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# Antibacterial properties of cassava starch/xanthan gum/zinc oxide nanoparticles coating solution and physicochemical analysis of coated banana (*Musa acuminata*) for shelf-life extension

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## Abstract

Bananas serve as a nutrient-rich energy source for human diets, and a valuable raw material for downstream products. Despite the high demand for bananas, they have a relatively short shelf-life. To mitigate these shortcomings, researchers have explored the use of edible coatings to extend the shelf-life of bananas. Nevertheless, only a few studies have assessed the integration of starch, gum, and zinc oxide nanoparticles (ZnONPs) in developing a coating solution that can preserve the qualities of bananas post-harvest. Therefore, the present work assessed the antibacterial properties of a novel edible coating formulation combining cassava starch, xanthan gum, and ZnONPs, and its impact on the physicochemical characteristics and shelf-life of bananas of the ‘Pisang Berangan’ variety (*Musa acuminata*). Firstly, coating solutions with compositions of cassava starch, xanthan gum, and varying concentrations of ZnONPs were prepared. Their antibacterial properties were then assessed using the disc diffusion method. Subsequently, *pisang berangan* were coated with the prepared coating solutions *via* the dip-coating method. Finally, the shelf-life study was performed to evaluate the effects of the coating solution on the physicochemical characteristics and shelf-life of coated *pisang berangan*. Based on the results, the coating formulation containing 0.5% ZnONPs exhibited the most potent antibacterial properties. Remarkably, coated *pisang berangan* recorded an extended shelf-life, lasting up to 15 days compared to only nine days for uncoated *pisang berangan*. Furthermore, coated *pisang berangan* demonstrated significantly lower weight loss and significantly higher total soluble solids (TSS), pH level, titratable acidity (TA), and firmness than uncoated *pisang berangan*. The synergistic effect was contributed by the combined protective role of cassava starch, the enhanced coating adhesion of xanthan gum, and the antimicrobial properties of ZnONPs. In summary, the formulated cassava starch/xanthan gum/ZnONPs edible coating solution (1.0%:0.5%:0.5% ratio) yielded the longest shelf-life of *pisang berangan*. The findings of the present work would boost the economic value of bananas, especially *pisang berangan*.

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## Introduction

‘Pisang Berangan’ (*Musa acuminata*) is a popular banana variety particularly in Malaysia, and available throughout the year. This type of banana is suitable for fresh consumption and as a dessert owing to its high sugar content that yields a sweet aroma, flavour, and tender texture (Sarduni *et al*., 2020).

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Similar to other banana varieties, *pisang berangan* is a climacteric fruit that rapidly ripens after being harvested. The ripening process is influenced by various factors, including the nutrient content, rapid ethylene release (Huang *et al*., 2024), carbon dioxide ($CO_2$) production (Mongkolchaiyaphruek *et al*., 2023), and moisture permeability within the fruit (Khanal *et al*., 2022). Ripened bananas can be identified through physical property changes, such as the appearance of black spots, browning, and loss of firmness (Chiwate *et al*., 2023). An edible coating solution can be applied to delay the ripening process (Majeed *et al.*, 2023).

An edible coating solution refers to a thin layer of an odourless, colourless, and tasteless substance (Farina *et al*., 2020) applied on the surface of a fruit. This layer forms a barrier that protects the fruit against natural volatile flavours, oxidation, moisture absorption/desorption, chemical reactions, and microbial attacks in the surrounding environment (Suhag *et al*., 2020). Additionally, edible coating solutions can enhance the visual and aesthetic qualities of the coated fruit. Essentially, the composition of edible coating solutions must be Generally Recognised as Safe (GRAS), and non-hazardous when consumed (Pirozzi *et al*., 2021). The composition has been studied as an edible coating solution in starch (Thakur *et al*., 2019a), protein (Li *et al*., 2019), lipid (Zhou *et al*., 2021), and natural gum (Salehi, 2020). The edible coating solution developed in the present work consisted of three key ingredients: cassava starch, xanthan gum, and zinc oxide nanoparticles (ZnONPs).

Cassava (*Manihot esculenta* C.) is a root crop grown in tropical climates, and rich in starch (Travalini *et al*., 2019) comprising amylose and amylopectin. Amylose, which makes up 16.04 - 26.95% of cassava starch (Raya *et al*., 2022), exhibits essential edible coating properties (Hatmi *et al*., 2020) due to its linear branch structure that contributes to the amorphous phase of the starch granule (Garces *et al*., 2021). Meanwhile, the percentage of amylopectin contributes mainly to the peripheral crystalline arrangement of starch granules (Lungoci *et al*., 2023), which yields a transparent, tasteless, and odourless coating with a robust oxygen barrier and high tensile strength (Hatmi *et al*., 2020). As such, amylose in starch is a better oxygen barrier for coating properties, and amylopectin provides an excellent film-forming property, especially when integrated with glycerol as a plasticiser (Basiak *et al*., 2017).

Xanthan gum is an exopolysaccharide synthesised aerobically by the bacterium *Xanthomonas campestris*. This non-toxic and food-grade substance appears as transparent and glossy, and is used as a thickener, stabiliser, and emulsifier in food products (Elella *et al*., 2021). Xanthan gum serves multiple functions, including regulating enzyme activity, slowing enzyme destruction, and inhibiting the growth of psychotropic microorganisms in fruits (Golly *et al*., 2019). Past studies have shown that using xanthan gum in edible coatings could reduce weight loss in coated foods (Salehi, 2020). The combination of cassava starch with xanthan gum also enhanced the properties of the edible coating solution (Sapper and Chiralt, 2018). Furthermore, the hydrocolloid properties of xanthan gum could overcome the retrogradation limitation of cassava starch, leading to enhanced mechanical strength and flexibility through the formation of a protective barrier against moisture, oxygen, and $CO_2$ (Su *et al*., 2023).

