

Characteristic and Stability of Chitosan-Based Polymer for Fruit Coating in the Presence of Ginger Essential Oil: Effect of Acetic Acid Concentration

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Chitosan-based polymer is ideal for coating fruits in post-harvest treatment to maintain their quality as they can create a film and is biocompatible. The mechanical strength of chitosan (CH) can be enhanced by forming ionic crosslinking between CH molecules through tripolyphosphate (TPP) and surfactants, Tween 80. The presence of ginger essential oil (GEO) increases antimicrobial activity and reduces the swelling of fruit. Selecting appropriate molecular weight of CH, concentration of acetic acid and Tween 80 is necessary for ideal chitosan-based coating properties and stability. This study examined the effect of the parameters on the physicochemical characteristics of final CH-TPP particles. Chitosan-based polymer coating was produced by ionic gelation and the formulated coating solution's particle size and chemical bonding were characterised using Malvern Mastersizer 2000 and Fourier Transform Infrared Spectroscopy (FTIR). The coating on papaya was studied for decay prevention over eight days. The particle size of the formulated coating solutions was found in the 107-221 nm range. The presence of crosslinking between CH and TPP was observed from FTIR analysis. Good stability of CH-TPP coating was found when utilising high molecular weight chitosan (HMW-CH), the concentration of acetic acid and Tween 80 at 1.0 % and 0.3 %, respectively. Papaya coated with CH-TPP, and GEO 0.3% had the slowest ripening process during eight-day storage. In conclusion, the formulated CH-TPP with 0.3 % GEO coating was effective for fruit coating applications, especially papaya.

1. Introduction

Papaya (*Carica Papaya L.*) is a local and non-seasonal fruit in Malaysia, known for its nutritional benefits with an annual export value of MYR 100-120 million, reflecting the importance of Malaysia's papaya industries. The post-harvest of papaya has become difficult in the tropical fruit's marketing and transportation. The papaya fruit's short shelf life adds to its poor look, texture, taste, and overall quality and can result in post-harvest losses of up to 40 % if not consumed promptly (Sekeli et al., 2018). The polymer coating is one of the methods of preserving fruit and keeping the quality of the fruit throughout the post-harvest storage period.

The demand for fruit coating has increased due to its convenience, improved barrier and mechanical properties, and increased shelf life. The available fruit coating in the market is expensive, non-edible, and some contain harmful ingredients. A safe, edible, biodegradable and cheaper fruit coating product with strong mechanical strength would be the consumer's desire. The chitosan-based polymer is a popular natural polymer widely used to produce edible fruit coating. Chitosan (CH) is non-toxic, biodegradable, biocompatible, and microbe resistant. This study aims to enhance the particle stability of chitosan and improve its ability as a moisture barrier. Polymer cross-linking is the most popular way to increase CH mechanical resistance by using chemical agents to create

covalent or ionic connections between polymer chains (Silvestro et al., 2020). Sodium Tripolyphosphate (TPP) is a cross-linking agent used in the ionic gelation process to cross-link polycationic polymers.

The molecular weight of chitosan can provide the different impacts of chitosan solution properties. Higher molecular weight chitosan is more viscous than lower molecular weight chitosan (Chattopadhyay and Inamdar, 2010). The molecular weight of chitosan also affected both its film-forming ability and thickness. The chitosan layer of high molecular weight chitosan (HMW-CH) on coated mangoes was the thickest, followed by medium molecular weight chitosan (MMW-CH) and low molecular weight chitosan (LMW-CH) (Jongsri et al., 2016). There is a lack of extensive research on preserving of papaya fruit through various molecular weights of chitosan coatings.

