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Development and Characterisation of Nano Bioplastic Infused with Lemongrass Essential Oil for Fruit Packaging Application

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ABSTRACT

In the present research, bioplastic films based on polylactic acid (PLA) were developed for food packaging applications. Food packaging protects food from environmental factors and extends its shelf life by preventing chemical and microbiological contamination. Notably, the limitation of PLA in brittleness was improved by incorporating nanoclay as a filler. To enhance the antimicrobial activity of the bioplastic films, lemongrass essential oil (NBLG) was infused into the solution. The nano bioplastic infused with NBLG films was evaluated for antimicrobial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The results reveal that NBLG films exhibited antimicrobial properties, especially against *S. aureus,* with an inhibition zone of 18.40 mm. The NBLG film was then used as plastic packaging for red grapes, and the quality of the grapes was assessed on day 1, day 7, and day 14 of storage. Interestingly, on day 14, grapes packed with NBLG film were observed to have less physical deterioration, and their weight was 14.55% higher compared to those packed with control PLA film, which demonstrated a 46.70% weight reduction from their initial weight. At the same time, the fruit firmness decreased by 37.5% and 17.4% for grapes packed with PLA and NBLG film, respectively. Overall, it was asserted that PLA bioplastic filled with nanoclay and infused with NBLG can be used as an alternative, eco-friendly packaging with antimicrobial properties to increase the shelf life of packed fruit.

Keywords: *Bioplastic; packaging; lemongrass essential oil; antimicrobial; nanoclay*

1. INTRODUCTION

Food packaging plays a vital role in preserving the quality of food products and acts as protection against external environmental factors. The main function of packaging is to store food in the best economical way, ensuring food freshness and safety, while minimising environmental effects to meet the quality expectations of consumers. Notably, rapid innovation in food packaging led to the development of sustainable packaging and packaging with special features such as antimicrobial activity. Many brands and retail chains have shifted from non-recyclable, difficult-to-recycle multilayer flexible packaging materials in favour of more eco-friendly and sustainable alternatives due to changing consumer preferences and emerging regulations. Sustainable packaging is also supported by the New Plastics Economy Global Commitment, led and launched in October 2018 by the Ellen MacArthur Foundation in collaboration with the United Nations Environment Programme [1].

On the other hand, microbial contamination in the packaging industry is a serious and common problem, particularly in food packaging, which could be harmful to human health [2]. Accordingly, antimicrobial packaging has been developed, primarily by adding antimicrobial agents (e.g., antibiotics) to traditional polyolefin-based packaging materials to prevent the development of bacterial decay that might compromise the quality and safety of the packaged goods. However, in most cases, the packaging itself remains non-biodegradable and can potentially lead to antibiotic abuse. As a result, it is critical to develop new antimicrobial packaging materials with high antimicrobial efficiency and durability without the use of antibiotics to reduce the environmental problems and to ensure food safety [3].

In recent years, polylactic acid (PLA) has been widely used in the research and development of biodegradable packaging materials [4,5,6] due to its biodegradability, biocompatibility, recyclability, compostability, and renewability. Nevertheless, PLA is a brittle material in nature [7]. Hence, nanomaterials such as nanoclay can be used as fillers to overcome the brittleness and low mechanical properties of PLA. Moreover, it has been reported that by adding nanoclay as a filler in PLA, the mechanical properties were improved [8,9,10,11].

It is crucial to consider packaging when considering the shelf life of foods. Thus, understanding the needs of the food is critical in selecting the appropriate package to maximise shelf life. Meanwhile, perishable, chilled products such as fruits require an antimicrobial package, as microbial or enzyme activity is the cause of spoilage. In line with this, antimicrobial agents can inhibit microbial growth on the surface of food [12]. To prepare antimicrobial packaging, several methods are available, including the addition of antimicrobials into packages, the direct incorporation of antimicrobials into selected polymers, the coating of polymer surfaces with antimicrobials, the immobilisation of antimicrobials onto polymers through ionic or covalent bonds, and the use of antimicrobial polymers with film-forming properties. Furthermore, essential oils are widely used in the food industry due to their natural antimicrobial, antioxidant, and bio-preservative effects, which help prolong the shelf life of foods, especially fruits and vegetables [13]. Perdana *et al*. [14] claimed that adding Lemongrass Essential Oil (NBLG) to starch/chitosan film may be useful for chilli preservation. Specifically, it reduced the microbial growth and delayed the ripening during storage. On a similar note, Shojaee-Aliabadi *et al*. [15] reported that the carrageenan film containing Satureja hortensis essential oil was highly effective against five selected bacteria. It could potentially be used as a packaging material with good degradability.