Although starch and gum can reduce moisture loss, ethylene rate, and respiration rate, and serve as a physical barrier against microbial growth (Salehi, 2019), they may not be sufficient to protect against all spoilage microorganisms. Remarkably, ZnONPs possess strong antimicrobial properties against bacteria, fungi, and viruses, as well as ultra-violet (UV)-blocking capabilities (Rosman *et al*., 2022). Furthermore, ZnONPs can enhance mechanical strength, improve UV resistance, and provide better adhesion to the fruit surface (Bahrami *et al*., 2019). Previously, La *et al*. (2021) found that the incorporation of 0.5% ZnONPs in edible coatings was optimal for achieving a homogeneous coating layer on banana peels. Hence, their addition to the edible coating solution may reinforce the protection of fruits against spoilage microorganisms (Jin and Jin, 2021). These hexagonal-shaped inorganic oxide crystals with sizes < 100 nm have also been approved by the United States Food and Drug Administration (USFDA) as safe and suitable for human consumption (Lian *et al*., 2021).

Despite its promising properties, developing an edible coating solution that combines cassava starch, xanthan gum, and ZnONPs to extend the shelf-life of bananas remains a challenging task. Moreover, prior research on combining starch, xanthan gum, and

ZnONPs for coating bananas is scarce. Realising this research gap, the present work assessed the antimicrobial properties of a novel cassava starch/xanthan gum/ZnONPs edible coating solution, and its effect on the physicochemical characteristics of coated *pisang berangan* for shelf-life extension. The proposed edible coating solution offers a cost-effective, safe, and sustainable approach to delay the rapid ripening process, and improve the shelf-life of *pisang berangan*, which would boost its accessibility in the local market.

## Materials and methods

### *Materials*

Fresh and damage-free *pisang berangan* (maturity index = 1) were purchased from a local market, and washed with distilled water before being left to dry at room temperature (25°C). Cassava starch (Cap Kapal ABC, Malaysia) and xanthan gum (Evachem, Malaysia) were also purchased from a local market. Meanwhile, stock cultures of *Escherichia coli* and *Staphylococcus aureus* were provided by UiTM (Kampus Kuala Pilah), Cawangan Negeri Sembilan, Malaysia. The chemicals used in the present work included glycerol (Sigma-Aldrich, Germany), sodium hydroxide (NaOH; RandM Chemicals, Malaysia), phenolphthalein indicator (RandM Chemicals, Malaysia), 3,5-dinitro-2-hydroxybenzoic acid (DNS) reagent (Sigma-Aldrich, USA), Muller Hinton broth (Oxoid, USA), and Muller Hinton agar (Becton Dickinson and Company, USA). Except for the food-grade ZnONPs (US Research Nanomaterials Inc., USA), all substances were chemical-grade, and used without further purification.

### *Preparation of edible coating solutions*

The edible coating solution was prepared based on the method described by Bahrami *et al*. (2019) with slight modifications. Accordingly, 1% (w/v) of cassava starch was mixed with 0.5% (w/v) of xanthan gum, 30% (v/v) of glycerol, and varying concentrations of ZnONPs (0.5, 1.0, and 1.5% (w/v)). Firstly, cassava starch was dissolved in distilled water at 90°C, and stirred for 30 min. Separately, xanthan gum was dissolved in distilled water at room temperature (25°C), and stirred for 30 min. Both solutions were then mixed using a rotor-stator homogeniser at 10,000 rpm for 2 min at room temperature (25°C). Concurrently, ZnONPs were dispersed in distilled water at room temperature (25°C) using an ultrasonic cleaner (60 Hz) for 10 min. Lastly, the cassava starch/xanthan gum mixture was combined with the ZnONPs solution, and stirred at 400 rpm for 2 h. The prepared edible coating solutions were labelled as: cassava starch and xanthan gum (CX); cassava starch, xanthan gum, and 0.5% ZnONPs (CX-0.5ZnONPs); cassava starch, xanthan gum, and 1.0% ZnONPs (CX-1.0ZnONPs); and cassava starch, xanthan gum, and 1.5% ZnONPs (CX-1.5ZnONPs).

### *Determination of antibacterial properties of edible coating solutions*

The antibacterial properties of the edible coating solutions were determined using the disc diffusion method, as outlined by Lungoci *et al*. (2023). Firstly, *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) were cultured separately in Muller Hinton broth at 37°C for 24 h. Afterwards, the bacterial cultures were inoculated in 0.9% (w/v) saline solution, and the turbidity of the colony suspension was adjusted equivalent to 0.5 McFarland standard, which contained approximately $1 \times 10^8$ CFU/mL (Asan *et al*., 2022). Next, 0.1 mL of the bacterial suspension was transferred onto a Muller Hinton agar plate, and spread evenly using sterile cotton swabs. Subsequently, a single antimicrobial disc (Oxoid, USA; diameter = 6 mm) was dipped into the edible coating solution, and placed at the centre of the agar plate. For comparison purposes, streptomycin was used as a positive control, while sterile distilled water was used as a negative control. The culture plates were then incubated at 37°C for 24 h. Lastly, the diameter of the inhibition zone that appeared on the culture plates was measured as an indicator of the antibacterial capabilities of the edible coating solutions (Lungoci *et al*., 2023).

