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Research Paper

Carbon Dots-based Antifungal Coating Film Against Pathogens *Colletotrichum* sp. for Active Coating Application of Mango

Idayu Safitri[1], Sri Sugiarti[1*], Noviyan Darmawan[1]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java, 16680, Indonesia*
***Corresponding author**: srisugiarti@apps.ipb.ac.id

Abstract
Mangos are one of horticultural products that are leading the Indonesian agricultural industry. Simple post-harvest handling leads to high damage. The most common causes is anthracnose disease caused by *Colletotrichum* sp. Therefore, it's necessary to modify the existing methods in postharvest handling such as coating. Adding active agents such as carbon dot (CD) in coating material to prevent anthracnose from growing and shorten the shelf life. This study aims to investigate the applicability and antifungal properties of CD as a composite coating. CDs were synthesized using chitosan as the carbon source in a one-pot hydrothermal technique. The CDs have blue-emitting luminescence caused by the functional group at the surface of CD. The CDs were used to prepare chitosan/pectin (Chi/Pec)-based composite coatings. The addition of CDs increased the viscosity and density of composites, increased the blue-emitting luminescence, and showed potential antioxidant activity. The CDs and composite coatings exhibited high antifungal activity against *Colletotrichum* sp. by agar well diffusion method and were classified as very strong antifungal agents. The chitosan/pectin/CDs-coating effectively reduced the growth of black spots on the surface of mangos and increased the fruit shelf life of the fruit by 24 days. The CDs are evidently safe, affordable, and value-added nanomaterials that can be used to prepare active packaging applications.

Keywords
Antifungal, Antioxidant, Carbon Dots, Chitosan, Coating, Mango

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1. INTRODUCTION

Horticultural products are leading the Indonesian agricultural industry. Indonesian Statistical Agency shows mangoes dominate the harvest by 2.83 million tons annually. Mangoes (*Mangifera indica*) are also included in the strategic plan of the Ministry of Agriculture of Indonesia 2020-2024 for the main commodity. However, simple post-harvest handling leads to high damage by 20-30% (Uddin et al., 2018). Postharvest losses can be caused by physical, physiological, or pathological factors (Palou et al., 2015). Physical losses such as rind wounds, bruises during harvest, wilting, or poor handling while packing. Other pathological losses are caused by pathogens such as fungi. The most common causes are anthracnose caused by *Colletotrichum* sp. and stem end rot (Tipre et al., 2022). Furthermore, some postharvest losses occur in the field, while others are the result of poor handling or storage conditions in the marketplace (Uddin et al., 2018). Postharvest treatment with common synthetic waxes or chemical fungicides such as benomyl, prochloraz, and thiabendazole has been alongside still to be used to prevent rot and improve fruit shelf life for many years (Kantanet and Chompoorat, 2022; Tipre et al., 2022). Nevertheless, continuous application can cause chemical residues or pathogenic resistance (Palou et al., 2015). Therefore, it's necessary to modify the existing methods in postharvest handling such as coating. Adding active agents such as carbon dot (CD) in coating material to prevent anthracnose caused by *Colletotrichum* sp. from growing and spreading to other crops (Ezati et al., 2022; Priyadarshi and Rhim, 2020).

Carbon dot (CD) is a nanomaterial with sp^2/sp^3 hybridization carbon structure and abundant functional groups on its surface that endow CD with fluorescent characteristics (Shen et al., 2020). Several studies conducted that CD has low toxicity, biocompatibility, great water solubility, antimicrobial properties, and extraordinary antioxidant activity so they are promising prospects for active food packaging film development and fruit spoiling prevention (Ezati et al., 2022; Li et al., 2018; Priyadarshini et al., 2018). Due to the strong interaction of the hydrophilic interaction among the polymer matrix and CD, composite coatings involving CD may increase physicochemical features such as barrier, mechanical, and UV protection

(Kousheh et al., 2020). Combining more than one polymer can increase the antifungal activity and mechanical properties of coating materials such as gelatin, pectin, and chitosan (Alvarez et al., 2022; Ezati et al., 2022). Gelatin, pectin, and chitosan are often used in food and pharmaceutical industries for coatings because of their high flexibility, biodegradable properties, high transparency, and inexpensive (Roy and Rhim, 2021; Yadav et al., 2020).

Regulatory aspects of nanotechnology CD as carbon-based nanomaterials in food has been established by several countries such as the USA since 2012, Switzerland since 2010, Japan since 2016, and Malaysia since 2013 (He et al., 2019). European Union countries along with Switzerland are known to have specific regulations on the use of nanomaterials in agriculture, feed, and food. Whereas in Asia, the existing regulations are more implicit and still in the early stage of guidance for the industrial sector (Amenta et al., 2015). Therefore, the advanced improvement of nanomaterials for safety in food packaging can enhance food quality, reduce unwanted waste, and enhance shelf-life may lead to a renewal of the regulations (Kumar and Gaikwad, 2023).

