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Potential of Chitosan-Encapsulated Mango Leaf Extract Nanoemulsion as Fruit Packaging

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Abstract*:* Fruits have a relatively short shelf life. One of the extensively researched preservation methods for fruits is edible coating. Edible coating is a method of coating food products with a polymer layer. Considering the environmental and sustainability aspects, edible coatings were synthesized from biodegradable polymers derived from polysaccharides. Chitosan is a widely developed and researched type of biopolymer due to its antimicrobial activity. In addition to antimicrobial activity, antioxidant properties are important in food packaging applications. Mango leaves (*Mangifera indica* L.) contain various secondary metabolites that play a role in antioxidant activity, such as mangiferin, gallic acid, flavonoids, and gallotanin. In this study, mango leaf extract nanoemulsion was synthesized to improve the antioxidant and anti-UV properties of biopolymer chitosan-based fruit packaging. Mango leaf extract nanoemulsion was synthesized using surfactant and ultrasonication methods. The results of Particle Size Analyzer (PSA) measurements showed that the size of all nanoemulsions was 83-99 nm. Observations with Transmission Electron Microscope (TEM) showed that the nanoemulsion was spherical with a 15-46 nm size range. The UV-Vis spectrophotometer instrument was used to test antioxidant properties using the DPPH reagent and anti-UV activity. These tests showed that adding mango leaf extract concentration to the nanoemulsion system increased DPPH inhibition and absorbance against UV light. The results of the performance test of mango leaf extract nanoemulsion encapsulated by chitosan have a high potential to be used as a bio-based edible coating matrix and a substitute for materials that are difficult to degrade.

Keywords: mango leaf, edible coating, packaging, chitosan, nanoemulsion

Abstrak: *Buah-buahan memiliki umur simpan yang relatif singkat. Salah satu teknik pengawetan buah yang sedang banyak diteliti adalah edible coating. Edible coating merupakan metode pelapisan produk pangan dengan menggunakan lapisan polimer. Dengan mempertimbangkan aspek teknologi berkelanjutan yang ramah lingkungan, edible coating dapat disintesis dari polimer biodegradable yang berasal dari polisakarida. Kitosan merupakan salah satu jenis polimer hayati yang banyak dikembangkan dan diteliti karena memiliki aktivitas antimikroba. Selain antimikroba, sifat antioksidan juga merupakan parameter penting pada aplikasi pengemasan produk pangan. Daun mangga (Mangifera indica L.) mengandung berbagai metabolit sekunder yang berperan pada aktivitas antioksidan seperti mangiferin, asam gallat, flavonoid, dan gallotanin. Pada penelitian ini, dilakukan sintesis nanoemulsi ekstrak daun mangga untuk meningkatkan sifat antioksidan dan anti-UV pada kemasan buah berbasis biopolimer kitosan. Nanoemulsi ekstrak daun mangga disintesis dengan menggunakan surfaktan dan metode ultrasonikasi. Hasil pengukuran Particle Size Analyzer (PSA) menunjukkan ukuran nanoemulsi semua variasi berada pada rentang 83-99 nm. Pengamatan pada Tranmission Electron Microscope (TEM) menunjukkan nanoemulsi berbentuk spherical dengan rentang ukuran 15-46 nm. Instrumen spektrofotometer UV-Vis digunakan untuk menguji sifat antioksidan menggunakan reagen DPPH dan aktivitas anti-UV. Pengujian ini menunjukkan bahwa penambahan konsentrasi ekstrak daun mangga pada sistem nanoemulsi akan meningkatkan inhibisi DPPH dan absorbansi terhadap sinar UV. Hasil uji performa nanoemulsi ekstrak daun mangga yang dienkapsulasi oleh kitosan berpotensi tinggi untuk dimanfaatkan sebagai matriks edible coating berbasis bahan alam dan pengganti material yang sulit terdegradasi.*

Kata kunci: *daun mangga, edible coating, kemasan, kitosan, nanoemulsi*

INTRODUCTION

Indonesia is a tropical country, often referred to as a maritime nation. Its abundant fisheries, agriculture, and plantations are invaluable assets for the Indonesian people. Generally, plantation products such as fruits commonly consumed by the public have a very limited shelf life. These products are highly susceptible to changes in sensory properties (such as smell, color, taste, and morphology) within a short period due to several factors, including oxidation reactions, enzymatic reactions, and microbial activity (Kumar *et al*. 2020). Therefore, a food preservation system is needed to maintain the quality of these products for a longer period.