It was reported in our previous study that the addition of 3 wt.% of nanoclay and 1.5 wt.% of NBLG in PLA film demonstrated optimum physico-mechanical properties [16]. Hence, in this current study, the films were further characterised for their physical, mechanical, and antimicrobial activity against pathogens. In addition, the quality of grapes packed by PLA film and nano bioplastic infused with NBLG film was also determined by their physical appearance, weight, and firmness.

2. MATERIALS AND METHODS

PLA granules (Nature Works LLC, product name Ingeo™ biopolymer, white in colour, 100% corn-based, density: 1.25 g/cm^3) and chloroform were obtained from Merck, USA. Nanoclay (Nanomer® 1.28E) was acquired from Nanocor, USA. The Australian-grown red grapes, Red Globe, were bought from a local supermarket.

2.1 Preparation of Films

Approximately 2 g of PLA granules were dissolved in 40 ml of chloroform using a magnetic stirrer at room temperature for 45 min until the PLA granules were fully dissolved. The solution was then poured into a Petri dish (15 × 15 cm). Subsequently, the solution was spread evenly using a bent glass rod and allowed to dry for about 48 h at room temperature. Before further characterisation, the films were preconditioned at 25°C and 50% Relative Humidity (RH) for at least 48 h. For the NBLG film, the same procedure was repeated. After the dissolving process, PLA solution was added with 3.0 wt.% nanoclay and 1.5 wt.% of NBLG. This is followed by sonication for 5 min using an ultrasonic probe before being poured into the Petri dish.

2.2 Film Characterisation

A UV-double beam spectrophotometer was used to measure the film's transparency. The light transmittance of the nano bioplastic films was measured in the visible region (400-700 nm) using ASTM D1746. For a clear comparison, light transmittance at 240 nm was measured.

Tensile tests were performed on films using an Instron Universal Testing Machine (Model 3366) in accordance with ASTM D882-97. The samples were cut into 2 cm × 6 × 0.02 cm. The edges of the film were attached to a piece of paper frame using adhesive tape. Prior to testing, both sides of the paper frame were clamped between the grips to hold the sample. The test was conducted at a crosshead speed of 5 mm min^{-1}. At least five replicates were assessed for each sample variation.

2.3 Antimicrobial Evaluations

The disc diffusion method was performed using Mueller-Hinton Agar (MHA) to determine the antimicrobial activity of the NBLG film, following the European Committee on Antimicrobial Susceptibility Testing (EUCAST), with MHA pH set at 7.2. Activity against *Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli)* was evaluated. The films were cut into 1 × 1 × 0.02 cm square sizes and were placed on MHA plates once the plating of the pathogen culture was completed using the spread plating method. The plate was incubated at 35°C for 20 h. Finally, the zone of inhibition was determined.

2.4 Determination of Physical Appearance, Weight Loss, and Firmness of Red Grapes After Being Packed with PLA Film and NBLG Film

Red grapes were used for the assessment of the packaging application. In particular, fresh red grapes without visual defects were packed on the same day of purchase, each pack containing six grapes. The grapes were first washed with tap water and drained for 1 h. The remaining water on the fruits was wiped off using a paper towel. The grapes were packed in a Styrofoam tray, and the films were used to seal the packs. After 14 days of storage at 20°C, red grape quality, including physical appearance, firmness, and weight, was evaluated according to the procedure described by Thakur *et al*. [17]. The physical appearance, which is a sign of wrinkles and fruit's plumpness, was observed by a digital camera, and the images on day 1, day 7, and day 14 were recorded. The weight loss of the fruits was calculated [18].
The firmness of red grapes packed in PLA and NBLG films was examined using a texture analyser (TA-XT2i) on days 1, 7, and 14 of storage, following the ISO 7505:1999 standard. At the same time, the fruit was probed with a stainless-steel probe, and the corresponding values were recorded. Accordingly, the maximum penetration force (N) was defined as the force required to push the probe into the grape surface to a depth of 2 mm. The procedure was repeated at an average of two readings from each side of the fruits.

