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Original Research Article

Antimicrobial Effect of Poly-Lactic Acid (PLA)/Poly-Butylene Succinate (PBS) Films Incorporated with Patchouli Essential Oil Nano emulsions Against Foodborne Pathogen

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Abstract: The rising development in the production of bio-based polymers has reduced the dependency on conventional plastics. Several revolutions have been initiated as society is increasingly concerned about the potential ecological consequences of plastics derived from petroleum. Utilising PLA biopolymer matrix for food packaging presents a viable solution for achieving environmental sustainability. Reinforcing the PBS into the PLA matrix could enhance the quality and properties of the packaging film. Active packaging is an innovative concept in the food packaging industry that enhances food quality and prolongs shelf life by integrating essential oils (EOs) nano emulsions. A study was conducted to produce a film based on PLA/PBS blends using the solvent casting method with the incorporation of Patchouli essential oil nano emulsions (PEO-NE) at different concentrations (1%, 2%, and 4%, w/v). The antimicrobial properties of the films against foodborne pathogens were then examined and tested on fresh chicken meat samples and stored for 15 days at a temperature of 4°C. The PLA/PBS/PEO-NE film demonstrated significant efficacy in suppressing the growth of *Escherichia coli*, *Streptococcus aureus*, and *Pseudomonas aeruginosa*, in comparison to *Salmonella typhimurium*, *Streptococcus mutans*, and *Klebsiella pneumoniae*. The most effective concentration of PEO-NE in PLA/PBS film was 4% (w/v). In addition, the utilisation of 4% PEO-NE in PLA/PBS film extended the shelf life of the chicken flesh sample from 6 days to 12 days. The results of this study suggest that incorporating PEO-NE into PLA/PBS can create an active packaging biopolymer that enhances food quality and freshness.

Keywords: active packaging, antimicrobial, patchouli essential oil, poly-lactic acid (PLA), poly-butylene succinate (PBS)

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1. Introduction

Petroleum-based conventional plastics are widely used in the world. To date, more than a trillion pounds of plastic have been manufactured, with an annual eighteen billion pounds having been disposed of into marine ecosystems (Gibben *et al.*, 2018). In addition, microplastic contaminants tend to have environmental issues when they start to appear in lands and marine ecosystems and are mistakenly consumed by birds, fish, and shellfish as food (Hassan *et al.*, 2019). Mitigating environmental challenges has been a key focus of recent initiatives. These efforts include using environmentally friendly and renewable materials that naturally break down. The production of biodegradable polymeric materials has been pursued for the benefits of environmental conservation, and the protection of biodiversity (Hassan *et al.*, 2019; Yap *et al.*, 2022).

The biodegradable polymers that are mostly employed in food packaging applications include polylactic acid (PLA) and polybutylene succinate (PBS). According to Taib *et al.*, (2023), PLA plastic is celebrated for its environmentally friendly properties and versatility. It is made from renewable resources like cornstarch or sugarcane. PLA is biodegradable and compostable, breaking down into natural components under industrial composting conditions. One of its key benefits is that it reduces reliance on fossil fuels, contributing to a more sustainable materials cycle. PLA is also non-toxic, making it safe for food packaging and medical applications. Additionally, it offers good clarity and strength, making it suitable for a wide range of products.

Polybutylene succinate (PBS) is a thermoplastic and biodegradable polymer known for its impressive rigidity, which stems from its high crystallinity. This structural characteristic gives PBS a robust and durable nature, making it suitable for a variety of applications, including packaging, agriculture, and automotive components. Its biodegradability allows it to break down in natural environments, reducing long-term waste and environmental impact. Additionally, PBS exhibits good thermal stability and mechanical properties, which enhance its performance compared to some other biodegradable polymers. Its ability to combine functionality with environmental responsibility makes PBS an attractive choice in the push for more sustainable materials (Karolina *et al.*, 2019).

Essential oils (EOs) are natural active agents extracted from plants, rich in bioactive compounds that exhibit antioxidative and antibacterial properties. Numerous EOs are available on the market, each demonstrating varying degrees of antibacterial efficacy. For example, the EO derived from *Eucalyptus globulus* leaves has been shown to possess

significant antibacterial activity against pathogens such as *Escherichia coli* and *Staphylococcus aureus*. These properties make EOs valuable for applications in food preservation, packaging, and medicinal formulations, contributing to the development of safer and more effective antimicrobial solutions (Mekonnen *et al.,* 2016). This antibacterial activity is attributed to phenolic compounds present in *E. globulus* leaves known as terpene carvacrol, geraniol, menthol, and thymol (Chouhan *et al*., 2017).