Using a coating material with antimicrobial properties is crucial to hindering harmful microbes' growth on fruit surfaces. The storage condition of the fruit, for example, papaya fruit, is commonly stored at room temperature and exposed to the air, which increases the potential of fungus and bacteria on its skin, thus leading to the decline of fruit shelf life. Edible fruit coatings with antimicrobial properties can be utilised to prevent rotting and reduce microbial growth to ensure food safety. Essential oil (EO) is a concentrated lipophilic volatile fragrance molecule that includes terpenes, terpenoids, and aromatic and aliphatic components produced from phenol. EO's phenolic chemicals reduce microbes and minimise lipid oxidation (Ataei et al., 2020). Adding an alginate edible coating with oregano essential oil nanoemulsion improved tomato fruits' quality and shelf life, according to a study by Pirozzi et al. (2022). Arabpoor et al. (2021) found that treating cherries with CH-TPP and Eryngium Campestre essential oil after harvest reduces microorganisms, titratable acid, and weight loss at 4 °C storage. Adding ginger essential oil with adequate concentration can act as an antibacterial agent.

The addition of acetic acid in the formulation can improve the physical stability of the polymer coating. It was shown that chitosan with acetic acid coating film had the highest tensile strength and the lowest elongation percentage, according to a study by Xing et al. in 2016. Incorporating Tween 80 surfactant was crucial for improving the wettability of the polymer coating solution. It is essential to emphasize that the surfactant plays a vital role in maintaining a stable balance between the water and oil phases, thus ensuring the consistent dispersion of oil droplets. This research focuses on the effects of CH-TPP coatings on papaya storage quality based on chitosan molecular weight, acetic acid concentration, and Tween 80 levels.

2. Materials and method

2.1 Preparation of coating solution

The chitosan-based polymer fruit coating was prepared using the method proposed by Al-nemrawi et al. (2018) and Mondéjar-López et al. (2022) with some improvements. The pure acetic acid Emsure® with ≥ 99.8 % assays were diluted in deionized water in three different concentrations in which 0.2, 0.6 and 1.0 %v/v. The chitosan flakes (>75 % deacetylated) with low molecular weight chitosan (LMW-CH: 50-190 kDa) and high molecular weight chitosan (HMW-CH: 10-375 kDa) from Sigma Aldrich® were dissolved in the prepared acetic acid solution to form 0.5 %w/v of chitosan solution and stirred overnight. A 0.3 % w/v of TPP solution was prepared by dissolving the Sigma Aldrich® TPP powder with 85 % purity in the deionized water and stirred overnight at room temperature followed by filtration using a syringe filter sized 0.45 µm.

A chitosan-based polymer coating was prepared using the ionic gelation method. Six samples of coating solution were prepared using different molecular weights of CH (LMW and HMW), acetic acid (0.2 - 1.0 %v/v) and Tween 80 concentration (0 % - 0.6 %) in a constant BF1 Malaysia manufactured ginger essential oil (GEO) and TPP concentration. The HMW of chitosan only tested in the 1.0 % of acetic acid since the previous study has proven that the HMW chitosan is highly soluble in 1.0 % acetic acid (Saisa Saisa et al., 2019). The CH solutions were sonicated using DSA300 ultrasonic water bath at 60° C for 10 min at a high frequency. The CH solutions were homogenized with Tween 80, GEO and TPP solution using Silverson L5 M-A High Shear Mixer. Tween 80 from EvaChem was preheated in the water bath at 60° C for 10 minutes and added to the solution. The GEO was added to the solution at room temperature while it was homogenized using the homogenizer at 8,000 rpm for 15 minutes. The TPP solution was added to the solution under continuous agitation. The pH was measured and adjusted to 5.7-6.1 using 2M sodium hydroxide to ensure the coating solution was suitable for papaya skin.

2.2 Physicochemical analysis of CH-TPP coating solution

The coating solution was characterized using Malvern Mastersizer 2000 with HydroMU (Catalog number: MAI1020331+MAL140679, Malvern, UK), a particle size analyser and Nicolet™ iS™ 5 FTIR Spectrometer (Catalog number: IQLAADGAAGFAHDMZA, Thermo Scientific™, USA). The analysis results of Malvern Mastersizer 2000 include surface mean area diameter, uniformity and span while FTIR was used to examine the chemical bonding on specimens.

