (Table 4.3). The Moisture Content (MC) analysis helps to determine the hydrophobicity of the films, in other words, the moisture content is a function of the film ability to retain water. One of the most fundamental properties of packaging films is to maintain optimum moisture levels within the packaged product, because too low or too high levels will damage the product (Hornung *et al*., 2020). From this study all the films have moisture content in the range of 25-26 % at $p<0.05$ % level. The starch film has the least moisture value, this therefore suggest that nanoparticles enhances moisture absorption capacity of biofilms. Moisture content is an important property which is in relation to the total water molecules occupied in the complex network of microstructures with nanoparticle embedded films (Mahuwala *et al*., 2021).

Glycerol or plasticizer content also increases the moisture content as reported by Wang *et al.,* (2018). The film thickness is also a factor to reckon with as it will affect the tensile strength of the film. The starch film without nanoparticle has a thickness of 0.02 mm and while there was significant increase ($P < 0.05$) after incorporation of the nanoparticles to

0.03 mm of thickness. The film thickness is affected by many factors such as dry mass, drying conditions, alignment and distribution of components in the films. The enhancement in the film thickness was mainly dependent on the dispersion of nanoparticles in the nanocomposite film (Ortega *et al.,* 2020).

Roy *et al.,* (2019) also show that film thickness of agar films enhanced by the incorporation of melanin nanoparticles. The swelling index (SI) values indicates the interaction between water and starch molecules, the swelling index of the starch nanocomposite films as shown in Table 4.3 indicates that titanium film has the highest moisture absorption capacity with value of 57.28±0.32 %, followed by zinc Oxide film which is 55.14±0.16 %, and silver film 53.51±0.01 %, while that of starch film without embedded nanoparticles was 52.01±0.11 % which diminished significantly ($p < 0.05$) compare the films with embedded nanoparticles.

The swelling capacity of the films is affected by incorporated nanoparticles, the higher the swelling index, the suitable the film for packaging of fresh foods as it will help resist the effect of moisture in the food. Similarly, the outcomes regarding swelling index has been described by Mahuwala *et al.,* (2021). Increase in swelling index of the film with the addition nanoparticles might be due to the interaction of the nanoparticles with the starch polymer, thereby improving the structural integrity of the film. This implies that that structural integrity and hydrophobicity of the nanocomposite films improved after the addition of nanoparticle.

From Table 4.3, the solubility values of the nanocomposite films show biodegradability of the various biofilms. This implies that nanoparticles lower the solubility of starch film as compare to the film without nanoparticles which has the highest solubility value, this indicates that starch film with embedded nanoparticles are more suitable for packaging in

humid atmosphere. Kumar *et al.,* (2020) reported that solubility decreases with addition of ZnO nanoparticles in starch film polymer matrix and the reduction in the solubility of nanocomposite film is directly connected to the improved electrostatic interactions, hydrophobic nature of nanoparticles, and interactions among components of plant extract and starch chains (Kumar *et al*., 2020).

The energy dispersive x-ray spectroscopy (EDX) spectrum reveals the elemental composition of the films. From Table 4.4, the EDX of biosynthesized silver, zinc oxide and titanium dioxide nanoparticles reveals the elemental composition of each film. All the films have carbon and oxygen in common with silver nanoparticle film have additional element of silver and iron. The zinc oxides nanoparticles film has zinc and iron and the Titanium nanoparticles film have Titanium and Silicon.

Scanning electron microscopy (SEM) is an effective tool use to study the surface morphology of materials. The SEM micrographs of the films shows a uniform blending of Silver, zinc oxide and titanium dioxide nanoparticles within the starch matrix in Plate IV. The SEM confirmed the shape, size, and morphology of the biosynthesized nanoparticles at 5 Kv. It is apparent that the silver nanoparticles have spherical and crystalline shapes of different sizes, while the zinc oxide and the titanium dioxide films have spherical shapes with undulating surfaces appearing on the film, all the particles sizes are in the range of 10 to 70 nm. Additionally, the presence of little clusters was observed, which may be due to the aggregation of nanoparticles formed during film preparation. All the films showed characteristics of a smooth surface without cracks and bubbles. The strong adherence of the nanoparticles to the starch matrix could be observed and this is probably a contributing factor responsible for the modification of the physical and chemical characteristics of the films. In work of Burduniuc *et al*., (2021), the

antifungal activity of Silver Nanoparticles embedded in Pullulan Matrices and the SEM shown the green synthesized nanoparticles with irregular morphology.

