

Synergistic Effects of Aloe Vera and Moringa Oleifera in Chitosan-Based Composites for Preservation of Banana Fruit

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Chitosan, derived from deacetylated chitin, is renowned for various industrial applications, including fruit coatings, due to its distinct biological and physicochemical properties such as film-forming capability, biodegradability, non-toxicity, biocompatibility, and antimicrobial attributes. Chitosan exhibits certain limitations, including hydrophilicity, low mechanical strength, and poor mass transfer barriers. This research leveraged chitosan's biocompatibility by using sodium tripolyphosphate (NaTPP) as a crosslinker to strengthen chitosan (CH) molecules via ionic gelation, with Tween 80 as an emulsifier. Adding Aloe vera (AV) and Moringa oleifera (MO) to the chitosan-NaTPP coating solution aimed to further limit the respiration rate and inhibit microorganism proliferation. The study examined the physicochemical stability and characteristics of chitosan-NaTPP coating solutions with varying concentrations of AV, MO, and both. The effect of the deposition method, including single and double layers, on banana fruit was also studied over 10 d of storage. The particle size was characterized using Malvern Zeta Sizer Nano ZS and chemical bonding of the coating solutions using Fourier Transform Infrared Spectroscopy (FTIR). The particle size of the CH-NaTPP coating solution was 3.2 nm, CH-NaTPP/AV was 3.6 nm, CH-NaTPP/MO was 3.1 nm, and CH-NaTPP/AV/MO was 2.7 nm. Bananas coated with CH-NaTPP containing 1 % AV and 3 % MO exhibited the slowest ripening process during 10 d of storage. In conclusion, the formulated CH-NaTPP/1 % AV/3 % MO composite proved effective for fruit coating applications, particularly for bananas.

1. Introduction

Tropical fruits significantly contribute to food security, with an increase of 8.3 Mt in 2022 and further growth projected (Olunusi et al., 2024). Bananas (*Musa acuminata*) are major tropical fruits, notable for their high export volume in the global trade market. In 2022, the economic value of bananas was nearly USD 10 billion, compared to the combined USD 11 billion generated by mango, pineapple, avocado, and papaya (OECD-FAO, 2024). This is due to their importance in human nutrition and their impact on economic growth and income, particularly in developing nations. Nutritional benefits of bananas include carbohydrates (22.84 g/100 g), vitamins C, A, B6, and B12, energy (approximately 370 kJ/100 g), potassium (358 mg/100 g), catechin, and resistant starch (Ranjha et al., 2022). Regular consumption of bananas as part of a healthy lifestyle offers therapeutic benefits, such as enhanced heart and intestinal health, prevention of leg cramps during pregnancy, ulcers and heartburn prevention, reducing menstrual bleeding and its peels can treat skin infections and bruises (Kumar et al., 2012). Bananas often face rapid senescence during storage due to postharvest challenges like chilling effects, mechanical handling, short shelf life, microbial attack, and metabolic activities (Tchinda et al., 2023).

Biopolymer-based coatings have emerged as sustainable alternatives to synthetic and refrigeration preservation methods due to the adverse effects these methods pose on human health and the environment, as well as the

rapid deterioration of fruits (Olunusi et al., 2024). Chitosan, a common derivative of partially deacetylated chitin, is renowned for its unique biological and physiological properties, including film-forming tendencies, non-toxicity, biodegradability, eco-friendliness, antimicrobial capacity, and biocompatibility (Nguyen et al., 2024). Researchers have employed chitosan-based and other biopolymer composites to mitigate postharvest challenges in bananas. Functionalized chitosan-based composites with natural extracts have enhanced coating efficiency on bananas (Nguyen et al., 2024), successfully delaying ripening, weight loss, titratable acidity, CO₂ synthesis, and total soluble solids. Das et al. (2023) investigated the effect of chitosan and caraway oil nanoemulsion (CCN) on bananas, finding that CCN reduced quality deterioration, exhibited strong antibacterial activities against *Escherichia coli* and *Salmonella typhi*, and increased antioxidant enzyme activity. Thakur et al. (2019) employed rice starch/carrageenan coating to preserve bananas at room temperature, extending shelf life by 40 %, delaying ethylene biosynthesis, and reducing weight loss, firmness, and chlorophyll degradation. Tchinda et al. (2023) optimized an edible coating from starch, Aloe vera, and Arabic gum at different concentrations, demonstrating that this combination prolonged banana shelf life by delaying the production of total soluble solids, weight loss, firmness loss, and chlorophyll degradation.

