Current Trends in Biotechnology and Pharmacy
Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Development of Biodegradable Tapioca Starch Films Incorporating Green Synthesized Zinc oxide Nanoparticles for Enhanced Preservation and Packaging of Sweet lime Segments: A Study of Their Physical and Antimicrobial Properties

Sangeetha V[1], Hemalatha S[2]*, Hemamalini A. J[3], Supriya V[4], Deepa Parvathi V[5], and Natarajan Chandrasekaran[6]

[1]SRIHER(DU), Porur, Chennai
[2]Department of Pharmacognosy, Sri Ramachandra Faculty of Pharmacy, SRIHER(DU), Porur, Chennai
[3]Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, SRIHER(DU), Porur, Chennai
[4]Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, SRIHER(DU), Porur, Chennai
[5]Department of Biomedical Sciences, Sri Ramachandra Faculty of Biomedical sciences, SRIHER(DU), Porur, Chennai
[6]Centre for Nanobiotechnology, Vellore Institute of Technology
*Corresponding email: hemacognosy@sriramachandra.edu.in

Abstract

Packaging has been on the environmental agenda for decades. It has been discussed and debated within the society as an environmental problem and the focus has been on the packaging material, including recycling options.In this research workgreen synthesized zinc oxide nanoparticle was performed using clove and cinnamon extract. Two types of tapioca starch based food packaging films (F1 and F2) were produced. In these F2 film had embedded zinc oxide nanoparticles in it whereas, F1 was used as control food packaging film without any nanoparticles. These films were used to wrap sweet lime segments and their advantages were tested via functional properties, characterization and quantitative assessments. The procedure was standardized, filmswere developed and physical properties were tested. Tapioca starch film F2 had the least moisture content (10.21%±0.25), swelling index (28.37%±0.14) & solubility (21.63%±0.42), was smooth, flexible and F1 film had the most transparency. F1 hadthe high values of moisture content (11.03%±0.78), swelling index (27.02%±0.35) & solubility (20.70%±0.74), was fine, flexible, and had better transparency.Film F2 also proved to have a significant antimicrobial activity. Thus, from the overall results tapioca starch film F2 was found to be the better option for food packaging applications when comparedtoF1 film.

Keywords: Zinc Oxide Nanoparticle, Tapioca Starch, Green Synthesis, Sweet Lime Segments

Introduction

Food packaging ensures the safety of food products, facilitates easy handling and transportation, and prevents chemical contamination, extending shelf life and offering added convenience to consumers. Food packaging has been made from a variety of materials, such as plastics, crystals, alloys, papers, and their complexes. The relevance of transferring dangerous elements from the packaging materials into the foods, majority, is of more worry as a result of customers' heightened health awareness. Most materials used for food

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

packaging today are not biodegradable, leading to environmental problems (1). Regarding residual monomer and other plastics' stabilizers, plasticizers, and condensation-related components like bisphenol A, there have been certain health worries raised. To create materials for eco-friendly food packaging, a number of biofilms have been investigated. Because of their significant advantages over plastics, such as biodegradability, environmental friendly, low toxicity, and biocompatibility, the practice of biofilms as food packaging materials is starting to gain popularity on a globalscale (3). These substances provide outstanding cohesive film-forming properties as well as thin film layers of protection. Currently, materials for food packaging employ biofilms such starch, cellulose, and polylactic acid (PLA).

Polysaccharides, proteins, and aliphatic polyesters make up the majority of biofilms used as food packaging materials. These materials help preserve food quality and lengthen a product's shelf life. These packaging materials can shield food products from the external environment and stop the harm of desirable elements like flavour and texture thanks to their barrier qualities that regulate the exchange of gases, moisture, fragrance, and lipids. The use of innovative, high-performing, light weight, and environmentally friendly composite materials is made possible by the use of biofilms, which can replace conventional non-biodegradable plastic packaging materials. Because they are biodegradable and non-toxic, polysaccharides found in biofilms such chitosan, carboxymethyl cellulose, and starch may be employed to address ecological problems. In addition to these benefits, typical biofilms have several drawbacks, such as weak mechanical qualities and low water resistance. In order to increase the heat stable, mechanical, and gas retaining qualities while maintaining their biodegradability and low toxicity, nano biofilms are utilized (2). When included in biofilms, nanoparticles have a proportionately higher surface area than their microscale counterparts, which favours the interactions between the filler and matrix and the functionality of the resultant materials. In addition to serving as nano reinforcements, nanoparticles can serve a variety of purposes in polymers, including biosensing, enzyme immobilization, antibacterial activity, and others.

