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Nano-chitosan coating on maintaining the quality of postharvest chili pepper (*Capsicum frutescens* L.)

Noor Laila Safitri[1], Erma Prihastanti[1*], Sri Widodo Agung Suedy[1], Agus Subagio[2]
[1]Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro
Jl. Prof. Sudarto No.13, Tembalang, Semarang, Central Java, Indonesia. 50275
*Email: eprihast@yahoo.co.id
[2]Department of Physics, Faculty of Science and Mathematics, Universitas Diponegoro
Jl. Prof. Sudarto No.13, Tembalang, Semarang, Central Java, Indonesia. 50275

ABSTRACT. Chili pepper (*Capsicum frutescens* L.) is a horticulture product with a limited shelf life due to quality degradation following harvest. One method of preserving the quality of chili peppers can be evaluated by using nano-chitosan, which combine chitosan and sodium tripolyphosphate (STPP) in certain ratio. The purpose of this study was to determine the impact of nano-chitosan on maintaining the quality of *C. frutescens* L., the optimal ratio of chitosan to STPP for preserving the quality of *C. frutescens* L. after harvest, and the shelf life of *C. frutescens* L. treated with nano-chitosan after harvest. This study employed a completely randomized design (CRD) and included four treatments: P0 (control), P1 (0.2% nano-chitosan, 1:3 ratio), P2 (0.2% nano-chitosan, 1:4 ratio), and P3 (0.2% nano-chitosan, 1:5 ratio), which conducted for 16 days. Weight loss, water content, texture, color, and percentage of damage are the research variables in this study. The ANOVA test was used to examine the data, followed by the DMRT test. The results indicated that nano-chitosan could maintain the weight, water content, texture, and color of chili peppers. The optimal ratio of chitosan to STPP to retain the quality of postharvest *C. Frutescens* L. is 0.2% nano-chitosan (chitosan: STPP= 1:5), and nano-chitosan can maintain the quality of *C. Frutescens* L. for up to 16 days.

Keywords: chitosan and sodium tripolyphosphate; chitosan ratio; chroma meter; postharvest storage; shelf life of chili pepper

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INTRODUCTION

Chili pepper (*Capsicum frutescens* L.) is a commonly cultivated agricultural plant in Indonesia due to its high demand by the public and is frequently used as a complement to Indonesian cuisine spices (Kementerian Pertanian, 2020; Zahara *et al*., 2021). According to Badan Pusat Statistik (2020), Indonesian chili peppers production reached 1.51 million tons in 2020, an increase of 9.76% over the previous year. Post-harvest handling of chili peppers is critical for quality preservation hence it extends shelf life, minimizes mechanical and physiological damage, and inhibits the growth of spoilage microorganisms (Rochayat & Munika, 2015). Post-harvest handling is critical to preserving the chili pepper's quality, including package and storage (da Silva *et al*., 2015; Ali *et al*., 2016).

The most common method of postharvest storage is to keep chili peppers at room temperature and to keep them for two-three days (Finger & Pereira, 2016; Maskey *et al*., 2021). Chili peppers have a limited shelf life due to their vigorous metabolism when ripe, and microbial spoilages (Edusei *et al*., 2012). Another way to extend the shelf life of chili peppers is to store them at a temperature of 5°C, which can keep them fresh for up to 14 days (Maharani *et al*., 2019). However, this storage strategy may cause chilling injury in *Capsicum* spp., resulting in the product becoming soft, decreased levels of vitamin C, the appearance of holes and coloured patches on the fruit's surface, increased susceptibility to rot, damage to the plasmalemma, and plastid degradation (Wulandari *et al*., 2012; Kong *et al*., 2018; O'Donoghue *et al*., 2018).

Nano-chitosan become solution to protect chili peppers during storage by coating. Nano-chitosan will bind to lipids in the fruit's cuticle layer, forming a biofilm that can limit the fruit's respiration rate by modifying the oxygen, carbon dioxide, and ethylene concentrations

(Gardesh *et al*., 2016; Xing *et al*., 2019; Nguyen *et al*., 2020). Nano-chitosan offers better nutrient uptake, antimicobial, and antifungal properties than regular chitosan (Van *et al*., 2013; Ramezani *et al*., 2015), and is also non-toxic and suitable for human consumption (Slamet, 2011; Sivakumar *et al*., 2021). Nano-chitosan is smaller than chitosan, which results in increased antibacterial activity (Pilon *et al*., 2014). The ratio of chitosan to sodium tripolyphosphate (STPP), which is used to synthesize nano-chitosan by ionic gelation, affects the particle size of nano-chitosan. STPP is a polyanion that reacts with chitosan to create nano-chitosan, not carcinogenic or mutagenic (Triwulandari *et al*., 2018). The size of the nanoparticles reduces as the amount of STPP utilized increases.