### *Application of edible coating solutions on pisang berangan peels*

*Pisang berangan* from the same comb with sizes ranging from 85 - 90 g were coated with CX, CX-0.1ZnONPs, CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs using the dipping method for 10 sec, and hung at room temperature (25°C) until dry. Uncoated *pisang berangan* peels were labelled as No-CXZnONPs for comparison purposes. Any physical and colour changes were then observed and recorded (Dwivany *et al*., 2020).

*Shelf-life study of coated and uncoated pisang berangan*

The shelf-life study was conducted to observe changes in the physical and colour of the coated and uncoated *pisang berangan* at room temperature (25°C) every 3 d for a total of 18 d. The physical appearance of *pisang berangan* was captured using a phone camera, and any changes that occurred were reported (Chowdhury *et al*., 2020). In addition, the maturity ratings for each *pisang berangan* were evaluated by comparing the colour of each banana peel with the standard colour chart of bananas (Lustriane *et al*., 2018). The 7-point scale of the banana maturity index was as follows: 1 = all green; 2 = green with traces of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with traces of green; 6 = fully yellow; and 7 = fully yellow with brown spots.

*Physicochemical determination*

*Colour intensity*

The colour of the coated and uncoated *pisang berangan* peels was determined every 3 d in triplicate for each treatment following the method described by Wani *et al.* (2021). A chromameter (Konica Minolta CR-400, Japan) was employed to measure the colour intensity of *pisang berangan* peels (Wani *et al*., 2021). Before the analysis, the chromameter was calibrated on the Hunter lab colour space system equipped with a standard white plate ($L^* = 93.70$; $a^* = -0.46$; $b^* = 3.56$) as a background for colour measurement. The colour was expressed in $L^*$ (lightness), $a^*$ (green-red), and $b^*$ (blue-yellow).

*Weight loss*

The weight loss of the coated and uncoated *pisang berangan* was determined using a digital balance (Adam Equipment's PGW precision balance, UK) following the method described by Saekow *et al*. (2019). The bananas were weighed at the beginning of storage ($W_0$) and every 3 d ($W_1$) until the end of the shelf-life study. The experiments were run in triplicate for each treatment (Saekow *et al*., 2019). The weight loss (%) was calculated using Eq. 1:

$$\text{Weight loss (\%)} = [(W_0 - W_1) / W_0] \times 100 \quad \text{(Eq. 1)}$$

*Total soluble solid (TSS)*

The TSS of the coated and uncoated *pisang berangan* was determined following the method established by Wani *et al.* (2021). Approximately, 10 g of *pisang berangan* flesh was blended in 100 mL of distilled water, and the juice produced was filtered using a vacuum filter. The filtrate was then used to determine the TSS using a hand-held refractometer, and the results were expressed in °Brix. The experiments were run every 3 d in triplicate for each treatments (Wani *et al*., 2021).

*Titratable acidity (TA)*

The TA value of the coated and uncoated *pisang berangan* was assessed based on the titration method, as outlined by Nguyen *et al*. (2021). Firstly, 10 g of *pisang berangan* flesh was blended in 100 mL of distilled water, and the juice produced was filtered using a vacuum filter. For the titration method, 10 mL of the filtrate was added with 1% phenolphthalein indicator, and titrated with 0.1 N NaOH. A change in the colour of the indicator represented the endpoint. The TA was expressed in the percentage (%) of malic acid. The experiments were run every 3 d in triplicate for each treatment (Nguyen *et al*., 2021).

*pH value*

The pH value of the coated and uncoated *pisang berangan* was evaluated following the method applied by Wani *et al.* (2021). After blending 10 g of *pisang berangan* flesh in 100 mL of distilled water, the juice produced was filtered using a vacuum filter. The filtrate was then used to determine the pH value using a digital pH meter (765 Laboratory pH meter, Europe). The experiments were run every 3 d in triplicate for each treatment (Wani *et al.*, 2021).

*Firmness*

The firmness of the coated and uncoated *pisang berangan* was determined using a texture analyser (Stable Micro Systems, England), as described by Saekow *et al*. (2019). *Pisang berangan* were pierced 5 mm deep using a 2 mm cylindrical probe at a 5 mm/sec rate, and the maximum penetration force (N) was recorded. The experiments were conducted every 3 d in triplicate for each treatment (Saekow *et al.*, 2019).

*Statistical analysis*

The IBM SPSS Software Version 24 (IBM, USA) was used in the present work. The data for the antibacterial analysis were expressed as the mean and standard deviation. The mean values were evaluated using the One-way analysis of variance (ANOVA) to determine the significant difference ($p < 0.05$)

between the samples. Likewise, the data for the physicochemical analysis were also expressed as the mean and standard deviation. The mean values were evaluated using the independent *t*-test to assess the significant difference ($p < 0.05$) between samples.

## Results and discussion

The present work evaluated the antibacterial properties of a novel edible coating solution containing cassava starch, xanthan gum, and ZnONPs, and its effectiveness in extending the shelf-life of *pisang berangan*. Firstly, the antibacterial properties of the four different edible coating formulations (CX, CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs) were examined. Based on the analysis, the CX-0.5ZnONPs recorded no significant inhibition zone ($p > 0.05$) compared to the CX-1.0ZnONPs and CX-1.5ZnONPs. Subsequently, the shelf-life of five edible coating formulations (No-CXZnONPs, CX, CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs) was assessed. Based on the antibacterial and shelf-life analysis, the CX-0.5ZnONPs were selected for the physicochemical analyses, which included weight loss, TSS, TA, pH, firmness, and colour. In addition, CX-0.5ZnONPs may be more cost-effective to produce, which is an important factor for large-scale production and commercialisation in the future. Lower production costs can make the formulation more accessible and affordable.