In this study, patchouli essential oils (PEOs) were utilised, extracted from the plant *Pogostemon cablin* (*Blanco*) *Benth,* which belongs to the *Lamiaceae* family (Yusibani *et al.,* 2019). PEO is produced by collecting and drying the leaves and stems of the patchouli plant, followed by extraction through a distillation method (Isnaini *et al.,* 2022). Patchouli EO has gained recognition for its potential as an antimicrobial agent that can encapsulate and release bioactive compounds, enhancing their efficacy against various pathogens. Studies have demonstrated that PEO can effectively inhibit microbial growth and improve the mechanical properties of the films making it a promising candidate for applications in food preservation and packaging. Patchouli EO is reported to have the ability to inhibit protein biosynthesis in bacteria. PEO has shown promising antimicrobial properties, particularly against *S. aureus*, by effectively preventing biofilm growth during the early adhesion phase. Similar studies have indicated that EOs can also inhibit biofilm formation in other pathogenic bacteria, including *Klebsiella* species and *Streptococcus* species. Among the compounds found in PEO, it is noted that pogostone and (-)-patchouli alcohol exhibit antibacterial properties (Bilcu *et al.,* 2014; Isnaini *et al.,* 2022).

EOs are extremely sensitive due to their volatile nature and complex chemical composition. Their high volatility means that many of the active compounds can evaporate quickly when exposed to air, light, or heat, leading to a loss of potency. Additionally, EOs are prone to oxidation, which can alter their chemical structure and diminish their effectiveness. Therefore, encapsulation-based emulsion methods of EO are developed to overcome the sensitivity and improve the stability of these bioactive compounds (Rakmai *et al.,* 2021). Based on Scartazzini *et al.* (2019), the antifungal activity of mint essential oil (MEO) was incorporated as an active agent in gelatine films.

Aside from that, the primary goal of nanosized emulsion EO is to control the release of bioactive chemicals in certain conditions while also protecting them from environmental aggressors like light and moisture. Nanosized emulsion can greatly improve their bactericidal, viricidal, fungicidal, anti-parasitic, and insecticidal actions, in addition to providing flavour protection. Furthermore, EO nanoparticles exhibit powerful antibacterial properties against multidrug-resistant organisms (Gupta *et al.,* 2016). Barrera-Ruiz *et al*. (2020) discovered that chitosan nanoparticles containing cinnamon, and thyme EOs had a higher inhibitory zone against *Enterococcus sp.* and *K. pneumonia*. In addition, Sara *et al*. (2016) employed the least minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to obtain antibacterial activity in oregano EO nanoparticles.

Hence, the present study aims to report on the development of PLA-PBS films integrated with nano emulsion patchouli EO (PEO-NE) via the solvent casting method. This study aims to observe the capability of PEO-NE to inhibit foodborne pathogens. The optimal film will proceed to the storage test with fresh chicken meat samples to determine the effects of PEO-NE on the antibacterial effects.

2. Materials and Methods

2.1 Preparation of EOs Nano Emulsion with Different Volume Concentrations

The nano emulsion was made with minimal changes to the procedure described by (Chang *et al*., 2013). In a nutshell, an organic phase comprised PEOs, Tween-80, and medium-chain triglycerides (MCT) oil, whereas an aqueous phase contained citrate buffer (pH 6.0). Firstly, 5 mL PEO, 5 mL MCT oil and 20 mL Tween-80 were mixed using a magnetic stirrer. The mixtures were then titrated at 2 mL/min into 70 mL of citrate buffer. The nano-emulsion was stored at 4℃ for further usage.

2.2 Preparation of PLA-PBS Bio-Polymer Films Using the Solvent Casting Method

PLA and PBS pellets were dried at 60℃ overnight, to eliminate the residual water. The ratio of chloroform to composite materials was 5:1. First, 0.96 g of PLA and 0.04 g of PBS were dissolved in a beaker that contained 50 mL of chloroform and stirred for 4 h. The mixture was stirred at 250 x g with strong agitation by a magnetic stirrer until aggregations of the compound disappeared. Next, the nano-emulsion of PEO was added to the PLA-PBS biopolymer mixture separately at the concentrations of (1.0% , 2.0 % and 4.0 %, w/v). The procedure was repeated for the control samples without the addition of PEO-NE. Finally, approximately 15 mL of film-forming solution was cast onto a 100 mm × 15 mm glass petri dish and dried in a laminar flow chamber without a blower, at room temperature for 2 h which was reported sufficient to achieve the complete evaporation of the chloroform (Hassan *et al*., 2019).