In the previous studies, nano-chitosan coating on *Capsicum annuum* L. could inhibit vitamin C loss and weight loss during storage (Slamet, 2011). Lustriane *et al.* (2018) reported that chitosan-nanoparticles extended the shelf life and maintain quality of *Musa acuminata* AAA group. In another research group, the application of chitosan nano-coating using STPP extended the shelf life of *Capsicum annuum* L. var. *grossum* (L.) Sendt without loss of weight, and sensory quality (Hu *et al*., 2020). Information regarding the use of nano-chitosan coatings containing various ratios of chitosan and STPP on *C. frutescens* L. has never been published. This study aims to determine the impact of nano-chitosan on maintaining the quality of *C. frutescens* L., the optimal ratio of chitosan to STPP for preserving the quality of *C. frutescens* L. after harvest, and the shelf life of *C. frutescens* L. treated with nano-chitosan after harvest. This study is expected to provide a preliminary data on the effect of edible nano-chitosan on *C. frutescens* L. Increased fruit storage life is associated with an increase in fruit quality, and hence is projected to contribute to national food security and economic prosperity.

MATERIALS AND METHODS

Chili peppers (*Capsicum frutescens* L.) **preparation.** *C. frutescens* L. is obtained from farmers in Temanggung Regency, Central Java. Harvesting was carried out on chili peppers aged 90 days after planting (DAP) and harvested in the morning. The chili pepper utilized as a sample was chosen based on the color and size of the fruit, which were similar. The chili pepper utilized is disease-free and has a reddish-orange color with a length of approximately 6 cm and a width of 0.90 cm.

Nano-chitosan preparation. This study session was conducted in Laboratory of Nanotechnology, Integrated Laboratory of Universitas Diponegoro. Nano-chitosan was prepared using the ionic gelation method. Chitosan 2 g was dissolved in 1 L 1% acetic acid, then homogenized for 2 h using a shaker, then 0.1% sodium tripolyphosphate (STPP) was added dropwise to form a nanoparticle suspension. Chitosan and STPP were mixed in a ratio of 1:3, 1:4, and 1:5; after STPP was added to the chitosan solution, stirring was continued for 1 h. The crosslinking process was complete the resulting particles were stable.

Nano-chitosan coating treatment. Chili peppers were dipped for 2 min in a basin containing a solution of nano-chitosan, after which they were removed and dried for approximately 15 min (Slamet, 2011). Treatment of P1, P2, and P3 were dipped in each 0.2% nano-chitosan using various ratio (chitosan:STPP) 1:3, 1:4, and 1:5, respectively. Control didn't get any coating treatment.

Chili peppers storage. Chili peppers were stored in perforated cardboard measuring 10×10 cm. Each cardboard box was packed with 30 g of chili peppers and then stored for 16 days at 29-30°C and 30% RH.

Calculation of weight loss. Weight loss was determined by weighing chili peppers every three days. The formula for calculating weight loss as follows (Meyer, 1932; Davis & Hofmann, 1973):

$$\text{weight loss (\%)} = \frac{\text{Wi} - \text{Wf}}{\text{Wf}} \times 100$$

Notes:
W0 = initial weight of storage (g)
Wn = weight on day n (g)

Determination of water content. A moisture balance tool (Hitachi, STA200RV) was used to determine the water content of chili peppers. After calibrating, the temperature was

set to 100°C, then loaded with 1 g of chili peppers extract. After 30 min, the water content findings were displayed on the moisture balance tool. The water content unit of a chili pepper was expressed as a percentage.

Texture determination. The texture analyzer tool (LLOYD Instruments/Ametek TA1) was used to determine the texture of chili pepper. The chili pepper was placed on the test table, followed by the probe installation, the computer's single hardness program was then selected, the probe pressure speed, pressure depth, and probe depth to the chili peppers were all set. Following that, the start button was hit, and the resulting texture was printed on the computer.

