Application of Shrimp Shell Waste Chitosan as Edible Coating to Extend the Shelf-Life of Tomato (*Solanum lycopersicum L.)*

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Abstract. Shrimp shell waste contains chitin compounds which are useful in the food sector. The synthesis of chitin into chitosan can be useful as an edible coating on tomatoes. The research was conducted in three stages: the chitosan manufacturing process, the antibacterial test of chitosan, and the application of chitosan as an edible coating on tomatoes. The manufacture of chitosan consisted of the isolation of chitosan (deproteination, demineralization, depigmentation, and deacetylation) and characterization of chitosan (moisture content, ash content, solubility, and spectrum test using FTIR). The yields obtained from the manufacture of chitosan were deproteination (66.66%), demineralization (57.5%), depigmentation (58.79%), and deacetylation (59.65%). Chitosan characterization includes water content of 3.2% and ash content of 0.41%. FTIR spectra test on isolated chitosan resulted in %DD which was 87.2%. Antibacterial activity of chitosan with four variations of chitosan (0.25%; 0.5%; 0.75% and 1%) produced chitosan with the best inhibitory power at a concentration of 1%. At the chitosan application stage, the average data for each test was obtained, namely the weight loss test of 1.60%; the organoleptic test showed a slower colour change of tomatoes than the control; the test for reducing sugar content of 12.46%; vitamin C test of 36.31 mg/100g; total acid test of 7.79%; so that the use of chitosan as an edible coating has a significant effect on the shelf life of tomatoes..

1 Introduction

Agricultural products are sources of important nutrients such as vitamins, minerals and bioactive compounds that provide many health benefits. [1,2] However, they are highly perishable products and require proper handling to reduce nutrient breakdown and extend shelf life. [3,4] Therefore, agricultural products are generally often packaged and stored at low temperatures during the post-harvest transport and storage process. This is expected to slow or reduce microbial growth and enzymatic reactions, improve overall product quality, reduce mass loss, and extend shelf life. [5]

Postharvest losses of fresh agricultural products are estimated to be 20-30%. [6,7] To overcome the perishable nature of agricultural products, it is generally necessary to use cold storage to overcome the rate of postharvest metabolic processes, which tend to accelerate ripening, softening, pigment changes, respiration rate, acidity changes, and weight loss. [8,9] Reducing post-harvest fruit and vegetable losses in the supply chain requires advanced technology. Reducing these losses increases the number of fresh foods that can be consumed. [10]

Tomatoes are a perishable commodity due to their climacteric nature. This means that tomatoes continue to ripen and continue to ripen after harvest. [11] Can be stored at room temperature for less than 7 days and refrigerated (13-15°C) for 14-20 days. [12] Factors affecting tomato shelf life include increased physiological responses to increased respiration and ethylene biosynthesis rates. Physiological disorders increased susceptibility to microbial infection; high water loss; physical and mechanical damage due to smooth texture. [13] These factors result in post-harvest losses of almost 20%–40% of the total product being wasted. [14]

The extension of the shelf life of fresh produce can be accomplished by the presence of a semi-permeable protective layer around the surface of the fruits and vegetables by modifying the gaseous environment (O_2

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and CO_2) which helps in the reduction of respiration rate, biosynthesis of ethylene, and finally delays the biochemical changes associated with ripening. [15] In addition, the semi-permeable protective layer of fruit or vegetables fills the gaps in the fruit pericarp, which results in the closure of stomata and lenticels which causes delays in developing physiological disorders such as decreased weight loss of agricultural products. [16,17]

Edible coatings are thin layers of polymeric films that are edible and have barrier properties that protect food from the external environment, extending the shelf life of agricultural products such as tomatoes. [14,18] Edible coatings are derived from polymers that are widely available in nature, environmentally friendly, non-toxic, biodegradable, and can be eaten together with food products. [19,20] Edible coatings are less than 10 μm thick and protect food from the external environment by controlling the transport of gases, moisture, and solutes, positively impacting the shelf life of fresh products. [21,22] They are classified based on their polymer matrix. Based on polysaccharides (starches, gums, chitosan), proteins (zein, gluten, gelatine), lipids (oils, waxes), hydrocolloids, or complexes combining lipids in several layers. [23]

Developing edible coatings based on natural polymers (lipids, proteins, polysaccharides, and their derivatives) offers a possible alternative. [24] Many researchers have explored the application of various biopolymer-based edible coatings to agricultural products. [25-30] One is based on chitosan from shrimp processing waste.