2.3 Mechanical Properties

The tensile properties of the blend films were assessed through tensile testing at ambient temperature. An Instron universal testing machine (model UK 404, NVLAP LAB, Norwood) was employed to measure the tensile strength and elongation at break for PLA, PLA/PBS, PLA/PBS/NFC, and PLA/PBS/NFC/EO films, following the ASTM D882-02 standard from the American Society for Testing and Materials. The films were cut into a standardised rectangular shape (100 mm × 15 mm) using a cutter press (GT-7016-A, GOTECH, Taiwan). Before testing, the films were conditioned for 24 h at 51% relative humidity. The specimens were positioned between grips set 50 mm apart, and testing was conducted at a speed of 5 mm/min, with five samples tested for each type to determine tensile strength and elongation at break.

2.4 Antibacterial Testing on PLA-PBS Films Incorporated with PEO

The films' antibacterial properties were analysed using the agar disk diffusion method as previously reported by Balouiri *et al*. (2015) with some modifications. The antibacterial properties of a PLA-PBS film integrated with PEO nanoparticles were tested against foodborne pathogens like *Salmonella typhimurium*, *K. pneumonia*, *S. aureus*, *E. coli*, *S. mutans*, and *Pseudomonas aeruginosa*. The bacterial culture was suspended in 10 mL nutrient broth and incubated at 37℃ for 24 hours. Next, the bacterial suspension was prepared using 0.5–0.6 OD UV Spectroscopy. Then, 100 µL of the bacterial suspension was poured and spread on a Mueller-Hinton Agar (MHA) aseptically with a sterile glass hockey stick.

The antimicrobial test was done by following the Kirby-Bauer disc diffusion method. A paper puncher was used to cut the films into 6 mm diameter discs. Then, by using sterile forceps the films were placed onto the Mueller-Hinton Agar (MHA) plates that were previously inoculated by the bacterial suspension. A 6 mm diameter sterile disc was dipped into autoclaved sterile water to be used as a negative control and kanamycin as the positive control. The plates were then placed in the incubator at 37℃ for 24 h. Then, the diameter of the inhibitory zone was measured.

2.4 Storage Test on Chicken Meat Using PLA/PBS Films.

The chicken meat for storage test was prepared as described by Higueras *et al*. (2013) with some changes. The chicken meat was cut into 2 cm x 2 cm, around 10 g, and placed in the chiller at 4°C for further use. The cut slices of chicken meat were wrapped with optimised film. The chicken meat wrapped with film and the control were kept in the chiller at 4°C until analysis. The chicken meat samples were kept for 15 days and analysed on day 0, 3, 6, 9, 12, and 15 (Luong *et al*., 2020).

The pH of the chicken meat wrapped with film and control was measured following the procedure of Fernandez-Pan *et al*. (2014) with some changes. Firstly, pH 4, 7 and 10 buffer solutions were used to calibrate the digital pH meter. The films were peeled off and the chicken meat was put into a bag. The chicken meat was then mashed with a pestle and 100 mL of sterile peptone water was added to the bag. The pH of the chicken sample before and after the storage analysis has been recorded by measuring the chicken mixture solution.

2.5 Statistical Analysis of Data

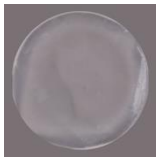
The experiment was conducted in triplicate (n=3). By using one-way ANOVA (Analysis of Variances), all the results were given as mean value ± standard deviation (Minitab19, 2019). The Tukey test has been used to determine if there was a significant difference between the different film formulations. A p-value of 0.05 was used to determine statistical significance.

3. Results and Discussions

3.1 Physical Properties of the PLA/PBS film

Table 1 shows the thickness and photographic images of the obtained PLA/PBS and PLA/PBS/PEO-NE films. The blending of PLA with PBS films renders them homogeneous and smooth appearances of films. According to Bilcu *et al.* (2014), the ductility of PBS is low, and various experiments involving PBS blends with other polymers have been conducted to improve its characteristics. In addition, Qiu *et al.* (2016), examined the characteristics of the PLA-PBS blends and discovered that PBS works as a nucleating agent, enhancing the degree of crystallinity of PLA.

Table 1. The tensile strength (TS) and elongation at break (EaB) of PLA (control), PLA/PBS and PLA/PBS film with concentrations of PEO-NE at 1%, 2% and 4%.