The most common food preservation techniques recently include refrigeration, freezing, drying, and smoking. However, these preservation methods do not fully inhibit microbial activity and oxidation processes over long durations, resulting in suboptimal shelf-life extension. Another food preservation method is packaging, which protects food products from various contaminants, including chemical substances and biological activities that can degrade their quality (Han *et al*. 2018).

A widely advancing food packaging method is coating technology. This preservation method involves applying a polymer layer to food products, suppressing microbial activity on their surface, minimizing oxidation, and shielding them from various contaminants (Arabpoor *et al*. 2021). Considering sustainable and environmentally friendly technology, coatings can be made using biodegradable polymers derived from polysaccharides, proteins, fats, and their derivatives (Haghighi *et al*. 2020). Various naturally derived polymer compounds utilized in coating technology include chitosan, pectin, alginate, and cellulose. These polysaccharides have been explored as potential substitutes for plastic-based packaging. Chitosan is the most extensively studied and developed among these materials (Priyadarshi & Rhim 2020).

Chitosan is a biopolymer compound synthesized through the deacetylation process of chitin. Chitosan is extracted from chitin using chemical extraction through two process stages: deproteinization and demineralization (Madhu *et al*. 2022). Chitin can be obtained from arthropods (spiders, beetles, scorpions), crustaceans (crabs, shrimp, lobsters), mollusks (squid), and microorganisms (fungi and cell walls) (Haghighi *et al*. 2020). Currently, the primary source of chitin production comes from seafood waste (exoskeletons of crabs, lobsters, and shrimp). By analyzing various parameters and material properties, chitosan is considered a suitable biopolymer for development into active packaging and intelligent packaging (Flórez *et al*. 2022).

Chitosan has good antimicrobial properties against various microorganisms, such as gram-positive bacteria, gram-negative bacteria, and fungi (Kumar *et al*. 2020). Chitosan's ability to enhance food quality as a coating improves when mixed with other substances, supported by the ability of chitosan to form complex compounds with metals (Varma *et al*. 2004). This characteristic enables chitosan to attach to other particles, leading to stronger antimicrobial effects (Priyadarshi & Rhim 2020). Research has shown that adding plant extracts, essential oils, and metal nanoparticles can highly boost chitosan's effectiveness in food packaging.

Natural extracts from various plant parts, such as leaves and fruit peels, have been extensively researched as additives in chitosan. Plant extracts can improve the quality of food packaging. For example, the addition of clove extract to chitosan has been shown to enhance antimicrobial properties against bacteria and pathogens in shrimp stored at low temperatures (Tayel *et al*. 2020). Besides clove extract, the addition of mango leaf extract has also been studied and found to increase the antioxidant properties and strength of chitosan coating films (Rambabu & Bharath 2019). Furthermore, mango leaf extract has been proven to increase tensile strength, enhance the hydrophobicity of the chitosan surface, and reduce water permeability (Rambabu & Bharath 2019).

Currently, extensive research is being conducted on the application of nanotechnology in food packaging. The role of nanotechnology in food products generally includes nanofood, nanocomposites, nanosensors, and nanoemulsions. In this study, the nanotechnology applied is nanoemulsion. Nanoemulsion is a colloidal dispersion consisting of two immiscible solutions, where one of the solutions is dispersed as tiny particles within the other (McClements *et al*. 2021). The formation of nanoemulsions requires the assistance of an emulsifier, which possesses both hydrophilic and hydrophobic groups, facilitating the creation of nanoemulsions and enhancing their chemical stability (Chakraborty & Dhar 2017).