### *Antibacterial properties of edible coating solutions*

The high nutrient and sugar contents, and low pH of bananas make them susceptible to microbial attacks, especially physically damaged banana peels, as a result of poor handling and storage (Kuyu and Tola, 2018). Microorganisms can also infect and spoil bananas through skin penetration, leading to undesirable changes, and affecting their quality. Thus, edible coating solutions must exhibit potent antibacterial properties to prevent such spoilage.

Figure 1 shows the antibacterial activity of the three edible coating solutions with ZnONPs as an antibacterial agent compared to the control (CX). The antibacterial activity was determined by measuring the diameter of the inhibition zone against *E. coli* and *S. aureus*. Both microorganisms are prevalent food-borne pathogens that pose a significant global public health concern. Specifically, *E. coli* can infect the gastrointestinal and urinary tracts, while *S. aureus* produces enterotoxins that result in food poisoning (Wang *et al*., 2020). Additionally, *E. coli* is a Gram-negative bacterium, while *S. aureus* is a Gram-positive bacterium. The antibacterial responses towards these two major categories of bacteria can provide a more comprehensive understanding of the CX-ZnONPs' inhibitory effects across these two types of bacteria (La *et al*., 2021).

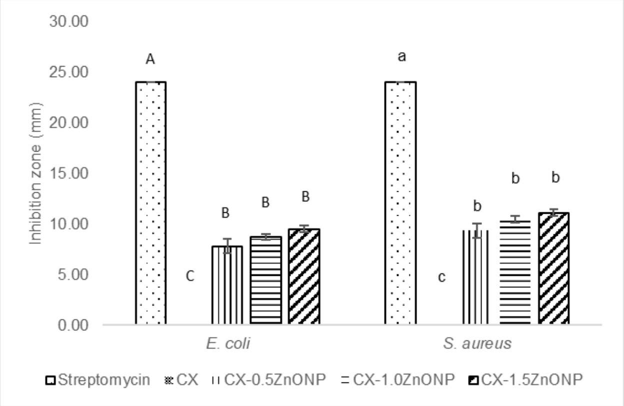


**Figure 1.** Antibacterial activity of CX, CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs against *E. coli* and *S. aureus*. Uppercase letters represent mean ± SD of different concentrations of ZnONPs against *E. coli*, showing a significant difference at $p < 0.05$. Lowercase letters represent mean ± SD of different concentrations of ZnONPs against *S. aureus*, showing a significant difference at $p < 0.05$.

Based on the results, an inhibition zone was detected against both bacteria for all three formulations (CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs), confirming the antibacterial properties of the ZnONPs-containing coating solutions. On the contrary, CX did not exhibit any inhibition zone. The lack of significant ($p > 0.05$) inhibition zone of CX-0.5ZnONPs compared to the CX-1.0ZnONPs and CX-1.5ZnOPs showed that it was sufficient at a lower amount to inhibit the growth of *S. aureus* and *E. coli*, and was thus further analysed. Edible coatings targeting food-borne pathogens, such as *E. coli* and *S. aureus*, require an appropriate concentration of antimicrobial agents to prevent contamination while maintaining quality and taste, enhancing food safety, and extending the shelf-life of the food (Wang *et al*., 2020).

The presence of ZnONPs triggers oxidative stress, and increases the penetration of $Zn^{2+}$ ions towards the negative charge of the cell wall, forming reactive oxygen species (ROS) (Krishnamoorthy *et* *al*., 2022). These harmful ROS can disrupt cellular components, such as DNA, proteins, and lipids, therefore effectively inhibiting microbial growth (Silva *et al*., 2019).

In brief, incorporating ZnONPs in the coating solution could enhance the function of edible coating as an antibacterial agent. The larger inhibition zone for *S. aureus* than for *E. coli* agreed with Silva *et al*. (2019), who stated that *S. aureus* (Gram-positive) possesses a thick peptidoglycan layer. The repeated carbohydrate and amino acid units in the peptidoglycan lead to a more intense interaction with ZnONPs. Conversely, *E. coli* (Gram-negative) contains a thinner peptidoglycan layer, and the external membrane contains lipopolysaccharides that reduce the antibacterial activity of ZnONPs (Silva *et al*., 2019).

### *Shelf-life study and colour of coated pisang berangan*

A shelf-life study is essential to determine the performance of the novel edible coating solution. Figure 2 shows that the shelf-life of *pisang berangan* was estimated to reach 18 d. However, the experiment showed that the shelf-life of coated *pisang berangan* was sustained up to 15 d in contrast to 9 d for uncoated *pisang berangan*. Thus, a cut-off period of 16 d was used for the physicochemical analysis. From the antibacterial analysis, the shelf-life study was carried out using the five formulations (No-CXZnONPs, CX, CX-0.1ZnONPs, CX-0.5ZnONPs, CX1.0ZnONPs, and CX1.5ZnONPs) to observe the colour change. Afterwards, the optimum formulation (CX-0.5ZnONPs) was used for physicochemical analysis.

