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Effect of Types of Cayenne Pepper and Chitosan Concentration of Shrimp Shell Waste as Biocoating to Extend Shelf Life

Roisatul Chumaidah [1], Meutia Maulina [2], Lulu Nurliyantini [3], Trisna Juliati [4], Nur Arifah Qurota A'yunin [5], Septian Cahya Azhari [6]
Universitas Siliwangi, Tasikmalaya, Indonesia

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ABSTRACT

This study aims to determine the effect of chitosan concentration on types of cayenne pepper and the interaction between the two as a respiration barrier during storage at room temperature. The research was conducted at the Basic Laboratory of the Faculty of Agriculture, Siliwangi University from June to August 2021. This research method used a Completely Randomized Factorial Design (CRD) consisting of two factors, repeated four times. The first factor was the type of cayenne pepper (A), namely green cayenne pepper (A1) and red cayenne pepper (A2), the second factor was the concentration of chitosan (C), namely 0% chitosan (C1), 1% chitosan (C2) and 2% chitosan (C3). The research data were analyzed using variance and further tested with Duncan's multiple range test with a significance level of 5%. The results showed that there was an interaction between the type of cayenne pepper and the concentration of chitosan on vitamin C content, phenolic content, and fruit weight loss of cayenne pepper.

Corresponding Author:

Septian Cahya Azhari
Universitas Siliwangi
Email: 192170004@student.unsil.ac.id

INTRODUCTION

Contains Indonesia is an area that has vast marine potential, has a water area of 3,110,000 km2 with abundant and potential natural wealth (Kemenkomaritim, 2018). According to BPS (2020) fishery commodity export activities in Indonesia during the COVID-19 pandemic in March 2020 reached USD 427.71 million, an increase of 6.34% compared to March 2019.

Shrimp is exported in the form of processed products, namely in the form of fresh frozen shrimp that have undergone cold storage after the process of separating the head and shell. The result of processing the shrimp industry still leaves waste that often causes pollution because it has not been used optimally and most of it is still being dumped into the environment which causes an unpleasant aroma. According to Hanafi (2000), the waste from industrial processing leaves the head, skin, tail, and legs which can reach 30-70% of the shrimp's weight. Until now, this waste has not been optimally utilized. As a result of the processing activities, the waste generated can reach 60,000 tons in the processing period.

Shrimp shell waste is abundant in number, and has several potential contents, namely 27.6% minerals, 34.9% protein, 18.1% chitin, and other components such as solutes, fat, and 19.4% digestible protein (Harjyanti, 2014). . Chitin content can be isolated and converted into chitosan through the deacetylation process. Chitosan has biodegradable, biocompatible, non-allergenic, low toxicity, and has biological activity. Based on research conducted by Malerba (2016), the use of chitosan can encourage tolerance to biotic and

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abiotic stresses in several commodities. The ability of chitosan as an antioxidant makes chitosan the most potent natural ingredient to reduce the use of chemical products in agriculture.

Post-harvest management in agriculture for cayenne pepper has not been developed much. Chili has characteristics that are easily damaged so maintaining the quality and quality of chili is difficult (Sembiring, 2009). This can be seen in the unstable price fluctuations of cayenne pepper. Post-harvest handling of chilies can be carried out based on the principles of Good Handling Practices (GHP). According to Masnun (2015), GHP includes the implementation of good and correct activities, so that product quality can be maintained, can suppress shrinkage, damage and extends the shelf life, and can maintain product status.

The purpose of this study was to determine the effect of chitosan concentration on the type of cayenne pepper and the interaction between the two as a respiration barrier (bio coating) during storage at room temperature.

IMPLEMENTATION METHOD

This research was carried out for 3 months from June to August 2021 at the Chemistry Laboratory and Production Laboratory, Faculty of Agriculture, Siliwangi University, Tasikmalaya. The tools used in this study were: Erlenmeyer, glass jars, hoses, plasticine, burettes, states, clamps, measuring flasks, measuring pipettes, measuring cups, beakers, container boxes, pumps, desiccators, ovens, Petri dishes, stir bars, thermohygrometer, thermometer, analytical balance, cuvette, 50 mesh sieve, UV-Vis KLab spectrophotometer, ostwarld capillary viscometer, sprayer and laboratory coat.

The materials used in this study were: shrimp shell waste, red bird's eye chilies, green bird's eye chilies, alcohol, technical NaOCl, NaOH, technical HCl, distilled water, phenolphthalein indicator, universal pH indicator, gallic acid, Follin C, methanol, ethanol, tamarind. acetate, KI, crystal I2, starch, concentrated H2SO4, clean water, general stationery, and label paper.

This research was divided into two stages, namely making chitosan from shrimp shell waste and coating it on red and green chili peppers. In the first stage, samples of shrimp shell waste were obtained from vannamei shrimp ponds in Cipatujah District, Tasikmalaya Regency. The waste is washed using running water and dried using an oven, then the chitosan production stage is carried out with the manufacturing process which includes; a) deproteination b) demineralization c) depigmentation, and d) deacetylation.