Shrimp is a major commodity and economic value as one of the most important fisheries in Indonesia, especially in Gorontalo province. Vannamei shrimp (*Litopenaeus vannamei*) is widely cultivated by communities because it is easy to keep, has high economic value, and is resistant to various diseases. [31,32] Approximately 80-90% of shrimp exports are headless frozen shrimp and 75% of total shrimp weight is shell and head. [33] Shrimp shells are raw materials for the production of chitin and chitosan. [34,35]

Chitin content in shrimp shell debris ranges from 20% to 50% of its dry weight and is a carbohydrate group classified as structural homoglycans. Chitin, the second most abundant biopolymer after cellulose, is the main component of the shells of crustaceans such as shrimp, crabs, lobsters, and krill. In natural materials, chitin is associated with minerals, mainly calcium carbonate, proteins, and lipids such as pigments. [36-38] Chitin and its derivatives are biomolecules with great potential, high economic value, diverse bioactivity, and biodegradability, and are widely used in pharmaceutical, agriculture, food and textile industries, cosmetics, and wastewater treatment. [39]

Chitosan is a natural biopolymer obtained by hydrolyzing and deacetylating chitin in an alkaline solution. [40] During deacetylation, an acetyl group (-$COCH_3$) is removed from chitin and converted to an amine group (-NH_2). [41] Chitosan is a chitin derivative with the [β-(1-4)-2-amino-2-deoxy-D-glucose] structure and a polycationic polymer. [42] The difference is determined by the nitrogen content, with chitin having less than 7% nitrogen and chitosan having more than 7% total nitrogen. [43]

Chitosan exhibits various physicochemical properties such as renewable natural resources, non-allergenic, antibacterial, biocompatible, non-toxic, and biodegradable. [44] These biopolymers have therefore attracted great interest in a variety of research areas, including applications in food, pharmaceutical, pharmaceutical, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and various chemical industries. [45-48]

The most important physicochemical properties of chitosan are the degree of deacetylation (DD) and molecular weight (MW). Because these parameters play a major role in the quality of chitosan in various applications.49 The purpose of this study was to investigate the edible quality of shrimp shell chitosan coatings affected by the chitosan extraction process to extend the shelf life of tomatoes.

2 Method

2.1 Materials

The materials used were shrimp shell waste, tomatoes, NaOH 3.5% (p.a), HCl 1 N (p.a), H_2O_2 2%, NaOH 50% (p.a), H_2O, concentrated HCl (p.a), HNO_3, CH_3COOH 1% (p.a), NH_3, Na_2SO_4, Nutrient Agar (NA), Aquadest, paper disk, Escherichia coli and Staphylococcus aureus bacteria, H_2SO_4 (p.a), 70% alcohol (technical), 20% KI, $CaCO_3$, luff-scroll solution, indicator starch, phenolphthalein indicator, 0.1M NaOH (p.a), iodine solution, $Na_2S_2O_3$ 0.1N, and sodium oxalate.

2.2 Isolation of Chitosan

2.2.1 Shrimp Shell Waste Preparation

The shrimp shell waste used came from the village of Botu Barani, Bone Bolango Regency with the type of vannamei shrimp. Shrimp shell waste that has been obtained is then washed and dried. After drying, the samples were ground and sieved using an 80-mesh sieve. The sample was continued at the isolation stage into chitosan.

2.2.2 Deproteination

Shrimp shell samples were poured into a beaker containing 3.5% NaOH solution, with a ratio of 1:10 (g/mL), stirring, and heated at 90 °C for 4 hours. Then filtered and rinsed with distilled water until neutral. The residue was dried at 60 °C in an oven for 24 hours after which it was weighed to a constant weight.

2.2.3 Demineralization

The residue from the protein separation was added with 1 N HCl solution in a ratio of 1: 7 and heated at room temperature with stirring for 1 hour. Subsequently, the residue was filtered and washed using distilled water. The resulting residue (chitin) was dried in an oven at 60 °C for 24 hours.

2.2.4 Depigmentation

The mineral-free residue (chitin) was added with a 2% H_2O_2 solution in a ratio of 1: 10, stirred for 1 hour, filtered, and washed with distilled water until the pH was neutral. The resulting residue (chitin) was dried in an oven at 60 °C for 24 hours.

2.2.5 Deacetylation

Depigmented residue was added with 50% (w/v) NaOH solution at a ratio of 1: 10, stirred for 1 hour at 120 °C then filtered. The residue was washed using distilled water and then heated in an oven at 60 °C for 24 hours

2.3 Characterization of Chitosan

2.3.1 Determination of the Degree of Deacetylation

Determination of the degree of deacetylation using FTIR was carried out to calculate how many acetyl groups were lost using the following formula:

$$\%DD = 100 - \frac{(A_{1650})}{(A_{3450})} \times 115 \quad (1)$$

Wherein, "A" represents absorbance = log(P_0/P). The subscripts "A_{1655}" and "A_{3450}" are absorbance of wavelength 1655 cm^{-1} for absorption of amide/acetamide group (CH_3CONH^{-1}) and wavelength of 3450 cm^{-1} for absorption of hydroxy group (-OH).