CD are expected to be utilized in active packaging applications due to their excellent functional qualities, antimicrobial and antioxidant activity, biocompatibility, and low toxicity. They are intended to be mixed with another matrix polymer to increase antibacterial activity and mechanical features (Alvarez et al., 2022; Ezati et al., 2022; Kousheh et al., 2020). The application of CD incorporated in polymers for food packaging or coating is often carried out as fruit coatings for avocados and strawberries (Ezati et al., 2022; Guo et al., 2024). The other applications as active packaging for peeled fruit and vegetables (Kumar and Gaikwad, 2023; Fan et al., 2019).

A few researchers focused on using gelatin for coating (Ezati et al., 2022; Roy and Rhim, 2021; Yadav et al., 2020). There have been limited studies concerned with using porcine and bovine gelatin for coating caused by limitations for certain consumers, particularly Muslims, Jews, and Hindus are concerned about religious issues (Fadzillah et al., 2019). Therefore, this research intends to prepare chitosan/pectin-based functional coating by incorporating CD, evaluating physicochemical and functional features of coatings, and testing the efficacy of the coating through the coating of mangos. The primary objectives of this research are to explore the potential for CD functional antioxidant and antimicrobial substances for active packaging applications.

2. EXPERIMENTAL SECTION

2.1 Materials and Instruments

This study used mango varieties of Harum Manis that are ready to be harvested. The chemicals acetic acid, orange pectin, chitosan (DD 75-85%), and glycerol. The microorganism *Colletotrichum* sp. (IPB CC 15 1268). The tools used are Fourier Transforms Infrared Spectroscopy (FTIR), Spectrophotometer UV-Vis, Spectrophotometer fluorescence, UV lamp, and Spectrophotometer visible.

2.2 Methods

2.2.1 Preparation and Characterization of CD

CDs were formed using one step hydrothermal process with chitosan solution as a reaction precursor, as described by Song et al. (2018) with few adjustments. As shown in Figure 1, 5 g chitosan from Merck with DD 75-85% was added to 100 mL of 1% acetic acid solution (Merck) and dissolved at room temperature using an ultrasonic technique. Secondly, 80 mL of chitosan solution was placed in a 100 mL autoclave and heated at 180°C for 8 hours. Thirdly, once the brownish product had reached to room temperature, it was filtered through a Whatman filter, and the supernatant was filtered again using a nylon membrane filter (25 mm diameter, pore size 0.22 μm). The filtrate was then dried in an oven at 105°C to produce a black powder CD, which was then refrigerator before further testing. The prepared CD were characterized using UV-Vis spectroscopy (Thermo Scientific Genesys 10) at 200-600 nm to obtained absorbance, irradiation under UV lamps (CAMAG UV cabinet) at 254 nm and 366 nm to determine fluorescent color of CD, fluorescent spectrophotometer (Horiba Fluoromax Hybrid Fluorescence) to determine the emission, and FTIR spectroscopy (Thermo Scientific-Nicolet iS50) to establish the functional groups on surface of CD.

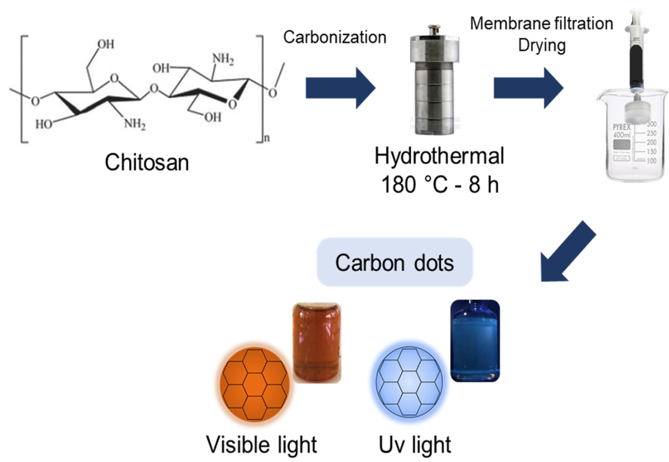

Figure 1. Schematic Illustration of the Preparation Procedure of CD by Hydrothermal Method

2.2.2 Preparation and Characterization of Composites

Modified chitosan/pectin-based coatings made according to a method by Ezati et al. (2022). With vigorous swirling, 2 g of each of the pectin (Andre Pectin) and chitosan (Merck) and 1.2 g of glycerol (Merck) were dissolved in 150 mL of distilled water and left to sit at 90°C for 45 minutes. after that, 0.04 and 0.08 g CD (1 and 2 w/w) were added gradually while being vigorously stirred for 30 minutes. Additionally, the same process was used without CD to generate Chi/Pec composites. The composites were stored in a closed jar at the refrigerator for further characterization. The prepared composites were measured with density. The composites were also characterized using UV-Vis spectroscopy (Thermo Scientific Genesys 10), UV lamps (CAMAG UV cabinet) at 254 nm and 366 nm, and FTIR spectroscopy (Thermo Scientific-Nicolet iS50).