The second stage, the chitosan coating process on cayenne pepper using an experimental method, namely a Completely Randomized Design (CRD) arranged in a factorial manner, with two factors, namely: a) the first factor is the type of cayenne pepper (A) which consists of two levels, namely green cayenne pepper (A1) and red cayenne pepper (A2). The second factor consisted of three levels of chitosan concentration (C), namely 0% chitosan (C1), 1% chitosan (C2), and 2% chitosan (C3). So 6 treatment combinations were obtained and repeated 4 times to obtain 24 experimental units. Cayenne pepper which will be given the coating treatment is first carried out by sorting and grading the chilies used, washed thoroughly with running water, then given a coating treatment with the dip method (dipping) for 30 minutes. Coating results were stored in plastic boxes at room temperature and observed up to 10 days after coating.

The tests carried out included testing the characteristics of chitosan and testing the characteristics of cayenne pepper based on Physico-chemical properties which included organoleptic assessment, testing for vitamin C levels, measuring respiration rate, testing for phenolic content, and testing for weight loss.

RESULTS AND DISCUSSION

Characteristics of Chitosan

Research/Devotion Chitosan produced in this study was carried out through the extraction method. Shrimp shell waste is processed through a deproteination stage using NaOH with a high-heating process. After the deproteination stage, followed by the demineralization stage using HCl solution. The next stage is through the deacetylation process to break the acetyl group (-COCH3) (Rahmawati, 2012). The process of making chitosan is shown in Figure 1.

Based on the results of the study, most of the chitosan produced had characteristics that met the quality standards of chitosan from Protan Laboratories, Inc. 1987 (Table 1). The purity of chitosan can be seen from the low water content but has a high degree of deacetylation. The higher the degree of deacetylation, the more ammonia (NH2) groups in the chitosan molecular chain so that it will be more reactive (Hossain and Iqbal, 2014). The value of water content obtained in chitosan is 7.69%, this result is to the standard recommendations of Protan Laboratories, Inc. 1987, namely, chitosan has a moisture content of <10%, according to Suptijah et.al (2011) that a good drying method will produce low water content.

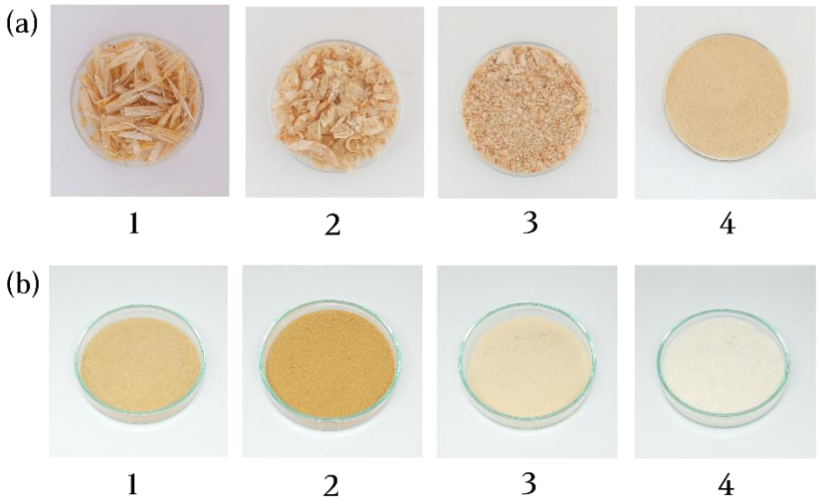

Figure 1. a. Preparation of shrimp shell powder (sorting and grinding) physically, b. Synthesis of chitosan powder (processed (1) deproteination; (2) demineralization; (3) depigmentation; (4) deacetylation) chemically
Source: Author's Research

Based on measurements, the particle size of the resulting chitosan is in the form of powder, this is to the chitosan quality standards (table 1), which are influenced by the raw materials used. The raw material comes from shrimp shells which have a smooth shape and are easily crushed in the chitosan manufacturing process. The solubility of chitosan in acetic acid is one of the parameters that can be used as a standard for assessing the quality of chitosan. The higher the solubility of chitosan, the better the quality produced (Zahiruddin, Aprillia, Ella, 2008).

Chitosan viscosity measurements were measured with an Ostwald Viscometer based on determining the time between the volume flowing between the two calibration marks. Based on the research results, it was found that the viscosity of chitosan was 6.48 cP with a molecular weight of 1x105 g/mol. According to the Mark-Houwing equation, molecular weight is directly proportional to intrinsic viscosity (Anugraini et al. 2008). Despite the increase, the viscosity of the chitosan obtained in this study was low when compared to the standard of Protan Laboratories Inc, namely > 200 cP. The low viscosity is thought to be due to the long demineralization process and the high deacetylation temperature. Based on research conducted by Siregar et al. (2016), the decrease in the viscosity of chitosan is in line with the addition of the demineralization process time.