2.3 Application of polymer coating on fruits

Carica Papaya L. were obtained from a local farmer in Segamat, Johor. The purchased papayas weighed between 0.9 to 1.0 kg, with free from defects and the yellow region covered around one-fourth (1/4) of the papaya size. The fruit was rinsed and disinfected with 2 % v/v sodium hypochlorite, NaOCl from R&M Chemicals. The dip coating method was used to coat the papaya fruit, which was dipped into the CH-TPP coating solution for 5 min. The coated papayas were stored at room temperature, and the evaluation of physicochemical and organoleptic observation was performed periodically. The fruit decay process was observed every 2 days on the appearance of the papaya in terms of weight, color, texture, fungal activities and Total Soluble solid (TSS) after the coating treatment. The TSS was analyzed using the method described in Ovando-Martinez et al. (2018). Three sets of papaya were used per experimental groups and repeated for three times.

3. Result and discussion

3.1 Characterization of acetic acid and chitosan solution: Particle size analysis

Table 1 shows the particle size of the CH solution in a range of 135-1,140 μm . Based on uniformity and span value, it was found that the highest uniformity of LMW-CH particles is contributed by the highest acetic acid concentration at 1.0 %. Hence base on particle size distribution data, chitosan solution LMW-CH in 0.2 %, 0.6 % and 1.0 % acetic acid, and HMW-CH in 1.0 % acetic acid there is less variability and greater consistency in the particle size. A greater acetic acid concentration may enhance chitosan powder solubility in solution with less aggregation of particles (Lukman Hekiem et al., 2021). However, due to droplet flocculation during the homogenization process, the size distributions in each sample were found to be multimodal (Bonilla et al., 2012). The solubility of CH is dependent on its molecular weight (MW). Chitosan's low molecular weight contributes to its high solubility by reducing intermolecular interactions such as hydrogen bonds and van der Waals forces (Qin et al., 2003). As the increase of MW of CH, the solubility of CH decreases. The effect of acetic acid concentration in LMW-CH can be seen from the surface area mean diameter in Table 1. The particle size of CH solution is smallest in the concentration of acetic acid at 1.0 %, followed by 0.2 % and 0.6 %, in which 1,121.439 μm , 1,135.543 μm and 135.203 μm . CH is more soluble in 1.0 % acetic acid at the highest pH value, 3.637, compared to 0.2 % and 0.6 % acetic acid. According to a previous study, more acid leads to a stronger protonation degree, making chitosan more soluble (Pavoni et al., 2019). Higher acid concentration and lower pKa value increase the molecules' tendency to give up a proton (H^+).

Table 1: Particle size in surface area mean diameter and the uniformity and span of the chitosan solution

CH solution (%)	Surface area mean diameter (μm)	Uniformity	Span	Range of particle size distribution (μm)
LMW-CH 0.2 %	1,121.439	0.174	0.571	563.667 – 2,000
LMW-CH 0.6 %	1,135.543	0.204	0.659	502.377 – 2,000
LMW-CH 1.0 %	135.203	0.357	1.118	5.024 – 632.456
HMW-CH 1.0 %	370.781	0.582	1.871	20 – 2,000

3.2 Physicochemical Analysis of CH-TPP Coating Solution: Particle size distribution

The inclusion of TPP caused a reduction in CH's microparticles, resulting in nanoparticle formation ranging from 107-221 nm, as shown in Table 2. The particle size is measured by surface area mean diameter. The effect of the molecular weight of chitosan was studied by comparing CH-TPP coating solution sets A and B in a constant concentration of acetic acid at 1.0 % and Tween 80 0.6 %. LMW-CH coating showed the lowest surface area mean diameter, uniformity and span value compared to HMW-CH coating. As the MW of chitosan increases, the particle size of chitosan nanoparticles becomes larger, possibly due to the increasing chain length of chitosan. HMW-CH coating provides a compact structure compared to LMW-CH. The higher polymerisation degree of the chitosan chain leads to a more compact structure (Leceta et al., 2013).

The effect of acetic acid concentration on the coating solution was studied by comparing CH-TPP coating solution sets B, C and D using LMW-CH and 0.6 % Tween 80. Based on Table 2, it is shown that the particle size increases with the concentration of acetic acid. Acetic acid concentration does not affect the particle size of composite chitosan-based coating solution with GEO, TPP, and Tween 80, as it is related to solubilising the chitosan flakes. Table 2 shows that the particle size increases as the concentration of Tween 80 increases. Çakır et al. (2020) reported that surfactant resulted in well-coated surfaces but, increasing Tween 80 led to an increase in particle size. Additionally, increasing the concentration of Tween 80 can increase the particle's stability and efficiency. The CH-TPP solution is the most stable at a 0.6 % concentration of Tween 80.

