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EFFECT COLD TEMPERATURE AND pH ON PERFORMANCE OF A STARCH-BASED WATERMELON SEED NANOCOMPOSITE FILM FOR LOCUST BEANS PACKAGING

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ABSTRACT

In this research, the performance of starch-based watermelon seed nanocomposite film was evaluated for locust beans packaging under varying pH and temperature conditions. The film was prepared by casting a blend of 15g potato starch, 0.3g watermelon seed nanoparticles with an average size 4.04 nm, 100g glycerol, and 300ml distilled water at 85ºC in 300× 150 mm plastic mold. It was characterized to determine its microstructural, thermal, and barrier properties using standard methods. Samples of the locust beans, prepared at 7.22– 11.13 pH range, were wrapped using the film and a low-density-polyethylene (LDPE) which serves as a control. The microbial loads were determined after 30 days of storage between 4– 10ºC temperatures. Results show ~ 4% loss in weight up to 300ºC, thus the film is thermally stable. The permeability coefficients were 0.7×10^{-10}, 2.15×10^{-10} and 22.0×10^{-10} cm^3 (STP) cm/cm^2scm Hg for nitrogen, oxygen, and carbon-dioxide, gases, respectively. Morphologically, the particles in the film are heterogeneously distributed within the matrix, revealing traces of elemental components with average pore size smaller than a water molecule but sufficient to allow exchange of the gases in the microsphere. There was significant difference ($p<0.05$) in the level of the microbial loads in the samples packaged using the nanocomposite film compared those packaged using the LDPE. The nanocomposite film is therefore a better packaging material than the LDPE for locust beans packaging under the same pH and temperature.

1.Introduction

A crucial step in the post-harvest processing of the Africa locust beans is packaging (Barak and Mudgil, 2014). This contributes to the product's containment and defense against microbial, fungal, and bacterial attack, consequently extending its shelf life (Yun *et al.,* 2022). The performance of the packaging material may be impacted by the application, the type of food being transported, the temperature, storage, and distribution, and other factors (Wu *et al.,* 2021). Typically, the type of packaging materials used for sea foods may not be suitable for other kind of foods because of the temperature and transportation effects (Alabi *et al.*, 2022). To ensure that the product is effectively secured for a prolonged shelf-life, packaging needs to be applied carefully for different foods and purposes.

Aluminum foil, paper, and leaves have all been used to contain the condiment over time to prevent it from deteriorating(Liu *et al.,* 2021). Although there has been improvement, the product still demonstrates signs of poor-quality degradation, which shortens its shelf life. Additionally, printing on these materials may be challenging, which may impair market communication between the product's makers and consumers (Liu *et al.,* 2020). Although nylon and other standard packaging can help with the problems, their long-term use is frequently discouraged because they are not biocompatible. In the meanwhile, bio-based packages have been suggested as a solution to the issue with conventional materials. This kind of package, which is typically made from sustainable by-products of plant and animal origin, can provide the product with thoughtful and long-lasting protection (Yun *et al.,* 2022). As a result, almost all the problems with locust bean packaging can be resolved by adopting bio-based packaging. Biodegradable nanocomposite films have generally been reported for use in food packaging. For instance, Fadeyibi *et al.* (2020) reported the packaging of cucumber and garden eggs using nanocomposite films. The film has also been successfully applied for the packaging of tomatoes (Fadeyibi *et al.*, 2017), sliced okra (Fadeyibi *et al.*, 2019; Al-Naamani *et al.*, 2018), strawberry (Barikloo and Ahmadi, 2018), pears (Bodaghi and Hagh, 2019), and many other food products (Farhoodi, 2015; Huang *et al.,* 2015). However, the application of the biodegradable film for locust beans packaging is only sparingly reported. Also, there was no reported research on the synthesis of a bioplastic from the mixture of potato starch and watermelon seed nanoparticles hitherto. This research was therefore carried out to produce and test a nanocomposite film from the blend of sweet-potato starch and watermelon seed nanoparticles for locust beans packaging under varying conditions of pH and low temperatures.