Several tests have demonstrated an improvement in the performance of chitosan as the main material for edible coating with the addition of mango leaf extract nanoemulsion. Research conducted by Rambabu & Bharath (2019) found that incorporating mango leaf extract into chitosan as a bioplastic enhances antioxidant activity, particularly against DPPH (*2,2-diphenyl-1-picrylhydrazyl*) and ABTS (*2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid*)). This study also showed that chitosan layers with mango leaf extract improve tensile strength and hydrophobicity. Analysis using Scanning Electron Microscopy (SEM) confirmed that chitosan layers combined with mango leaf extract are denser and thicker compared to pure chitosan layers (Rambabu & Bharath 2019). In another study, the addition of mango seed and peel extracts in biopolymer-based coatings was also found to enhance antioxidant and mechanical properties (Adilah *et al*. 2018a; Adilah *et al*. 2018b). The experiment involving mango leaf extract in nanoemulsion form expands the exploration of mango plants as active ingredients for edible coatings. Similar to previous studies, antioxidant properties remain the key advantage of mango leaf extract. Therefore, mango leaves and other parts of the plant can be considered suitable active materials for food packaging applications.

Unlike previous experiments, this study does not focus on food packaging in the form of films or layers but rather on edible coating. Mango leaf extract is formulated into a nanoemulsion and combined with chitosan to create an edible coating liquid that has the potential to slow down the fruit ripening process. Various tests were conducted to evaluate the performance of chitosan-encapsulated mango leaf extract as an edible coating. Antimicrobial activity tests were performed on gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Bacillus cereus*. Additionally, tests were conducted to assess its ability to block UV light, which can prevent food quality deterioration caused by photo-oxidation (Botalo *et al*. 2024). Moreover, antioxidant tests were carried out, as high antioxidant activity helps prevent browning, a common issue in cut fruits (Robles-Sánchez *et al*. 2013).

MATERIALS AND METHOD

Materials

The powder of arum manis mango (*Mangifera indica* L.) leaf was obtained from local producers in Indonesia. Ethanol, Tween 80, Span 80, Sodium Tripolyphosphate (TPP), and chitosan powder were obtained from chemical supply stores in West Java. All experimental equipment and testing instruments used in this study were located at the Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung.

Mango Leaves Extraction Method

Mango leaf extract was obtained from an extraction method using 96% ethanol with a soxhlet column. A total of 20 grams of dried mango leaf powder, as the simplicia, was placed into filter paper, formed into a tube, and then inserted into the soxhlet column. The soxhlet column was assembled with a 500 ml boiling flask and a condenser column. Approximately 180 ml of ethanol was added to the boiling flask, which was equipped with a magnetic stirrer. A set of stands and clamps were attached to the soxhlet column to provide support so that the column system would not easily fall. A chiller was connected to the condenser column as a cooling system to facilitate the condensation of vapor formed from heating by the heating mantle on the heating flask. Extraction was carried out for at least three circulations for approximately three hours until a dark green mango leaf extract was obtained.

Mango Leaves Extract Nanoemulsion Synthesis

Mango leaf extract is heated using a water bath until a concentrated paste is obtained, which takes approximately six hours. A total of 0.5 grams of mango leaf extract is mixed with two types of surfactants: 4.25 ml of Tween 80 and 0.75 ml of Span 80. The mixture is then dissolved in water until the total solution volume reaches 100 ml. This combined solution is homogenized using an ultrasonic homogenizer for 30 minutes or until no sediment remains.

Chitosan-Encapsulated Mango Leaves Extract Nanoemulsion Synthesis

Sodium Tripolyphosphate (TPP) is dissolved in water using a magnetic stirrer. Chitosan powder is then added to 1% acetic acid and dissolved in water until a homogeneous solution is achieved. The TPP solution and chitosan solution are mixed at a 1:2 volume ratio of chitosan to TPP. The chitosan-TPP solution is then combined with the previously synthesized mango leaf extract nanoemulsion at a 1:1 volume ratio.