Color determination. The color of the chili pepper was determined using a chroma meter (Konica Minolta, CR-400 Head). Using white calibration, the chroma meter was produced and calibrated in advance. Chili peppers had been prepared and placed directly in front of the detector. The start button was pressed to initiate the test, and the results will be displayed on the device's display screen. The total color can be calculated as follows (CIE, 1976; Lamona *et al*., 2015; Putri *et al*., 2020):

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

Notes:
ΔE* = Total color change
ΔL* = Difference between dark and light
Δa* = Difference between red and green
Δb* = Difference between yellow and blue

Damage percentage. The level of damage was obtained by calculating the number of chili peppers that were damaged and the number of chili peppers stored. The percentage of chili peppers damage as follows (Abou-Aziz *et al*., 1974; Putri *et al*., 2020):

$$\text{Damage (\%)} = \frac{\text{Number of damaged chili peppers}}{\text{Number of chili pepper stored}} \times 100$$

Shelf life of chili peppers. To determine the shelf life of chili pepper that is suitable for consumption is assessed qualitatively by examining the performance of chili pepper, which still looks fresh and has not faded. In addition, the age estimation test was terminated when the chili peppers were still fresh, as shown by a crack sound when they were broken (Kementerian Pertanian, 2019).

Data analysis. The observational data were then examined using analysis of variance (ANOVA) at a 95% confidence level. If there is a significant effect, additional testing using Duncan's Multiple Range Test is performed (DMRT).

RESULTS AND DISCUSSION

The nano-chitosan coating on *Capsicum frutescens* L. had a significantly different effect on the quality during storage ($p \leq 0.05$) than the control (Table 1). This layer acts as a barrier between the outer atmosphere and the internal gas composition, inhibiting and modifying gas exchange. *C. frutescens* L. stomata with a diameter of ±10 µm, and when it is dipped, nano-chitosan can enter and close the stomata pores. The smaller the nano-chitosan particle size, the greater the absorption into the fruit cells and the more effectively the nano-chitosan particles cover the stomatal pores. Following that, the nano-chitosan coats the cuticle layer, allowing the fruit to have a slower rate of respiration, transpiration, and microbial growth. This coating has a beneficial effect on a variety of *C. frutescens* L. quality parameters, including weight, water content, texture, color, and damage.

Effect on weight loss of *C. frutescens* L. Fresh fruit and vegetable weight loss is mainly impacted by the loss of water through respiration and transpiration (Castellanos *et al*., 2016; Romanazzi *et al*., 2017). According to table 1, chili peppers lost 68.2%, 70.8%, and 66% of their weight after 16 days in treatments P1, P2, and P3, respectively. P0 loses a lot of weight due to the lack of a barrier between the product and the environment. The respiration and transpiration processes are accelerated, resulting in rapid weight loss for the product. Sugars and other substrates such as lipids and proteins are transformed to carbon dioxide, water vapor, and energy during respiration, while the by-products of respiration are removed through evaporation (transpiration)(Tkemaladze & Makhashvili, 2016; Otoni *et al*., 2017). Treatments P1, P2,

and P3 resulted in less weight loss than P0 due to the nano-chitosan coating acting as a barrier to gas entry and escape. The nano-chitosan coating may reduce the rate of respiration and transpiration. Our findings corroborate with Slamet (2011), red chilies that were not treated with nano-chitosan lost the most weight when compared to curly chilies that were coated with nano-chitosan. Previous studies reported similar results regarding the weight loss of fruit coated with nano-chitosan (Eshghi *et al*., 2014; Meena *et al*., 2020).

Table 1. Effect of nano-chitosan coating on the quality of *Capsicum frutescens* L. after storage.

Treatment	Weight loss (%)	Water content (%)	Texture (gf)	Damage (%)
P0	79.8[a]	33.6[a]	3304.2[b]	46.0[a]
P1	68.2[b]	26.0[b]	4522.4[a]	24.0[b]
P2	70.8[b]	21.3[b]	4877.6[a]	26.0[b]
P3	66.0[b]	27.3[b]	4351.6[a]	20.0[b]

Notes: Numbers followed by the same letter in the same column are not significantly different based on the DMRT test at the level of 95%.

Effect on water content of ***C. frutescens*** **L.** *C. frutescens* L. water content decreased when stored at 29-30°C, as high temperatures and low humidity accelerate product respiration and water loss. On the first day, the water content of chili pepper was 80%t, but by the 16th day, P1, P2, and P3 had water content values of 26%, 21%, and 27%, respectively, while P0 (control) had a water content value of 33% (Table 1). It demonstrates that the *C. frutescens* L. coated with nano-chitosan contained less water than the control. P0 contains more water than P1, P2, or P3 due to increased respiration. The process of respiration involves oxygen reacting with organic molecules in the tissue, resulting in the production of carbon dioxide and water. Because the nano-chitosan layer covering the *C. frutescens* L. pores was selectively permeable to oxygen, the nano-chitosan coating slowed down their respiration process. The DMRT test findings indicate that P1, P2, and P3 are not statistically different. This is achievable because the nano-chitosan employed in each experiment has the identical concentration of 0.2%, rendering the results insignificant in comparison to one another. Our study discovered the best outcomes in P3 due to the fact that the nano-chitosan utilized was 200 nm smaller than the nano-chitosan used in P1 and P2. The more the amount of STPP added, the smaller the nano-chitosan size. According to Kumar *et al*. (2017), chitosan applied as a fruit coating will cover the pericarp and stomata layers, hence reducing the rate of respiration and transpiration through the pores. In line with Lustriane *et al.* (2018), coating fruits with chitosan results in the formation of a layer on the fruit's surface that is selectively permeable to carbon dioxide and oxygen gases. This layer functions as a barrier, preventing and altering gas exchange between the external atmosphere and the internal gas composition, hence inhibiting the transpiration process.