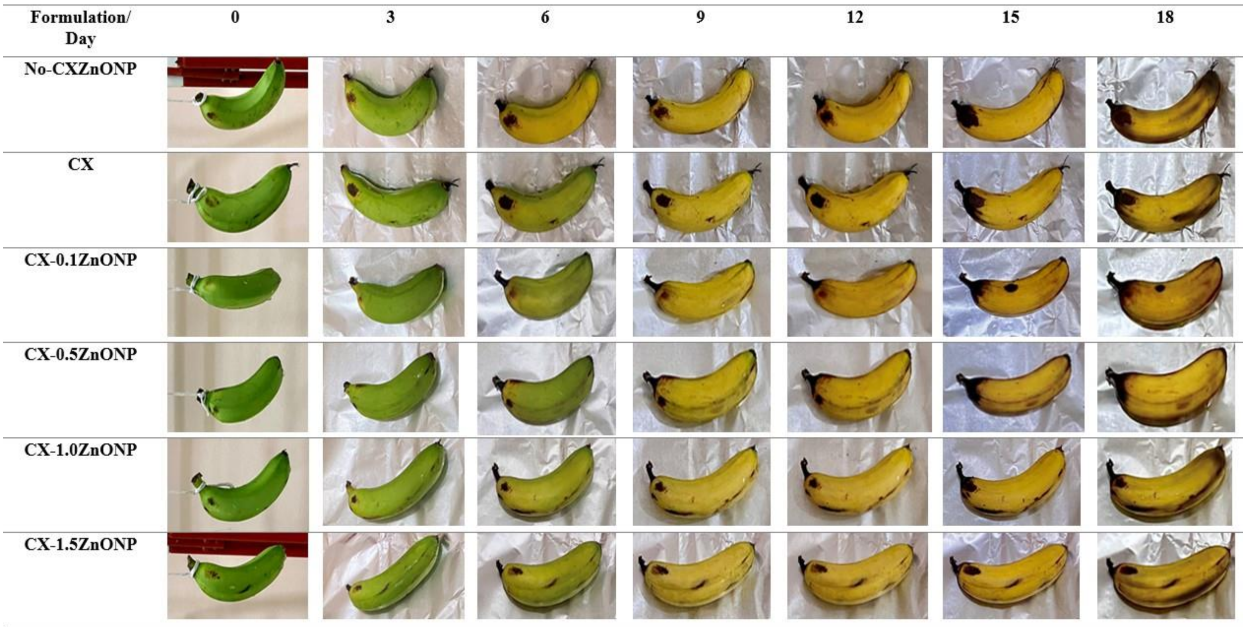


**Figure 2.** Physical appearances of No-CXZnONPs, CX, CX-0.1ZnONPs, CX-0.5ZnONPs, CX-1.0ZnONPs, and CX-1.5ZnONPs throughout 18 days of storage at room temperature (25°C).

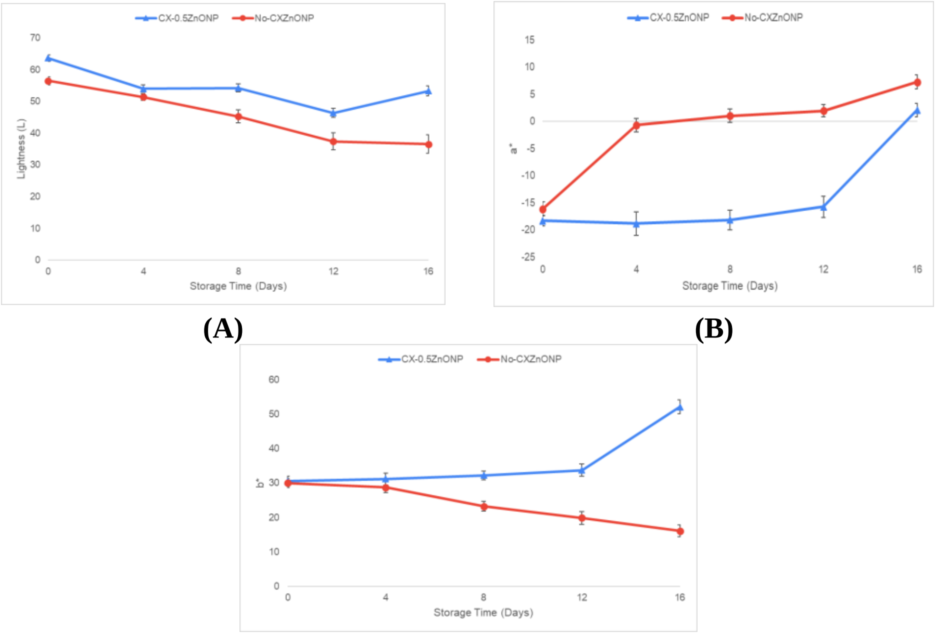
The shelf-life of bananas refers to the duration of the fruit which maintains its quality from the initial ripening stage until it becomes inedible. As shown in Figure 2, the No-CXZnONP (control) *pisang berangan* reached maturity index 5 (yellow with traces of green) on day 6 compared to the coated *pisang berangan* at maturity index 3 (more green than yellow). By day 9, the No-CXZnONP (control) *pisang berangan* reached maturity index 6 (fully yellow), while the coated *pisang berangan* reached maturity index 5 (yellow with traces of green). Further observations revealed that the No-CXZnONP, CX, and CX-0.1ZnONP *pisang berangan* shrank over time in contrast to 0.5-CXZnONP, 1.0-CXZnONP, and 1.5-CXZnONP *pisang berangan* that maintained their size from day 0. By day 12, all *pisang berangan* reached maturity index 6 (fully yellow), and the shrinkage of the three formulations was evident.

However, the No-CXZnONP, CX, and CX-0.1ZnONP *pisang berangan* reached maturity index 7 (fully yellow with brown spots) by day 15, while the 0.5-CXZnONP, 1.0-CXZnONP, and 1.5-CXZnONP *pisang berangan* remained at maturity index 6 (fully yellow). On final day 18, more brown spots appeared on the No-CXZnONP, CX, and CX-

0.1ZnONP *pisang berangan* peels. Meanwhile, the 0.5-CXZnONP, 1.0-CXZnONP, and 1.5-CXZnONP *pisang berangan* reached maturity index 7 (fully yellow with brown spots). These results agreed with the findings of Thakur *et al*. (2019b), who formulated a rice starch coating blended with sucrose ester that effectively controlled and extended the post-harvest physiology and quality of Cavendish bananas.