2. Materials and methods

2.1. Starch preparation

A 1000 g tuber of fresh harvested sweet potatoes was bought at the main market of Malete and processed to extract the starch needed. This was done by peeling the potato and crushing it to reduce the size before grating it into a purée. The material was then passed over a prepared sieve and water was added to the filter. The filtrate was maintained intact for 1 hour to allow the starch to settle before the supernatant was decanted. The starch formed was dried in an air circulation oven for 24 hours and then packaged in a polyethylene bag for further experiment.

2.2. Preparation of the starch-based water-melon seed nanocomposite film

A 1000 g of watermelon fruits were bought from the Malete market, Kwara State, Nigeria. It was then divided into small pieces, crushed to make paste, then carefully cleaned with fresh water. The seeds were then extracted from the mixture and poured into a sieve. The seeds were bleached in an acid solution of 10% (w/w) hydrogen chloride. They were then thoroughly rinsed with tap water and dried in an air circulated oven for 24 hours. An electric mixer was then used for milling, and a mechanical sieve shaker was used for pruning into fine particle size. The fine materials in the shaker pan were collected and subjected to a particle size experiment with a Zetasizer (version 7.01). This gives an average particle size of 4.04 nm. Based on preliminary research and previous literature reports, the film was prepared by mixing 15 g of the sweet-potato starch, 0.3 g of the watermelon seed nanoparticles and 100 g of glycerol in 300 ml of distilled water (Farhoodi, 2015; Huang *et al.*, 2015). The mixture was placed on a burner and heated to 80°C and stirred continuously until it became gelatin-like in consistency. The gelatinous liquid was then poured into a mold that measured 300 mm by 150 mm, and it was dried for 24 hours at 60 ^{o}C and 65% R.H. in an air-circulated oven dryer. The dried film was packed in a nylon for a later investigation.

2.3. Characterization of the starch-based water-melon seed nanocomposite film

The titration method described by Hadassah and Sehgal (2006) and Fadeyibi *et al.* (2023) was used to determine the methane, oxygen, hydrogen, nitrogen, and carbon dioxide gas permeabilities of the starch-based watermelon seed nanocomposite film. The method described by Kviesitis (1971) and Schöffski and Strohm (2000) was used to measure the water vapor permeability of the film. Thermal properties were determined using the procedure described by Fadeyibi *et al.* (2017) and Shanks (2010). The procedure described by Nikov *et al.* (2020) for Scanning electron microscopy (JEOL JSM-7600F model electron microscope) in conjunction with the Energy-Dispersive x-ray Spectroscopy was used to analyze the microstructural property of the nanocomposite film at 50 and 100nm resolutions.

2.4. Evaluation of the starch-based water-melon seed nanocomposite film

The performance of the nanocomposite film was assessed using 1000 g of fresh locust beans, which were purchased at Malete Market. To change the pH in the range of 7.22–11.13, a buffer solution of sodium chloride at a concentration of 3% (w/w) was applied to the sample of locust beans. The products were then packaged using the film and the LDPE and stored between 4– 10^{o}C for 30 days. A total 12 main samples were thus obtained at the end of storage, as shown in Table 1, and the microbial loads were determined using the methods reported by Fadeyibi *et al.* (2017) and Sunmonu *et al.* (2020). The experiment was replicated three times, and the average value and standard deviation were recorded as the microbial loads of the locust beans condiment.

Table 1: Sample of packaged locust beans

		Variables	
	Sample	Temp (^{o}C)	pH
Film	A	4	7.22
	B	4	9.34
	C	4	11.13
	D	8	7.22
	E	8	9.34
	F	8	11.13
	G	10	7.22
	H	10	9.34
	I	10	11.13
LDPE	control 1	4	7.22
	control 2	8	9.34
	control 3	10	11.13

2.5. Statistical analysis

The results obtained from the barrier, mechanical, thermal, and structural characterizations were illustrated graphically. To reduce experimental error, the data were also examined in triplicate, and average values and standard deviations were established. At $p< 0.05$, a two-way multivariate analysis was utilized to determine the significance of temperature, pH, and their interaction on microbial growth in the 12 samples of packing material. We utilized the Duncan Multiple Range (DMRT) test to determine the degree of significance for each sample treatment compared to the control (LDPE) for various pH and cold storage temperatures at $p< 0.05$.