Characterizations and Performance Tests

The particle size of the formulated mango leaf extract nanoemulsion was analyzed using a Particle Size Analyzer (PSA). The size and morphology of the nanoemulsion were confirmed through observation with a Transmission Electron Microscope (TEM). Its stability was assessed using a Zeta Potential Analyzer (ZPA). Key tests for evaluating fruit packaging performance include antimicrobial activity and antioxidant tests. The antimicrobial test involved *Bacillus cereus* as a gram-positive bacteria and *Escherichia coli* as a gram-negative bacteria, using the disc diffusion method and measurement of the inhibition zone on agar media. The antioxidant test was conducted using DPPH reagent, which reacts with samples at varying concentrations to determine absorbance values. Additionally, absorbance testing was performed within the 200–400 nm wavelength range using a UV-Vis spectrophotometer to evaluate the anti-UV potential of mango leaf extract.

RESULT AND DISSCUSION

Characterization of Mango Leaf Extract Nanoemulsion

The nanoemulsion characterization was analyzed using PSA and ZPA instruments based on the Dynamic Light Scattering (DLS) principle. The test results for particle size, polydispersity index, and zeta potential are listed in Table 1. According to the test results, the particle size of both the mango leaf extract nanoemulsion and the chitosan-encapsulated

Tabel 1. Result of particle size, polidispersity index, and zeta potential testing

Sample	Particle size (nm)	Polidispersity index	Zeta potential (mV)
Nanoemulsion	83.30 ± 0.08	0.45 ± 0.15	-2.60 ± 0.15
Nanoemulsion : chitosan (2 : 1)*	84.28 ± 0.08	0.40 ± 0.32	-21.00 ± 0.32
Nanoemulsion : chitosan (1 : 1)**	94.38 ± 0.08	0.48 ± 1.31	-13.76 ± 1.31
Nanoemulsion : chitosan (1 : 2)***	99.34 ± 0.04	0.47 ± 0.34	-12.32 ± 0.34

Notes :
**Ratio of mango leaf extract nanoemulsion solution volume to chitosan volume is 2:1*
***Ratio of mango leaf extract nanoemulsion solution volume to chitosan volume is 1:1*
****Ratio of mango leaf extract nanoemulsion solution volume to chitosan volume is 1:2*

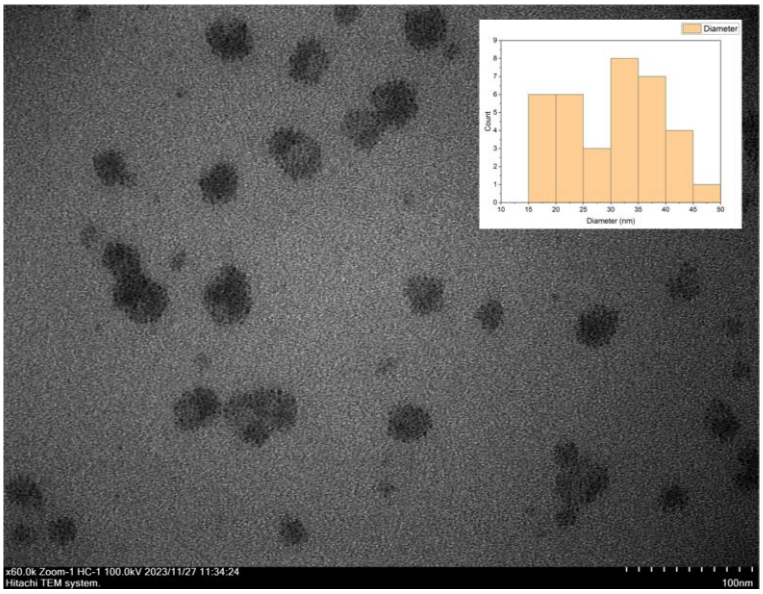

Figure 1. TEM result of chitosan-encapsulated mango leaf extract nanoemulsion at 60k magnification.

