

Analysis Effect of Nano Chitosan Coating on The Quality of Shallot Bulbs (*Allium ascalonicum* L. var. Bauji)

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Abstract. Shallots are bulb-shaped commodities that are difficult to maintain in new conditions because they are easily damaged. An effective effort to prevent deterioration of food quality during storage is by coating it using nano chitosan. The effectiveness of nano chitosan is influenced by particle size which related to the ratio of chitosan and STPP (sodium tripolyphosphate). This study aimed to determine the effect of nano chitosan coating with the addition of different ratios of chitosan and STPP and the appropriate ratio of chitosan and STPP on nano chitosan as a coating to maintain the quality of shallot bulbs (*Allium ascalonicum* L. var. Bauji). This study used a completely randomized design (CRD) method with four treatments and five replications. The treatments of this research were P0= Control, P1= Nano chitosan ratio of chitosan: STPP 1:3, P2= Nano chitosan ratio of chitosan:STPP 1:4, and P3= Nano chitosan ratio of chitosan:STPP 1:5. The variables of this study were the percentage of damage, the percentage of diameter shrinkage, weight loss, color, hardness, and moisture content. Data were analyzed using the ANOVA test and continued with the DMRT test. The application of nano chitosan coating on shallot bulbs could reduce damage, shrinkage of tuber diameter, weight loss, color, hardness, and decrease in water content better than the control treatment. The best results were shown by treatment P3 (1:5) with a percentage of damage of 8%, diameter shrinkage of 20.20%, weight loss of 18.40%, total color change of 54.45, hardness of 226.23 N, and a decrease in water content of 4.65% at the final water content of 79.09%.

Keywords: Shallots, nano chitosan, storage, postharvest, sodium tripolyphosphate

Citation

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INTRODUCTION

Shallots are one of the ingredients for cooking spices and medicines (Mutia et al., 2014), which require special handling after harvest. In addition, shallot bulbs are easily damaged and difficult to maintain in a new form because they will change due to phys-

iological, biological, physicochemical, and microbiological processes (All et al., 2016). High levels of water content drive decay and microbial growth during the storage process (Mitra, 2012). Temperature and humidity in the storage room of shallot bulbs are factors for germination, root growth, weight loss, and shelf life (Nega et al., 2015). According

to Mutia et al. (2014), post-harvest damage to shallot bulbs is caused by pests or diseases, mechanical and physiological.

Pati Regency contributed to the national production of shallots of 22,101 tons or contributed 1.80% of the total national output in 2015. However, from 2012 to 2015 the productivity of shallot bulbs in Pati Regency tended to decrease (Nurjati et al., 2018). Subiyono Citro, one of the shallot farmers and collectors in Pati Regency, explained that the quality of shallot bulbs is influenced by the drying and storage processes which are part of the post-harvest process. Damage to shallot bulbs is generally caused by high water content during storage, which can cause rot, mushy tubers, and caterpillars. In addition, shallot bulbs stored for more than 14 days will experience weight loss, and damage due to the presence of brownish-white to black microbes that cause the bulbs to hollow, emit a pungent stench, and microbes will attack other bulbs around them. This condition will reduce the quality standard of shallot bulbs (Personal Communication, 19 September 2021). According to Amiarsi et al., (2019) the quality of shallot bulbs is determined by color, moisture content, hardness, and durability during storage.

The method of preserving shallot bulbs widely used in Indonesia is usually through storage at low temperatures ranging from 7-10°C. However, there is still a lot of damage to the shallot bulbs in this storage, such as budding (Mutia et al., 2014). Jasmi, et al., (2013) describe sprouting at 10°C due to an increase in enzyme activity and gibberellins in cells; these conditions cause increase in the cell division process and the breaking of dormancy, causing changes that trigger bud formation.

Another effort to prevent quality degradation during storage is by coating. The

coating is an effective method to extend shelf life and maintain the quality of food products (Jiang et al., 2014). The most widely used material as a coating is chitosan extracted from crustacean shells (Gavhane et al., 2013). According to Elsabee & Abdou, (2013) coatings using chitosan as a base material have selective permeability to gases (CO₂ and O₂), so they can act as an oxygen barrier. Marlina et al. (2014) stated chitosan can inhibit the rate of respiration, reduce the rate of transpiration, and can reduce the contamination of microorganisms in salak fruit. Research conducted by Nagamanuamma et al. (2019), reported that coating chitosan on peeled shallot bulbs affected the improving quality and prolonging shelf life. Besides that, it can delay ripening, reduce respiratory pathways, regulate gas exchange, suppress transpiration rate, maintain fruit firmness, and suppress weight loss in shallot bulbs.