Due to its trivial, affordable, visible, adaptable, and simple-to-process qualities, plastic, a petroleum-based, diversified, and pervasive material, is frequently employed in food packaging. An estimated 20% rise in plastic consumption from current levels of 6% by 2050. Plastic garbage accumulates over time owing to extended degradation, harming terrestrial ecosystems and polluting marine ones (5). During abiotic and biotic breakdown, landfill plastics produce hazardous chemicals that contaminate soil and water. While the decomposition of plastic in water releases compounds like polystyrene, chlorinated plastics leach hazardous chemicals that harm ecosystems. Global warming is caused by methane and CO2 emissions produced by microbes that break down plastic. Plastic garbage exposes animals by ingestion and entanglement, which has negative effects.

Zinc oxide nanoparticles have a variety of shapes and effectively prevent the growth of a wide range of bacterial species (4). In ready-to-eat poultry meat, zinc oxide nanoparticles have been shown to have antibacterial activity against *Salmonella typhimurium* and *Staphylococcus aureus.* These zinc oxide-Nanoparticles may also have the ability to shield food against bacterial contamination (12). Studies have shown that compared to other metal oxides, zinc oxide nanoparticles are more effective against *Escherichiacoli, Staphylococcus aureus, Pseudomonas aeruginosa* species. Zinc oxide nanoparticles are more attractive for packaging applications than silver nanoparticles since they are less expensive and unharmful to both humans and animals. Additionally, due to the antibacterial properties exhibited by zinc oxide nanoparticles, they can generate a substantial amount of hydrogen peroxide upon exposure to UV irradiation. This leads to oxidative stress in bacterial cells. (4*).*

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Several natural materials can be used to create different types of biofilm packaging. The polysaccharide family has succeeded in creating fresh origin materials that may now be used in place of their nonbiodegradable petrochemical-based equivalents by adding hardness, viscosity, and gel-forming capacity, it has the ability to create and contribute to the production of a range of polysaccharide films (9). Because they are harmless, they may biodegrade and do not leave behind any damaging byproducts for the environment. Additionally, it possesses exceptional gas permeability qualities that increase the product's shelf life. Materials being explored for biodegradable packaging films include polysaccharides like starch, cellulose, and chitosan. These polysaccharides can create films that are effective at blocking the exchange of gases like oxygen and carbon dioxide. Tensile strength and elongation percentage, on the other hand, are crucial mechanical attributes because it depends on them to maintain the quality of the packed food (8). The three main sources of starch for commercial production are potatoes, corn, and wheat. One of the most prevalent and commonly utilized polymers in the packaging business is cellulose and its derivatives. A crucial polymer in the food sector is cellulose. It is the well-known polysaccharide that degrades naturally. Chitosan is the second most prevalent polysaccharide in the world after cellulose. Commercial supplies of these biofilms are currently in abundance and are thus inexpensive because it is mostly made from waste products in the shellfish industry (12).

Starch is a polysaccharide that is derived from maize, potato, cassava, and cereal grains. It is made up of linear (amylose) and branching (amylopectin) sections. Because of its numerous advantageous characteristics, such as biodegradability, affordability, abundance, transparency, colorlessness, flavorlessness, tastelessness, diminished water sensitivity, outstanding oxygen barrier properties, renewability, edibility, and its capability to form excellent biofilms, it is considered one of the most promising biodegradable polymers for applications in food packaging. It is frequently regarded as a viable alternative to plastic for food packaging.

In food packaging industry, the use of starch in food wrapping and covering is vindicated due to its non-toxic, odour-free, and neutral nature, along with its excellent film-forming capabilities. Furthermore, starch can serve as a carrier for natural antioxidants and antibacterial agents, enabling the production of intelligent packaging resources. Starch exhibits notable properties such as exceptional barrier and film-making abilities, making it a promising candidate for the production of environmental food packaging resources that can potentially replace petroleum-based synthetic alternatives in the future. Nonetheless, the hydrophilic nature, brittleness, and low mechanical strength of starch-based packaging materials have limited their claim in the food business, particularly in packing and covering (8). To address the issues, various starch alterationmethods are employed to progress functional properties such as package thickness, water content, solubility (S), and swelling index (SI) of starch films (3).