2.2.3 Antioxidant Activity of the CD and Composites

The antioxidant activity of CD was assessed using DPPH• radical scavenging methods. An ethanolic solution of DPPH 0.1 mM purchased from Merck was prepared and stirred vigorously before being used. Ascorbic acid (Merck) was used as a positive control with 0, 1, 2, 3, 4, and 5 ppm concentration series. The concentration series with 0, 20, 40, 60, 80, and 100 ppm was prepared using CD and composites solution. The absorbance at 517 nm was measured using a visible spectrophotometer (Jenway 7300) after a 2:3 ratio of DPPH solution with the sample was applied and incubated in a dark room for 30 minutes at room temperature.

The free radical scavenging activity was calculated as follows:

$$\text{Free radical scavenging activity}(\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c and A_s were the absorbances of the DPPH solution of control and test solutions.

Concentration values of sample or the antioxidant comparator (DPPH solution) and percent inhibition were plotted respectively on the x and y axes of the linear regression equation. The formula was obtained from Equation (2).

$$y = a + bx \quad (2)$$

The formula used to calculate the value of IC50 (50% inhibitor concentration) of each sample with a y set to 50 and the x value derived from IC_{50}. The IC_{50} value states the concentration of the sample solution required to reduce DPPH free radicals by 50%.

2.2.4 Antifungal Activity of the CD and Composite Coating

Through the agar well diffusion method, the antifungal activity of the coatings and CD was examined against *Colletotrichum* sp. (IPB CC 15 1268) in media. On the agar surface, an inoculum was applied. After that, 6 mm diameter wells in agar media were punched, filled with 100 μL of composite solution, and cultured for three days at 37°C before the diameter of the inhibitory zone was determined. Positive control is used with ketoconazole (Ezati et al., 2022; Kousheh et al., 2020). The diameter of the inhibition zone was then measured with ImageJ software. Table 1 shows the determination of growth inhibition response groups.

2.2.5 Coatings Application

Mangos 'harum manis' variant harvested from Bogor, West Java was used to test the efficacy of the coating in extending life fruit based on the Ezati et al. (2022) method with modification. The fruit was left to dry at room temperature after being submerged in the coating solution for a minute. The uncoated control and thinly coated fruits were placed in a tray and stored at room temperature for 24 days based on growth of fungi. Throughout the storage time, a smartphone is used to record each group's visual changes every four days.

Changes in fruit weight in each mango sample were calculated by the difference in fruit weight at the beginning and after storage days during the storage interval (Belwal et al., 2020). The percent weight loss of mangoes is calculated by the Equation (3).

Table 1. Classification of Fungal Growth Inhibition Responses

Clear Zone Diameter	Categories
>20 mm	Very strong
11-20 mm	Strong
6-10 mm	Intermediate
≤ 5 mm	Weak

$$\text{Weight loss } (\%) = \frac{(\text{Initial weight -Weight at measurement})}{\text{Initial weight}} \quad (3)$$

3. RESULTS AND DISCUSSION

3.1 Characterization of CD

Highly luminous CDs were created by 180°C hydrothermal carbonization of chitosan for 8 h. The CD solution excited at 366 nm, it turned a brownish color and emitted a brilliant blue light when exposed to a UV lamp. Whereas chitosan has no absorption band beyond 220 nm, CD exhibits a broad absorption band at 285 nm due to the n-π* transition of C=O (Song et al., 2018) (Figure 2(a)). The CD fluorescence spectrum exhibits an emission peak at 466 nm and an excitation peak at 360 nm (Figure 2(b)). Based on the literature, the emission is caused by of functional group at the surface of CD that consists of carbonyl and hydroxyl (Lin et al., 2019).

The properties of CD are emission wavelength and size-dependent photoluminescent behavior (Tang et al., 2023). These properties are also influenced by the source of CD precursor and the concentration of CD (Yan et al., 2021). These properties are similar with to the properties of silver nanoparticles that also size dependent absorbance (Alim-Al-Razy et al., 2020). The lower the absorbance it has, the smaller the CD particle size and the lower concentration of CD. Therefore, the particle size of CD can be determined based on the distribution of the absorption spectrum of silver nanoparticles. Based on the literature, the absorption spectrum ranges from 250-280 nm with emission ranging from 400-470 nm the particle size of the synthesized CD ranges from 1-10 nm (Lee et al., 2017; Singh et al., 2013).

The FTIR spectra of synthesized CD from chitosan are shown in Figure 3. The peaks at 3407 cm^{-1} in the FTIR spectrum are attributed to −OH stretching and overlaps with N−H stretching in the same area. The peak at 1600 cm^{-1}