Nano chitosan is liquid chitosan with nanoparticle size (10-1000 nm), which has better absorption and ability as antibacterial and antifungal than chitosan with regular size (Komariah, 2014). Based on research conducted by Nguyen & Nguyen (2020), coating strawberries with chitosan concentrations of 1%, 1.5%, 2%, and nano chitosan concentrations of 0.2%, 0.4%, and 0.8% was able to maintain the quality during the shelf life. The best results were shown by the 0.2% concentration of nano chitosan, which could maintain the shelf life of strawberries up to 21 days.

The particle size of nano chitosan is influenced by the ratio of chitosan and STPP or sodium tripolyphosphate (Al-Nemrawi et al., 2018). Sodium tripolyphosphate (STPP) is a source of polyanion used as a crosslinker when making chitosan nanoparticles using the ionic gelation method (Mardliyati et al., 2012). The size of the nanoparticles formed becomes smaller as the STPP increases. The smaller

the particle size, the larger the surface area to increase its stability (Sundar et al., 2010). According to Marei et al. (2018), chitosan nanoemulsions can be applied as preservatives for fruits and antimicrobial substances.

This study aimed to determine the effect of nano chitosan coating with the addition of different ratios of chitosan and STPP and the appropriate ratio of chitosan and STPP on nano chitosan as a coating to maintain the quality of shallot bulbs (*Allium ascalonicum* L. var. Bauji). Information regarding nano chitosan coating with the addition of chitosan and STPP ratios 1:3, 1:4, and 1:5 on shallot bulbs has never been reported, so researchers were interested in conducting this study to obtain information about this.

MATERIALS AND METHODS

The research was conducted from January to July 2021. Samples of shallot bulbs (*Allium ascalonicum* L. var. Bauji) were taken from rice fields in Pati Regency, Central Java Province, which were harvested at the age of 60 days after harvest, and had been dried for four days, clean from dirt, cut leaves and has a uniform weight, color, and diameter. The nano chitosan solution used was a ready-to-use nano chitosan solution with a concentration of 0.2% with the addition of chitosan and STPP ratios of 1:3, 1:4, and 1:5, which were obtained directly from the Nanotechnology Laboratory at the Integrated Laboratory of Universitas Diponegoro, Semarang.

Nano chitosan solution was made by the ionic gelation method because the process is simple and easy to control (Nadia et al., 2014). The mechanism of the ionic gelation method was carried out by combining the simple method of Prahmila (2016), where 2 grams of chitosan were dissolved in 1% acetic acid which then homogenized or mixed

using a shaker for 2 hours, after which 0.1% sodium tripolyphosphate (STPP) was added dropwise to form a nanoparticle suspension. The nanoparticle suspension formed was added with chitosan and STPP with various ratios of 1:3, 1:4, and 1:5, then stirring continued for 1 hour so that the crosslinking process was perfect and the resulting particles were stable.

Nano Chitosan Coating Treatment

Ten bulbs of shallots were prepared for each treatment. The shallot bulb samples were immersed for 2 minutes in the nano chitosan solution with different ratios of chitosan and STPP. Treatment P1 was dipped in nano chitosan with a proportion of chitosan and STPP 1:3, treatment P2 was added to the ratio of chitosan and STPP 1:4, and P3 was added to the ratio of chitosan and STPP 1:5. In contrast, the control treatment (P0) was not treated with any coating.

Shallot Bulb Storage

Samples of shallot bulbs were packaged using a plastic mesh bag (poly net) and stored at room temperature $\pm 30^{\circ}\text{C}$ with a relative humidity of $\pm 90\%$ for nine weeks.

Determination of Damage to Shallot Bulbs

The level of damage was obtained by calculating the number of damaged shallot bulbs for the number of shallot bulbs stored.