Materials and Methodology

Preparation of plant extract using clove and cinnamon

In this method flower buds of clove and barks of cinnamon were used for the preparation of plant extract. 25 g of cinnamon barks and 25 g of clove flower buds were collected cleaned in running tap water and shade dried. It was then made into coarse powder. 1 liter of distilled water was added to coarsely powdered cinnamon and clove.The mixture was then placed in a shaker at 20°C and agitated at 100 rpm for 24 hours. The resultant extract was then filtered and kept at 4°C.

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Green Synthesis of zinc oxide nanoparticles

25 ml of the plant extract was heated at 60°C for 10 mins. 3g of zinc nitrate hexahydrate was added to the extract and the solution was left for one hour until white precipitate is formed. The resultant solution was then shifted to a crucible, tailed by aeration for 12 hours at 65°C to form a creamy paste. This paste was washed several times with solution of distilled water and ethanol to eliminate impurities. The paste was dried in a furnace at 300°C for 1 hour to synthesize green zinc oxide nanoparticles Table 1.

Development of Zinc oxide nanoparticles incorporated Food Packaging film

Methodology: Zinc oxide nanoparticles embedded starch films were produced and casted on a petri plate to form the food packaging films. For this procedure, 200 ml of deionized water and 6 g of tapioca starch were combined while continuously being stirred at 300 rpm for 15 minutes. 200mg of green synthesized zinc oxide nanoparticles were then added to this solution. 1.8 g of glycerol as plasticizer and 0.5 ml of 1% acetic acid solution (to sustain the pH of the starch mixture) was mixed and heated at 85°C till the solution became gelatinous for 45 minutes. The produced starch content was immediately subjected to a 5-minute sonication at 90°C, followed by vacuum oven degassing. Finally, prepared homogeneous solutions were distributed evenly on the sterilised plates and set aside in an incubator for 24 hours at 25°C for drying purpose. Two types tapioca starch based food packaging films were produced and the description is given in Table 2.

Functional Propertiesof the developed tapioca starch based food packaging films (F1, F2):

The packaging material's functions include physical protection and stability of the film, hence protection of the contained food matter inside. The following properties are tested for both F1 and F2 packaging films.

Moisture content (MC)

Weight loss was used to gauge the films' moisture content (MC). From each film, equal ratio of 2cm wereused, and the results were evaluated. The samples were then dehydrated for 24 hours at 105°C before being evaluated once again.

$$\text{Moisture Content} = (w_a/w_b - 1) \times 100$$

Where w_a is the weight of the films before drying and w_b is weight of the films after drying.

Swelling index (SI)

Swelling index describes how starch and water molecules interact. Briefly stated, samples measuring 2 x 2 were kept for drying

Table 1: Chemicals used for green synthesis of zinc oxide nanoparticles

S. No	Chemical Name	Quantity
1	*Syzygium aromaticum* L (clove)	25 g
2	*Cinnamomum zeylanicum* (cinnamon)	25 g
3	Zinc nitrate- hexahydrate	3 g
4	Ethanol (for washing)	50 ml

Table 2: Synthesized films

Films	Film characteristics
F1	Tapioca starch food packaging film without Zinc Oxide nanoparticles
F2	Tapioca starch food packaging film incorporated with Zinc Oxide nanoparticles

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

at 105°C for 24 hours before being measured for weight. Dried samples were submerged in distilled water for two minutes before being taken out. The swollen samples had extra water removed before being weighed.

$$\text{Swelling Index (SI)} = [(A_2 - A_1)/A_1] \times 100$$

Where, A_2, A_1 is the weights of increased samples after the removal of surplusdistilled water and mass of dehydratedfilms respectively.

Solubility (S)

F1and F2 film was split into samples having a 2*2 cm^2 dimension. All prepared samples were weighed (w_0) after drying for 24 hours at 105°C. During storage at 25°C for 24 hours, dehydrated samples was submerged in a container with 15 ml of deionized water. The swollen samples were then taken out, dehydrated at 105°C for another 24 hours weighed (w_1) once more.

$$\text{Solubility (S)} = [(M_1 - M_2)/M_1] \times 100$$

Where, M_1 is the mass of dehydratedfilms before water immersion and M_2 is the dry mass of the unsolvablefilm after absorption.