Effect on texture of ***C. frutescens*** **L.** According to Table 1, *C. frutescens* L. coated with nano-chitosan had a lesser texture drop than controls due to its capability to inhibit cell wall disintegration on the fruit surface. P0 has a lower texture value than P1, P2, and P3, which all have a texture value of 3304 gf, since P0 softens more quickly due to pectin degradation and oxidation. Wibowo *et al*. (2020) stated that pectin oxidation results in the release of additional water, causing the texture of the red chili to become soft and wringkled. The texture value increased by 2.6% in P1, from 4404.8 to 4522.4 gf, and by 10.7% in P2, from 4404.8 to 4887.6 gf, indicating that the texture of treatments P1 and P2 is becoming harder. This is because P1 and P2 dry out more quickly than P3 due to increased transpiration. The particle size of the nano-chitosan used in P1 and P2 is larger than in P3, 6075nm and 247nm, respectively. As a result, the texture diminishes and becomes dry. Additionally, because the nano-chitosan coating on P1 and P2 did not completely cover the cell surface, their transpiration rates were higher than those of P3. Marganingsih & Putra (2021) reported that after 15 days of storage, cherry tomatoes coated with 2.5% shrimp chitosan increased in hardness (texture) value from 35.17 to 37.33 N. The texture value fell by 1.2% in P3, from 4404.8 to 4351.6 gf. While the textural value of P3 decreases, it still creates a "crack" sound when broken, indicating that the *C. frutescens*

L. is safe for consumption and marketing. P3 has the most excellent texture compared to P0, P1, and P2 because the nano-chitosan employed is very small, around 200 nm. The smaller the nano-chitosan, the greater its absorption into cells, preventing chitosan's effectiveness in suppressing excessive CO_2 production and cell wall disintegration. Shiekh *et al.* (2013) assert that the texture of the fruit is related to the cell wall structure. During fruit ripening, the cell wall is destroyed. According to Liu *et al.* (2014), pectin methylesterase (PME) is involved in cell wall breakdown. The activity of the PME is related to CO_2 production; once it is inhibited, the movement of cell wall-degrading enzymes will be reduced, allowing the fruit texture to be preserved during storage.

Effect on color of *C. frutescens* **L.** C. *frutescens* L. changes color during storage due to the rate of transpiration and respiration. Table 2 shows the brightness values of C. *frutescens* L. treated with and without nano-chitosan. The findings of the Hunter L, a, b method color test in Table 2 indicate that P1, P2, and P3 have ΔE values of 33.62, 24.99, and 21.37, respectively. Because a greater ΔE value indicates a darker sample color, the data indicates that P3 has a lighter color. P2 and P3 in Fig. 1 have brighter hues than P0 and P1. C. *frutescens* L. are reddish-orange in color due to the presence of carotenoid and anthocyanin pigments, where carotenoids will be degraded by oxodation during storage. Since nano-chitosan is selectively permeable to oxygen, it inhibits the carotenoid breakdown process (Salinas-Roca *et al*., 2018; Haghighi *et al*., 2020), hence slowing the color shift in C. *frutescens* L.

Table 2. The color of *Capsicum frutescens* L. after storage.

Treatment	L	a	b	ΔE
P0	26.15^{c}	21.25^{c}	19.18^{b}	38.11
P1	29.66^{b}	24.68^{b}	21.03^{b}	33.62
P2	34.81^{a}	27.01^{b}	27.97^{a}	24.99
P3	37.95^{a}	31.25^{a}	29.55^{a}	21.37

Notes: L*= brightness; a*= red (+a*) and green (-a*); b*= yellow (+b*) and blue (-b*); ΔE*= a total color change. Numbers followed by the same letter in the same column are not significantly different based on the DMRT test at the level of 95%.