2.3.2 Moisture Content

Moisture content was analyzed using a hot air oven as per the AOAC method (2000). The samples were dried for 3 hours at 100 °C. Determination of moisture content can be done with the following equation:

$$Moisture\ content = \frac{c-(b-a)}{c} \times 100\% \quad (2)$$

wherein, "a" represents the weight of the empty porcelain cup. The subscripts "b" and "c" are the weight of the porcelain cup + sample and the weight of the sample, respectively.

2.3.3 Ash Content

Determination of ash content can be done with the following equation:

$$Ash\ Content = \frac{b-a}{c} \times 100\% \quad (3)$$

wherein, "a" represents the weight of the empty porcelain cup. The subscripts "b" and "c" are these weight of the porcelain cup + sample and the weight of the sample, respectively.

2.3.4 Solubility

The chitosan solubility test aims to determine the solubility of a substance in the solvent H_2O, HCl, HNO_3, 1% CH_3COOH, and NH_3. The testing phase was carried out by dissolving 1 gram of chitosan with 10 mL of solvent each. The solubility of chitosan is seen from the change in the colour of the solution and the solubility that occurs when added to each solvent.

2. 4 Anti-Bacterial Activity

2.4.1 Making Nutrient Agar (NA) Media

20 g of NA was dissolved in 1 L of distilled water and homogenized with a magnetic stirrer in a water bath until it boils. The homogenized NA medium was sterilized in an autoclave at 121°C for 15 minutes and cooled to ± 45-50 °C.

2.4.2 Anti-Bacterial Testing

Test bacteria culture is taken as much as 100 µl inoculated on NA medium by pour plate method. Paper discs were soaked in a chitosan solution for 30 minutes and then placed on 5 paper discs symmetrically on NA medium. The test solution used was chitosan solution with various Chloramphenicol as a positive control. The Petri dishes were then incubated at 37 °C for 24 hours.

After incubation, the zone of inhibition was observed, measured, and documented. The clear area indicates the sensitivity of bacteria to antibiotics or other antibacterial materials used as a test material expressed by the diameter of the inhibition zone.

2.5 Chitosan Application as Edible Coating

2.5.1 Weight Loss Test

Changes in weight loss were measured by weighing the same tomatoes every day of observation, namely at a time of 5 days for 20 days using an analytical balance, the formula used was:

$$Weight\ Loss = \frac{Ba-Bb}{Ba} \times 100\% \quad (4)$$

wherein, "Ba" and "Bb" represent initial fruit weight and fruit weight after storage, respectively.

2.5.2 Organoleptic Test

Organoleptic testing was carried out by using a colour test using a scoring test by 13 panelists. Assessment is done through filling out a questionnaire.

2.5.3 Reducing Sugar Test

This test aims to determine the amount of sugar in tomatoes, calculated as reducing sugar. In this test, the Luff-Scroll method is used. The sample was added with Luff-Scroll reagent and aquadest and then heated by closing the funnel with wet cotton over an Erlenmeyer to produce a red precipitate. Added 7.5 mL of 20% KI and 12.5 mL of 26.5% H_2SO_4, titrated using 0.1 N $Na_2S_2O_3$ solution by adding starch indicator until the solution turns milky brown. The reducing sugar content (RSC) can be determined using the following formula:

$$\%RSC = \frac{mg\ reducing\ sugar}{mg\ sample} \times 100\% \quad (5)$$

2.5.4 Vitamin C Test

Vitamin C content is measured by titration with an iodine solution. As much as 5 grams of tomato sample is diluted to 100 mL then the starch indicator is added and titrated until it turns blue. Calculation of vitamin C content using the equation:

$$Vit\ C = \frac{mL\ iod \times \frac{0{,}88\ mg}{mL} \times dilut.factor \times 100}{W\ (g)} \quad (6)$$

wherein, mL iodine represents the amount of iodine used for the titration (1 mL ion=0.88 mg vitamin C) while the subscript "w" represents the weight of the paste (g).

2.5.5 Total Acid Test

As much as 5 grams of tomato fruit is diluted to 100 mL then phenolphthalein indicator is added and titrated with 0.1 M NaOH solution until it turns pink. Calculation of total acid uses the following formula:

$$\%\text{Total Acid} = \frac{\text{mL NaOH} \times \text{N NaOH} \times \text{dilut.Factor}}{\text{W (g)}} \times 100\% \quad (7)$$

wherein, mL NaOH represents the number of mL NaOH used forthe titration and N NaOH is the normality of NaOH (mEq/mL). The subscript "w" represents the weight of the pasta (g).

3 Result and Discussion

3.1 Chitosan Application as Edible Coating

3.1.1 Deproteination

Deproteination is the step of separating chemical bonds between chitin and protein by using chemicals that depolymerize biopolymers. Removal of protein in shrimp shell waste using a strong base of 3.5% NaOH with a ratio of 1:10 (g/mL). The protein will dissolve in the base so that the protein covalently bound to the chitin functional group will be separated. Na^+ ions will bind to proteins, causing the thickening of the solution, characterized by forming a few bubbles and a reddish colour change.