Determination of the percentage of damage to the shallot bulbs is as follows:

$$\text{Damage} = \frac{\text{Number of damaged shallot bulbs}}{\text{Number of shallot bulbs are stored}} \times 100\%$$

Determination of Weight Loss

Determination of weight loss was done by measuring the weight of the shallot bulbs once a week. Weight loss is expressed in percent (%), which can be calculated using the

following formula:

$$\text{Weight loss} = \frac{B_0 - B_n}{B_0} \times 100\%$$

B₀= initial weight of storage (g)

B_n= weight on day n (g)

Determination of Water Content

Determination of the water content of shallot bulbs was carried out using the Moisture Balance tool. The tool was calibrated first, and then the temperature was set to 100°C. The shallot bulb extract was inserted into the device. Then after 30 minutes, the results were displayed on the monitor in percent (%).

Determination of Shallot Bulb Diameter

Shallot bulbs' diameter is determined once a week with five repetitions using a caliper in millimeters (mm). The decrease in tuber diameter was calculated by the difference between the initial and final diameters, then expressed as a percent (%).

Determination of Outer Skin Color

The exterior skin color of shallot bulbs was determined using a Konica Minolta CR-400 chromameter, three repetitions. The instrument was first calibrated using white calibration. The color system used was the Hunter's Lab Colorimetric System. The Hunter color notation system was characterized by three values, namely L (lightness), a* (redness), and b* (yellowness). The notation L (lightness) expresses the brightness parameter with a value range from 1-100, indicating from dark to light. The notation a* (redness) with a value range from (-80)–(+100) indicates from green to red. The b* (yellowness) notation with a range of values from (-70)–(+70) indicates from blue to yellow (Indrayati et al., 2013).

The formula then calculates the deter-

mination of the total color:

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

Information:

ΔE^* = Total color change

ΔL^* = The difference between dark and light

Δa^* = The difference between red and green

Δb^* = difference between yellow and blue

Determination of Hardness Level

Researcher determined shallot bulbs' hardness level before being treated with nano chitosan coating and at week 9. Determination of the hardness value of shallot bulbs using the LLOYD texture analyzer with three repetitions.

Statistical Analysis

The observed data were then analyzed using the Analysis of Variance (ANOVA) with a 95% confidence level to prove whether the results had a significant effect or not. If the results significantly impact, proceed with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Nano chitosan coating on shallot bulbs had a significantly different effect from the control ($p \leq 0.05$) on the parameters of the reduction in the quality of shallot bulbs. Coating using nano chitosan has selective permeability to gas. They can act as a barrier that inhibits gas exchange, suppresses respiration rates, reduce transpiration rates, and reduce microbial contamination. Nano chitosan coating has a good influence on the freshness parameters of shallot bulbs, including weight, water content, damage, tuber diameter size, hardness, and exterior skin color of shallot bulbs.

Table 1. Effect of nano chitosan coating with the addition of different ratios of chitosan and STPP on the quality of shallot bulbs at the 9th week of storage

Treatment	Damage	Weight Loss (g)	Water Content (%)	Diameter (mm)	Hardness
P0	36 ^b	28 ^c	71.48 ^a	29.40 ^b	216.19 ^a
P1	18 ^a	21 ^b	76.29 ^b	20.75 ^a	220.74 ^b
P2	14 ^a	20 ^b	77.5 ^b	20.67 ^a	221.50 ^b
P3	8 ^a	18 ^a	79.09 ^c	20.20 ^a	226.23 ^c

Note: Numbers followed by different letters in the same column show a significant difference based on the DMRT test at the 95% confidence level

Effect on Damage to Shallot Bulbs

Based on the study results (Table 1), shallot bulbs coated with nano chitosan had a lower percentage of damage than P0. The forms of damage that occurred at P0 were hollow tubers, shriveled and rotten tubers that started with the growth of white, brown to black microorganisms (Figure 1). Diseases of shallot bulbs are caused by *Aspergillus niger* in the form of black mycelia and *Fusarium fungus* (Mutia et al., 2014).

P3 gave the best effect on the damage of shallot bulbs during nine weeks of storage related to the size of nano chitosan with the addition of a ratio of chitosan and STPP 1:5, which resulted in a nanoparticle size of 200 nm. The use of sodium tripolyphosphate or STPP as a polyanion can produce smaller nanoparticles. The resulting nano chitosan has a better penetration ability and increases specific functions in cells. Based on research conducted by Triwulandari et al., (2018) the syn-

thesis of chitosan nanoparticles using sodium tripolyphosphate polyanion has the smallest size compared to other polyanions.