*Colour analysis*

Colour is the most prominent visual characteristic of bananas (Silva *et al.*, 2019). In view of this, the colour development of uncoated and coated *pisang berangan* peels was measured during the 16-day storage (Nguyen *et al.*, 2021). Figure 3 shows that the L* value of CX-0.5ZnONPs significantly slowly decreased ($p < 0.05$) compared to CXZnONPs with increased storage time. In particular, the L* value of No-CXZnONPs declined steadily from day 0 (56.52) until day 16 (36.56). In comparison, the L* value of CX-0.5ZnONPs was significantly higher ($p < 0.05$) from day 0 (63.68) until day 16 (53.34) than the uncoated *pisang berangan*. This indicated that the lightness of the No-CXZnONPs was more reduced than the CX-0.5ZnONPs. These results agreed with the visual observation of *pisang berangan*, in which the brown patch or senescent marking on ripe *pisang berangan* might have been due to cell necrosis as a result of chlorophyll breakdown, thus decreasing the L* value (Dwivany *et al.*, 2020). Besides, browning is contributed by phenol oxidation catalysed by polyphenol oxidase, phenylalanine, and peroxidase activity (Zhou *et al.*, 2021). The oxidation of phenolic compounds in banana peels by the polyphenol oxidase produces melanin, leading to the emergence of brown spots (Wohlt *et al.*, 2021).



(A) (B)

(C)

**Figure 3. (A)** L*, **(B)** a*, and **(C)** b* values of No-CXZnONPs and CX-0.5ZnONPs throughout 16 days of storage at room temperature (25°C).

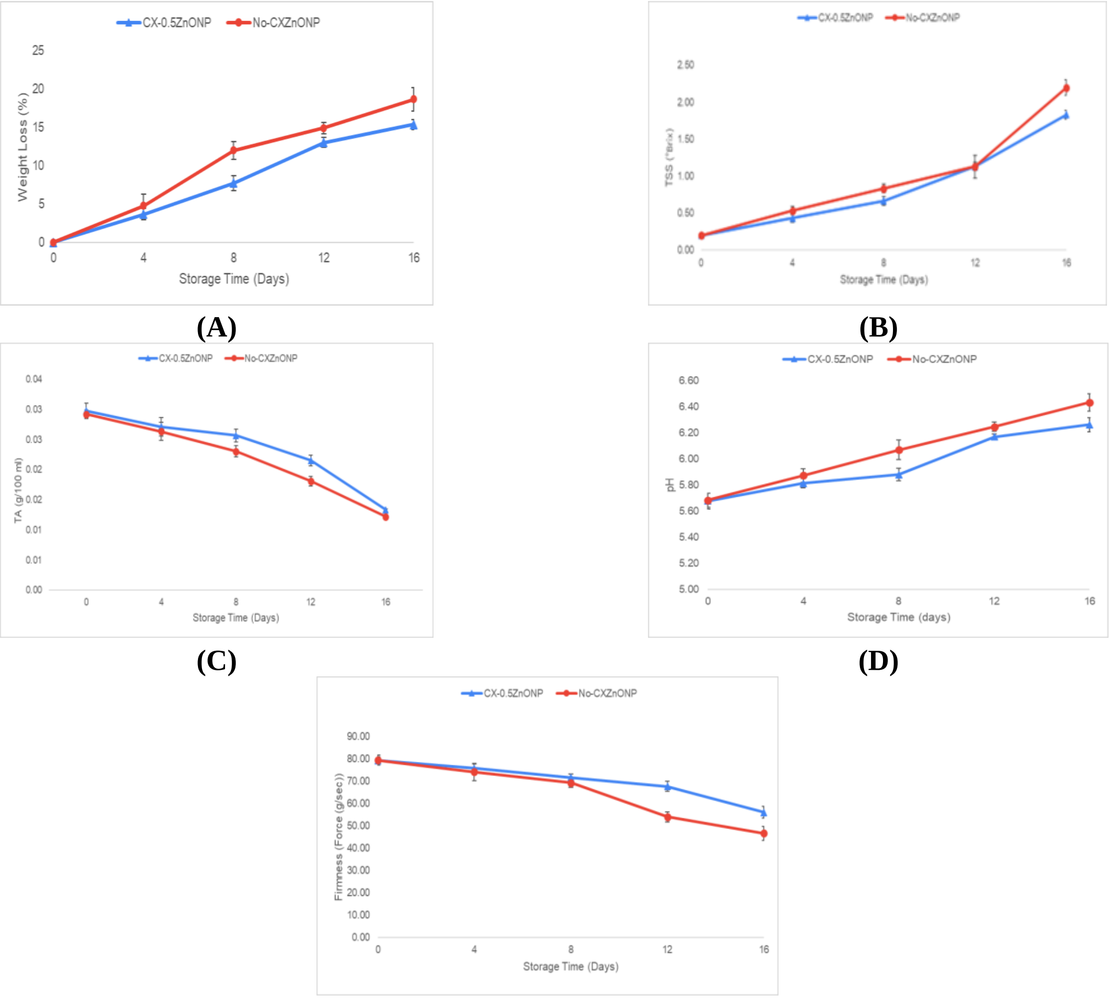
The a* values indicated the change in colour of *pisang berangan* peels from green to red. Figure 3 shows that the a* values increase in both treatments with prolonged storage time. The a* value of No-CXZnONPs increased rapidly from day 4 (-0.68). However, the a* value of CX-0.5ZnONPs was significantly lower ($p < 0.05$) than the uncoated *pisang berangan* with increased storage time. The colour of CX-0.5ZnONPs *pisang berangan* peels remained green from day 0 (-18.30) to 12 (-15.73), and started to increase, which began to change to red until day 16 (2.12).