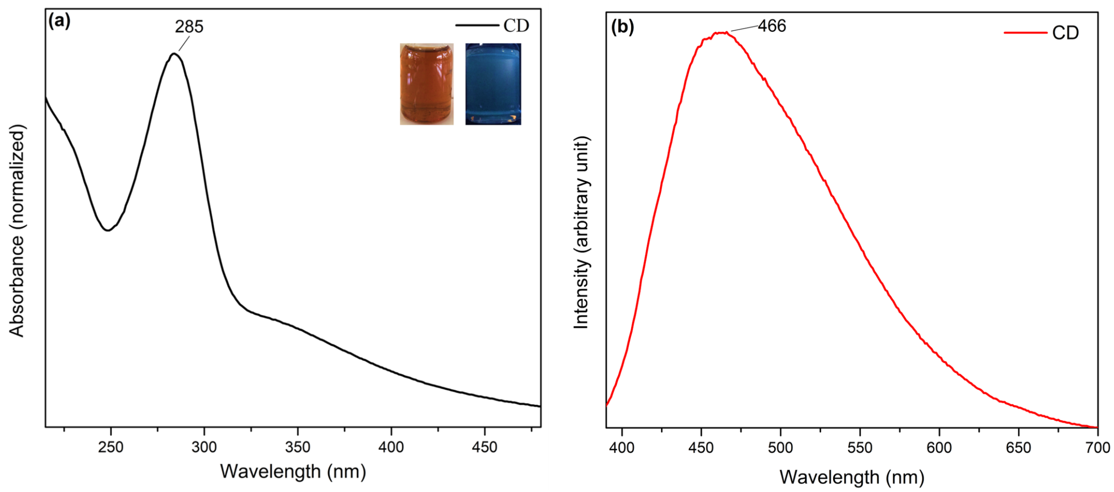

Figure 2. (a) Absorption Spectra of CD, (b) Emission Spectra of CD

may be attributed to C=O stretching, which overlap with CD surface $-NH_2$ stretching. The peaks at 1392 cm^{-1} and 2752 cm^{-1} indicate −OH bending. Due to the structure of chitosan the CD contained with -NH groups. The peak at 2940, 2752, 2666, and 2572 cm^{-1} assigned to graphitic structure in CDs core (Janus et al., 2019; Song et al., 2018).

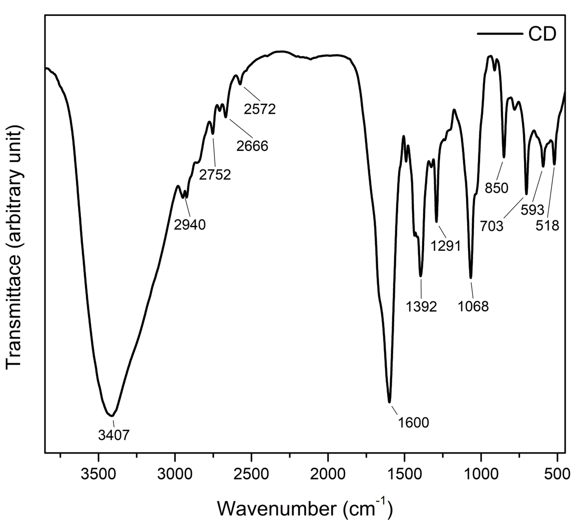

Figure 3. FTIR Spectrum of CD from Chitosan

3.2 Characterization of Composite Coatings

The optical characteristics and apparent photo image of the composites based on Chi/Pec are displayed in Figure 4. The CD were uniformly distributed throughout the polymer matrix regardless of concentration. While displayed under a UV lamp with excitation at 366 nm it showed bright blue color emission. The higher the concentration of CD the darker color and luminescence it gets. The CD is incorporated with chitosan and pectin because CD tends to clump and reduce its fluorescent properties by itself (Yan et al., 2021). Therefore, mixing with chitosan and pectin matrix facilitates application as a fruit coating and can improve the properties of the composite.

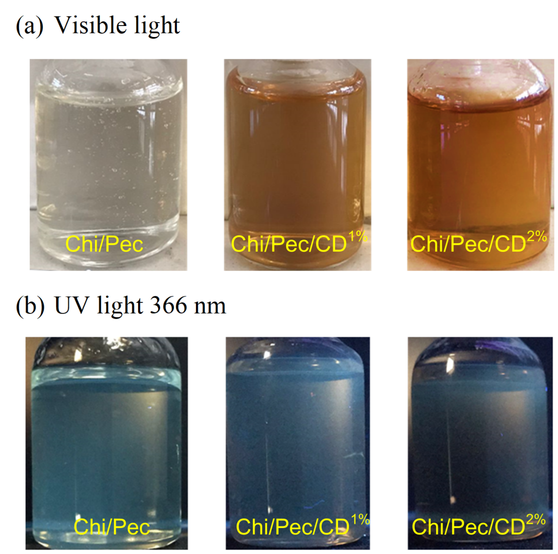

Figure 4. Photographic Images of Composites (a) Visible Light and (b) UV Light 366 nm

Based on the UV-Vis spectrum, Chit/Pec coatings without CD addition have one weak peak at 281 nm. Composite coatings with the addition of 1% and 2% of CDs showed similar peaks at 254 and 286 nm and 253 and 285 nm (Figure 5(a)). Peak at range 250-270 nm caused by transition π-π^* from aromatic structure C=C from CD core with sp^2 hybridization. Whereas peak at range 280-320 nm caused by transition n-π^* from functional group C=O and NH_2 at the surface of CDs. The FT-IR spectrum shows stretching −OH at 3276 cm^{-1}, stretching $-CH_2$ from CDs core at 2874 and 2431 cm^{-1}, stretching $-NH_2$ also found at 1500 cm^{-1} that overlaps with