Table 2: Particle size in surface area mean diameter and the uniformity and span of the CH-TPP Coating Solution.

Set	Molecular weight of Chitosan	Concentration of acetic acid (%)	Concentration of Tween 80 (%)	Surface area mean diameter (nm)	Uniformity	Span
A	HMW	1.0	0.6	221	85	211.431
B	LMW	1.0	0.6	158	13.5	34.741
C	LMW	0.2	0.6	107	0.88	3.939
D	LMW	0.6	0.6	180	79.8	208.302
E	LMW	1.0	0.3	154	15.9	2.349
F	LMW	1.0	0	129	1.35	5.235

3.3 FTIR analysis of CH-TPP Coating Solution

The FTIR analysis confirmed the presence of Chitosan and TPP in six different formulations with their peak assignment. The strong and broad peak in the 3,500-3,300 cm^{-1} region of the chitosan spectra is assigned to hydrogen-bonded O-H stretching vibration. In contrast, the peaks of N-H stretching from primary amine and type I amide overlap the same region (Mohammadpour et al., 2012). The sample coating solutions are shifting peak wavenumbers to a higher value, as shown in Table 3, compared to the solution before adding TPP, GEO and Tween 80. For example, the peak of the LMW-CH solution in 0.2 % acetic acid (set C) at 3,314.99 cm^{-1} and 1,636.49 cm^{-1} it has shifted to 3,318.11 cm^{-1} and 1,636.47 cm^{-1} . In coating set D, the peak of the LMW-CH solution in 0.6 % acetic acid at 3,294.81 cm^{-1} and 1,634.02 cm^{-1} has shifted to 3,311.12 cm^{-1} and 1,636.42 cm^{-1} .

The CH solution in acetic acid 1.0 % also shows shifting peak wavenumbers. The peak of the LMW-CH solution at 3,312.54 cm^{-1} and 1,636.46 cm^{-1} has shifted to 3,284- 3,312 cm^{-1} and 1,636.22-1,636.27 cm^{-1} in coating sets B, E and F. In the HMW-CH solution, the peak wavenumber at 3,261.3 cm^{-1} and 1,636.53 cm^{-1} has shifted to 3,314.47 cm^{-1} and 1,636.2 cm^{-1} in coating set A. When a peak wavenumber shifts to the increasing value, it shows that bond length reduces. The bond length grows as the peak wavenumber reduces. Bond length alterations can arise due to an atom's electronegativity changing. The higher the peak wavenumber, the stronger the bond. It takes more energy to stretch and bend the N-H bond.

The spectrum gets broader as the relative intensity increases, suggesting an increase in hydrogen bonding. The 1,634-1,636.5 cm^{-1} peak of NH bending vibration changes to 1,636.2-1,636.47 cm^{-1} . A new peak around 1,500 cm^{-1} has developed in the CH-TPP coating solution, attributed to N-O-P stretching vibration. This means the TPP anions were cross-linked with the CH ammonium groups to create CH particles. These findings have been linked to the interaction of phosphoric and ammonium ions. As a result, we believe that TPP's tripolyphosphoric groups are linked to chitosan's ammonium groups. Chitosan particles have improved inter- and intramolecular interactions. Other studies had similar outcomes (Loutfy et al., 2016).

Table 3: FTIR peak value of the coating solution.