Films	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Visual Appearance
PLA/PBS (Control)	0.08±0.01[a]	17.0±0.69[a]	13.9 ±0.31[a]	
PLA/PBS/PEO-NE1	0.12±0.01[b]	14.3±0.42[b]	6.8±2.19[b]	
PLA/PBS/PEO-NE2	0.13±0.01[b]	14.8±0.60[b]	6.6±0.40[b]	
PLA/PBS/PEO-NE-4	0.14±0.01[b]	15.0±0.68[a]	6.4±0.38[b]	

PEO-NE= Patchouli essential oil nano emulsions, PLA= Polylactic acid, PBS=Polybutylene succinate. The data was presented in triplicate with a mean ± standard deviation. According to Tukey's test, a-b different superscripts within the same column indicate significant differences between formulations ($p < 0.05$).

Based on Table 1, the texture of the PLA/PBS film was improved with the addition of PEO-NE. The PLA-PBS film integrated with PEO-NE was observed to be more translucent, smooth appearances, not brittle, and slightly yellowish. Hence, it was noticed the physical appearance of PLA/PBS films with PEO-NE was slightly better as compared with neat PLA and PBS films. Peng *et al*. (2014), observed similar results as this study that the physical characteristics and antibacterial activities of EO-chitosan films have been considerably improved as compared to control chitosan films.

Thicker films provide more security for food products (Silva *et al.*, 2019). But since thickness impacts the mechanical characteristics of films, thickness is an important parameter in film characterisation. The thickness of PLA-PBS film was affected by the concentration of the PEO-NE. According to Table 1, the thickness of the films increased from 0.05 mm to 0.14 mm with an increasing concentration of PEO-NE from 0 % to 4 %. The results show a significant difference ($p \leq 0.05$) in terms of film thickness from 0% to 4% (v/v) of the PEO-NE. The thickness of the film was slightly varied when the concentration of PLA-PBS integrated with PEO-NE was increased. When the addition of 1% of PEO-NE in PLA-PBS film increased, the thickness increased from 0.05 to 0.12 mm. The thickness of the PLA-PBS film integrated with 2% of PEO-NE was enhanced from 0.05 to 0.13 mm whereas for concentration 4% is 0.05 to 0.14 mm.

Khaleque *et al.* (2016) highlighted similar research that film thickness increased when different amounts of clove oil and cinnamon oil were added, respectively. According to Luzi *et al*. (2016), incorporating a chitosan matrix with EO increased the thickness value. The PLA-PBS integrated with PEO nanoparticles was thicker compared to the PLA-PBS control films. Therefore, the elasticity of the film increased as the concentration of the PEO-NE in PLA-PBS film increased. According to Rossi-Marquez *et al*. (2023), discussed a similar study on film thickness affects the elasticity (E%), with the thicker film having more elasticity than thinner film due to a higher amount of material per unit length, meanwhile Ghasemlou *et al.* (2011) stated that thinner film is tougher to handle.

3.2 Mechanical Properties

Tensile strength, a critical property for film integrity, reveals a nuanced response to PEO-NE incorporation. The mechanical properties of PLA/PBS at different ratios are presented in Table 1. The tensile strength (TS) and elongation at break (EaB) of the films were significantly ($p<0.05$) affected by the loading of PEO-NE. Specifically, incorporating PEO-NE at 1%, 2%, and 4% concentrations reduces the tensile strength of PLA/PBS films, deviating from the control film's baseline of 17.0 MPa. This trend aligns with studies on various EOs in polymer matrices, where a decrease in tensile strength is observed due to the plasticizing effect of the oils, which weakens intermolecular interactions and enhances the mobility of the polymer chains Zabidi *et al*. (2023). Research involving nettle EO nano emulsions added to corn starch-chitosan films demonstrated a decrease in tensile strength, supporting the notion that essential oils disrupt polymer chain interactions, thereby increasing flexibility and reducing mechanical strength (Kalateh-Seifari *et al*., 2021).

A similar trend by Rui *et al*. (2024), in the chitosan films with clove EO nano emulsions. While these films experienced reduced tensile strength, they exhibited enhanced properties such as improved moisture resistance. This further confirms that the introduction of EOs, regardless of the specific polymer matrix, generally results in reduced tensile strength due to the plasticizing nature of the oils. These consistent findings reinforce the understanding that EO nano emulsions interact with polymer structures in a way that modifies

their mechanical properties, leading to decreased tensile strength across different types of films, including PLA/PBS.