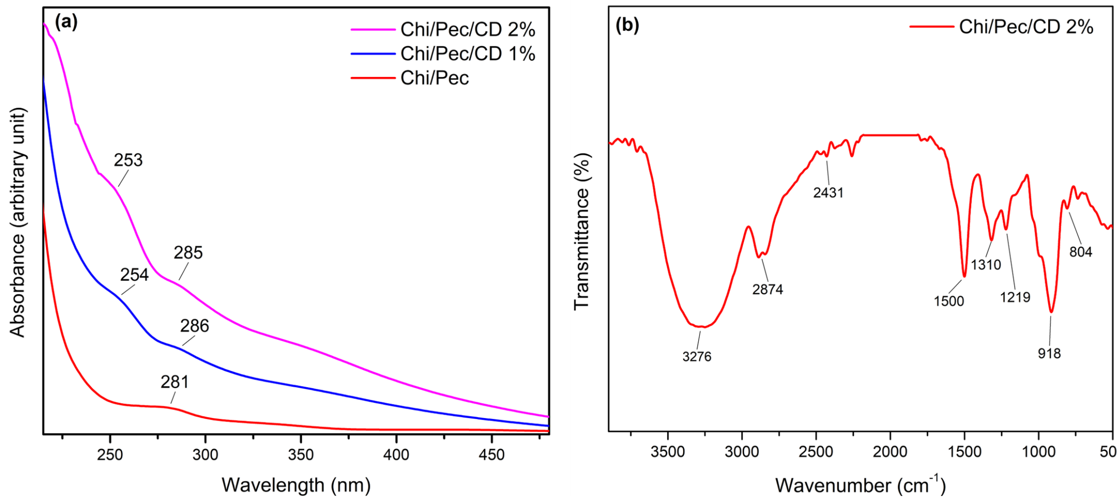

Figure 5. (a) Absorption Spectra of Coating Composites and (b) FT-IR Spectra of Chi/Pec/$CD^{2\%}$

aromatic structure C=C, and bending OH at 1310 and 1219 cm^{-1} (Figure 5(b)). Specific peaks from glycosidic bonds and pyranose rings from chitosan were also found at 918 and 804 cm^{-1} (Janus et al., 2019).

The interaction between chitosan-pectin and CD form hydrogen bonds that prevents aggregation. The aggregation from CDs can lead to the loss of luminescence. CDs can be well-dispersed in a chosen polymer matrix that reduces the agglomeration problem, promoting stability, and easier application (Feng et al., 2021). When compared to other nanomaterials such as silver nanoparticles, CD have functional groups on their surface that prevent them from agglomerating as occurs with metal nanoparticles that form metallic bonding to stabilize themselves (Alim-Al-Razy et al., 2020; Rafique et al., 2020). The viscosity and density of coatings were measured. As the concentration of pectin increased, the viscosity and density of the pectin solutions increased slightly. The result was analyzed using one-way ANOVA and showed that CDs-added composites significantly increased density compared with no CD (carbon dot) (Table 2).

3.3 Antioxidant Activity of CD and Composites

Through DPPH techniques, the antioxidant activity of the CD was assessed. Resilient free radicals found in DPPH react with antioxidants to shift color from purple to yellow or colorless. Figure 6 illustrates the DPPH free radical scavenging activity that is dependent on CD concentration. The % inhibition activity was calculated using Equation (1). The regression Equation of the CD and composite samples respectively are, y = 2.3851x + 14.126; y = 0.4128x + 9.439 for Chi/Pec; y = 0.3781x + 16.704 for Chi/Pec/CD 1%; and y = 0.6615x + 3.511 for Chi/Pec/CD 2%. The classification of antioxidant is categorized into 5, which are <50 μg/mL (very strong), 50-100 μg/mL (strong), 100-150 μg/mL (medium), 150 – 200 μg/mL (weak), and >200 μg/mL extremely weak (Floegel et al., 2011) (Equation (2)).

Based on the classification of antioxidants, CD has an IC_{50} value of 95.01 belongs to strong antioxidants, all composite Chi/Pec/$CD^{2\%}$, Chi/Pec/$CD^{1\%}$, and Chi/Pec has IC_{50} respectively at 70.30; 88.06; and 98.2 considered strong antioxidants (Figure 6(a)). The CD added-composites s have lower inhibitory concentration than only CDs. The lower inhibitory concentration meaning it has higher antioxidant activity. By adding the CD at 1% and 2% in composite, the lower the IC_{50} value and indicates there is a synergistic effect between CD with chitosan and pectin polymer. Chitosan and pectin polymer increases the number of hydroxyl functional group so the composite capture more DPPH radicals. Thus, the incorporation of CD into the composite does not inhibit the antioxidant activity of the composite.

Using the DPPH technique, the CD exhibited strong antioxidant activity. According to Zhang et al. (2018), CD's surface with hydroxyl groups, -NH_2, and -CONH, may contribute to free radical scavenging in DPPH assays to yield more stable products, which might be responsible for its greater antioxidant activity. The H transfer from the CD's functional groups from DPPH-H can diminish and quench the free radicals DPPH• (Bhattacharya et al., 2023). Food oxidation is a serious issue for the food industry, consequently, functional coatings with antioxidant features are used to prevent the oxidation. The antioxidant activity of the Chi/Pec coatings was 50.15%. The scavenging activity of the coatings did, however only slightly increase to 56.78%, and 68.53% for 1% and 2% w/w additional CDs, respectively (Figure 6(b)).