3. Results and discussions

3.1. Permeability of the starch-based water-melon seed nanocomposite film

The findings of the permeability of the starch-based watermelon seed nanocomposite film are shown in Figure 1. This shows that the film is more permeable to carbon dioxide and hydrogen gases and less permeable to oxygen, methane, and nitrogen gases. According to Fadeyibi *et al.* (2023) and Kim *et al.* (2014) locust beans typically produce oxygen, and carbon dioxide gases during packaging. Over accumulation of these gases can lead to imbalance in the storage structure thereby decreasing the shelf life. Therefore, it is essential to control the gas concentration to prevent this from happening (Ghosh and Singh, 2022; Mohammadpour and Naghib, 2021). During locust beans packaging, a stable microsphere can be created with high carbon dioxide and low oxygen and methane gas concentrations because of the inherent property of the nanocomposite film. Due to the limited amount of oxygen gas present in the microsphere, the product is likely to undergo anaerobic respiration, which will cause the concentration of the carbon dioxide to increase thereby extending the shelf life of the locust beans. This supports the findings of Qin *et al.* (2021), who used graphene oxide nanosheets to extend the shelf life of maize cellulose, and Zhang *et al.* (2020), who used lignin to improve the water vapour transmission rate of the film by incorporating it into polyvinyl acetate. In other similar works, Raja and Xavier (2021) functionalized a silane nanoparticle on nanoclay to improve the maintaining quality of the film during packing, while Fadeyibi and Osunde (2021) added a zinc nanoparticle to cassava starch. Considering the foregoing, it follows that during food packing, the nanocomposite film can monitor and regulate the gaseous concentration within the microsphere.