In contrast to tensile strength, the elongation at break (EaB) of the films is significantly affected by the addition of PEO-NE, with a significant decrease in flexibility across all PEO-NE concentrations. The control film displays an elongation at a break of 13.9%, indicative of a more ductile material. However, the incorporation of PEO-NE reduces this value significantly (p<0.05), with films containing 1%, 2%, and 4% PEO-NE achieving elongation percentages of 6.8%, 6.6 % and 6.4%, respectively (see Table 1). This reduction by nearly 50% suggests that PEO-NE imparts rigidity to the PLA/PBS matrix, potentially by restricting polymer chain mobility or increasing matrix density. Such a decrease in flexibility is an important consideration, as it may limit the use of PEO-NE-modified PLA/PBS films in applications requiring high elasticity or resilience where dimensional stability and resistance to stretching are priorities, such as in packaging that requires structural integrity. EO nanoemulsions often reduce elongation at break, indicating increased rigidity. Cinnamon oil nanoemulsions in corn starch films, for instance, led to lower tensile strength but increased elongation, thus offering more flexibility but at the cost of strength (Amiri *et al*., 2019). Films with clove oil also showed that flexibility could be either enhanced or reduced depending on the specific interaction with the polymer matrix (Vahedikia *et al*., 2019).

3.3 Antibacterial Testing of The PEO Nanoparticles Against Foodborne Pathogens

Tables 2 show the antibacterial activity of PLA-PBS film integrated with PEO-NE at various concentrations against the major foodborne pathogens; *P. aeruginosa*, *S. aureus*, *E. coli*, *S. typhimurium*, *K. pneumonia*, and *S. mutans*. These bacteria were chosen to represent a diverse range of species commonly encountered in both foodborne and environmental settings, facilitating a comprehensive study of their interactions and responses to antimicrobial agents. This study shows that the incorporation of PEO-NE can inhibit foodborne pathogens. Bilcu *et al.* (2014), discussed that patchouli and ylang-ylang EO primarily prevented *S. aureus* biofilm growth during the early adhesion phase. PEOs have been shown to have antibacterial properties and repellent activities against *S. aureus* (Yang *et al.*, 2013).

Furthermore, the prepared PLA-PBS film alone does not have any antibacterial properties hence with the addition of the PEO-NE, the film was shown to inhibit the foodborne pathogens (Table 2). The PEO-NE can inhibit foodborne pathogens, especially *S. aureus* (Bilcu *et al.*, 2014). The PEO-NE might become degraded when incorporated into the film if the oil is not encapsulated. According to Maes *et al.* (2019), the primary reason for encapsulating emulsion-based was to protect the EO from outside aggression and degradation. The inhibitory property against the foodborne pathogens of PEO-NE oil is reduced when incorporated into the PLA-PBS film in this study.

Table 2: Inhibition zone of various concentrations of PEO nanoparticles and its effects on *E. coli*, *S. aureus*, *P. aeruginosa*, *S. Typhimurium*, *S. mutans*, and *K. pneumonia*.

Films	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*	*S. mutans*	*K. pneumonia*
PLA/PBS (control)	-	-	-	-	-	-
PLA/PBS/ PEO-NE1	5.50 ± 0.50^{a}	4.00 ± 0.50^{bc}	4.67 ± 0.58^{ab}	3.17 ± 0.29^{cd}	3.83 ± 0.29^{bc}	2.17 ± 0.29^{d}
PLA/PBS/ PEO-NE2	7.17 ± 0.76^{a}	5.83 ± 0.58^{ab}	5.67 ± 0.58^{b}	4.67 ± 0.29^{b}	4.83 ± 0.29^{b}	4.67 ± 0.29^{b}
PLA/PBS/ PEO-NE-4	10.17 ± 0.76^{a}	7.17 ± 0.76^{b}	6.50 ± 0.50^{bc}	5.33 ± 0.58^{cd}	5.17 ± 0.29^{cd}	4.50 ± 0.50^{d}

PEO-NE= Patchouli essential oil nano emulsions, PLA= Polylactic acid, PBS=Polybutylene succinate. The data was presented in triplicate with a mean ± standard deviation. According to Tukey's test, a–d superscripts within the same row indicate significant differences between measurements of the inhibition zone ($p<0.05$).

As shown in Table 2, the inhibition zones increased with higher concentrations of PEO-NE. For instance, the inhibition zones for *E. coli* ranged from 5.50 mm to 10.17 mm, while for *S. aureus*, it increased from 4.00 mm to 7.17 mm. Similar trends were observed for *P. aeruginosa, S. typhimurium, S. mutans*, and *K. pneumonia* as the concentration of PEO-NE increased from 1% to 4%. According to Yang *et al.*, (2013), the MIC test for PEO revealed a value of 4.5 mg/mL against *S. aureus*, indicating antimicrobial activity at this concentration. The study also tested the individual components of PEO, such as patchoulol and pogostone, which are known to contribute to its antimicrobial properties. The results highlight the potential of PEO as a complementary therapeutic option, particularly in managing infections. In conclusion, further research, including additional MIC tests, is needed to fully explore the potential of PEO in this area (Yang *et al.*, 2013; Isnaini *et al.*, 2022).