Characterization of Zinc oxide nanoparticles incorporated tapioca Starch Films

Scanning Electron Microscope analysis (SEM)

The microscopic structure of altered starch and compound films was analyzed via Scanning Electron Microscopy (SEM) with the OMD 2x2 model. The standard procedure was employed for the test, utilizing anfast-tracking voltage of 10 kV. Picturesare captured at intensifications ranging from 300 to 2500 cm^{-1}.

Antimicrobial activity of synthesized zinc oxide nanoparticles incorporated tapioca starch films

a) Antibacterial activity of the film was determined by well diffusion method on Muller Hinton agar medium. The antibacterial activity of Zinc oxide nanoparticles incorporatedtapioca starch film against *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria) was investigated using the standard agar diffusion method. The antibacterial test was conducted according to CLSI guidelines (M02-A12) against two bacterial strains.The medium was prepared by dissolving of Muller Hinton agar in deionized water. After cleansing, the media was transferred to petri plates and allowed to solidify for one hour. Once solidified, the inoculum was spread onto the solidified media using a sterile swab moistened with the bacterial suspension. Wells were created using a cork borer. Samples and the positive control, Streptomycin (1 mg/ml - 20 µL), were loaded into the respective wells. The plates were then incubated at 37°C for 24 hours. Microbial growth was assessed by measuring the diameter of the zones of inhibition.

b) The antifungal activity of the sample was assessed using the well diffusion method on Potato Dextrose Agar (PDA) medium. The medium was prepared by dissolving 4.4 g of PDA in 100 ml of distilled water. After sterilization, the medium was poured into sterile petri plates and allowed to solidify for 1 hour. Once solidified, the inoculum was spread onto the plates using a sterile swab moistened with the fungal suspension. Wells were created using a cork borer *candida albicans* and *aspergillus niger* were the two test organisms used. The sample and the positive control, ketoconazole (10 mg/ml - 20 µL), were loaded into the respective wells. The plates were then incubated at 37°C for 24 hours. Antifungal activity was determined by measuring the diameter of the zones of inhibition.

Quantitative analysis of synthesized tapioca Starch based packaging film, wrapped Over Sweet lime segments (F1 and F2 films)

pH

The pH of sweet lime was observed during the storage period using a pH meter during the initial and post storage period of 28 days at 4°C.

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Brix

The pulp was separated from the sweet lime segments and crushed to remove the pulp. The pulp was further squeezed to form filtered fruit juice which was used to measure total soluble solids, which has a series of 0-32%.

Acidity

Acidity was determinedby following the A.O.A.C (2016). Briefly, six grams of sweet lime juice was addedto 25 ml of deionized water in a flask. The juice was titrated with a 0.01 N NaOH solution, with 1% phenolphthalein as an indicator. A pale pink colour in the juice indicated the endpoint of the titration. The acidity was stated as percentage of ascorbic acid per 100 grams of sweet lime pulp.

Vitamin C

10 g of crushed sample was added to 50 ml of the metaphosphoric acid-acetic acid solution to stabilize the ascorbic acid. The mixture is then filtered paper to obtain a clear extract. This solution was then titrated against DCPIP solution until pink colour appears as endpoint. The vitamin C was reported as mg per 100 ml of sweet lime pulp.

Reducing sugars

The fruit juicewasneutralized precisely with concentrated sodium hydroxide (NaOH) and phenolphthalein as an indicator, then diluted to a volume of 100 ml. Thesolution was titrated against the mixture of Fehling's solutions A and B, titrant value was calculated.

Non reducing sugars

10 ml of Fehling's reagent was added to dextrose solution to complete the titration. The solution was boiled for 2 minutes with addition of 1 ml of methylene blue indicator solution. The remaining standard dextrose solution was added until the blue color of the indicator disappears and non reducing sugars value was calculated.

Total sugars

Total sugars is calculated as Total reducing sugar – Non reducing sugar x 0.95 + Reducing sugar (AOAC 2000 16TH edition).

Weight:

The weight of all sweet lime segments in both F1 and F2 packaging was measured initially and finally after 28 days stored at 4°C. This was done to determine the weight loss or weight gain of the packaging materials.

Results and Discussions

Functional properties of the synthesized films (F1 and F2)

The functional properties of tapioca starch based packaging films (F1 and F2) are shown in Table 3.