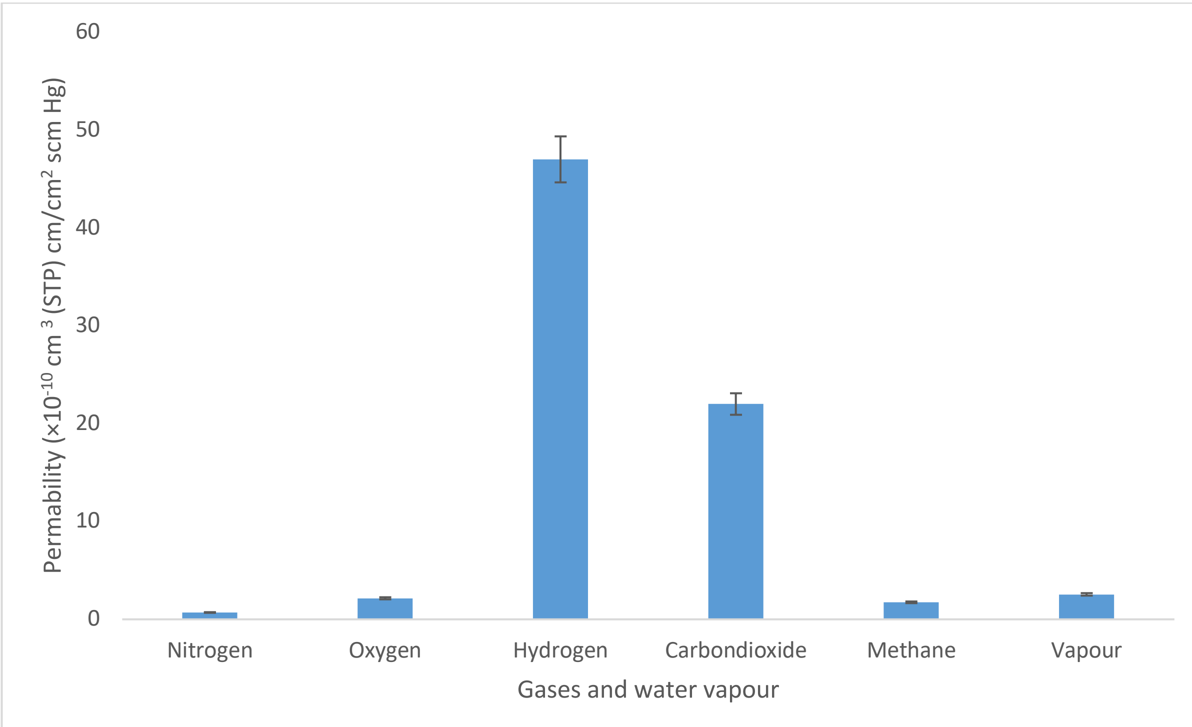

Figure 1. Permeability of the starch-based water-melon seed nanocomposite film

3.2. Microstructural properties of the starch based water-melon seed nanocomposite film

The results of the microstructural properties of the water-melon seed nanocomposite film are shown in Figure 2. A heterogeneous arrangement of the film matrix can be seen which indicates inconsistencies in the particle size between 10.55 to 11.77nm. This arrangement can be associated with the different particle sizes of the glycerol and starch molecules used in forming the film. Consequently, gas molecules smaller than 11.77 nm like oxygen, nitrogen, carbon-dioxide, and methane can easily permeate the film due to their inherent smaller molecular sizes in comparison with the pore sizes in the film. Thus, this allows gaseous exchange when applied for food packaging. In other related investigations, Merritt *et al.* (2020) reported that a polystyrene encapsulation will enhance the gas permeability of graphene-based nanocomposite film. Also, Figure 2 shows the EDS profile of the watermelon seed-based nanocomposite film with a high signal for oxygen gas (30.3% by weight). This means high oxygen and other gas permeability for the film. The findings of Nikov *et al.* (2020) on the SEM characterization of a polyvinyl alcohol and clay-based nanocomposite film agree with the present research.

The results of the microstructural properties of the nanocomposite film as viewed from the TEM micrograph (50nm and 100 nm resolutions) are shown in Figure 3. White and dark clusters of particles are seen converging around some spots within the film matrix. Areas with dark clusters depicts the spots where the watermelon seeds are mostly concentrated while the areas with white patches show the spots where the nanoparticles are less concentrated. This variation can be associated with the high surface area of the nanoparticles which provided the bonds needed to concentrate the particles around some spots more than the others (Chenwei *et al.,* 2018; Feng *et al.,* 2018). At 50 nm resolution, this disparity is even clearly seen than at 10 nm resolution; and the pattern observed can further give information about the particle size arrangement and suggest the permeability level of the watermelon seeds nanocomposite film.