The study by Bilcu *et al.* (2014), focused on the use of magnetic nanoparticles combined with EOs, including patchouli oil, to create novel nano biosystems for medical applications, specifically targeting the adhesion and biofilm development of *S. aureus* and *K. pneumoniae* on catheter surfaces. Their results showed that PEO, when stabilised by iron oxide@C14 nanoparticles, was particularly effective at inhibiting the initial adherence phase of *S. aureus* biofilm formation, aligning with previous studies on the antimicrobial potential of patchouli EO. Thus, the findings underscore the multi-faceted antimicrobial properties of patchouli EO, which not only inhibits bacterial growth but also plays a crucial role in preventing biofilm formation, a major challenge in chronic infections and medical device-related issues. These nano biosystems, which combine patchouli oil with nanoparticles, represent a promising approach to improving the resistance of medical surfaces to microbial

colonisation, opening new possibilities for biomedical coatings that are both anti-adherence and anti-biofilm (Isnaini *et al.*, 2022).

The highest effective concentration of PEO-NE against *E. coli, S. aureus, P. aeruginosa, S. typhimurium, S. mutans,* and *K. pneumonia* was 4 %. However, there was no significant difference ($p>0.05$) between PEO-NE into PLA-PBS film of 1 % and 2 % for *E. coli* and *P. aeruginosa,* 2 % and 4 % for *S. aureus, S. typhimurium, S. mutans* and *K. pneumonia* (Table 2). In comparison to all other foodborne bacteria, the PLA-PBS film integrated with PEO-NE were observed to have the biggest inhibitory zone in *E. coli* at all concentrations (10.17 mm). Thus, as the concentration of PEO-NE in PLA-PBS increases, the zone of inhibition of pathogenic bacteria has a bigger diameter. PEO-NE is less effective against *K. pneumoniae* and *S. typhimurium*. This could be attributed to several factors, including the inherent resistance mechanisms of these bacteria, their biofilm-forming capabilities, and the specific chemical composition of patchouli oil, which may not target the pathways or structures crucial for inhibiting these pathogens. To conclude, the inhibition zone of PEO-NE was not as large as the standard inhibition zone measurements, but it proves that PEO-NE had the potential to inhibit foodborne bacteria.

According to Swamy, M.K., *et al.* (2015), PEO demonstrated strong antibacterial effects, according to molecular docking technologies and in vitro antimicrobial tests. Due to strong antimicrobial effects, the particularly potent antibacterial activity of pogostone and (-)-patchouli alcohol, patchouli oil has broader therapeutic prospects in bacterial infection (Abdul *et al.*, 2022).

Liu *et al.* (2016) stated that chitosan has antibacterial properties against gram-negative bacteria like *S. typhimurium, E. coli, Vibrio parahaemolyticus,* and *P. fluorescens,* as well as gram-positive bacteria like *Listeria monocytogenes, S. aureus, Bacillus cereus, Bacillus megaterium, Lactobacillus brevis, Lactobacillus bulgaricus,* and *Lactobacillus plantarum*.

3.4 Physical Condition of The Chicken Meat Storage

The condition of the chicken meat storage was observed for 15 days at 4℃ shown in Figure 1.

Based on Figure 1, the chicken meat wrapped in control film was easily spoiled compared to the chicken meat wrapped in 4% PLA-PBS film integrated with PEO-NE. Furthermore, from day 12 to 15, the chicken meat wrapped in PLA-PBS film with PEO-NE showed slight stickiness and a mild odour. In contrast, the control samples began showing a strong odour, yellowing, and slimy texture as early as day 6. According to El Kadri *et al.* (2020), explained that chicken meat will easily spoil if kept in the "danger zone" of 4℃ to 60℃ for more than a few days. This is a temperature range where bacteria grow exponentially, further increasing the risk of foodborne illness. Similar studies focused on using PLA film to test the effectiveness of composite films against *S. typhimurium* in chicken

samples. The results showed that films containing silver-copper nanoparticles (Ag–Cu NPs) and 50% clove essential oil (CEO) exhibited antibacterial action over 21 days when stored under refrigerated conditions (Ahmed *et al.*, 2018).