Moisture content (MC)

The films' ability to contain moisture was expressed by means of moisture content. It also has an impact on the practical qualities including mechanical strength and water vapor penetrability, making it a crucial film quality. *Seligra et al.,* 2014 reported that the MC also improves with an increase in starch concentration. According to *Ghanbarzadeh et al.,2011*, acetic acid passed in between the starch polymer and lowered the contact, which led to an improvement in the MC. The MC is also increased by glycerol or plasticizer concentration, as reported by *Wang et al*. in 2017.

Swelling Index (SI)

According to *M.A. Bertuzzi et al*., 2007, when starch concentration increases,

Table 3: Results of functional properties of the films

Tester films	Moisture content %	Swelling index %	Solubility %
F1	10.21± 0.25	28.37±0.14	21.63 ±0.42
F2	9.98±0.54	27.02±0.35	20.70 ±0.74

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

so does the swelling index. According to *Ghanbarzadeh et al., 2011* the contact between thestarch molecules like amylopectin and amylose and the water fragments changes with the quantity of acetic acid and plasticizer in the tapiocastarch packaging film. The green synthesized Zinc Oxidenanoparticles embedded tapioca starch film F2 was found to have lower SI (27.02 ±0.35) than the other starch film F1 without nanoparticles. This might be as a result of the hydrophilic properties that the Zinc Oxidenanoparticles capping on the tapioca starch film has generated.

Solubility

Solubility process provides information on how films interact with water molecules; solubility is crucial in the choice of the right matrix for food. According to research by *Maryam Adilah et al., 2017* the presence of hydrophilic material in the starch films boosted the films' solubility. *Seligra et al., 2016* investigated how the addition of acetic acid reduces the solubility of the tapioca starch film. It has been found that the solubility reduces as Zinc oxide nanoparticles are added in the tapioca starchfilm. Evaluation between the two films i.e., F1 and F2, it was detected that F1 exhibits higher solubility (26.37 ±0.63) than the F2 film. F2 had the minimum solubility i.e., 20.70±0.74(%), because of low hydrophilic nature.

Characterization of the Zinc oxide nanoparticlesnanoparticles embedded starch films

Scanning Electron Microscope Analysis: The microscopic graphs of the starch and nanocomposite films were examined using SEM. (as shown in Fig.1 (a), (b). Fig. 2 (b) at various resolution, showed that the Zinc oxide nanoparticlesincorporated in the starch solution comprising acetic acid (as cross-linker) and glycerol (plasticizer) resulted insmoothscattering of Zinc oxide nanoparticles. SEM images showed Tapioca starch film had small holes on the surface of the film as a result of Zinc oxide nanoparticles adhering to the film's surface. Micrographs also demonstrated that when Zinc oxide nanoparticles were introduced to the starch solution, the film surface formed was found to be uniform and smooth.

Antimicrobial activity of synthesized zinc oxide nanoparticles incorporated tapioca starch films

The starch solution containing ZnO nanoparticles (at a concentration of 20, 40, 60, and 80 mg) was supplemented to wells

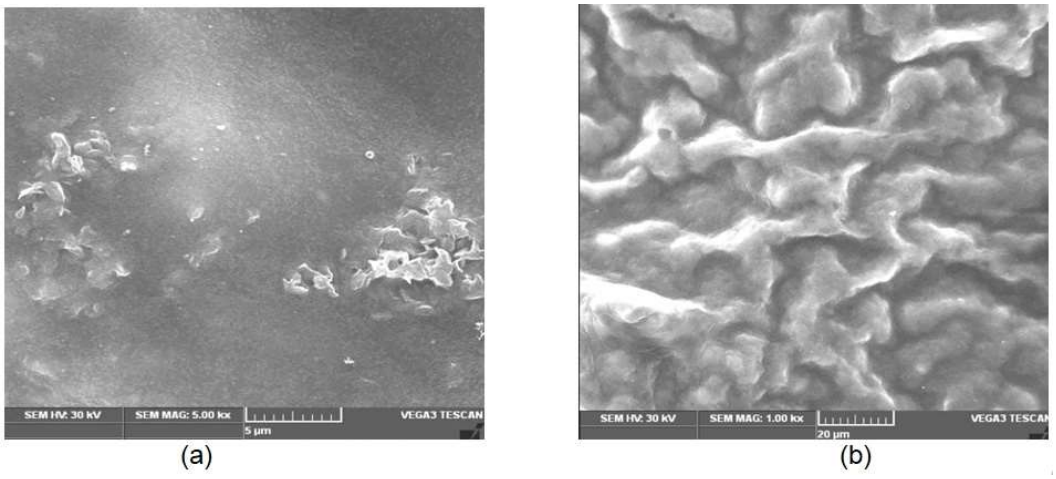

Figure 1: (a) SEM Images of tapioca starch packaging film without Zinc oxide nanoparticles; (b) SEM Analysis of tapioca starch packaging film embedded with Zinc Oxide nanoparticles