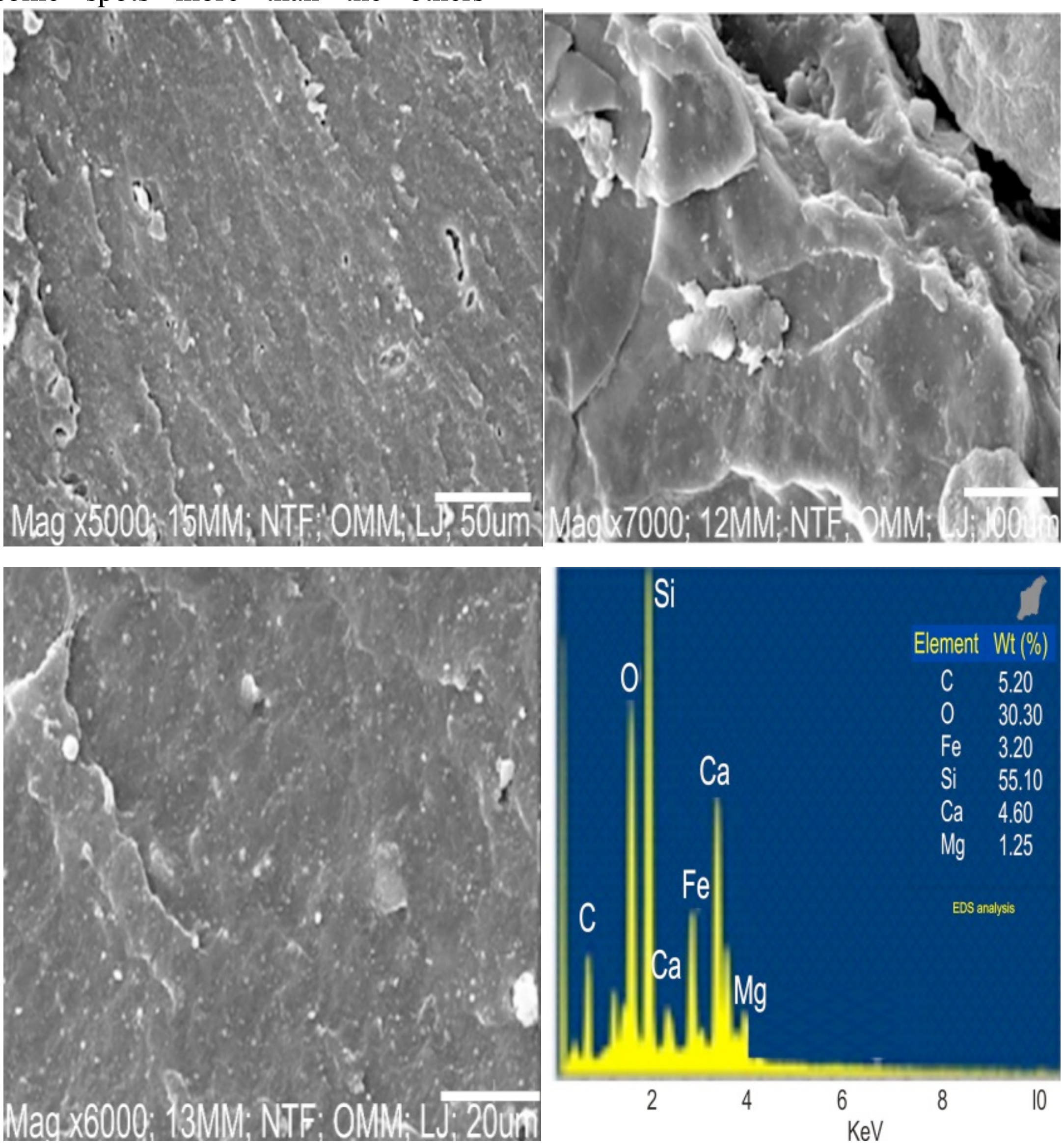

Figure 2. SEM micrographs of the starch-based water-melon seed nanocomposite film

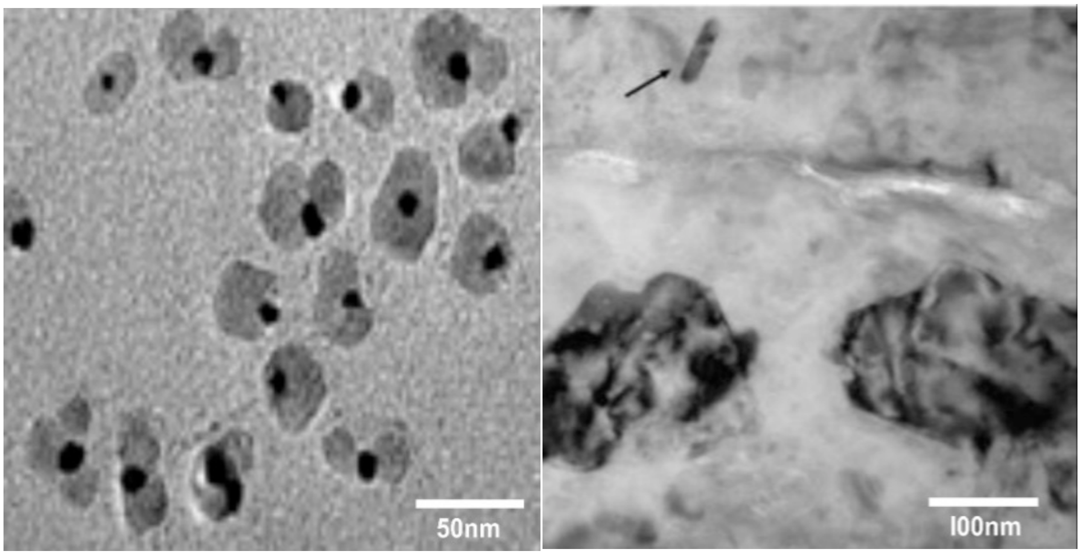

Figure 3. TEM microtopography of the starch-based water-melon seed nanocomposite film