Set name	Assignment based on wavenumber (cm^{-1})	
	N-H stretching overlapped with O-H Stretching	N-H bending vibration
A	3,314.47	1,636.2
B	3,284.7	1,636.25
C	3,318.11	1,636.47
D	3,311.12	1,636.42
E	3,311.98	1,636.27
F	3,298.75	1,636.22

3.4 Papaya decay process evaluation and Total Soluble Solid (TSS)

The analysis of CH-TPP coating solution on papaya fruit was done by evaluating the papaya decay process and Total Soluble Solid (TSS). Fruit ripens when ethylene gas is released to signal for the fruit to turn yellow. This gas is produced naturally as the fruit ages (Jongsri et al., 2016) The ripening process of papaya depends on the molecular weight of chitosan, acetic acid concentration and Tween 80 concentration. Based on Figure 1, the ripening process of papaya is at the slowest rate when it is coated with HMW-CH compared to LMW-CH due to the properties such as water vapour permeability (WVP) of HMW-CH being lower than LMW-CH. Based on a previous study conducted by Bof et al. (2015), presented that WVP of LMW chitosan films was nine times greater than HMW films. Compared to LMW-CH, HMW-CH showed the greatest potential to delay ethylene formation. Hence, the acetic acid concentration at 1.0 % slowed the ripening process compared to the acetic acid concentration at 0.2 and 0.6 %. This shows that the CH flakes are well dissolved in acetic acid at 1.0 %, thus

high CH dispersing on the coating. The concentration of Tween 80 significantly impacts the papaya ripening process. A surfactant was added to improve water vapour resistance by attaching its polar end to the polar region of the chitosan molecule. The non-polar part is kept away from the chitosan molecule. The exact ratio of Tween 80 with GEO at coating set E (0.3:0.3) shows fungus growth in the papaya peel. This may be due to insufficient surfactant presence to solubilize the GEO. The TSS values in Table 4 appear to slightly difference on the ripening process sequence presented in Figure 3. The visual observations on coated papaya in this study might have weakness when detecting microbial activity on papaya, however it could potentially used as preliminary studies on the potential of this coating approach. Microbial activity can be determined by counting colony formation units (CFU) per mL on the papaya's surface to improve the experimental data in the future. Coating solution set F without Tween 80 shows the highest TSS value. The effect of acetic acid concentration seems inconsistent with papaya's TSS value. CH solution in acetic acid at 0.2% (set B) has the lowest TSS at $6.58 \pm 0.24\%$ Brix, followed by 1.0% acetic acid (set C) with $6.78 \pm 0.17\%$ Brix and 0.6% acetic acid (set D) with TSS value of $9.72 \pm 0.11\%$ Brix.

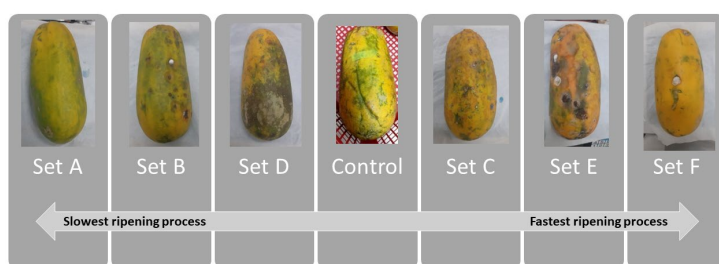



Figure 1: Ripening process line of observed papaya on day 8.

Table 4: Total Soluble Solid of observe papaya on day 8.

Set	Mean Total Soluble Solid, TSS (% Brix)	Ripening Process Sequence
A	4.88 ± 0.32	Slowest  Fastest
C	6.58 ± 0.24	
B	6.78 ± 0.17	
Control	6.80 ± 0.32	
D	9.72 ± 0.12	
E	9.78 ± 0.07	
F	10.02 ± 0.20	

4. Conclusions

This study found that HMW-CH and acetic acid concentration at 1.0 % gave coating solutions the best stability and mechanical strength. Dipping papaya fruit in HMW-CH aided with ginger essential oil extended its shelf life and resulted in a more compact structure of particles. It was discovered that these properties are able to slow down the ripening process of papaya. The coating solution was not stable enough to produce a protective layer when the same ratio concentration of Tween 80 to GEO at 0.3 %:0.3 % exhibited the existence of agglomeration particles. The optimum formulation for post-harvest treatment was determined to be chitosan-based coated polymers with HMW-CH that dissolve in acetic acid concentrations of 1.0 % and 0.6 % Tween 80 CH-TPP coating solutions with GEO addition provide a feasible option for improving papaya preservation and potential for research and innovation in the food science and fruit storage industries.

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