The mechanism that takes place in the coating of nano chitosan against damage to the integrity of microbes refers to Xing et al. (2019), the electrostatic interaction between the positive charge or cation of chitosan (NH₃⁺) and the negative amount or anion of the microbial cell membrane that can damage the integrity of microbial cells. When the enclosure's integrity is disturbed, it can increase lipid peroxidase and leakage of proteins to undergo lysis. These conditions can affect the permeability and the presence of holes in the microbial cell membrane. Nano chitosan can then penetrate microbial cells to form Reactive Oxygen Species (ROS) or free radicals and interfere with protein synthesis, inhibiting RNA and DNA transcription mechanisms from causing death in microbial cells.

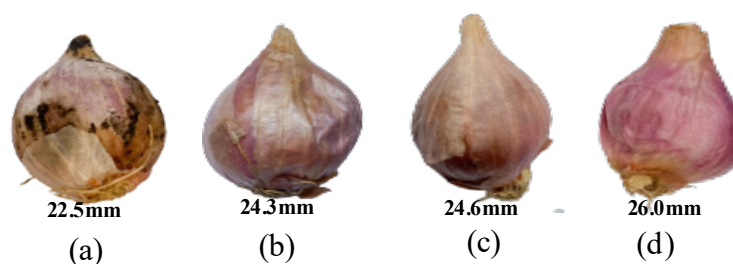


Figure 1. Differences in the form of damage to shallot bulbs coated with nano chitosan and not coated with nano chitosan at the 9th week of shelf life (a) damage to P0; (b) damage to P1; (c) damage to P2; (d) damage to P3)

Effect on Weight Loss of Shallot Bulbs

Based on table 1, shallot bulbs P1, P2, and P3 at week 9 had a final weight loss of 21%, 20%, and 18%, while the control treatment (P0) was 28%. Treatments P1, P2, and P3 showed lower weight loss than P0; this is related to the nano chitosan layer, which can be a barrier to the entry and exit of gases so that nano chitosan can slower the rate of respiration and transpiration, and also can suppress the loss of weight loss.

Red chili fruit coated with chitosan nanoparticles can reduce the transpiration rate in the fruit so that the rate of weight loss can be slowed down (Slamet, 2011). According to Mutia et al., (2014) weight loss in fresh fruit and vegetables is affected by the loss of nutrients and minerals usually absorbed from the soil. The smaller nano chitosan particle size makes it easy to penetrate and close the pores of the outer layer of the fruit tightly so that the respiration process that causes weight loss can be reduced.

Xing et al., (2019) explained that the nano chitosan coating mechanism for decreasing fruit weight loss occurred because of the positive charge on chitosan, which comes from NH_3^+ , which binds to the cuticle on the outer layer of the fruit, which has a negative charge of glycerol. Nano chitosan will cover the cuticle on the outer layer of the fruit and cover the damage or injury caused by mechanical damage during the post-harvest process. Nano chitosan, which acts as a barrier, will suppress the gas exchange of oxygen and carbon dioxide to stop weight loss.

Effect on Water Content

The water content of shallot bulbs with and without nano chitosan coating can be seen in Table 1. The final results of the water content of shallot bulbs at the 9th week of storage, P1, P2, and P3 were 76%; 77.50%; 79.09%

respectively. The process of water loss in shallot bulbs is related to the process of respiration where the exchange of oxygen (O_2) in the outside air with organic molecules in the tissues of shallot bulbs will produce carbon dioxide (CO_2) and water (H_2O). The selectively permeable nature of the gas owned by the nano chitosan layer can suppress respiration and clog the pores on the outer layer of shallot bulbs so that nano chitosan can slow down the process of water loss during storage.

The best results were shown by P3 because the nano chitosan used had a size of 200 nm, smaller than P1 and P2. The smaller the size of the nano chitosan, the greater the absorption into the cells so that it can cover the pores of the outer layer of the fruit tightly. Chitosan as a coating can protect the pericarp layer of the fruit so that the rate of respiration and transpiration through the pores can be reduced (Kumar et al., 2017).

Effect on Diameter of Shallot Bulbs

Based on table 1, the diameter shrinkage of shallot bulbs after nine weeks of storage at P0, P1, P2, and P3 were 29.40%; 20.75%; 20.67; 20.20%, respectively. It shows that the shallot bulbs treated with nano chitosan have a lower diameter shrinkage rate than the control. P3 displayed the best results. It is suspected that this is influenced by the smaller nano chitosan size, which is 200 nm. It is more stable, quickly penetrates the outer pores of the shallot bulbs, and can suppress the process of respiration and transpiration.

