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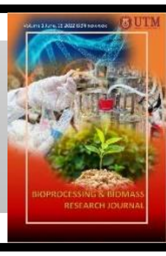

Research Article

Formulation and Characterization of Antimicrobial Packaging from Chitosan and Curry Leaf (*Murraya Koenigii*) Essential Oil for Vegetables Packaging

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ABSTRACT

This study formulated chitosan-based antimicrobial films incorporating curry leaf (*Murraya koenigii*) essential oil using the film casting method. The films were characterized with 0%, 1%, and 2% concentrations of essential oil, and evaluated through physical, mechanical, and biological tests. Physical and mechanical tests included tensile testing, film thickness, pH, and water solubility. The film thickness ranged from 0.04 mm to 0.08 mm, and pH values ranged from 3.84 to 4.93. Water solubility was 22.7%, 20.56%, and 40.17% for films with 0%, 1%, and 2% essential oil, respectively. Tensile strength was assessed alongside antimicrobial properties using the zone of inhibition method against *E. coli* and *Bacillus cereus*. The films showed significant inhibition zones for the gram-negative *E. coli* and minimal inhibition for the gram-positive *B. cereus*. Shelf-life tests on dwarf pak choi (*Brassica rapa chinensis*) and baby kailan (*Brassica oleracea L.*) demonstrated an extended shelf-life of up to 14 days using common storage methods such as on-top placement and wrapping. This study concludes that chitosan-based films infused with curry leaf essential oil exhibit promising antimicrobial properties, making them a viable food packaging material with considerable strength, elongation, and flexibility, effectively inhibiting foodborne pathogens.

INTRODUCTION

The reliability of the food supply plays an important role in voyages and cruise ships. During voyages and cruises, passengers rely on the ship's food supply to meet their nutritional needs. A reliable and consistent food supply ensures that passengers have access to regular meals, maintaining their health and well-being throughout the journey. One specific aspect that plays a significant role in meeting this challenge is the need for antimicrobial packaging to increase the shelf life and freshness of vegetables (Hosier, 2023).

However, the conventional vegetables packaging has its own problem that is the limited antimicrobial effect on the vegetables which prevents them from browning and keeps them fresh. This is because the conventional packaging only uses normal plastic film and does not release any bio-active substances to maintain the freshness of the vegetables. This can lead to the microbial contamination and accelerated spoilage of vegetables, reducing their shelf life and freshness. Therefore, the incorporation of a biodegradable

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with antimicrobial properties packaging in the food supply chain on cruise ships is essential to ensure the freshness and quality of vegetables. By inhibiting microbial growth, it helps maintain the nutritional value of the food, reduces the risk of foodborne illnesses, minimizes food waste, and ensure the health of the crew members and passenger during their journey.

Chitosan, derived from chitin found in the shells of crustaceans, is a natural polymer with antimicrobial properties. It exhibits broad-spectrum activity against bacteria, fungi, and viruses (Raafat & Sahl, 2009). Chitosan is biodegradable, non-toxic, and renewable, making it an excellent choice for sustainable antimicrobial packaging. Among the antimicrobial packaging materials, research on the chitosan-based films incorporated with the essential oils is among the most popular combinations to achieve substantial antimicrobial activity (Casalini & Baschetti, 2022). Chitosan exhibits potent antimicrobial properties against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. It can inhibit the growth and survival of various pathogens that cause food spoilage and foodborne illnesses. The antimicrobial activity of chitosan is attributed to its ability to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with cellular processes (Atay, 2019). In addition, chitosan exhibits biodegradable properties. Being a natural material, chitosan is considered safe for food contact applications. It is also biodegradable, meaning it can be broken down by natural processes and does not contribute to long-term environmental pollution. Furthermore, chitosan's biodegradability adds to its environmental benefits. When chitosan-based packaging reaches the end of its useful life, it can be broken down by natural processes, such as the action of microorganisms or enzymes, into simpler, non-toxic components. This degradation process is environmentally friendly and reduces the accumulation of persistent waste in landfills or ecosystems.

Curry leaf (*Murraya koenigii*) essential oil, derived from the curry tree, is known for its strong antimicrobial properties. This essential oil effectively inhibits the growth of foodborne pathogens such as *Escherichia coli* and *Bacillus cereus* by disrupting their cell membranes. Its potent antibacterial, antifungal, and antioxidant activities make it a valuable natural preservative. Integrating curry leaf essential oil into chitosan-based films enhances their antimicrobial efficacy, extends the shelf-life of perishable foods, and offers a sustainable, chemical-free solution for food packaging (Rajendran et al., 2014).