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

(a) (b)

Figure 2: (a) F1 film wrapped over sweet lime segments, (b) F2 film wrapped over sweet lime segments- initial packaging

Table 4: Antibacterial activity of Zinc oxide nanoparticles incorporatedtapioca starch film					
Microorganisms	Zone of Inhibition (mm)				
	1	2	3	4	5
Staphylococcus aureus	1.2	1.8	4.2	5.7	30
Escherichia coli	7	8.9	10.5	11	22
Note: 1- 20 µl, 2- 40 µl, 3-60 µl, 4- 80 µl 5 -Streptomycin (control)					

Table 5: Antifungal activity of Zinc oxide nanoparticles embedded tapioca starch film					
Microorganisms	Zone of Inhibition (mm)				
	1	2	3	4	5
Aspergillus niger	0.8	0.8	1.2	2.4	30
Candida albicans	0.1	0.3	1.1	1.8	20
Note: 1- 20 µl, 2- 40 µl, 3-60 µl, 4- 80 µl 5 ketoconazole (control)					

(6 mm) on the agar plate. This Zinc oxide nanoparticles embedded starch solution was used as it deliverscomparable results to testing the film directly.

The results, shown in Table 4, indicated that Zinc oxide nanoparticles incorporated tapioca starch packaging films exhibited significant inhibitory activity against *E. coli* (gram-negative bacteria) when compared to *S. aureus* (gram-positive bacteria).

The antifungal activity of the tapioca starch film incorporated with zinc oxide nanoparticles was assessed using the well diffusion method on Potato Dextrose Agar Ketoconazole (1mg/ml -20 microlitre)was used as controlfor comparison.The results, shown in Table 5, indicated that Zinc oxide nanoparticles embedded starch films exhibited higher inhibitory activity against *Aspergillus niger*when compared to *Candida albicans*.

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Quantitative analysis of tapioca starch based packagingfilm (F1 and F2), wrapped over sweet lime segments

The sweet lime segments were separated from the peel individually and then placed over the developed films F1 and F2. These segments were wrapped and stored at 4°C for 28 days. Post 28 days samples were tested for pH, brix, acidity, vitamin C, reducing sugars, non-reducing sugars, total sugars, and weight. weight of test packets were found to be increased both in F1 and F2 packaging after 28 days (Fig. 2A & B, Fig. 3A & B, and Table 6). Theacidity, vitamin-C, total sugars, reducing sugars, non reducing sugars and total sugars contents were recorded to be increased in both F1 and F2 film packaging and was comparatively high in F2 film packaging after 28 days compared to initial fresh fruit segments, An opposite trend was observed for the pH and brix levels of sweet lime segments in different packaging.

(a) (b)

Figure 3: (a) F1 film wrapped over sweet lime segments, (b) F2 film wrapped over sweet lime segments- post 28 days stored at 4°C

Table 6: quantitative parameter of sweet lime segments wrapped over F1 and F2 packaging films

Parameters	Initial (before packing)	28 days in refrigerated storage (4^0C) control (tapioca starch)	28 days in refrigerated storage (4^0C) experimental (tapioca starch with nano ZnO)
pH	4.74	4.80	4.50
Brix	10	10.9	10.3
Acidity %	1.518	1.218	1.483
Vitamin C (mg/100ml)	48.8	24.1	24.9
Reducing sugars %	6.65	6.56	6.62
Non reducing sugars %	6.4	5.5	5.9
Total sugars %	13.21	12.18	12.86
Total weight (sample and packaging) g	Initial	18.8096	19.573
	final	19.1190	20.4501

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

Table 7: Antimicrobial activity of sweet lime segments wrapped over F1 and F2 packaging films stored at 4°C for 28 days

Packaging materials	Total bacteria count (CFU/ml)	Total yeast and mould count (CFU/ml)
F1	29×10^{-3} CFU	2×10^{-3} CFU
F2	12×10^{-3} CFU	2×10^{-3} CFU