It is worth noting none of the previous studies reported the combined effect of chitosan-NaTPP composites involving Aloe vera and *Moringa oleifera*. The addition of sodium tripolyphosphate (NaTPP) as a cross-linking agent was intended to improve the mechanical strength of chitosan and encourage microparticle formation (Ramli et al., 2023), while Aloe vera (AV) and *Moringa oleifera* (MO) are natural extracts used to inhibit microbial attack on banana surfaces and enhance fresh produce quality and safety after harvesting (Tchinda et al., 2023). This is owing to the presence of high levels of antioxidants and antibacterial capacity against a wide range of microbes, making the two-leaf extracts an attractive material for the edible coating. This research aimed to evaluate the synergistic effects of these combinations (Odetayo et al., 2022).

2. Materials and method

2.1 Preparation of stock solution

The chitosan-NaTPP polymer fruit coating was prepared following the methods outlined by Odetayo et al. (2022) and Ramli et al. (2023), with several improvements. Glacial acetic acid (99.5 % purity) was diluted with distilled water to a 0.1 % v/v concentration to create the acetic acid stock solution. Medium molecular weight chitosan flakes (190-310 kDa) from Sigma Aldrich® were dissolved in this acetic acid solution at a concentration of 0.2 % w/v and stirred overnight. A solution of NaTPP (85 % purity) at 0.5 % w/v was prepared by dissolving NaTPP from Sigma Aldrich® in deionized water and stirring overnight at room temperature. The solution was then filtered using a 0.45 µm syringe filter. A 0.5 % Tween 80 from EvaChem was preheated in a water bath at 60 °C for 10 min. The CH solution was homogenized with Tween 80 and the NaTPP solution using a Silverson L5M-A high-shear laboratory mixer at 8,000 rpm for 30 min. With constant stirring, the NaTPP solution was added to the CH solution. The pH of the mixture was measured and adjusted to 5.7 - 6.1 using 2 M sodium hydroxide to ensure suitability for banana skin. AV and MO were prepared by dissolving AV and MO powder at different concentrations in deionized water. The AV and MO were then added to the CH-NaTPP solution and homogenized for an additional 15 min at 8,000 rpm. The edible coating matrix was combined as shown in Table 1 according to the previous findings with adjustments. The formulated matrix was reported to lower respiration and decay rates, improve firmness and retention capacity, when compared to using chitosan solution alone (Odetayo et al., 2022).

Table 1: Formulated edible coating matrix

Formulation Type 1	Formulation Type 2	Formulation Type 3
CH-NaTPP (SC/DC)	CH-NaTPP (SC/DC)	CH-NaTPP (SC/DC)
CH-NaTPP/0.6 %AV (SC/DC)	CH-NaTPP/1 %AV (SC/DC)	CH-NaTPP/1.2 %AV (SC/DC)
CH-NaTPP/1 %MO (SC/DC)	CH-NaTPP/3 %MO (SC/DC)	CH-NaTPP/6 %MO (SC/DC)
CH-NaTPP/1 %AV/3 %MO (SC/DC)	CH-NaTPP/0.6 %AV/1 %MO (SC/DC)	CH-NaTPP/1.2 %AV/6 %MO (SC/DC)

SC = Single coating; DC = Double coating. Note: Formulation Type 1 was discussed in the results because of its better performance over others.

2.2 Physicochemical analysis of CH-NaTPP coating solution

The coating solution characterization was determined using the Delsa Max PRO Zeta Potential DLSA (B29164) and Nicolet™ iS™ 5 FTIR Spectrometer (Thermo Scientific™, USA). The Delsa Max PRO Zeta Potential DLSA (B29164) was used to analyze the particle radius with the assistance of infrared light scattering. Meanwhile FTIR analyses chemical bonding and functional groups in materials by absorbance of transmitted infrared light

as spectra, and the wavelength whose wavelength can be measured with the standards. The wavelength was recorded between 4,000 to 1,000 cm^{-1} .

2.3 Application of polymer coating on fruits

Banana (*Musa acuminata*) was obtained from the local supplier with uniform size and color without any signs of mechanical damage or fungal decay. The banana fruits were rinsed with tap water and then immersed in a 2 M sodium hypochlorite solution, NaOCl from R&M Chemicals for 3 min. For the single-layer coating method, the bananas were immersed once into the coating solution beaker. For the double-layer coating method, the bananas were immersed twice into the coating solution, drying in between. The coated bananas were left to dry completely and then stored at room temperature. The fruits were visually inspected every 3 d throughout a total storage period of 10 d.