The use of NaOH solution with high concentration and temperature is more effective in removing protein and causing the deacetylation process. The stirring process during and heating aims to accelerate the binding of the end of the protein chain with NaOH so that the protein degradation and deposition process takes place perfectly. [50]

The yield of deproteinized shrimp shell waste was 66.66%. This indicates that the protein content in the shrimp shell has been released and reacts with NaOH to form amino acids with the reaction equation shown in Figure 1.

To determine the presence or absence of protein, the Biuret test was then carried out to detect the presence of peptide bonds obtained from the reaction in the form of purple colour in the solution. The results showed no colour change, meaning the sample was protein-free.

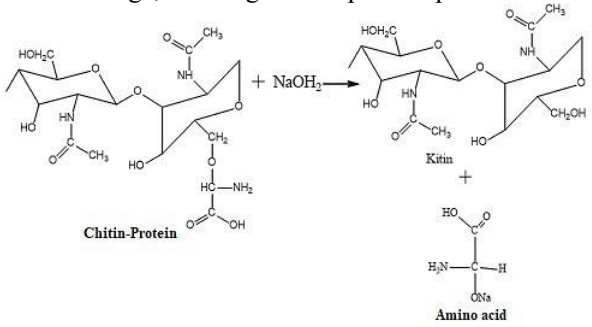


Fig 1. Breaking reactions between chitin and protein

3.1.2 Demineralization

The demineralization process aims to remove inorganic compounds in shrimp shell waste. In addition to containing chitin, shrimp shell waste resulting from the deproteination process also contains minerals. Shrimp shell waste generally contains 30–50% mineral carbonate ($CaCO_3$). In addition, there is also calcium phosphate ($Ca_3(PO_4)_2$) with levels of 8-10% of the total inorganic material depending on the species. [51]

These minerals can be removed from the matrix by using a 1 N HCl solution. The minerals become dissolved salts as a form of a reaction with acid, so they are easily separated during filtration and washing. The more minerals are removed, the better the chitin produced.

The indicator of the mineral release process in shrimp shell waste is indicated by the formation of CO_2 gas bubbles when mixing the sample with HCl solution. Mineral salts in $CaCl_2$ are soluble in solvents, so they are easily removed during the washing process. The residue

resulting from demineralization is a dark brown powder with a coarse texture. The reactions that occur are:

$CaCO_3(s) + 2HCl(aq) \longrightarrow CaCl_2 + CO_2(g) + H_2O(l)$

$Ca_3(PO_4)_2(s) + 6HCl(aq) \longrightarrow 3CaCl_2(aq) + 2H_3PO_4(aq)$

Chitin yield decreased from 66.66% (deproteination result) to 57.50% after demineralization. The reduced mass of chitin solids was due to the mineral content lost in the shrimp shell waste during the demineralization process. Furthermore, to determine the presence or absence of mineral content in the sample, an anion cation test was carried out using several reagents in the form of HCl, NH_3, NaOH, KCN, and $AgNO_3$ which showed negative results.

3.1.3 Depigmentation

Depigmentation removes chitin's colour (pigment) by immersing it in a 2% H_2O_2 solution. The dark-coloured pigment in shrimp shell waste is crustacyanin which is a lipoprotein compound, whereas the lipid group is a carotenoid compound known as astaxanthin. [52]

The process of changing the colour of the chitin residue from brown to brownish-white is due to the presence of H_2O_2 solution, which can remove the pigment in chitin. [53] This is because H_2O_2 is a strong oxidizing agent capable of oxidizing the colour pigments of shrimp shell waste.

The yield from the depigmentation stage was 58.79% and further processing was needed to produce chitosan, namely the deacetylation process.

3.1.4 Deacetylation

Deacetylation is breaking the acetyl groups of chitin to produce chitosan. An acetyl group ($-COCH_3$) is removed from chitin using an alkaline solution and converted to an amine group ($-NH_2$). In general, the reaction that produces chitosan from chitin is an amide hydrolysis reaction with a base, with chitin acting as an amide and NaOH acting as a base. Chitin has a long crystal structure with strong hydrogen bonds between the nitrogen atoms and carboxylate groups of adjacent chains. A commonly used method is to use an alkaline NaOH solution. [54] Using highly concentrated and hot alkaline solutions during deacetylation can affect the degree of deacetylation produced. This proves that the higher the concentration, the more substances will react and the greater the chance of collisions. This process also causes the loss of acetyl groups in chitin, resulting in positively charged chitosan, making it soluble in organic acids such as acetic and formic acids.[55] The loss of the acetyl group in chitin is called the deacetylation process.