The purpose of this research is to study the formulation and characterization of antimicrobial packaging from chitosan and curry leaf essential oil (EO) for vegetables packaging by film casting method. The film is evaluated based on the physical, mechanical and biological test. The physical and mechanical test of the film included tensile test, film thickness, pH and water solubility.

MATERIALS AND METHOD

Materials

Medium molecular weight (MW) chitosan (⩾ 75% deacetylated) purchased from Sigma-Aldrich, Tween20 (QReC), distilled water, glacial acetic acid purchased from Supelco, *Bacillus cereus* ATCC 10876, *E. coli* ATCC 10536, curry leaf (*murraya koenigii*) essential oil purchased from R.K's Aroma, dwarf pak choi (*Brassica rapa chinensis*) purchased from local supermarket, baby kailan (*Brassica oleracea* L.) purchased from local supermarket, knife, beaker, heating plate, magnetic stirrer, water bath, thermometer, incubator (Memmert, Peltier-cooled incubator IPPeco), oven (Memmet UM400), petri dish, electronic balance (HR-250AZ), digital vernier calliper (150 mm), digital micrometer (Mitutoyo, QuantuMike IP65) and universal testing machine (Zwick/Roell Z020).

Preparation of Chitosan Film

Chitosan of 1.5% (w/v) was dissolved in 1% (v/v) of glacial acetic acid solution. The solution was heated at 60 °C in a water bath shaking incubator at 100 oscillation/min for 60 min. The chitosan solution was filtered through a coarse sintered glass filter to remove undissolved impurities. The solution was cooled to room temperature and EO was added with Tween to obtain the final concentrations of 0, 1 and 2 (w/v) of EO in the chitosan film-forming solution. The solution was stirred for 15 min (or with the help of homogenizer). The filmogenic mixture was casted on plastic petri dishes with the weight of 10 g. The film was dried in the chamber of 55 °C with humidity of 50% for 24 h. The process was repeated with different EO (Elshamy et al., 2021).

Vegetable Shelf-life Test

The vegetables brassica rapa (dwarf bok choy) and brassica oleracea (baby kailan) were freshly prepared and the vegetables was placed onto the 1% chitosan film and wrapping method in refrigerator under temperature 5 ºC. The browning intensity and surface appearance of fresh-cut vegetables were analysed and recorded. The observation is last for 14 days (Vieira et al., 2022).

Water Solubility Test

The film was cut into small pieces, 1 x 4 cm in size, and was dried at 100 °C in an oven weighed to the nearest 0.01 g for the initial dry weight. The film was then placed in a beaker with 100 ml of distilled water and gently shaken for 2 h. The remaining parts of the film were dried at oven. The weight of film before and after was recorded.

Water solubility (percent) was calculated using the following (**Eq. 1**):

$$\text{Solubility\%} = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100\% \quad \textbf{(Eq. 1)}$$

Film Thickness and Tensile Test

The thickness of the film was measured using a digital micrometer (Mitutoyo, QuantuMike IP65) The micrometer has a precision of ±0.001 mm. To obtain accurate measurements, five random positions on each film were measured, and the mean thickness with the standard deviation was reported.

Tensile tests were carried out using a material testing machine. Prior to testing, the samples were prepared with dimensions of 80 mm (length) and 10 mm (width). Each sample was clamped between grips, and force and deformation were recorded during extension at a rate of 10 mm/min, with an initial grip distance of 30 mm. Tensile strength (TS), elongation at break (EB), and Young's modulus (YM) were measured in five replicates for each sample. The average values from five repetitions of each sample were reported, along with their respective standard deviations (Tee et al., 2016).

Antimicrobial Activity Test

The bacteria strain used in this study was *Bacillus cereus* ATCC 10876 and *E. coli* ATCC 10536. All bacterial strains have been inoculated in blood agar and incubated for 24 h at 37 °C. Agar well diffusion method was used to determine the antimicrobial activity of chitosan film. One hundred microliters of bacterial culture were spread on Muller-Hinton agar (MHA) plates. A 6 mm diameter size of film was cut and placed into plates were incubated in oven at 37 °C for 24 h. The diameter of the growth inhibition zone around the tested film was measured and recorded (Balouiri et al., 2016).