3. RESULT AND DISCUSSION

3.1 Physical and Mechanical Properties

Table 1 Physical and mechanical properties of PLA film and NBLG film

	Thickness (mm)	Light transmittance (%)	Tensile Strength (MPa)	Young Modulus (MPa)	Elongation at Break (%)
PLA film	0.22 ± 0.05	100	30.36 ± 1.77	1164 ± 20.0	3.93 ± 0.11
NBLG film	0.24 ± 0.07	83.95	41.02 ± 1.80	2147 ± 26.5	10.40 ± 0.73

Table 1 tabulates the thickness, light transmittance, tensile strength, Young's modulus, and elongation at break for PLA film and nano bioplastic film. The NBLG film exhibits good optics with 83.95% light transmittance from UV light transparency analysis. Furthermore, it can be observed that the addition of nanoclay to PLA has reduced the film transparency due to the whitish colour of nanoclay. However, the addition of nanoclay resulted in an improvement of brittleness. Ahmad *et al*. [19] noted that montmorillonite nanoclays are commonly used with polymers in packaging applications, as the inclusion of montmorillonite with polymers at low concentrations significantly improves film rigidity and tensile strength. In line with this, the tensile strength and elongation at break of NBLG film are 35% and 165% higher than those of the PLA film, respectively. Meanwhile, the increment in tensile strength could be attributed to the strong interfacial bonds formed between PLA and nanoclays. During the transfer of stress between the PLA matrix and nanoclay, interfacial interaction plays a critical role [20]. In essence, the elongation at break of a nanocomposite with nanoclay increases compared to the pure PLA, which might be due to the enhanced interfacial interactions and reinforcement effects provided by the nanoclay. Studies, such as those by Ray and Okamoto [21] on polymer-layered silicate nanocomposites, have demonstrated that optimised nanoclay dispersion leads to an improved balance of stiffness and toughness, resulting in higher elongation at break. Notably, the mechanical properties of plastic packaging are one of the essential criteria due to the forces encountered during food handling, shipping, and transportation [22].

3.2 Antimicrobial Evaluation

The disk diffusion method was performed to investigate the antimicrobial activity of the films against indicator strains, including *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria). Figure 1 displays the antibacterial analysis of pure PLA film and NBLG film against *E. coli and S. aureus*. The observation demonstrates that the PLA film has no antimicrobial activity against either of the bacteria. Previous research discovered that PLA has poor antimicrobial activity [23]. On the other hand, NBLG film performed an inhibitory effect against *S. aureus* and *E. coli*, which could be attributed to the effect of NBLG on the cell membrane surface structure and loss of intracellular material. The essential oil of lemongrass and its major component, citral, have been demonstrated to inhibit the growth of a wide range of pathogens, including S. *aureus* and Candida species. A wide spectrum of microorganisms can be inhibited by essential oils, which are rich in volatile terpenoids and phenolic compounds. In general, plant essential oils inhibit microorganisms by disrupting cytoplasmic membranes, disrupting proton motive force, electron flow, and active transport, and inhibiting protein synthesis [24]. Moreover, Gao *et al.* [25] claimed that citral can downregulate the hyphal adhesins and reduce the gene expression involved in *S. aureus* fatty acid biosynthesis.

The size of the inhibition zone in Figure 1 demonstrates that lemongrass has a greater antimicrobial ability against *S. aureus* than *E. coli*. This could be related to gram-positive bacteria's cell walls, which have a thin lipopolysaccharide outer membrane [26]. It was reported that toxic drugs and hydrophilic antibiotics are more resistant to Gram-negative bacteria than hydrophobic compounds [9]. Table 2 outlines the diameter of the zone of inhibition for both samples. The inhibition zone of the *E. coli* sample is approximately 14.30 mm, while the *S. aureus* sample demonstrates a larger inhibition zone, which is 18.40 mm. Meanwhile, pure PLA film exhibits no zone of inhibition for both plates.

Table 2 Inhibition zone analysis of pure PLA film and NBLG film

Types of film	*E. coli* (mm)	*S. aureus* (mm)
Pure PLA film	No inhibition zone	No inhibition zone
NBLG film	14.30	18.40

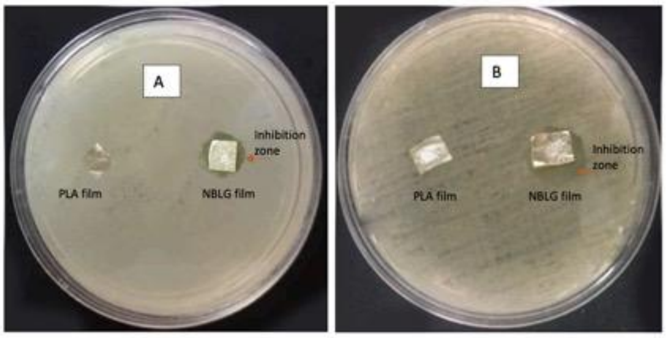

Figure 1. Antimicrobial activity of PLA film and NBLG film oil against (A) *E. coli* and (B) *S. aureus* bacteria.