The b* values indicated the change in colour of *pisang berangan* peels from yellow to blue. Figure 3 shows that the b* values in both treatments decrease with increasing storage time ($p < 0.05$). The b* value of No-CXZnONPs rapidly decreased from day 4 (28.78) and above (16.12), potentially due to the emergence of black spots and brown pigments (Nguyen *et al.*, 2021). As the banana ripens, the chlorophyllase enzyme breaks down chlorophyll, changing the peel's colour from green to yellow and then brown (Maduwanthi and Marapana, 2019). Comparatively, the b* value of CX-0.5ZnONPs was significantly higher ($p < 0.05$) than the uncoated *pisang berangan* from day 4 onwards. This indicated that the colour of CX-0.5ZnONPs *pisang berangan* peels remained yellow from day 0 (30.52) to 16 (52.16), which agreed with the visual observation of *pisang berangan*.

### *Weight loss*

As a climacteric fruit, bananas undergo rapid weight loss during storage. Hence, weight loss is one of the vital parameters used to determine the quality of bananas. Based on Figure 4, the weight loss percentage of *pisang berangan* in both treatments gradually increased with prolonged storage time. The No-CXZnOPs exhibited rapid weight loss from day 4 (4.81%) until day 16 (18.67%). However, the weight loss percentage of CX-0.5ZnONPs was significantly lower ($p < 0.05$) from day 4 (3.69%) until day 16 (15.37%). The rate of weight loss for CX-0.5ZnONP decreased, which was similar to the decreased weight loss in bananas coated with starch-based film (Thakur *et al*., 2019b).

This suggested that the coating solution on *pisang berangan* peel might have reduced the water loss due to the modified internal atmosphere and the blocking of pores on its peel (Nguyen *et al*., 2021). The presence of hydroxyl groups forms hydrogen bonds inside the coating matrix and on the fruit peel, effectively reduced water loss (Saberi *et al*., 2018). The chemical interaction between the compounds in the film coating could also reduce water vapour permeability and limit water loss (Pinzon *et al*., 2019). Furthermore, the low oxygen permeability of cassava starch provides an oxygen barrier that reduces the respiration rate, and minimises weight loss in coated bananas (Martins *et al*., 2022). In



**(A)** **(B)**

**(C)** **(D)**

**(E)**

**Figure 4. (A)** Weight loss, **(B)** TSS, **(C)** TA, **(D)** pH, and **(E)** firmness levels of No-CXZnONPs and CX-0.5ZnONPs throughout 16 days of storage at room temperature (25°C).

contrast, uncoated *pisang berangan* lost weight due to the conversion of starch to sugars, which led to fruit softening. Therefore, the coating solution provided an efficient and prolonged preservation of *pisang berangan* compared to uncoated ones.

### *Total soluble solid (TSS)*

The TSS measures the total concentration of dissolved substances in a matrix such as fruits and vegetables, primarily sugars, amino acids, and other soluble materials. As shown in Figure 4, the TSS of *pisang berangan* increased in both treatments with extended storage time. The TSS value of No-CXZnONPs increased from day 4 (0.43 °Brix) until day 16 (2.20 °Brix). However, the TSS value of CX-0.5ZnONPs was significantly lower ($p < 0.05$) than uncoated *pisang berangan*, except at day 12 (1.13 °Brix). This agreed with Chettri *et al.* (2023), who utilised lima bean starch as an edible coating to preserve sapota fruit. This indicates the hydrolysis of starch into sugars, such as glucose, sucrose, and fructose (Nguyen *et al.*, 2021). The applied surface coatings may also reduce the respiratory rate, which decreases the rate of starch hydrolysis (Nguyen *et al.*, 2021). The starch degradation into sugar causes bananas to ripen faster with an increased respiration rate, leading to a high TSS value (Thakur *et al.*, 2019b).

### *Titratable acidity (TA)*

The TA measures the amount of organic acids of natural substances that contribute to fruit acidity. Based on Figure 4, the TA in both treatments decreased gradually with increased storage time. The TA of No-CXZnONPs decreased from day 4 (0.026 g/100 mL) until day 16 (0.012 g/100 mL). Comparatively, the TA of CX-0.5ZnONPs was significantly higher ($p < 0.05$) and consistently decreased than the No-CXZnONPs from day 4 (0.027 g/100 mL) until day 16 (0.013 g/100 mL).

Malic and citric acids are the most abundant organic acids in bananas at maturity index 1, and decrease as they ripen (Maduwanthi and Marapana, 2019). During ripening, malic acid is used as a substrate for the enzymatic reaction for the respiration process (Shahbazi and Shavisi, 2020). The decrease in acidity is associated with the decrease in organic acids, which corresponds to the accumulation of sugars in bananas (Thakur *et al.*, 2019b). The consistent decrease in acidity during ripening results from starch hydrolysis, which leads to an increase in total sugars. Malate and oxaloacetate are dicarboxylates generated from the decarboxylation of tricarboxylates, which undergo further decarboxylation into phenol pyruvate and activate gluconeogenesis (Zhou *et al.*, 2023). This suggested that the surface coating might have reduced the fruit respiration rate, and hindered the consumption of TA (Nguyen *et al.*, 2021), thus extending the shelf-life of *pisang berangan*.