4.2.5 Shelf life evaluation of packaged maize

The quality of the maize store depends on the storage facility. Two fungi contaminants identified from the maize in this study are *Aspergillus terreus* and *Mucor Pusillus.* Birgin *et al*., (2020) reported that stored maize stored maize is not only infected by *Aspergillus* but also *Mucor*, *Penicillium* and *Fusarium*. However, poorly constructed and unmaintained structures may allow the entry of rodents and insects which may mechanically damage the maize by nibbling and holing respectively, thereby predisposing the grains to fungal infection (Birgin *et al.,* 2020). The starch film produced without incorporating any active nanoparticles as shown in Plates II-V indicate maize deteriorating overtime for a period of 12 weeks above due to fungal contaminants in the maize. This is due to the fact that the fresh maize without active packaging material makes the film atmosphere conducive for microbial growth.

The synthetic petroleum based polymer (Plate III) was use for comparative shows maize seeds germinate after a week before deteriorating, this might be due to the trap moisture which aids the seed germination process (Hou *et al*., 2022). The starch film embedded with silver nanoparticles preserve the physical integrity of the maize with little or no noticeable change in colour of the maize. This can be attributed to antimicrobial properties of silver nanoparticles as reported by Huang *et al*., (2015). Moya *et al.,* (2017) who found that silver nanoparticles applied at 50 ppm concentration by pressure impregnation significantly suppressed the decay activity of the white-rot fungus *T. versicolor* in tropical woods *Acacia magnium, Cedrela odorata* and *Vochysia guatemalensis*, after 4 months. Also, Moya *et al.,* (2017) shows the effectiveness of silver nanoparticles against brown

and white-rot fungi acting on nine tropical woods. Nanoparticles are known to disturb fungal cell membrane integrity as one of their fungicidal mechanism (Kumari *et al*., 2020). The zinc oxide nanoparticles embedded film also preserved the physical integrity of the maize seeds against the action of decaying microbes as compare to the films produced without nanoparticles.

This observation behavior of zinc oxide nanoparticles is fine with study of Kumar and Gautam (2019) which shows the antimicrobial effect of zinc oxide nanoparticle embedded starch biofilm on fresh grapes and observed that the embedded biofilms wrapped grapes showed the least changes over the freshness grapes when compare to the non-wrapped grapes for the period of 7 days. Navale *et al.,* (2021) also observed the activity of zinc oxide nanoparticles against molds *Aspergillus flavus* and *A. fumigatus* and the study confirmed zinc oxide antifungal activity. Wu *et al*., (2019) used protein isolatebased biocomposite films and zinc oxides nanoparticles and reported that zinc oxides nanoparticles prevent deterioration of the study food thereby extending the food shelf life. The titanium dioxide nanoparticles embedded film shows the highest the seeds without any deterioration in the maize sample packaged.

This observation was similar to the study of Sani *et al.,* (2017) who use titanium dioxidenanoparticles and rosemary essential oil reported antimicrobial activity against *Pseudomonas spp., Enterobacteriaceae, S. aureus, L. monocytogenes, E. coli, and lactic bacteria* in inoculated lamb meat. Also, the shelflife of meat samples packed with this biodegradable film was extended up to 6 days as compared to controls. Besides ensuring the microbial quality of food, the titanium dioxide biopolymer composite preserved the sensory quality of the meat samples (Sani *et al.,* 2017).