nanoemulsion has an average size of less than 100 nm. The chitosan-encapsulated mango leaf extract nanoemulsion increased in size proportional to the varied volume ratios. This indicates that the more chitosan added, the larger the nanoemulsion size. The polydispersity index values for all variations range between 0.40–0.48, showing no significant differences. This suggests that the detected nanoemulsion distribution is relatively uniform. These values are also close to 0.3, which is considered optimal for nanoemulsion systems in water (Yazgan 2020). The zeta potential analysis results indicate that the nanoemulsion without chitosan addition has the lowest negative value compared to the other samples.

To confirm the results presented in Table 1, morphological and particle size analysis was conducted using TEM. The TEM results are shown in Figure 1. Based on Figure 1, the globular shape of the chitosan-encapsulated mango leaf extract nanoemulsion is evenly distributed with varying sizes. According to TEM observations, the formed nanoemulsion size ranges from 15–46 nm. The size values obtained from TEM measurements differ from those observed using PSA, which employs hydrodynamic analysis. Hydrodynamic measurements result in larger particle sizes due to the presence of the dispersing medium used.

Performance Testing of Mango Leaf Extract Nanoemulsion

Antimicrobial, antioxidant and anti-UV tests were conducted to evaluate the potential of mango leaf extract nanoemulsion as fruit packaging. The antimicrobial test results were assessed based on the measurement of the inhibition zone formed on agar media. The test samples included mango leaf extract, mango leaf extract nanoemulsion, chitosan, and chitosan-encapsulated mango leaf extract nanoemulsion, all of which were evaluated for their effect on microbial activity against gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Bacillus cereus*). The antimicrobial test results can be seen in Figure 2 and Table 2.

Based on Table 2, it was found that at the same concentration ratio, mango leaf extract nanoemulsion exhibited better antimicrobial activity than the extract without nanoemulsion formation. This indicates that

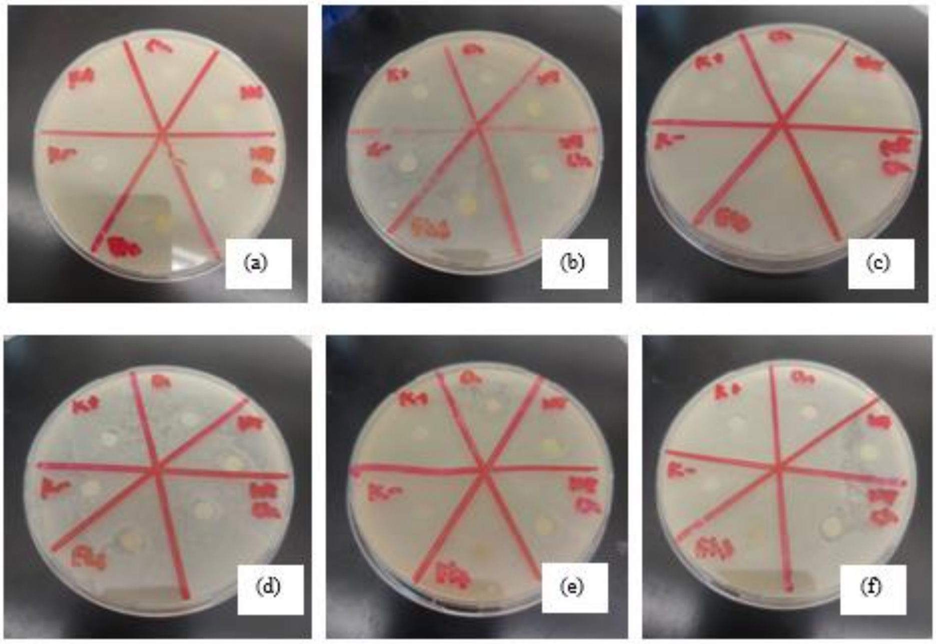

Figure 2. Antimicrobial test results for *Escherichia coli*: (a) plate 1, (b) plate 2, (c) plate 3, and for *Bacillus cereus*: (d) plate 1, (e) plate 2, (f) plate 3