3.3. Thermal properties of the starch based water-melon seed nanocomposite film

The results of thermal properties of the water-melon seed nanocomposite film obtained from the TGA/DTA analysis are shown in Figure 4. There was only a 4% weight degradation when the film was heated to 300°C, which correspond to the threshold or glass transition temperature where it was stable thermally. At this threshold, the heat of sublimation was 4.55 J/g which gradually increased with an increase in the temperature. Thus, above the glass transition temperature the degradation maybe enormous thereby rendering the film thermally unstable. The findings of Thomas *et al. (*2021), Manikandan *et al.* (2019) on natural rubber and nano-cellular composite films, respectively, reported high weight degradation above the glass transition temperature. Hence, the starch-based watermelon seed nanocomposite film can be recommended for packaging food at temperatures below 300°C.

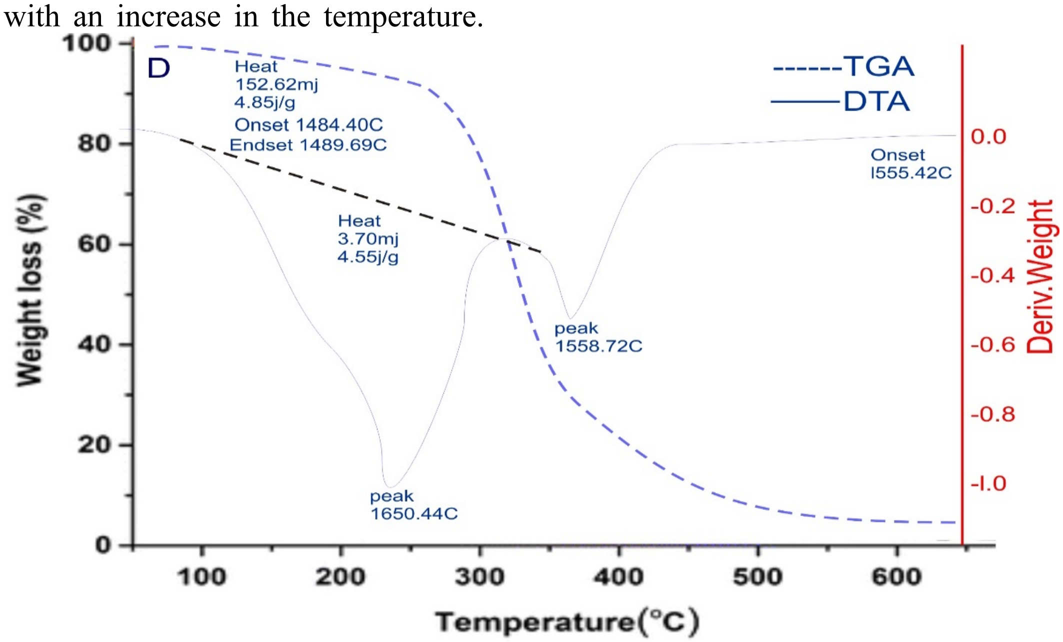

Figure 4. Effect of temperature on weight degradation of the starch-based water-melon seed nanocomposite film

3.5. Effects of cold temeprature and pH on microbial loads of packaged locust beans

The results of the influence of pH and cold temperature on the microbial load of the packaged locust beans are shown in Table 2. This shows high performance of the nanocomposite film compared with the LDPE at the same temperature and pH. There is no significant difference between the pH and temperature interaction or their individual effects on the microbial loads ($p< 0.05$) as shown in Table 3. Also, there is no significant difference in the level of the microbial loads between the packaged samples, partly due to the presence of the nanoparticles in the matrix of the film, and partly due to the surface area between the molecules in the film mix. In related studies, Fadeyibi *et al.* (2020) and Shi *et al.* (2022) reported a low level of microorganisms on some fruits and vegetables when nanocomposite films are used to package them. This means a low pH and temperature can lower the number of microorganisms in the stored locust beans. Furthermore, the DMRT in Table 4 indicates no significant different between the fungi counts in B and G samples, but A, C, D, E, F, I samples were significantly different from each other and from the control ($p< 0.05$). Similarly, all the film samples are significantly different from each other and from the control ($p< 0.05$) for the bacteria counts. Thus, the starch-based watermelon seed nanocomposite film can be suitable for the locust beans packaging at all pH and temperature.