3.3 Assessment on The Physical Appearance, Weight Loss, and Firmness of Red Grapes Packed with PLA Film and NBLG Film

The quality of red grapes after packing and storing was evaluated to determine the potential of NBLG film as a packaging material. Notably, PLA film is used as a control. On day 7, grapes packed by both films presented physical deterioration and were less plump, as summarised in Table 3. It could be observed that grapes packed with NBLG film display less skin wrinkling as compared to grapes packed with PLA film. On day 14, the surface of the grape packed with PLA film was evidently wrinkled, a sign of water loss, and its final weight had reduced by approximately 46.70%. Meanwhile, grapes packed with NBLG film presented a better physical appearance, and the calculated weight loss was about 32.15%. That indicates that the NBLG film keeps the grapes 14.55% juicier than the PLA film. According to Koide and Shi [27], even a 5.0% weight loss would reduce the market value of vegetables and fruits.

Table 3 Physical appearance of the red grapes on day 1, 7, 14, and the total weight loss after 14 days of packing with (a) PLA film and (b) NBLG film

Type of film	Day-1	Day-7	Day-14	Weight loss after 14 days (%)
(a) PLA film				46.70
(b) NBLG film				32.15

It is believed that PLA films filled with nanoclay and NBLG are able to reduce the speed of deterioration of grapes during storage. This has caused minimal weight loss and maintained a fresh appearance for a more extended period, and therefore was more effective for increasing the shelf life of the fruits. According to Bangar *et al.* [28], incorporation of essential oil as an antimicrobial agent also forms a semi-permeable layer, which allows passage of certain small molecules but acts as a barrier to others. It also acts as a protective barrier, reducing respiration and transpiration on the fruit surface and conferring a physical barrier against O_2, CO_2, moisture, and solute movements.

Based on the results obtained in Figure 2, a decrease in firmness was observed with time for both sample types. After 14 days of storage, the firmness of packed grapes decreased by 37.5% and 17.4% for grapes packed with PLA and NBLG film, respectively. This finding reveals that the reduction in firmness contributed by weight loss and degree of injury due to decay or microbial growth has been delayed by the addition of NBLG [29]. NBLG may enhance the shelf life of the grapes as it prevents bacterial growth on the fruits [30]. Research by Perdana *et al.* [14] also suggested that incorporating NBLG into a polymer starch may be a useful alternative for vegetable preservation, such as chilies, as it reduces the water loss and delays the ripening during storage. The firmness of fruit is a critical quality attribute associated with overall consumer acceptability. Firmer fruit tends to be juicier, crispier, crunchier, and less mealy [31].

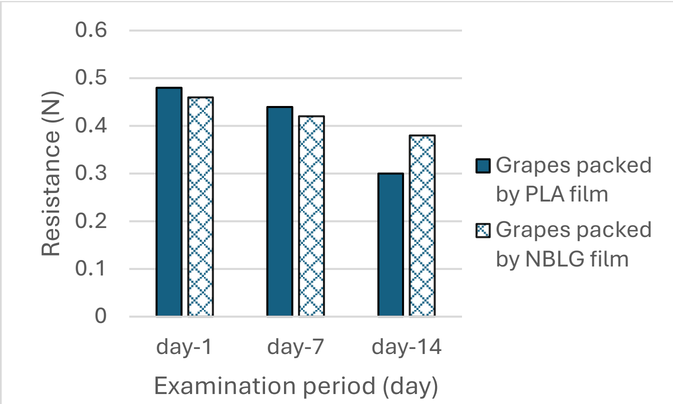

Figure 2. Firmness of grapes packed by PLA film and NBLG film.

4. CONCLUSION

The addition of nanoclay particles to the PLA matrix has improved its physical and mechanical properties by overcoming the brittleness of PLA. NBLG provides the antimicrobial ability to the packaging film. Hence, it could prevent microbial growth on the fruit's surface. Antimicrobial evaluation of the films against *E. coli* and *S. aureus* reveals clear antimicrobial activity for the nano bioplastic films with inhibition zones of approximately 14.30 mm and 18.40 mm, respectively. The addition of both nanoclay and NBLG was proven to prolong the shelf life of packed fruit while maintaining its physical integrity under storage conditions. The NBLG film was demonstrated to be more effective than pure PLA film in preserving the physical (firmness and texture) stability of the grapes. After 14 days of observation, the surface of the grapes packed by PLA film was obviously wrinkled, its final weight was reduced by 46.70%, and the firmness was reduced as much as 37.5%. Meanwhile, grapes packed by NBLG film demonstrated better physical appearance, and the calculated weight loss was about 32.15% with only a 17.4% reduction in fruit firmness. Overall, the results demonstrated that incorporating nanoclay and NBLG into PLA has significantly improved the antimicrobial properties, effectively protecting grapes from physical deterioration and prolonging their shelf life. Nevertheless, this work may lead to future research in antimicrobial polymer films, which are beneficial to food packaging applications.

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