The process of shrinking the diameter of shallot bulbs during post-harvest is related to respiration events which also determine the occurrence of weight loss and decrease in water content. According to Iswahyudi et al. (2015), post-harvest weight loss is caused by an overhaul of the structure of the fruit tissue. After being harvested and separated from the

parent, the fruit is still undergoing a metabolic process using nutrients and mineral reserves. If the metabolic process continues, the loss of nutrients and mineral reserves will be higher.

Effect on Changes in the Outer Skin Color of Shallot Bulbs

Viewed from table 2, the results of the color test using the Hunter L*, a*, b* method indicate that the treatments P1, P2, and P3 have E values of 56.36; 55.65; 54.45 and a* value of 19.78; 21.56; 25.76, respectively. The higher the a* value, the more red the color will be. E indicates the accumulation or total color change in the shallot bulbs during the 9-week shelf life. P3 has a lower rate of change than P0, P1, and P2. Based on this, it can be seen that P3 has a better color at week nine compared to other treatments (Figure 2).

The results of the L*, a*, b* color test was supported by the physical shape of the shallot bulbs at week 9 (Figure 1), P3 had a purplish pink color, did not show much color change, and the compact tubers were slightly wrinkled, and the outer skin feels stiff and thick. P0 underwent many color changes where the purple color began to fade, the tubers were wrinkled, soft, and turned white to brownish, microorganisms were also attached to the 9th week of storage. P1 and P2 have color changes that slightly different. The color change was visible in the 9th week of storage where purple color, which indicated the freshness of the shallot bulbs had begun to fade, the tubers were wrinkled, and the out-

er skin felt hard and thick, but the growth of microorganisms caused no color change. According to Nurhasanah (2012), a good shallot bulb has a fresh and healthy appearance, compact or dense and not wrinkled and brightly colored. Based on the outer skin color change parameters, P3 maintained the quality of the shallot bulbs at the 9th week of storage.

P3 has a better effect on the final color change after storage because it has a smaller 200 nm nanoparticle size than P2 and P3. The smaller nanoparticle size will make it easier to enter the network and close the pores. The layer formed will be denser so that the nano chitosan layer that becomes an oxygen barrier is maximized. The smaller the particle size, the larger the surface area to increase the stability (Sundar et al., 2010).

The mechanism of discoloration of the shallot bulb's outer skin and the coating of nano chitosan starts from the content of anthocyanins which can be oxidized by oxygen when in direct contact with the outside air in the atmosphere. Oxidation of anthocyanins by oxygen will result in a reduced content of the purplish-red pigment in shallot bulbs. Chitosan is semipermeable and capable of modifying the internal conditions of the fruit by reducing the permeability of the fruit to water, oxygen, and carbon dioxide. As a result, it can reduce the rate of transpiration and the degradation process of anthocyanins (Esyanti et al., 2019). It slows down the discoloration of shallot bulbs during the storage period.

Table 2. Alteration in the color of the outer skin of the shallot bulbs at the 9-week shelf life

Treatment	L	a	b	ΔE
P0	55.21 ^c	16.54 ^a	3.59 ^a	57.75
P1	52.45 ^b	19.78 ^b	5.82 ^b	56.36
P2	50.80 ^b	21.56 ^b	7.15 ^b	55.65
P3	47.83 ^a	25.76 ^c	3.76 ^a	54.45

Note: L* indicates brightness, a* indicates red (+a*) and green (-a*), b* indicates yellow (+b*) and blue (-b*), ΔE* indicates total color change (numbers followed by different letters in the same column show a significant difference based on the DMRT test at a 95% confidence level).