RESULTS AND DISCUSSION

Antimicrobial Test of Chitosan Film incorporated with Curry Leaf

The antimicrobial activity of the film had been evaluated based on biological antimicrobial properties using zone of inhibition method on gram-negative bacteria, *E. coli* and gram-positive bacteria, *Bacillus cereus*. The original size of the tested film is 6 mm. From the **Table 1**, there is no zone of inhibition on control film from both microbes. Analysis of the results presented in **Table 2** shows that zone of inhibition of gram-negative bacteria is greater than gram-positive bacteria. The results obtained is complimentary to other study that also propose that Gram-negative bacteria might be more vulnerable to chitosan compared to Gram-positive bacteria (Liu et al., 2021). This is due to Bacillus cereus is a Gram-Positive Bacteria, and it has thick layer of peptidoglycan (murein) that protecting the cell from bursting while Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Chitosan with its polycationic structure interacts with negatively charged components found on the surface of Gram-negative bacteria, such as lipopolysaccharides and proteins (Guarnieri, 2022).

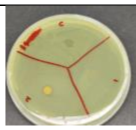
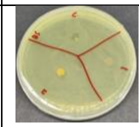
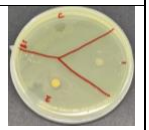
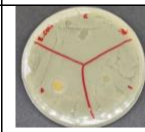
On the other hand, a greater zone of inhibition was observed with increased essential oil concentration. At lower concentrations, there was only a minimal inhibitory effect on microbial growth compared to the lower essential oil concentration and control samples (Bachir et al., 2012). Generally, chitosan film incorporated with curry leaf essential oil shows a reduction on the growth colonies and play important role in the antimicrobial activity (Schweitzer et al., 2022).

Table 1 Effect of essential oil-based chitosan film based on different concentrations of curry leaf essential oil.

Curry Leaf Oil Concentration**	Zone of inhibition (mm)	
	Gram-Positive Bacteria	Gram-Negative Bacteria
	Bacillus cereus	*Escherichia coli*
CL-EO-0	4.50 ± 0.41*	5.50 ± 0.92*
CL-EO-1	5.97 ± 0.31*	7.87 ± 1.41*
CL-EO-2	6.03 ± 0.40*	11.23 ± 0.33*

* Means ± standard deviation
**Curry leaf(CL)-Essential oil (EO)- (concentration of EO%)

Table 2 Zone of inhibition on *Bacillus cereus* and *Escherichia coli.*

Test	1	2	3
Bacillus cereus			
Escherichia coli			

Physical appearance

From the findings of physical appearance of chitosan films based on different concentrations of curry leaf essential oil, the control with 0% chitosan essential oil shows a slightly yellowish coloration, however, in **Table 3**, with the increasing concentration of essential oil, the colour of the film is turning darker yellowish and thicker texture.

Table 3 Appearance of chitosan film with different concentration of curry leaf essential oil.

Control	1% curry leaf essential oil	2% curry leaf essential oil
		

Film Thickness and Tensile Test

The mechanical strength of the chitosan film prepared was presented on **Table 4**. The tensile modulus, Et, is varied among the samples. In 0%, 1%, 2% essential oil concentration, the tensile modulus is 1126.22 MPa, 1033.15 MPa, and 1297.68 MPa, respectively. The p-value in t-test between the data from control and 1% EO is 0.322 which indicates the tensile modulus between them has 67.8% significant difference. While on the other hand, the p-value in t-test between the data from control and 2% EO is 0.256 which means the tensile modulus between the control and 2% EO has 74.4% significant difference. Tensile Modulus (stiffness) is the ratio of its tensile stress (force per unit area) to its strain (relative deformation). The highest stiffness is from the 2% essential oil concentration. This behavior is related to the higher thickness obtained with average thickness of 0.084 mm on 2% concentration essential oil, 0.047 mm on 1% concentration essential oil and 0.036 mm on 0% concentration essential oil.

In 1 % curry leaf essential oil chitosan film, it shows the highest mechanical properties in yield strength, tensile strength, and stress at break, that is 15.02%, 32.54 MPa and 32.46 MPa, respectively. It shows that 1% curry leaf essential oil can incorporate better in chitosan forming solution and exhibit better elongation and maximum load that film can support (Thakhiew et al., 2013).

Table 4 Mechanical properties of the chitosan film incorporated with different concentration of curry leaf essential oil, tensile modulus, yield strain, tensile strength, stress at break and strain at break.