### *pH level*

Figure 4 shows that the pH level in both treatments gradually increased with prolonged storage time. The pH of No-CXZnONPs constantly increased from day 0 (5.68) to day 16 (6.43). However, the pH of CX-0.5ZnONPs was significantly lower ($p < 0.05$) over time (pH 5.68 - 6.26) than the No-CXZnONPs. The increase in pH is due to the utilisation of acid contents as a substrate during respiration. This situation proved that CX-0.5ZnONPs could reduce respiration rate by delaying the breakdown of organic acids. This further indicated the protective role of the coating solution, which slowed down ripening (Nguyen *et al*., 2021).

### *Firmness*

Based on Figure 4, the firmness of *pisang berangan* in both treatments decreased throughout the storage period. The No-CXZnONPs recorded a rapid loss of firmness after day 8 (69.27 g/sec) until day 16 (46.60 g/sec). Remarkably, the firmness of CX-0.5ZnONPs was significantly higher ($p < 0.05$) on day 12 (67.70 g/sec) and day 16 (56.07 g/sec). The softening of *pisang berangan* during ripening could have been due to sugar breakdown, which then led to the degradation of pectin, hemicellulose, polysaccharides, and starch (Shahbazi and Shavisi, 2020). Ripening also triggers the depolymerisation of the matrix glycan and pectin at the cell wall, resulting in the loss of neutral sugars from the pectin lateral chains (Pan *et al*., 2021). Eventually, the pectin-rich layer binds with the cell wall, contributing to the decreased rigidity and firmness of the fruit (Nguyen *et al*., 2021). Besides, the moisture in bananas is lost and dispersed into the external environment due to respiration and transpiration during post-harvest storage, resulting in the loss of water and banana weight (Li *et al*., 2019).

The retained firmness of coated *pisang berangan* indicated that cassava starch coating effectively reduced the metabolic and enzymatic activities in them, resulting in the slower degradation of pulp tissue (Thakur *et al*., 2019b). Cassava starch serves as a semi-permeable barrier that facilitates the formation of hydrogen bonds between the hydroxyl groups within the coating matrix and on the banana peel (Arayaphan *et al*., 2020). The addition of glycerol as a blended composite improved the compactness and homogeneity of the coating, and reduced pore size and film cracks (Saberi *et al.*, 2018). This also enhanced the adhesion of the coating solution to the banana surface (Zhou *et al*., 2021). Overall, the semi-permeability of the cassava starch regulated the fruit's respiration process, leading to slower ripening and gradual changes in TSS, pH and TA values, weight loss, and firmness (Chettri *et al*., 2023).

This agreed with previous research on the preservation of 'Cripps Pink' apples using rice starch and carrageenan coatings (Thakur *et al*., 2019a), verifying that the colourless and transparent starch-based coatings are biodegradable and safe for consumption (Wilfer *et al.*, 2021; Martins *et al*., 2022). Economically, cassava starch is also a renewable, abundant, and reliable material for the development of edible surface coating (Wilfer *et al*., 2021).

Furthermore, xanthan gum was incorporated into the coating solution to overcome the negative impact of starch, and improve the overall characteristics of the coating (Wilfer *et al.*, 2021). The hydrocolloid property of xanthan gum enhances the mechanical strength of the coating, providing flexibility, elasticity, and tear resistance (Salehi, 2019). It also strengthens the barrier properties against moisture, oxygen, and $CO_2$. Besides, the plastic and transparent characteristics of xanthan gum make it a desirable component in the coating solution.

Apart from that, the presence of ZnONPs improved the moisture barrier properties of the coating by interacting with hydrogen bonds in the composite solution. This promotes smooth and well-dispersed adhesion of the coating solution to the surface of the banana peel, limiting the transfer of oxygen and $CO_2$ through the peel, and preventing aerobic bacterial penetration (Alamdari *et al.*, 2020). Consequently, the banana's colour degradation and enzymatic activity were slowed down. The enhanced moisture barrier also facilitated the controlled release of ethylene gas, which is a crucial step for ripening in bananas as climacteric fruits (Santhosh and Sarkar, 2022).

Furthermore, the nano-sized ZnO particles played a crucial role in reducing the pore size of the composite coating, leading to a more uniform distribution of the coating ingredients (Li *et al.*, 2019). ZnONPs are also classified as GRAS by the USFDA, given their desirable thermal and mechanical properties that contribute to the stability of biocomposite coating solutions. In addition, the colour of the ZnONPs did not impact the overall aesthetic value of coated *pisang berangan*, resulting in an appealing appearance. However, using an optimal amount of ZnONPs is essential to avoid excessive levels of inorganic ingredients in food products.

## Conclusion

The present work demonstrated a novel edible coating solution formulated through the combination of cassava starch, xanthan gum, and ZnONPs that effectively extended the shelf-life of *pisang berangan* up to 15 days. The findings were supported by the significantly lower weight loss, pH level, and TSS, as well as a considerably higher TA level and firmness of the coated *pisang berangan* compared to uncoated *pisang berangan* within the same storage period. The colour intensity also corresponded with the maturity index level of *pisang berangan*. Moreover, the addition of a minimal ZnONPs concentration of 0.5 mg/L (CX-0.5ZnONPs) served as a potent antibacterial agent against *E. coli* and *S. aureus*. In conclusion, the newly developed cassava starch/xanthan gum/ZnONPs edible coating solution is a promising, environmentally friendly, and economical edible coating material that can effectively extend the shelf-life of *pisang* berangan, and preserve its quality post-harvest. The outcome of the present work has the potential to significantly contribute to *pisang berangan* industry, meeting local market demands, facilitating exports, and preventing food waste.

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