The brix values were relatively lower, measuring 10.9 in F1 film packaging and 10.3 in F2 film packaging. The slights changes in reducing sugar, (6.56 mg/ml), total sugar (12.18 mg/ml) and non reducing sugar (5.5 mg/ml) of F1 packaging were recorded in comparison to initial fresh segment juice as 6.65, 13.21, and 6.4 mg/ml, respectively. The changes in reducing, non-reducing, and total sugars in the juice were greater in F1 packaging compared to F2 packaging. Both packaging types provided a shelf life of 28 days for the segments under refrigerated storage at 4°C. However, due to overlapping of the segments in the test packages, they became watery and lost their shelf life within 28 days.Total bacterial count on nutrient agar from sweet lime segments juice after 28 day of storage was found to be 290 CFU/ml in F1 packaging filmand 120 CFU/ml in F2 packaging film.Similar findings were reported by *Raccachet al., 2007* on quantitative analysis of Nagpur mandarin segments (Table 7).

Conclusion

The present study involved developing of two starch based food packaging films F1 (tapioca starch based food packaging film), F2(tapioca starch based food packaging film embedded with greensynthesized Zinc oxide nanoparticles) Films functional properties, structural morphology and quantitative assesments were tested. Functional analysis and characterization of synthesized starch films-F1and F2 was performed.Thus, from the whole study, it can be concluded that biofilm developed from tapioca starch (F2) was found to be the better option for food packaging as provided better functional properties and better quantitative and anti microbial results when wrapped over sweet lime segments.

Future Scope of Work

Various biologically synthesised nanoparticles can be studied since green synthesis is more ecological, economical, and useful than chemical synthesis. Moreover, starch films using an improvedblend of cross-linking agents (such as genipin, glutaraldehyde, glyoxal, etc.), plasticizers (such as HPMC, PVA, etc.), and other stabilising agents (such as agar, xanthan gum proteins, emulsifiers, etc.) shall be investigated. The qualitative analysis of films can performed with various forms of highly perishable food products.

References

1. M.A. Del Nobile, A. Conte, C. Scrocco, I. Brescia, B. Speranza, M. Sinigaglia, R. Perniola, D. Antonacci, Postharvest Biology and Technology 51 (2009) 21–26.
2. Liu, San Jose State University Packaging Engineering 13 (2006) 1348– 1368.
3. P. G. Seligra, C. M. Jaramillo, L. Fama, S. Goyanes, Carbohydrate polymers 138 (2016) 66–74.
4. S. Xu, Z. L. Wang, Nano Research 4 (2011) 1013–1098.
5. H. Lennernas, B. Abrahamsson, Journal of pharmacy and pharmacology 57 (2005) 273–285
6. AOAC. (2016). Official Methods of Analysis. Association of Analytical Chemists, 15th Ed. Washington DC.
7. Y. D. Adjouman, C. Nindjin, F. A. Tetchi, A.-C. Dalcq, N. G. Amani, M. Sindic, Journal of Food Processing and Technology 8 (2017) 665.
8. A. E. C. Fai, M. R. A. de Souza, S. T. de Barros, N. V. Bruno, M. S. L. Ferreira, E. C. B.

Vol. 17 (Supplementry Issue 3s) 257 - 267, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.20

de Andrade Goncalves, Postharvest Biology and Technology 112 (2016) 194–204.
9. A. Sorrentino, G. Gorrasi, V. Vittoria, Trends in Food Science & Technology 18 (2007) 84–95.
10. Z. Luo, W. Cheng, H. Chen, X. Fu, X. Peng, F. Luo, L. Nie, Journal of agricultural and food chemistry 61 (2013) 4631–4638.
11. Jones, N., Ray, B., Ranjit, K. T., and Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiology Letters, 279, 71−76.
12. V. K. Sharma, R. A. Yngard, Y. Lin, Advances in colloid and interface science 145 (2009) 83–96.
13. Y. D. Adjouman, C. Nindjin, F. A. Tetchi, A.-C. Dalcq, N. G. Amani, M. Sindic, Journal of Food Processing and Technology 8 (2017) 665.
14. J. Santhoshkumar, S. V. Kumar, S. Rajesh kumar, Resource-Efficient Technologies 3 (2017) 459–465.
15. S. Rana, P. Kalaichelvan, Antibacterial Activities of Metal Nanoparticles 11 (2011) 21–23.