First, the double bond between C and O is broken, making C positively charged and O negatively charged. The OH- of highly electronegative NaOH attacks the highly electropositive C, and Na binds to the O of $NHCOCH_3$. In addition, the lone pair of -NH binds to the H of OH, thus electron delocalization occurs and the -NH missing electron gets a donor from C. This leaves C lacking electrons. To achieve stability, electrons from O are used to attach to C, breaking the acetyl bond with this amide to form a $-NH_2$ group. [56] The deacetylation mechanism of chitosan is shown in Figure 2. [57]

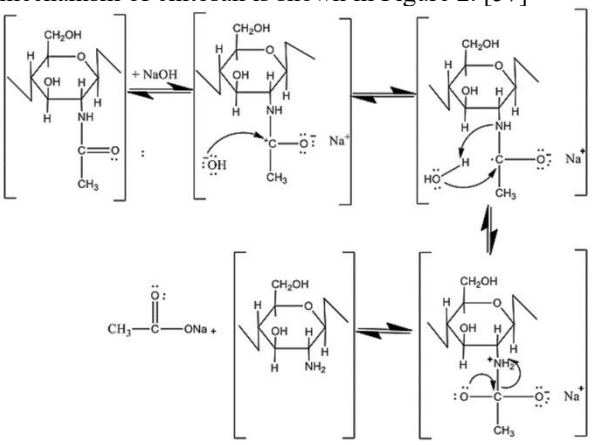


Fig 2. Mechanism of the chitosan deacetylation reaction

This process is the final step that removes the acetyl group to separate chitin into chitosan. The yield obtained from this step was 59.65% and the weight of chitosan was 78.90 g. The deacetylated chitosan was then characterized by FTIR to determine the functional groups and degree of deacetylation.

3.2 Characterization of Chitosan

3.2 1 Determination of the Degree of Deacetylation

The degree of deacetylation (DD) is one of the most important chemical properties affecting chitosan's usefulness in various applications. This is calculated using the baseline method. Figure 3 shows the FTIR spectrum results of chitosan.

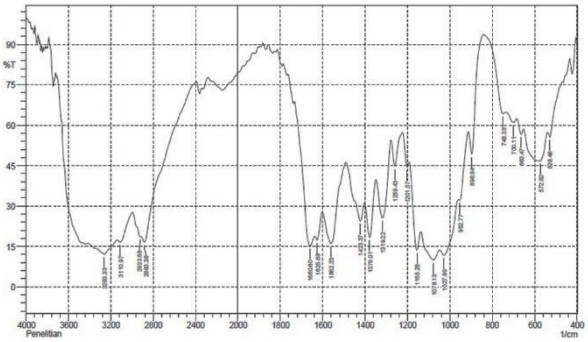


Fig 3. FTIR spectrum of chitosan

In the infrared spectrum of standard chitosan, an absorption band appears in the region of 3274.90 cm^{-1}, indicating the overlapping absorption of the OH and N-H stretching modes. The absorption band at wavenumber 2881.45 cm^{-1} indicates a C–H functional group stretcher, and the absorption band at wavenumber 1652.88 cm^{-1} indicates a weakly intense C=O stretcher group. The absorption band at a wavenumber of 1593.09 cm^{-1} indicates a kink NH group, and the absorption band at a

wavenumber of 1427.23 cm^{-1} indicates a kink C—H group. At a wavenumber of 1029.92 cm^{-1}, it exhibits a stretched C-O group.

On the other hand, the isolated chitosan also shows almost the same absorption band as standard chitosan. Wavenumber 3263.33 cm^{-1} shows the absorption overlap of the OH and N-H stretching modes. The absorption bands at wavenumbers 2923.88 cm^{-1} and 2883.38 cm^{-1} indicate stretching C-H groups. The wavenumber at 1660.60 cm^{-1} indicates the stretching vibration of the C=O group. The bending modes of the N-H group appear at wavenumbers 1625.8 cm^{-1} and 1562.23 cm^{-1}. The absorption bands at wavenumbers 1423.37 cm^{-1} and 1379.01 cm^{-1} indicate bending vibrations of the C-H group. Stretching vibration of the C-O group at wave numbers 1078.13 cm^{-1} and 1027.99 cm^{-1}.

Based on the FTIR results, standard chitosan was 84.66% and isolated chitosan was 87.2%. In addition, the produced chitosan are chemically characterized with respect to water content, ash content, and solubility, as shown in Table 1.

Moisture content is one of the important parameters determining the quality of chitosan. In this study, the average water content of the obtained chitosan was in the range of 3.32%. This water content value can meet the quality standard of commercial chitosan, which is ≤10%. [58].

Table 1. Test results of Physico-chemical properties of chitosan isolation results

Specifications	Chitosan
Moisture (% dry weight)	3.32 %
Ash (% dry weight)	0.41 %
Degree of deacetylation	87.2 %

Low water content can limit or reduce damage to chitosan, such as by avoiding moisture-induced microbial activity. The water content of chitosan plays a very important role in its stability and durability. The moisture content of chitosan is affected by the relative humidity of the air around the storage area. This is because it easily absorbs water vapor from the surrounding air. Chitosan polymer groups (amine, N-acetyl, and hydroxyl groups) form hydrogen bonds with H_2O in the air.