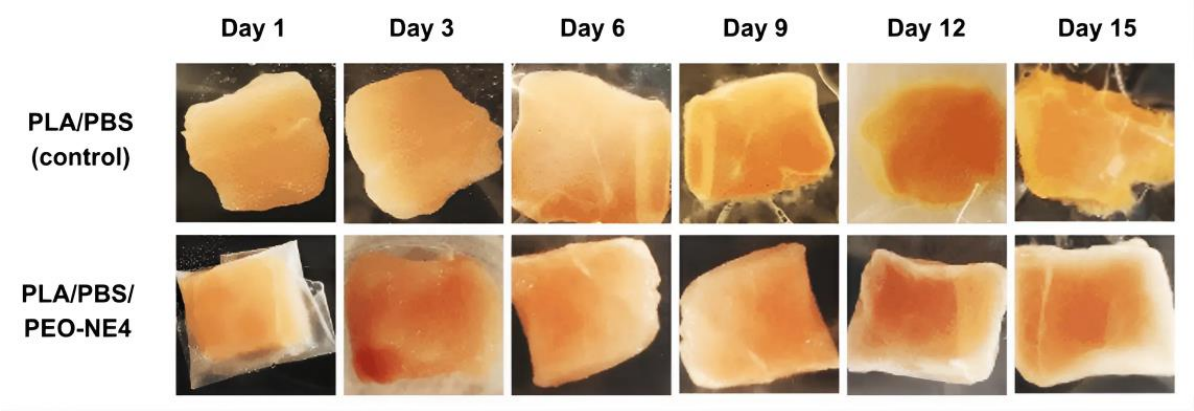


Figure 1. The physical condition of the storage of fresh chicken with and without 4% PLA-PBS film integrated with PEO-NE.

Therefore, by the addition of the PEO-NE into the PLA-PBS film, the shelf life and texture of the chicken were enhanced. This is due to the EO's ability to suppress lactic acid bacteria, which are known to spoil chicken meat. Lucera *et al.* (2012) observed similar research that the EOs of balm and thyme effectively reduced lactic acid bacteria, which are frequent spoilage-related anaerobic microorganisms that occur in fresh meats under humid circumstances and improved their shelf life. It is interesting to note that PEO-NE could reduce chicken meat spoilage by inhibiting the spoilage bacteria. According to Vilela *et al.* (2016), differences in the biochemical properties of the meat, as well as the composition of the microbiota during storage, are caused by variances in EO content and the synergistic effect of their constituents.

3.5 Storage Test of Chicken Meat Based on pH Level.

The pH level of meat is important in determining its quality. At rigor mortis, an unstressed animal would have a pH of 5.4 to 5.8, but a stressed animal would have DFD (dark, firm, dry) meat with a pH of 5.9 to 6.5 (Yu *et al.*, 2005). Table 3 shows the trend of chicken meats' pH value of the unwrapped film, control film, and film integrated with various PEO-NE (treatment groups) during storage for 15 days at 4°C.

Table 3. The pH of the storage of fresh chicken with and without PLA-PBS film integrated with different concentrations of PEO nanoparticles.

Days	Unwrapped	PLA/PBS	PLA/PBS/PEO-NE1	PLA/PBS/PEO-NE2	PLA/PBS/PEO-NE4
0	5.60 ± 0.02^{A}	5.62 ± 0.02^{A}	5.61 ± 0.01^{A}	5.62 ± 0.01^{A}	5.60 ± 0.01^{A}
3	6.07 ± 0.15^{A}	5.87 ± 0.05^{AB}	5.71 ± 0.10^{BC}	5.63 ± 0.10^{BC}	5.63 ± 0.05^{C}

Days	Unwrapped	PLA/PBS	PLA/PBS/PEO-NE1	PLA/PBS/PEO-NE2	PLA/PBS/PEO-NE4
6	6.43±0.15^{A}	6.37±0.11^{A}	6.13±0.30^{AB}	5.80±0.10^{B}	5.73±0.15^{B}
9	7.07±0.11^{A}	7.00±0.05^{A}	6.93±0.05^{A}	6.30±0.30^{B}	6.10±0.20^{B}
12	7.23±0.15^{A}	7.17±0.11^{A}	7.17±0.10^{A}	6.87±0.15^{AB}	6.63±0.20^{B}
15	7.53±0.11^{A}	7.37±0.11^{AB}	7.00±0.30^{BC}	7.03±0.11^{BC}	6.90±0.10^{C}

PEO= Patchouli essential oil, PLA= Polylactic acid, PBS=Polybutylene succinate. The data was presented in triplicate with a mean ± standard deviation. According to Tukey's test, A-C different superscripts within the same column indicate significant differences between formulations (p<0.05).