CD-added coating had stronger antioxidant activity than without CDs because the OH groups at the CD surface dispersed in the solution of DPPH. When compared with a similar study, the results of antioxidant activity of CD reached 95% with the same concentration at 100 μg/mL. The difference is caused by the long measurement process that causes the DPPH solution exposed to room temperature which affects the quality of the reagent. DPPH is known to be sensitive and easily

Table 2. Comparison of Viscosity and Density Composite Solutions

Composites Coatings	Viscosity (cp)	Density (g/cm^3)
Chitosan/Pectin	3100 ± 14.4337^a	1.02 ± 0.0007^a
Chitosan/Pectin/$CD^{1\%}$	3400 ± 25.1661^b	1.03 ± 0.0068^{ab}
Chitosan/Pectin/$CD^{1\%}$	3550 ± 13.2287^c	1.04 ± 0.0024^b

Remarks: Numbers followed by the same letter showing no difference based on Duncan's test ($\alpha = 0.05$)

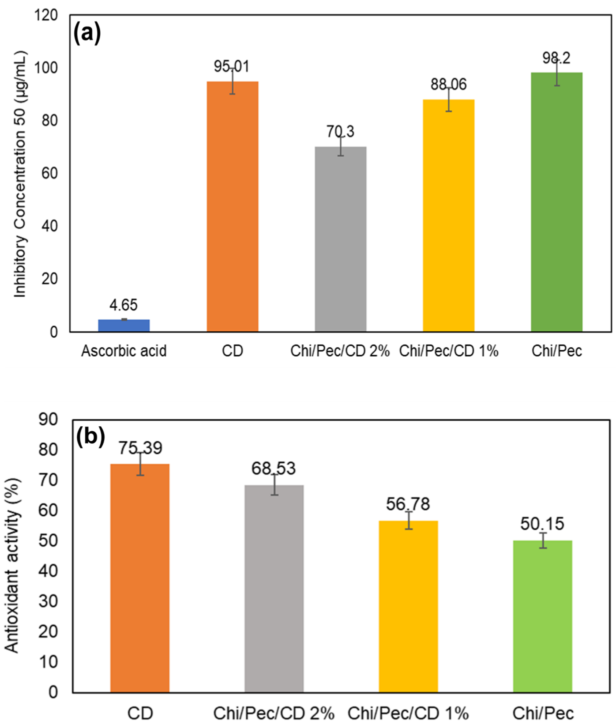

Figure 6. (a) Inhibitory Concentration 50 (μg/mL), and (b) Antioxidant Activity of the Composites and CD at 100 μg/mL

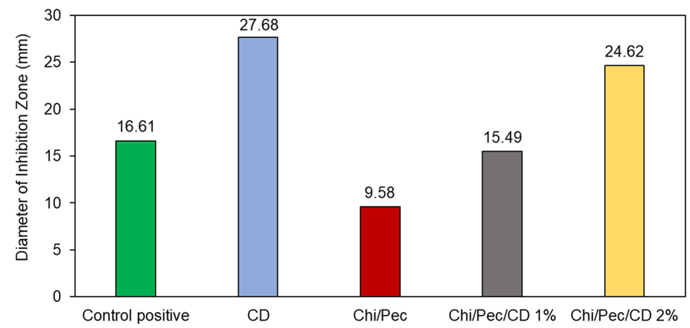

Figure 7. Comparison of the Diameter of Inhibition Zone of the Chitosan/pectin-based Coatings Against *Colletotrichum* sp.

damaged by light and must be stored at low temperatures to maintain its quality (Kedare and Singh, 2011).

3.4 Antifungal Activity of the Composites

In food-active coating applications, antifungal activity is a regularly employed functional characteristic. The antifungal activity of Chi/Pec-based coatings was evaluated using the agar well diffusion method (Figures 7 and 8). The agar well diffusion method was used to test the coating's inhibitory effect on microbial growth against *Colletotrichum* sp. Figure 7 shows the comparison in diameter of the inhibition zone. The Chi/Pec composite coatings showed an inhibition zone of 9.58 mm which classified as intermediate antifungal activity. The Chi/Pec/$CD^{1\%}$ composites have similar inhibition zones with positive control at 15.49 and 16.61 mm, respectively which classified as strong inhibition activity. The Chi/Pec composite added 2% w/w of CD also has a similar inhibition zone with only CD at 24.62 and 27.68 mm respectively, and is classified as very strong inhibition activity (Table 1).

CDs can inhibit fungal growth because they accumulate in the nucleus of hyphal cells directly entering the cell and destroying the cell nucleus, thereby inhibiting fungal growth (Li et al., 2018). Other literature also states that after CD binds noncovalently to fungal DNA and RNA and at the same time changes the conformations of DNA and RNA structure. Concurrently, the different conformation of DNA and RNA can inhibit the metabolism of fungus to expression of non-ribosomal peptide synthase gene. These genes play a role in secondary metabolites that cause the fungus to become dangerous and transmit disease (Le Govic et al., 2019).