Table 2. Effects of temperature and pH on the microbial loads in packaged locust beans

		Variables		Microbial loads ($\times10^5$ cfu/g)	
	sample	Temp (°C)	pH	Fungi count	Bacteria count
Film sample	A	4	7.22	0.46± 0.02	1.15± 0.06
	B	4	9.34	0.66± 0.03	1.90± 0.10
	C	4	11.13	0.62± 0.03	3.35± 0.17
	D	8	7.22	0.36± 0.02	1.50± 0.08
	E	8	9.34	0.76± 0.04	2.65± 0.13
	F	8	11.13	0.14± 0.01	3.20± 0.16
	G	10	7.22	0.67± 0.03	1.40± 0.07
	H	10	9.34	0.92± 0.05	2.60± 0.13
	I	10	11.13	1.62± 0.08	4.25± 0.21
Control	LDPE 1	4	7.22	3.41± 0.17	9.34± 0.47
	LDPE 2	8	9.34	4.14± 0.21	10.14± 0.51
	LDPE 3	10	11.13	5.04± 0.25	12.37± 0.62

Table 3. Analysis of variance of the microbial loads influenced by pH and temperature conditions

Dependent Variable	**Source of Variation**	**SS**	**df**	**MS**	**F-value**	**p-value**
Bacteria counts (cfu/g)	Corrected Model	69.32	8	8.66	0.28	0.94^{n*}
	Intercept	153.23	1	153.23	4.86	0.12^{n*}
	Temperature (T)	0.68	2	0.34	0.01	0.99^{n*}
	pH (P)	9.12	2	4.56	0.15	0.87^{n*}
	T× P	56.37	4	14.09	0.45	0.71^{n*}
	Error	94.56	3	31.52		
	Total	405.52	12			
	Corrected Total	163.87	11			

Fungi counts (cfu/g)	Corrected Model	14.48	8	1.81	0.34	0.90^{n*}
	Intercept	16.38	1	16.38	3.01	0.18^{n*}
	Temperature (T)	0.91	2	0.46	0.09	0.92^{n*}
	pH (P)	0.32	2	0.16	0.03	0.97^{n*}
	T× P	12.52	4	3.13	0.59	0.70^{n*}
	Error	15.91	3	5.30		
	Total	59.85	12			
	Corrected Total	30.39	11			

*Significant at $p < 0.05$

Table 4. Duncan Multiple range test for microbial loads

		Microbial loads ($\times 10^5$ cfu/g)	
	sample	Fungi count	Bacteria count
Film sample	A	0.46± 0.02^{i}	1.15± 0.06^{l}
	B	0.66± 0.03^{g}	1.90± 0.10^{i}
	C	0.62± 0.03^{h}	3.35± 0.17^{e}
	D	0.36± 0.02^{j}	1.50± 0.08^{j}
	E	0.76± 0.04^{f}	2.65± 0.13^{g}
	F	0.14± 0.01^{k}	3.20± 0.16^{f}
	G	0.67± 0.03^{g}	1.40± 0.07^{k}
	H	0.92± 0.05^{e}	2.60± 0.13^{h}
	I	1.62± 0.08^{d}	4.25± 0.21^{d}
Control	LDPE 1	3.41± 0.17^{c}	9.34± 0.47^{c}
	LDPE 2	4.14± 0.21^{b}	10.14± 0.51^{b}
	LDPE 3	5.04± 0.25^{a}	12.37± 0.62^{a}

*Sample with the same letter indicate no significant difference at $p < 0.05$.

4. Conclusions

A novel nanocomposite film was developed from the blend of starch and watermelon seed nanoparticles for locust beans packaging. The film was more permeable to carbon dioxide and hydrogen gases and less permeable to oxygen, methane, and nitrogen gases. A heterogeneous arrangement of the film matrix was seen which indicates inconsistencies in the particle size between 10.55 to 11.77nm. There was only a 4% weight degradation when the film was heated to 300^{o}C, which correspond to the threshold or glass transition temperature where it was stable thermally. There was also no significant difference ($p<0.05$) in the level of the microbial loads between the packaged samples, partly due to the presence of the nanoparticles in the matrix of the film, and partly due to the surface area between the molecules in the film mix.

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