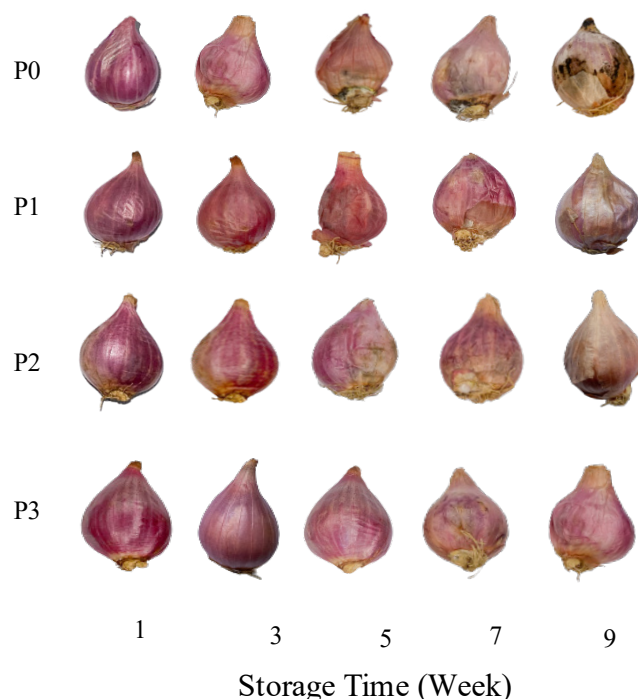


Figure 2. The color changes of shallot bulbs, (P0) control, (P1) Nano chitosan coating with the addition of chitosan ratio and STPP 1:3, (P2) Nano chitosan coating with the addition of chitosan ratio and STPP 1:4, (P3) Nano chitosan coating with the addition of chitosan ratio and STPP 1:5

Effect on Hardness of Shallot Bulbs

The hardness number of shallot bulbs (Table 1) before being treated with nano chitosan coating was 251.37 N, while after being given a nano chitosan coating at the 9th week of P1, P2, and P3, respectively, were 220.74; 221.50; 226.23, and P0 of 216.20 N. This indicated that the nano chitosan coating was better in maintaining the hardness of the shallot bulbs compared to the control. These results were supported by the physical form of shallot bulbs at P1, P2, and P3, which were still quite firm and compact at the 9th week of storage. The best results were shown by P3, which had a higher hardness number than other treatments. P3 showed the best results allegedly because it has a smaller nano chitosan size of 200 nm compared to the particle size at P1 and P2, the smaller the size of nano

chitosan, the higher uptake into the cell so it can reduce the rate of production of CO_2 and the rate of degradation of the cell wall.

An enzyme that plays a role in changing the cell wall structure that causes softening of shallot bulbs is pectin methylesterase. Chitosan coating effectively maintains fruit hardness because chitosan can slow down metabolism and enzymatic activity so that the degradation process of components in plums can be inhibited (Kumar et al., 2017). Chitosan has the properties capable of inhibiting carbon dioxide production (CO_2), which is responsible for methylesterase pectin enzyme activity that can improve the softening of the fruit, so the fruit softening can be inhibited (Liu et al., 2014).

The hardness of the shallot bulbs during the shelf life is related to the softening process

caused by spoilage, oxidation of pectin, and the loss of some minerals and nutrients in the shallot bulb tissue. It will affect the change in the composition of the cell wall during storage. Pectin oxidation causes the chili texture to become soft, wrinkled and lose more water (Rachmawati et al., 2014). It is related to modifications or changes in the cell wall composition during the fruit ripening process (Valero & Serrano, 2010). Plums that are not coated with chitosan have a rapid decrease in hardness compared to chitosan (Kumar et al., 2017).

Based on the research that has been done, the coating of nano chitosan on shallot bulbs (*Allium ascalonicum* L. var. Bauji) needs special attention, especially further research on the effect of nano chitosan on the content of fruit or vegetables. Besides, the production of nano chitosan on a large scale needs to be done to reduce the price of liquid nano chitosan so that all elements of farmers can obtain and apply the nano chitosan coating directly. The approach and introduction of nano chitosan to the public as a coating material that aims to maintain the quality of shallot bulbs during postharvest also needs to be done because nano chitosan still feels foreign in Indonesia.

CONCLUSION

Nano chitosan coating with different ratios of chitosan and STPP has an effect on reducing the percentage of damage, weight loss, diameter reduction, water content reduction, color change, and can maintain the texture of the bulbs of shallot (*Allium ascalonicum* L. var. Bauji) during 9-week shelf life. The best results were shown on 0.2% nano chitosan coating with the addition of a 1:5 ratio of chitosan and STPP.

AUTHOR CONTRIBUTION

D.W.P. designing, collecting, and analyzing data and writing scripts. E.P. and S.W.A.S. design research and oversee all research processes. A.S. helps prepare raw materials used for research.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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