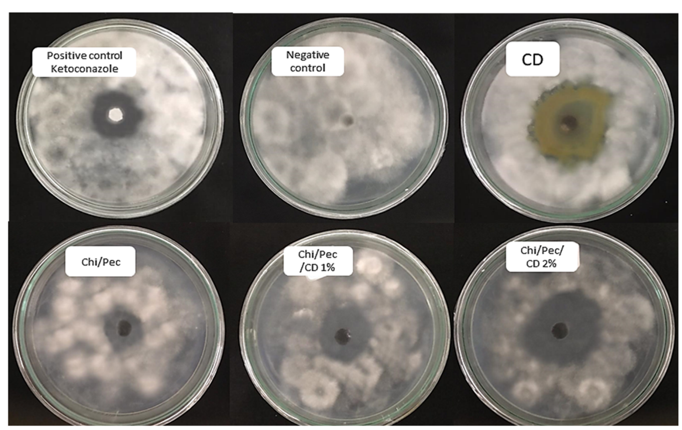

Figure 8. Antifungal Activity of the CD and Composite Coatings Against *Colletotrichum* sp. by the Diffusion Method

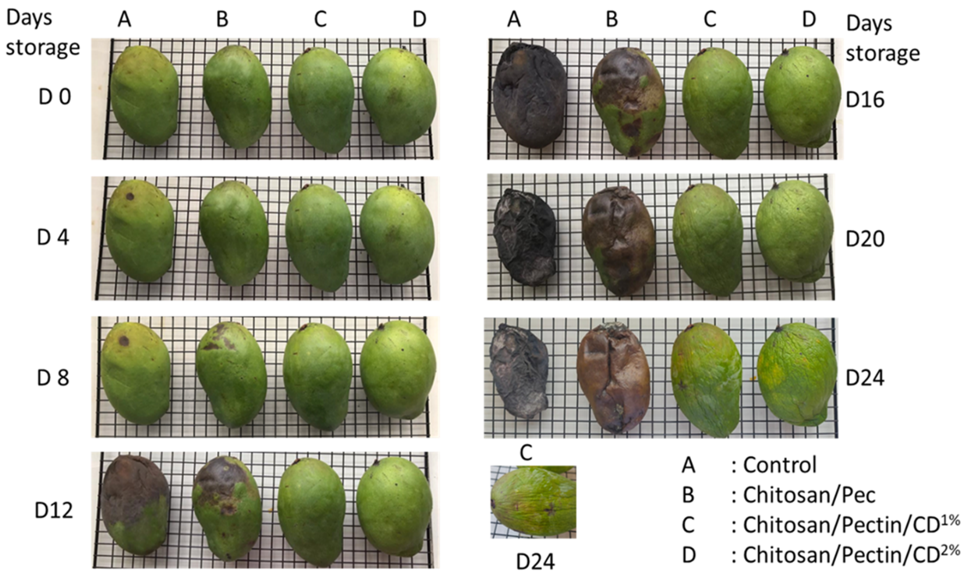

Figure 9. Change in Appearance during Storage at 25°C of Mangoes Coated with Chitosan/pectin-based Coatings

3.5 Packaging Test on Mango

Mango (*Mangifera indica*) is one of Indonesia's main horticultural commodities. Mango is classified as a seasonal fruit that can be stored for 3-9 days (Hoque et al., 2018). The short life period makes it difficult to distribute in distant places, thus postharvest treatment such as coating is important to maintain the quality and increase the shelf life of mangoes for the wider target market. Mangoes were coated with a Chi/Pec-based coating solution to increase the shelf life of fruit by more than 9 days. The mango coating process uses the dipping method which has the advantage easy to apply and does not require additional tools (Ezati et al., 2022).

The dipping process lasts for 1 minute to maximize the attachment process to the fruit skin (Ezati et al., 2022). The dipping process was done twice to make the composite more evenly distributed. The mango fruit appears to be more radiant than the control fruit (Figure 9). All of the fruits were fresh at first, but after 8 days, the uncoated fruits developed visible black patches on the surface, which got more severe after 12 and 16 days of storage (Figure 9). However, even after 8 days of storage, the fruits coated with Chi/Pec composite showed no symptoms of black spots on the fruit surface. Nonetheless, after 12 days of storage, black spots on the surface of the coated fruit began to develop, indicating mold growth. Mold growth was slowed due to chitosan's antifungal action, as revealed in composite microbiological tests (Figure 8).

In CD-coated fruits, black spots were observed only after 12 days of storage. Black spots were observed in Chi/Pec/$CD^{1\%}$ coated fruits after 24 days, but there are no black spots were observed in Chi/Pec/$CD^{2\%}$ coated fruits even after 24 days of storage. As a result, in the mango fruit application test, the treatment of mango fruit coating with composite materials exhibited significant antifungal activity, and the antifungal activity was directly related to CD concentration. CD-infused Chi/Pec coatings form an active coating on fruits, potentially preserving quality and shelf life during post-harvest distribution. When compared to similar studies, these results have a better shelf life of 24 days. According to Hoque et al. (2018), mangoes were successfully stored for 15 days using the washing method with the addition of mambi (neem) leaf extract. Thus, the application of composites with the addition of CD at 2% w/w is more effective in extending the shelf life of fruit at room temperature.