During the storage process, the amount of water absorbed increases. This means that their ability to bind water is reduced. This happens due to the formation of hydrogen bonds between particles. [59] Ash indicates the mineral content in chitosan, i.e. the amount of mineral residue remaining after the desalting process. Ash content is therefore one of the key parameters that indicate the effectiveness of the desalination process, as evidenced by the ash reduction. The lower the ash content, the higher the chitosan purity.

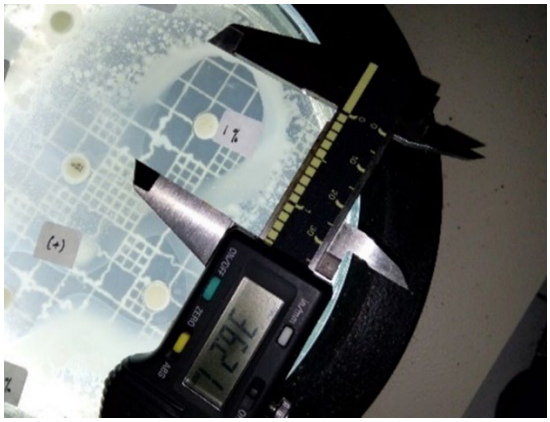
Deacetylation time also affects chitosan ash content. The higher the temperature and the longer the deacetylation time, the less ash content of chitosan. This is because more minerals are dissolved in the NaOH solution and the more chitosan used, the longer the washing time to neutralize the pH of the chitosan.

The solubility of chitosan in acetic acid is also a parameter that can be used as a benchmark to evaluate chitosan quality. The higher the solubility of chitosan in acetic acid, the better the quality of chitosan produced. [60] The resulting chitosan is completely soluble in 2% acetic acid. Solubility was monitored by comparing the clarity of the chitosan solution with that of the solvent.

3.3 Anti-Bacterial Activity

In testing the antibacterial activity of isolated chitosan, four concentrations of chitosan were used. 0.50%; 0.75%; 1%. Using the pour-over method, this test was performed on his two strains of bacteria, both Gram-positive and Gram-negative *Escherichia coli* and *Staphylococcus aureus*. The pore plate method is a method of fertilizing cultures in cups.

Chitosan test results against *Escherichia coli* and *Staphylococcus aureus* showed the presence of antibacterial activity. This is indicated by the formation of a clear zone around the disc paper treated with various concentrations of chitosan. ate As shown in Figure 4, the maximum zone of inhibition for *S. aureus* was 33.63 inches at 1% concentration.



(a)

(b)

Fig 4. Inhibition zone of 1% chitosan; (a) E coli; (b) S. aureus

Based on the zone of inhibition measurements, the stoppage of Gram-positive bacteria is greater than that of Gram-negative bacteria. This indicates that chitosan is more sensitive to Gram-positive bacteria. This difference in activity is due to differences in the structure and components of the bacterial cell wall. Gram-negative bacteria have a thin peptidoglycan layer in their cell walls, while Gram-positive bacteria have a thick peptidoglycan layer. The components of the cell wall of Gram-negative bacteria are more complex because they have an extra outer membrane layer, and they penetrate the cell wall of Gram-positive bacteria more easily than Gram-negative bacteria. [61,62]

Overall, the following are true: The higher the concentration of the respective extract, the larger the diameter of the zone of inhibition formed. Therefore, the next analysis is chitosan's ability as an edible coating and applied to plants, namely tomatoes.

3. 4 Chitosan Application as Edible Coating

The concentration of antimicrobial chitosan test results with the zone of greatest inhibition continued at the stage of applying edible coatings to tomatoes. Test parameters to determine tomato shelf life in terms of weight loss, organoleptic, reducing sugars, vitamin C, and total acidity. The tomatoes used in this study were at three degrees of ripeness: 0-10% (R1), 30-60% (R2), and 70% or higher (R3).

Weight loss is an indicator of tomato quality. Weight loss is due to physicochemical changes occurring during the storage process up to cooking. Horticultural products lose weight the longer they are stored, due to metabolic processes that continue after harvest. [63]

Fig. 5 shows the results of weight reduction after storing tomatoes with a maturity level of 3 at 5-day intervals for 20 days from day 0 to day 20.