4.2.6 Post packaged fungi load evaluation

The post packaging fungal load profile shown in Table 4.4 indicate that all the three films containing Ag, ZnO and TiO_2 nanoparticles inhibit/suppress the growth of the contaminating fungi when compared to the rapid fungal growth observe in the film without any nanoparticles embedded. This result agrees with several novel works that have been reported on the antimicrobial properties of these nanoparticles. Therefore, the use of nano-starch based plastic packaging materials to extend the shelf life of fresh foods may be considered as an environmentally healthy alternative method of fresh food storage at ambient conditions as reported by Tunma (2018).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

Hyptis suaveolens leaf extract was able to reduce the aqueous salts of silver, Zinc Oxide, Titanium dioxide to their nanoparticles size. The UV-Spectral and Zetasizer result confirms the synthesis of these nanoparticles. The SEM micrographs confirms the different sizes and shapes of the nanoparticles, while the EDS result confirms the presence of silver, zinc and titanium atoms in the starch film matrix. Maize is a staple food and a major source of income to farmers. The nature of the storage structures used by farmers to store maize determines the preservation quality of the maize during the storage period. The most important agent of maize spoilage in the store is the presence of storage fungi.The produced nanocomposite starch films inhibit the growth activities of *Aspergillus terreus* and *Mucor pussilus* contaminants of maize in the packaging films containing Silver, zinc oxide and titanium dioxide nanoparticles. This study demonstrated that the produced silver, Titanium dioxide and Zinc Oxide nanoparticle embedded starch films exhibited potential antifungal properties and thereby extending the shelf life of the maize. Therefore, these nanoparticles could be suggested for postharvest packaging of grains.

5.2 Recommendations

i. The government should established regulatory body that will be tasked with ensuring that safe foods are sold in the markets for consumption.

ii. The government should encourage the production of active biofilms for food packaging to prevent microbial deterioration of grains thereby ensuring the health of the consumer and extending food shelf-life.

iii. I recommend the use of bio packaging materials over petroleum based conventional packaging materials because of their biodegradable nature as they pose no threat to environmental pollution.

iv. Further research should be done the on the toxicity of these films to determine their health implications on consumers of the packaged foods.

5.3 Contribution to Knowledge

The need for fulfilling the food demand of world increasing population remains a major global concern, more than one-third of food is lost or wasted in postharvest operations. The increased fungal infection and cross-contamination of maize grains are hazards associated with the globalization of cereal trade. The contamination of maize with fungi (moulds) and mycotoxins represents a major problem for its use in human and animal nutrition. Reducing the postharvest losses by fungal contamination, especially in developing countries like Nigeiria, could be a sustainable solution to increase food availability, eliminate hunger and improve farmers livelihoods. Cereal grains are the basis of staple food in most of the developing nations, and account for the maximum postharvest losses on a calorific basis among all agricultural commodities. As much as 20–30 % of maize grains can be lost during the storage stage due only to the lack of storage inefficiency.

Use of scientific storage methods can reduce these losses to as low as 1–2 %. This research work sheds more light on postharvest losses in developing countries like Nigeria, the causes of storage losses and discusses the technological interventions to reduce these

losses. The basics of of biofilms embedded nanoparticles packaging films for food storage and their effectiveness on preserving maize grains during storage, at ambient conditions.

This research work has bring to lime light the cheapest and safest way of producing food packaging biofilms by incorporating active nanomaterials (silver, Zinc oxides and Titanium dioxide nanoparticles) into starch based biodegradable packaging fresh maize without the need for sun drying, to prevents microbial exposure and contamination, thereby ensuring food safety and extending the shelf of foods. Three biofilms were produced and embedded with nanoparticles of silver, zinc oxides and titanium dioxide nanoparticles and used to package fresh maize seeds for a period of three months. The nanofilms extend the shelf life of the maize package for over a period of three months, while the starch biofilms without embedded nanoparticles has its maize deterioration after two weeks. The use of these nano biofilms will help reduce the risk of mycotoxin contamination of stored grains, which make the food unsafe for consumption and reduces environmental pollution posed by the current conventional non-biodegradable films use for storing grain foods.

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APPENDIX

Plate VI: Postharvest Losses of Maize by Fungal Deterioration