Table 2. Inihbition zones from microbial testing of *Escherichia coli* and *Bacillus cereus*

Sample	Inhibition zone against *Escherichia coli* (mm)	Inhibition zone against *Bacillus cereus* (mm)
Positive control (*Chloramphenicol*)	0.05 ± 0.08	0.69 ± 0.41
Chitosan	0.10 ± 0.08	0.57 ± 0.15
Mango leaf extract nanoemulsion	0.09 ± 0.08	0.56 ± 0.32
Mango leaf extract nanoemulsion + chitosan*	0.11 ± 0.08	1.92 ± 1.31
Mango leaf extract	0.03 ± 0.04	0.44 ± 0.34
Negative control (aquadest)	0.00 ± 0.00	0.00 ± 0.00

Note :
**Using a ratio of mango leaf extract nanoemulsion solution volume to chitosan volume of 1:1*

the secondary metabolites in mango leaves, which act as antimicrobial agents, can be enhanced through nano-scale formulation. The mango leaf extract nanoemulsion combined with chitosan demonstrated the best antimicrobial activity among all samples, as evidenced by the largest inhibition zone. Chitosan, a unique biopolymer, has proven antimicrobial properties. It contains positively charged amine functional groups, which can interact with anionic peptidoglycan in bacterial cell walls, leading to cell wall disruption, intracellular fluid leakage, and bacterial death (Latou *et al*. 2014). Additionally, chitosan forms a protective coating, similar to cellophane, over food surfaces, physically preventing microbial attacks (Kumari *et al*. 2015). Other studies indicate that chitosan creates a water-resistant layer around bacterial cells, blocking solute exchange and disrupting bacterial metabolism (Díez-Pascual & Díez-Vicente 2015). Furthermore, low molecular weight chitosan can penetrate the cell nucleus, inhibiting DNA-to-RNA translation by binding to DNA molecules (Youssef *et al*. 2015).

The antioxidant activity of mango leaf extract nanoemulsion was analyzed in vitro using DPPH reagent. The DPPH test results are presented in Figure 3. According to the study, the percentage inhibition of DPPH in both the mango leaf extract and its nanoemulsion form exceeded 90%. This means that mango leaf extract exhibits excellent antioxidant activity even at low concentration variations (ranging from 20–100 ppm). This is due to mango leaves containing various secondary metabolites that effectively neutralize free radicals, such as mangiferin, gallic acid, flavonoids, and gallotannins (Barreto *et al*. 2008)

Additionally, the DPPH test can be used to evaluate the IC_{50} value, which indicates the concentration of the sample solution required to