According to Hertanto *et al.* (2018), the pH of fresh chicken meat ranges from 5.30 to 6.50. The accumulation of amines and ammonia due to bacteria growth can be attributed to changes in the pH value of chicken meat. As the bacteria degrade amino acids, ammonia accumulates and raises the pH of the chicken meat (Radha *et al.*, 2014). In addition, studies on chicken breast meat storage have shown that pH and bacterial growth have a positive correlation (Cortez-Vega *et al.*, 2012). Besides that, it was suggested by Bazargani-Gilani *et al.* (2015) that the rise in the pH value of chicken meat during storage may be because of a rise in volatile bases caused by the activity of endogenous enzymes such as protease and lipase.

The initial pH value of chicken meat in the present study was 5.60 (Table 3). According to Kim *et al*. (2018), similar findings stated the initial pH value of fresh beef is 5.87. The pH of chicken meat increased considerably after 15 days of storage at 4°C. Based on Table 5, the chicken meat wrapped with PLA-PBS film integrated with various concentrations of PEO-NE spoiled later than the control PLA-PBS film as well as unwrapped chicken meat. In the control group, the pH of chicken meat rose greater than the pH of chicken wrapped with PLA-PBS integrated with PEO-NE.

The pH value of chicken meat samples in the control group varied from the value of 5.62 to 7.37 from day 0 to day 15 as shown in Table 3. In contrast, the pH values of chicken meat samples wrapped in PLA-PBS film integrated with different concentrations of PEO-NE increased during the storage period. The pH ranged from 5.60 to 6.90 for samples with 4.0% PEO-NE, while samples with 1.0% and 2.0% PEO-NE showed a pH increase from approximately 5.60 to 7.00. The pH value of unwrapped chicken meat varied from 5.60 to 7.53 from day 0 to day 15. However, the current findings were consistent with Kim *et al.* (2018), who found an elevation in pH value in both the control and treatment groups over the storage period.

The unwrapped chicken meat and chicken meat wrapped with control PLA-PBS film started to deteriorate from day 9 onwards. The chicken meat wrapped with 4 % of PEO-NE in PLA-PBS film spoiled after day 12 onwards. Then, both 1 % and 2 % of PLA-PBS films integrated with PEO-NE deteriorate from day 9 onwards (Table 3). This indicates that as the

storage duration increases, the pH of the chicken meat also rises, which is closely related to bacterial activity. The pH of meat is a critical parameter that reflects its freshness and microbial activity; an increase in pH typically indicates spoilage due to microbial growth and the production of alkaline compounds such as ammonia and amines.

In our study, the pH of chicken meat treated with PEO-NE was consistently lower than that of the unwrapped chicken meat and wrapped with control film, PLA/PBS. This suggests that PEO-NE effectively inhibits microbial growth, thereby delaying spoilage and maintaining lower pH levels. Research supports the notion that EO nano-emulsions can exert antimicrobial effects, which is crucial in maintaining meat quality. This pH data supports the findings that PEO-NE contributes to the preservation of the chicken samples compared to the control. Thus, the PLA-PBS film integrated with 4% of PEO nanoparticles enhanced the shelf life of chicken meat from day 9 to 12. The control sample and unwrapped chicken meat were unsafe for consumption as a pH value above 7 from day 9 of storage onwards.

4. Conclusions

In conclusion, given their remarkable antibacterial properties and ability to extend shelf life, PLA-PBS films incorporated with patchouli EO nano emulsion have the potential to revolutionise commercial food packaging. The PEO-NE had a significant impact on the PLA-PBS film's antibacterial properties and the shelf life of chicken meat. The PEO-NE had the potential to inhibit foodborne pathogens. The incorporation of PEO-NE was proven to enhance the film’s thickness and antibacterial properties against foodborne pathogens. Moreover, the PEO-NE integrated film is an important tool for preserving the quality of chicken and extending its shelf life. The PLA-PBS film integrated with PEO-NE had improved antibacterial properties and can prolong the shelf life of chicken meat, which might have the potential to be used as active food packaging. While the PLA-PBS films with PEO-NE show great potential for active food packaging, further research is essential to optimise production methods and enhance overall performance. Future studies should explore the application of these innovative films with various food products and investigate different EOs. Each EO may offer unique active compounds that could broaden the spectrum of targeted pathogens. By examining a variety of EOs, researchers can identify optimal formulations that maximise both performance and consumer acceptance, paving the way for more effective and sustainable food packaging solutions.

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