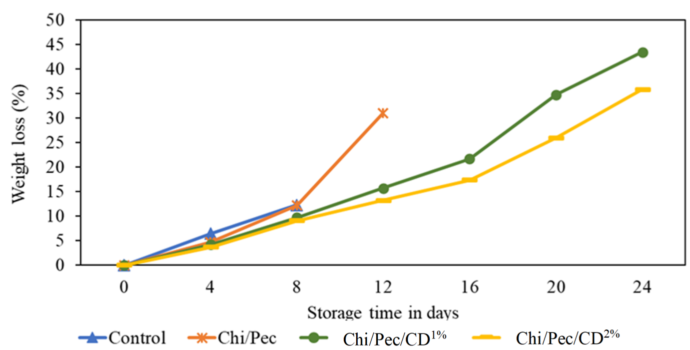

Figure 10. Weight Loss Rate of Mango Fruit

Weight loss is one of the parameters that must be considered in determining the quality of fruit shelf life. Weight loss shrinkage occurs due to the reduction of water content in the fruit and happens during fruit storage which is influenced by humidity, air temperature, and fruit moisture content (Jovanović et al., 2021). During the observation, all mangos were kept under the same conditions simultaneously in the laboratory at room temperature (±25°C), without direct light, and placed in a ventilated room with air circulation. The weight loss rate of

Table 3. Weight Loss Measurement (%) of Mango Fruit

Storage Days	Treatment			
	Control	Chi/Pec	Chi/Pec/CD 1%	Chi/Pec/CD 2%
4	6.44±1.67[a]	4.71±0.57[ab]	4.17±0.35[b]	3.64±0.35[b]
8	12.30±2.70[a]	12.21±1.72[ab]	9.69±0.86[b]	9.05±0.54[b]
12	NA	30.98±3.66[a]	15.69±9.44[b]	13.23±0.97[b]
16	NA	NA	21.72±8.93	17.33±1.56
20	NA	NA	34.80±9.16	25.99±4.16
24	NA	NA	43.46±8.25	35.86±1.19

Remarks: Numbers followed by the same letter showing no difference based on Duncan's test (α= 0.05)
NA*= Not Applicable (Fruit already rotten and not tested)

mangos that were treated with coating and control in shown in Figure 10 and calculated using Equation (3). Statistical analysis of weight loss measurement of mango fruit is shown in Table 3. Weight loss at control and coating treatment showed significant difference at 4 days of storage. Mangos with coating treatment at day 8 seemed to restrain the weight loss rate and indicated that coating treatment able to withstand the shrinkage of mangos by inhibiting the transpiration and respiration rates from the fruit surface. Other literature also states that transpiration and respiration are linked with the process of fruit weight loss (Kumar et al., 2023).

ANOVA analysis showed a significant difference at 12 days of storage between Chi/Pec/CD$^{1\%}$ and Chi/Pec/CD$^{2\%}$. Whereas the control fruit was not measured because of rotten, at 16, 20, and 24 days both fruits with CD-s added coating treatment showed no sign of rotten, and the composites with 2% w/w added CDs showed better restrain to weight loss because the data more acceptable and have smaller standard deviation. According to Kumar et al. (2023), significant weight loss is a sign of early decay followed by the appearance of black dots. In mangos fruit, black dots indicate decay due to anthracnose disease.

The main characteristic of anthracnose disease is characterized by a black spot at the stem end which will spread to all parts of the fruit (Uddin et al., 2018). Significant weight loss usually occurred on day 5 in control fruit by 10-15% and with coating fruit took place on day 9 by 10-15% (Hoque et al., 2018). This result is similar to the result of the study with control fruit weight loss on day 4 by 6.44% and with coating treatment fruit on day 8 by 9-12%. The difference is caused by the composite material which consists of a mixture of chitosan and pectin. The combination of more than one polymer can increase the mechanical properties and antifungal activity of coating (Alvarez et al., 2022; Kumar and Gaikwad, 2023). Therefore, the fruit coating treatment with chitosan/pectin/CD composite successfully prevented significant weight loss during storage and inhibited the decay process due to contact with air.

4. CONCLUSION

The hydrothermal approach was used to successfully synthesis carbon dots (CDs) with blue fluorescence from chitosan, and the CDs exhibited high antifungal and antioxidant activity. The addition of CD increases the viscosity, density, and blue-emitting luminescence of the coating composite. CD-added coatings were effective in coating mango against *Colletotrichum* sp. on the surface of the fruit, which was characterized by black spots and improved shelf life by 24 days. The coating treatment on the mango with CDs added composite showed a better result in holding weight loss than fruit control. As a result, the Chi/Pec/CDs coating compositions have a significant potential for properly packaging items and increasing post-harvest fruit shelf life.

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