3. Result and discussion

3.1 Characterization of coating solution: Particle size analysis

Table 2 shows that the particle size of the coating solution ranges from 2.7 to 3.2 nm for formulation type 1. Smaller particle sizes result in larger surface areas, facilitating better adhesion to the fruit surface and promoting the formation of a more uniform and durable coating (Bekele and Emire, 2023). This enhanced adherence can improve barrier properties by minimizing moisture loss, gas exchange, and the transfer of taste and aroma molecules between the fruit and its surroundings. These qualities are particularly beneficial for prolonging the fruit's shelf-life (Nguyen et al., 2021) and maintaining its overall quality during storage and transit (Bekele and Emire, 2023).

Table 2: Particle size for the formulations

Sample	Radius, nm
CH-NaTPP	3.2
CH-NaTPP/0.6 %AV	3.6
CH-NaTPP/1 %MO	3.1
CH-NaTPP/1 %AV/3 %MO	2.7

3.2 FTIR analysis of CH-TPP coating solution

Table 3 observed peaks at 3,346 cm^{-1} in the CH-NaTPP formulation, shifting to 3,354.34 cm^{-1} in CH-NaTPP/0.6 % AV, 3,371.45 cm^{-1} in CH-NaTPP/1 % MO, and 3,351.73 cm^{-1} in CH-NaTPP/1 % AV/3 % MO. This spectral variation strongly suggests the presence of hydrogen bond interactions between chitosan and the components of aloe vera or moringa. The observed shifts in peak positions indicate changes in the molecular environment, specifically in the vibrational modes associated with hydrogen bonding (Yan et al., 2023). This spectral analysis serves as compelling evidence of dynamic intermolecular interactions in the CH-NaTPP/AV and CH-NaTPP/MO formulations, highlighting the potential influence of aloe vera and moringa on the hydrogen bonding characteristics within the chitosan matrix (Yan et al., 2023). The degree of shift varied depending on the specific formulation, indicating that the type and extent of interactions differed among the CH-NaTPP/AV, CH-NaTPP/MO, and CH-NaTPP/AV/MO composite coatings. This variation is likely due to differences in the substituted functional groups from the natural extracts in place of the amino group of chitosan (Nguyen et al., 2024). According to FTIR spectrum analysis, the addition of natural extracts to chitosan impacts intermolecular hydrogen bonding energies in the coating matrix, altering the supramolecular structure and properties of the chitosan-based edible films.

Table 3: FTIR peak value of the coating solution

Sample	N-H stretching overlapped with stretching (cm^{-1})	O-HN-H bending vibration (cm^{-1})
CH-NaTPP	3,346.00	1,636.90
CH-NaTPP/0.6 %AV	3,354.34	1,636.55
CH-NaTPP/1 %MO	3,371.45	1,636.83
CH-NaTPP/1 %AV/3 %MO	3,351.73	1,636.40

3.3 Weight loss

Based on Figure 1, the control group experienced greater weight loss of 25.54 % than the coated group CH-NaTPP/1 % AV/3 % MO of 18.1 % after 10 d. This difference of 6.44 % is relatively high compared to previous findings of a 2.05 % difference of the control higher than the coated fruit, after 10 d of investigation (Thakur et

al., 2019). Low weight loss is preferable for maintaining fruit freshness, as it indicates better moisture retention and improves overall quality. The CH-NaTPP/1 % AV/3 % MO coating effectively prevents weight loss, suggesting reduced moisture escape. The inclusion of chitosan nanoparticles in the coating likely contributes to this effect by inhibiting CO₂ release during the banana's respiration process (Odetayo et al., 2022). While CH-NaTPP/0.6 % AV performed better than the control, it exhibited higher weight loss than both CH-NaTPP/MO and CH-NaTPP/AV/MO. Despite aloe vera's moisturizing properties, it may not be as effective as moringa in providing a water vapor barrier due to moringa's lipid and protein content, which enhances the coating's water vapor barrier capabilities (Pop et al., 2022). CH-NaTPP/1 % MO ranked between CH-NaTPP/AV and CH-NaTPP/MO, underscoring the multifunctional effects of *Moringa oleifera*'s proteins, polysaccharides, lipids, and bioactives. These components synergistically enhance chitosan's water barrier properties, making moringa a valuable addition (Tchinda et al., 2023). Fruits coated with *Moringa oleifera* extracts exhibited minimal moisture loss, demonstrating the potential of these bioactive compounds as natural components in edible fruit coatings. *Moringa oleifera*'s diverse bioactive constituents and beneficial properties make it a promising candidate for applications aimed at improving fruit quality and preservation (Pop et al., 2022).