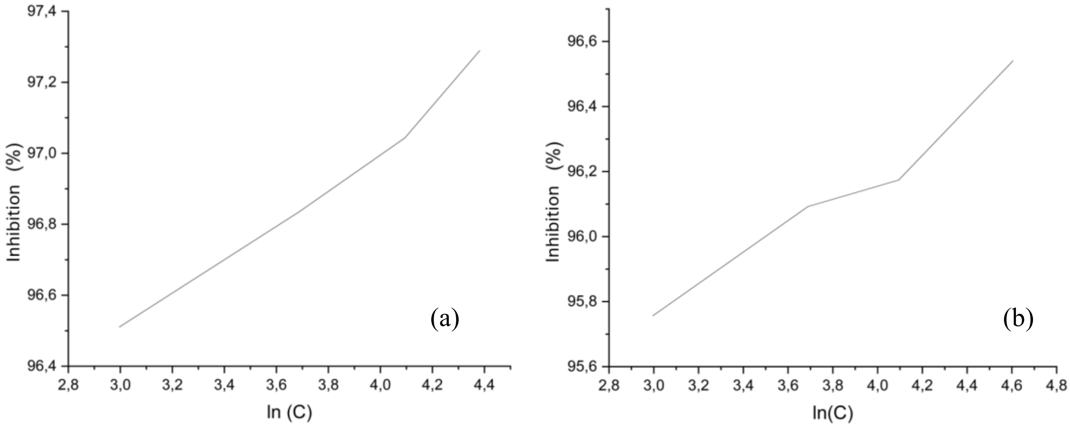

Figure 3. Antioxidant test results: (a) mango leaf extract and (b) mango leaf extract nanoemulsion

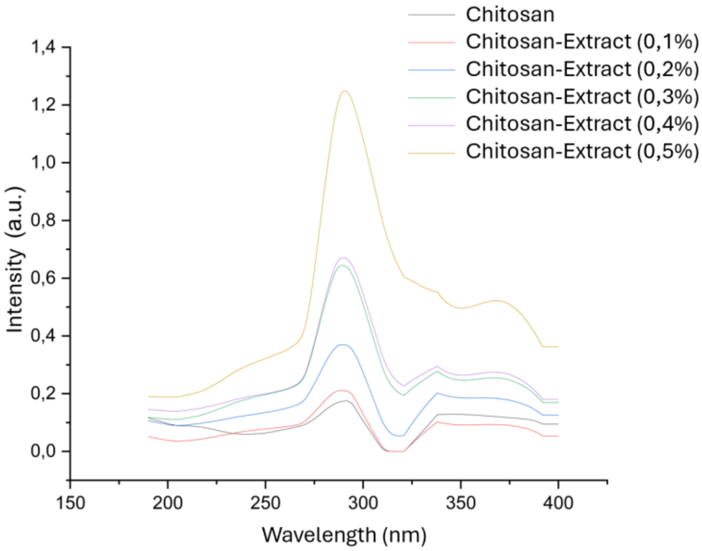

Figure 4. Absorbance test results of chitosan and chitosan-encapsulated mango leaf extract nanoemulsion

inhibit 50% of free radicals in DPPH. Figure 3 shows that at the same initial concentration of 20 ppm (ln C=3), the inhibition value for mango leaf extract is higher than that of the nanoemulsion. Consequently, the IC_{50} value of the nanoemulsion will be greater than that of the mango leaf extract without nano-scale formulation. This suggests that the nanoemulsion formation, encapsulated with the surfactant, delays the release of the extract, preventing an immediate reaction with DPPH. However, further evaluation and analysis of this study are still necessary.

The optical properties of the edible coating based on mango leaf extract nanoemulsion and chitosan were also tested by measuring absorbance using a UV-Vis spectrophotometer. Light within the 200–400 nm wavelength range was tested for each solution to determine the highest absorbance intensity. The UV-Vis spectrophotometer test results are shown in Figure 4.

Based on Figure 4, it was found that the chitosan solution without the addition of mango leaf extract nanoemulsion exhibited the lowest absorbance intensity. Other variations show that as the concentration of mango leaf extract nanoemulsion increases in the chitosan solution, the absorbance value also rises. The UV absorption peak for all solution variations falls within the 280–300 nm wavelength range, indicating anti-UV B activity (Jaisin *et al*. 2020).

CONCLUSION

Mango leaves have a high potential for use as additives in fruit packaging due to their rich secondary metabolites. These components enable mango leaves to serve as antioxidant and antimicrobial agents. This is demonstrated by their strong antioxidant activity against DPPH free radicals. Additionally, the antimicrobial properties of mango leaf extract can be enhanced through nanoemulsion formation using surfactants and encapsulation with chitosan. The results of this study indicate that chitosan-encapsulated mango leaf extract nanoemulsion has great potential as a raw material for edible coating in fruit packaging. This is due to its ability to improve antimicrobial properties, exhibit high antioxidant activity, and effectively block UV radiation.

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