Additionally, the delayed color shift of chitosan-coated fruit is mediated by metabolic activity inhibition, which slows anthocyanin breakdown (Kumar *et al*., 2017). The degradation of anthocyanin is affected by light, temperature, oxygen, and enzymes, which provided by polyphenol oxidase (Cavalcanti *et al*., 2011). The polyphenol oxidase enzyme catalyzes the hydroxylation of monophenols to produce o-diphenols and the oxidation of o-diphenols to produce o-quinones. The nano-chitosan coating functions as an oxygen barrier, limiting the action of the polyphenol oxidase enzyme, hence slowing anthocyanin degradation (Zambrano-Zaragoza *et al.*, 2014).

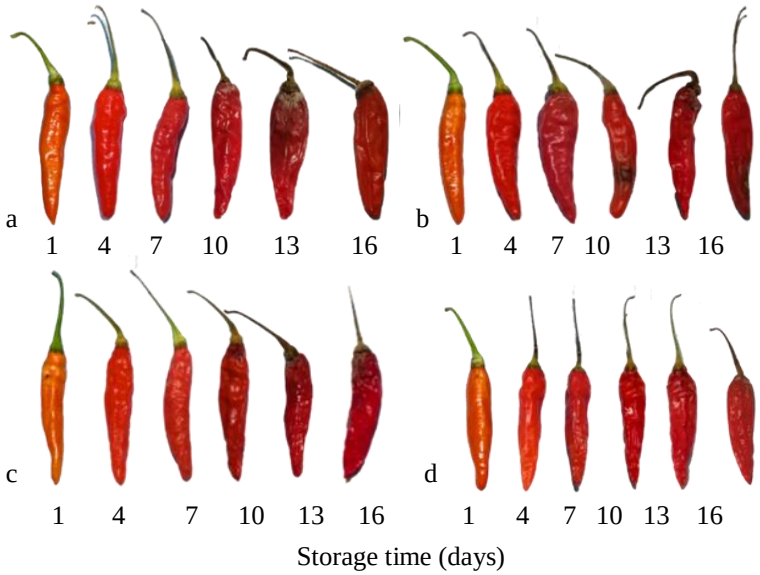

Fig. 1. The color change of *Capsicum frutescens* L. during 16 days of storage: a. Control; b. P1; c. P2; d. P3.

Effect on damage percentage of *C. frutescens* L. Our study discovered that *C. frutescens* L. causes chemical and microbial damage. The deterioration began on the 7th day, but after the P3 treatment, the deterioration began on the 10th day. *C. frutescens* L. decreased by 24%, 26%, and 20% on the 16th day of P1, P2, and P3, respectively. This result is preferable to the control, which causes 46% of the damage. Due to the antibacterial properties of nano-chitosan, covering fruit with nano-chitosan can help prevent deterioration. According to Raliya & Tarafdar (2014), nano-chitosan has a greater ability to penetrate cells, hence boosting chitosan's performance in cells. P0 treatment obtained the most damage since it lacked any coating to protect quality, allowing microorganisms to quickly colonize the surface of the fruit. Because the nano-chitosan employed as a coating has a small size (200 nm), P3 can minimize damage by 51% compared to control. In line with Pilon *et al.* (2014), the smaller the particle size, the greater the surface interaction with microbial cells, resulting in an increase in antimicrobial activity. In addition, Gad *et al.* (2016) stated, chitosan's antibacterial activity is caused by the positive charge of amino acids binding to the negative charge of the microbial cell membrane, increasing its permeability and ultimately causing cell death.

Based on the quality parameters after storage, nano-chitosan can extend the shelf life of *C. frutescens* L. by up to 16 days, specifically using P3. Storage was halted when one of the treatments continued to meet the fresh fruit criteria, specifically when it was damaged and still sounded "crack." It demonstrates that coating with 0.2% nano-chitosan in a 1:5 ratio can extend the shelf life of *C. frutescens* L. by 13-14 days. The coating with nano-chitosan has an effect on all observation parameters (weight loss, water content, texture, color, percentage of damage) of *C. frutescens* L. However, the layer containing 0.2% nano-chitosan in ratios of 1:3, 1:4, and 1:5 had no discernible effect on the other layers. Therefore, further research with nano-chitosan at various concentrations and ratios of chitosan to STPP less than 1:3 is necessary.

CONCLUSION

Nano-chitosan can help maintain the quality of *Capsicum frutescens* L. after harvesting, including weight, water content, texture, and color. *C. frutescens* L. coated with 0.2% nano-chitosan in a 1:5 ratio produced the greatest results and retained their quality for up to 16 days after storage. It is preferable to conduct additional research on the antimicrobial activity of nano-chitosan, which inhibits the growth of fungi in *C. frutescens* L.

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