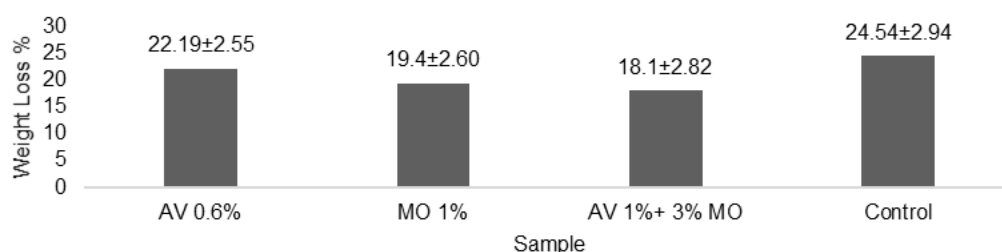


Figure 1: Histogram data of weight loss on bananas with different coating formulations

3.4 Decay process of Banana and Total Soluble Solid (TSS)

The development of a brownish color in fruits and vegetables is linked to the activity of polyphenol oxidase, which converts phenols into quinones through polymerization, leading to brown pigment formation (Nguyen et al., 2024). This complex reaction not only alters the structure of phenolic compounds but also results in larger molecules. On the other hand, bananas coated with CH-NaTPP/1 % AV/3 % MO exhibited the best outcomes, showing fewer dark spots. Du et al. (2023) highlighted that these coatings effectively reduced water vapor and slowed down banana respiration and retards the aging process. While CH-NaTPP/AV coatings provide moisture retention and antibacterial protection, they may not adequately defend against all deterioration factors (Tchinda et al., 2023). Chitosan coatings delayed ripening and extended the storage lifespan of bananas by creating an impermeable barrier that regulated gas exchange and reduced respiration rates (Thakur et al., 2019). This barrier significantly reduced metabolic activities such as ethylene biosynthesis and chitosan's antibacterial properties inhibited the growth of bacteria that induce spoilage.

As fruits ripen, their total soluble solids (TSS) increase due to the conversion of starches into simpler sugars like sucrose, fructose, and glucose, making the fruit sweeter (Du et al., 2023). Table 4 further affirmed the effectiveness of the coating formulations (CH-NaTPP/AV/MO < CH-NaTPP/MO < CH-NaTPP/AV < CH-NaTPP) in delaying ripening, which is interpreted as the higher the TSS, the quicker the ripening, and the faster the deterioration.

Table 4: Total Soluble Solid of observed banana on day 10

Sample	Refractometer (% Brix)
Control	3.7±0.56
CH-NaTPP/0.6 %AV	3.0±0.46
CH-NaTPP/1 %MO	2.9±0.47
CH-NaTPP/1 %AV/3 %MO	2.4±0.54

Furthermore, Figures 2, 3, and 4 illustrate that CH-NaTPP/1 % AV/3 % MO coatings resulted in the least weight loss, followed by CH-NaTPP/1 % MO and CH-NaTPP/0.6 % AV. This coating effectively slows water loss, respiration, and delays ripening by inhibiting starch breakdown (Du et al., 2023). Moringa extracts enhance the moisture barrier, exhibit antimicrobial effects, and possess antioxidant properties (Pop et al., 2022). The strong bonding between hydrophobic proteins and polysaccharides with chitosan chains prevented the mobility and diffusion of substances, moisture, and gases. Although the sugar content in CH-NaTPP/AV was higher than the

control, it was still lower than that in CH-NaTPP/MO and CH-NaTPP/AV/MO. The high water content in AV may result in a more open structure and reduced barrier properties when added to the CH coating.