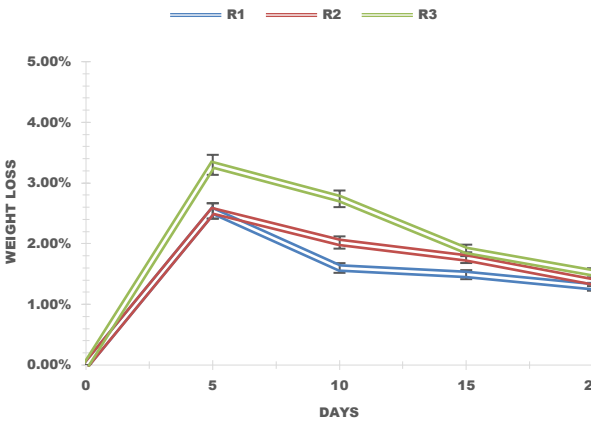


Fig 5. Weight loss results

The analysis showed that the average weight loss of tomatoes after 20 days of storage with treatment was 1.60%, which was lower than the loss of tomatoes without coating (control) which was 1.95%. This shows that chitosan can reduce the occurrence of the transpiration process so that the decrease in weight loss of tomatoes can be suppressed in such a way. Weight loss in tomatoes tends to be erratic along with storage time and maturity level.

As a chitosan coating, it has a good effect in inhibiting the diffusion of O_2 so that the respiration process is slightly hampered, as a result, less CO_2 gas which is the result of the respiration process will be produced so that evaporation or transpiration in the fruit is inhibited. Transpiration in fruit causes cell bonds to become loose and air spaces to become large like shrivelled up, this cell condition causes changes in air volume, turgor pressure, and fruit hardness. [64]

Organoleptic testing has an important role in tomato quality. This test is a test method using the human senses as the main tool for measuring product acceptance. Sensory analysis is subjective. The appearance of tomatoes is done visually (colour) with a descriptive test using a scale of 1 to 5. The appearance of the colour of tomatoes, namely scale 1 indicates the full green colour, scale 2 shows a yellowish-green colour, scale 3 indicates yellow to orange, scale 4 indicates reddish yellow, and scale 5 shows a full red colour. The assessment data of 13 panellists can be seen in Table 2.

Based on the sensory test results, it was concluded that uncoated tomatoes changed colour faster than tomatoes coated with edible chitosan. Uncoated tomatoes get more colour day by day than tomatoes with a chitosan edible coating. This indicates that chitosan can slow down the colour change process, indicating that the ripening (colour change) process in tomatoes is slow.

Table 2. Organoleptic results of tomato colour for 20 days by 13 panelists

Maturity	Day	Control					Treatment				
		Color scale					Color scale				
		I	II	III	IV	V	I	II	III	IV	V
Raw Red skin 0-10% (K1)	0	1	-	-	-	-	1	-	-	-	-
	5	3	6	2	-	-	3	5	-	-	-
	10	5	3	8	-	-	7	6	4	-	-
	15	2	5	8	-	-	3	9	4	-	-
	20	-	-	9	4	-	-	3	9	1	-
Mengkal, Red skin 30-60% (K2)	0	-	13	-	-	-	-	13	-	-	-
	5	-	5	8	-	-	-	9	4	-	-
	10	-	2	11	-	-	-	7	6	-	-
	15	-	-	9	4	-	-	3	7	3	-
	20	-	-	2	7	4	-	-	9	3	1
Ripe Red skin >70% (K 3)	0	-	6	7	-	-	-	4	9	-	-
	5	-	-	5	6	2	-	-	6	5	2
	10	-	-	2	8	3	-	-	4	6	3
	15	-	-	-	8	5	-	-	-	9	4
	20	-	-	-	1	12	-	-	-	5	8

Information:
1-13 = Number of Panelists
Colour scale:
I= Full green
II= Green yellow
III= Reddish yellow
IV= Red
V= Full red

A reducing sugar analysis aims to determine the number of sugars contained in tomatoes calculated as reducing sugars. As shown in Figure 6, the average total sugar reduction of tomatoes with edible chitosan coating was 12.46%, which was 12.19% higher than that of uncoated tomatoes.

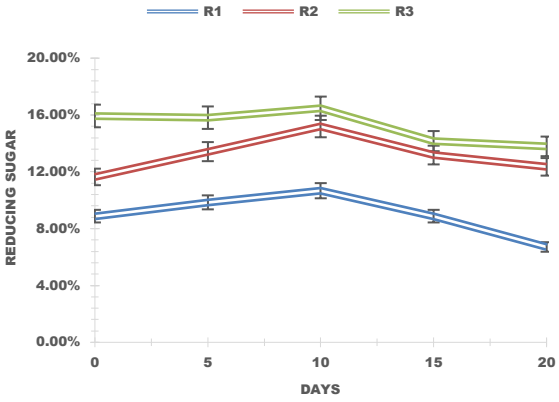


Fig 6. Reducing sugar results

The highest total sugar solids were obtained at ≥70% ripeness with a value of 16.47%, which is different from other ripeness levels. This indicates that ripe tomatoes have a higher sugar content. This is because starch (a carbohydrate) is broken down into simple sugars (glucose and fructose), increasing the sugar content.