PARAMETER	Type of film		
	Control*	CL-EO-1*	CL-EO-2*
Tensile Modulus, E_t (MPa)	1126.22 ± 272.52	1033.15 ± 215.73	1297.68 ± 295.14
Yield Strain, ε_Y (%)	14.67 ± 1.56	15.02 ± 0.71	12.73
Tensile Strength, σ_M (MPa)	19.28 ± 4.39	32.54 ± 12.00	30.00 ± 8.88
Stress at break, σ_B (MPa)	19.22 ± 4.46	32.46 ± 12.10	29.92 ± 8.98
Strain at break, ε_B (%)	8.02 ± 2.93	16.69 ± 12.95	21.00 ± 36.03

*Means ± standard deviation

Water solubility Test

Water solubility is important in determining biodegradability and environmental impact. The water solubility of chitosan film influences its rate of degradation when exposed to environmental conditions. Films with higher water solubility may degrade more rapidly, making them suitable for applications where quick biodegradation is desirable. However, films with lower water solubility can maintain its original mechanical properties when the films are exposed to moisture and vapour. Therefore, understanding and controlling the water solubility of chitosan films are essential for optimizing their performance in various conditions. In the study, the water solubility of 0%, 1% and 2% concentration curry leaf chitosan essential oil are 22.70%, 20.56% and 40.17%. The 1 % EO has the lowest water solubility might be due to the strong interaction between the phenolic compounds in the curry leaf essential oil and the polysaccharide chain of chitosan. This interaction might reduce the accessibility of the polymer's amine and hydroxyl groups to interact with water, resulting in decreased water solubility of the film when supplemented with the extract. This decrease indicates the strong cohesion between the 1% curry leaf essential oil and chitosan solution.

Table 5 Water solubility test of chitosan film incorporated with different concentration of curry leaf essential oil.

	Concentration of curry leaf EO					
	Control		1%		2%	
	Before	After	Before	After	Before	After
1	0.0317g	0.0244g	0.0329g	0.0262g	0.0710g	0.0415g
2	0.0331g	0.0257g	0.0287g	0.0228g	0.0758g	0.0464g
Difference	-22.70%		-20.56 %		-40.17%	

Vegetable Shelf-life Test

Shelf-life test involves monitoring the quality of vegetables over 14 days to determine how long they remain acceptable for consumption when packaged with chitosan films. Vegetables brassica rapa (dwarf bok choy) and brassica oleracea (baby kailan) were tested using 1% concentration of curry leaf chitosan film. The choice of film tested was due to overall performance including physical texture, mechanical and antimicrobial properties. The method to test on the shelf-life of both selected vegetables were wrapping method and place-on-top method. The temperature is set at 5 °C. Based on the result shown in **Table 5** and **Table 6**, both vegetables shown better visual appearance (less browning) and possess better texture than in control. Although the browning effect of vegetable might be due to enzymatic reaction, but the microbial contamination is one of the important factor that affect the shelf-life of vegetable. The comparison of the control (without the chitosan film) with 1% curry leaf essential oil chitosan film is significant at 14 days. There are less browning and dark spot (spoilage) on both wrapping method due to antimicrobial effect from the chitosan film.

Table 5 Shelf-life test on vegetable brassica rapa (dwarf bok choy) using place-on-top and wrapping method for 14 days.

Control / Methods	Days		
	0	7	14
Control			
On-Top-Method			
Wrapping			

Table 6 Shelf-life test on vegetable brassica oleracea (baby kailan) using place-on-top and wrapping method for 14 days.

Control / Methods	Days		
	0	7	14
Control			
On-Top-Method			
Wrapping			

CONCLUSION

In conclusion, the research conducted on formulation and characterization of chitosan film incorporated with curry leaf essential oil has yielded promising results. Through meticulous experimentation and analysis, it has been established that this particular combination offers a synergistic blend of desirable traits essential for effective food packaging. The optimal 1% concentration of curry leaf essential oil in chitosan film has been identified based on criteria such as highest mechanical properties in yield strength, tensile strength and stress at break, that is 15.02%, 32.54 MPa and 32.46 MPa, respectively, greatest antimicrobial activity, water solubility, and shelf-life extension of vegetables up to 14 days. The findings suggest that the developed film holds great potential as a next-generation active food packaging material. With its ability to enhance food preservation, inhibit microbial growth, and maintain product quality over an extended period, this innovative solution stands poised to address the evolving needs of the food industry for sustainable and efficient packaging solutions. Further exploration and refinement of this technology could pave the way for its widespread adoption, contributing significantly to the advancement of food packaging and preservation practices.

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