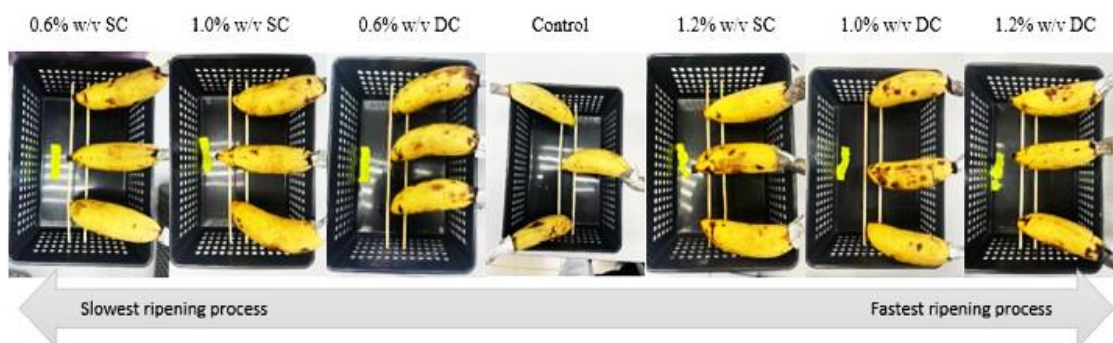


Figure 2: Ripening process line of observed banana on day 10 (CH-NaTPP/0.6 %AV)

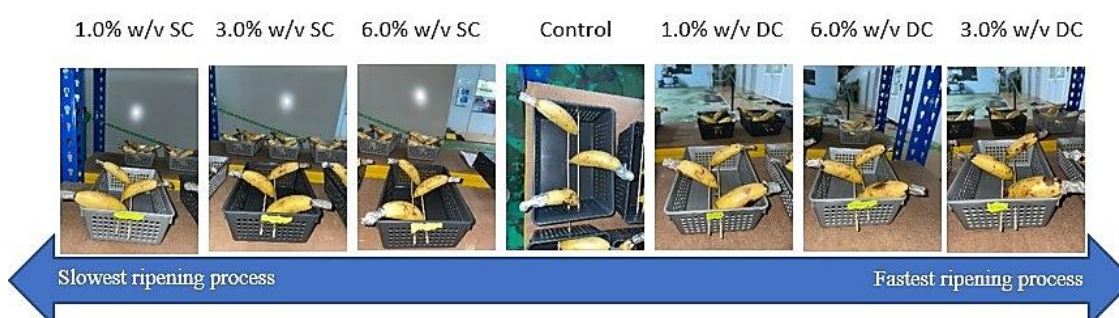


Figure 3: Ripening process line of observed banana on day 10 (CH-NaTPP/1 %MO)

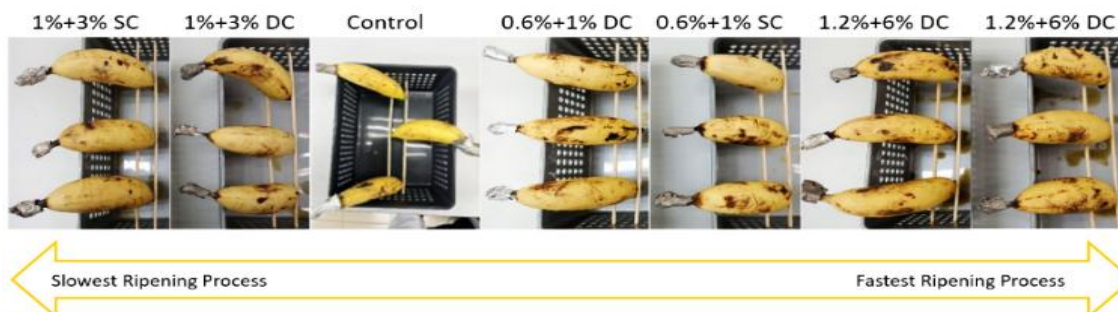


Figure 4: Ripening process line of observed banana on day 10 (CH-NaTPP/1 %AV/3 %MO)

4. Conclusions

Based on this study, the CH-NaTPP/1 % AV/3 % MO formulation demonstrates the best performance in extending the shelf life of bananas by achieving lower weight loss and reduced total soluble solids (TSS) compared to other coating formulations. This protective coating acts as a barrier, minimizing water loss through evaporation from the banana's surface. By slowing down the moisture loss, the coating ensures that the bananas retain more water, preventing dehydration and extending their shelf life.

Nomenclature

CH – Chitosan

NaTPP – Sodium Tripolyphosphate

AV – Aloe vera

MO – Moringa Oleifera

nm – nanometer

m – meter

FTIR – Fourier Transform Infrared Spectroscopy, cm^{-1}

cm^{-1} – per centimeter

rpm – revolution per minute

$^{\circ}\text{C}$ – degree celcius

M – molar concentration

kDa – kiloDalton

w/v – weight per volume

v/v – volume per volume
 USD – United States Dollar
 kJ – kilo Joule
 g – grams

mg – milligram
 SC – Single coating
 DC – Double coating

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