During the ripening process during fruit storage, starch is completely hydrolyzed to sucrose and converted to reducing sugars as substrates during the respiration process. Coating tomatoes with chitosan slows their respiration rate and prevents the loss of total sugars during storage. The reduction of total reducing sugars in tomatoes during storage was caused by the tomato respiration process, so reducing sugars were degraded to pyruvate to produce CO_2 and H_2O. [65] Tomatoes are one of the menopausal fruits that show an increase in respiration at the start of storage, followed by a decreasing trend with storage time. Ripeness also affects a tomato's total sugar solids. [66]

Vitamin C levels were determined using the iodometry method. The principle of iodometric titration is a redox reaction in which vitamin C acts as a reducing agent (reducing agent) and I2 acts as an oxidizing agent (oxidizing agent). Figure 7 shows the relationship between vitamin C content and shelf life in coated tomatoes.

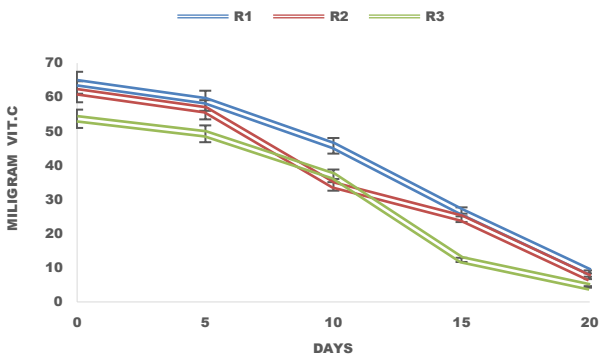


Fig 7. Vitamin C results

Vitamin C levels tend to decrease with storage time. Decreased vitamin C levels are related to its easily oxidizable nature. The vitamin C content ranges from 4 to 64mg/100g and the average content of uncoated tomatoes is 34.53mg/100g. This value is lower than chitosan-coated tomatoes. This is probably because the chitosan coating layer inhibited the penetration of oxygen into the tomato from the surface and damaged the vitamin C through an oxidation reaction. Chitosan edible coatings have excellent barrier properties against O_2 and CO_2 diffusion. [67]

A medium-sized tomato can supply him with 28% of his daily need for vitamin C. This vitamin is an important antioxidant that helps maintain immunity. [68] The body needs them both to form collagen and to participate in reactions with certain amino acids. [69] It is also readily activated in the upper small intestine, presumably absorbed by diffusion, and enters the bloodstream via the portal vein. The average absorption rate is 90% and the daily intake is 20-120 mg. When vitamin C consumption reaches 100 mg per day, the body can store up to 1500 mg of vitamin C.[70]

Total acidity measurement is to measure the concentration of total acidity contained in tomatoes. This value includes measurements of both dissociated and undissociated total acidity, whereas pH typically measures only dissociated total acidity. Therefore, measuring total acidity rather than pH is a better way to determine the organic acid content of tomatoes. Chitosan-coated tomatoes averaged 7.79% total acidity compared to 6.45% without coating. Figure 8 shows the relationship between total acidity and storage time for coated tomatoes.

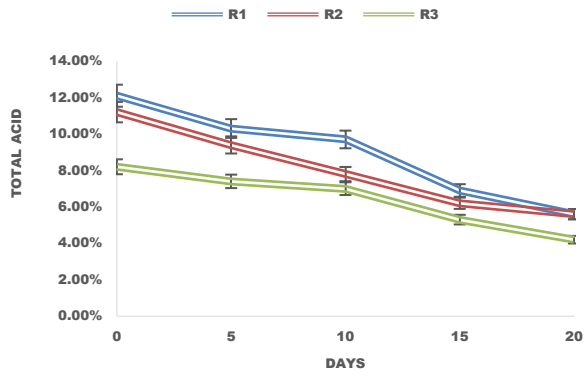


Fig 8. Total acid results

The total acidity of chitosan-coated tomatoes was higher than that of uncoated tomatoes. The higher the fruit acidity, the longer the fruit shelf life. The total acidity of tomatoes decreased during storage. This is related to respiration rate, uncoated tomatoes have a higher respiration rate, so organic acids are quickly broken down. It is commonly used as respiration energy during storage. The longer the storage period, the lower the acidity of the fruit.

Overall climacteric fruit the number of organic acids will decrease rapidly during storage, and there will be an increase in respiration rate which requires a lot of energy so organic acids are used as energy source substrates. However, the total acid in tomatoes coated with chitosan tends to be higher when compared to the control, this indicates that the tomato coating with chitosan can restrain the respiration rate so that the use of organic acids can be suppressed which in turn can maintain the total tomato acid during storage.

4 Conclusion

The antibacterial activity test of chitosan with a concentration of 1% has the highest inhibitory value so that it can be used as an edible coating to extend the shelf life of tomatoes. The isolated chitosan had a moisture content of 3.32% and an ash content of 0.41% with a deacetylation degree of 87.2%. Based on the data obtained, the overall testparameters showed that tomatoes with chitosan edible coating could extend their shelf-life

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