The Ninhidrin test was carried out to identify the presence or absence of amine groups in chitosan. Tests showed that the resulting chitosan powder was positive, indicated by a change in color to purple, meaning that chitin had changed to chitosan (Agustina, 2013).

Table 1. Quality Characteristics of Standard Lab Inc. Protan and Chitosan Powder Test

Parameter Analysis	**Standard Protan Laboratories Inc**	**Powder Test Chitosan**
Water content (%)	< 10	7,69
Particle Size	Granular-powder	Powder
Acetic Acid Solubility 2%	Late	Late
Acidity (pH)	Neutral	Neutral
Color	White to pale yellow	White
Aroma	No smell	No smell
Viscosity (cP)	>200	6,48
Ninhidrin Test	Positive	Positive

Source: Protan Laboratories, Inc. 1987

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The Quality of Chitosan Coating Cayenne Pepper

Organoleptic Test

Organoleptic testing in this study was conducted to determine the feasibility of testing the consumption of cayenne pepper based on panelist preferences. Organoleptic test parameters include color measurement, degree of freshness, level of damage, and level of preference for the treated cayenne pepper without coating, 1% and 2% chitosan coating which are presented in Figure 1 and Table 2.

Observations were made at t10 = the 10th day after coating on 20 panelists, the resulting data showed that the coating effect on color change, level of freshness, level of damage, and level of preference of the panelists.

Figure 2. Quality of Green Cayenne Pepper (a = 0% Chitosan concentration, b = 1% Chitosan concentration, c = 2% Chitosan concentration) and Red Cayenne Pepper (d = 0% Chitosan concentration, e = 1% Chitosan concentration, f = Chitosan concentration 2%) at day interval t0 = day 0; t2= 2nd day; t4 = 4th day; t6 = 6th day; t8= the 8th day; t10 = the 10th day.

Source: Author Research

Table 2. Organoleptic test of green cayenne pepper and red cayenne pepper on day 10

Type of Chili	Color	Freshness Level	Damage Rate	Levels of pleasure
A_1C_1	Reddish green	Not fresh	0-5%	Do not like
A_1C_2	Yellowish green	Somewhat wilted	0-5%	Enough like
A_1C_3	Dark green	Not fresh	0-5%	Enough like
A_2C_1	Brownish red	Not fresh	51-75%	Very dislike
A_2C_2	Yellowish red	Somewhat wilted	0-5%	Enough like
A_2C_3	Red	Somewhat wilted	0-5%	Enough like

Notes : A1 (green cayenne pepper); A2 (red cayenne pepper); C1 (chitosan 0%); C2 (chitosan 1%); and C3 (chitosan 2%).

Based on table 2. the color change occurred significantly in green cayenne pepper which was treated without chitosan coating and with 1% chitosan treatment, while the 2% treatment could maintain the color of chili until the 10th day. The freshness level of green cayenne pepper also changed, especially in the treatment without coating in the 1% treatment, the chili became not fresh, while in the 2% chitosan treatment could maintain the freshness level of the chili. This is because the chili coating with a concentration of 2% produces a thick enough coating that can maintain chili respiration conditions so that it can affect the physical appearance, especially the freshness level of chili. Meanwhile, the level of damage to green cayenne pepper can be maintained by all treatments. Based on this, the panelists quite liked the chitosan 1% and 2% chitosan coating treatment and did not like the treatment without chitosan coating.

In red chili, the 2% chitosan treatment can maintain the color of the chili until the 10th day, while the treatment without coating causes the chili to experience a significant color change. Chitosan treatment of 2% and 1% can maintain the freshness of chili, but the treatment without coating causes the chili to be not fresh. The level of damage to red cayenne pepper can be maintained well in the 1% and 2% chitosan coating treatment, while the treatment without coating causes damage to the chili up to 51-75%. Based on this, the panelists quite liked the 1% and 2% chitosan treatment and didn't like the treatment without chitosan coating.

Vitamin C content

Table 3. Vitamin C Levels in Cayenne Pepper

Types of Cayenne Pepper (A)	Chitosan Concentration		
	0%	1%	2%
Green (A1)	3,60 b A	2,94 b B	2,90 b B
Red (A2)	4,85 a A	4,56 a B	5,01 a A
Average	4,22	3,75	3,95

Note: The numbers followed by the same capital letters horizontally and lowercase letters vertically show no significant difference according to Duncan's multiple-distance test at a 5% significance level.

Based on the results of statistical tests on vitamin C content measured on the 10th day of storage, shows that there is an interaction between the type of cayenne pepper and the concentration of chitosan. The concentration of chitosan in green cayenne pepper did not significantly affect the vitamin C content in cayenne pepper. The content of vitamin C in red chilies is higher than in green chilies, according to Husna (2010) green cayenne pepper contains 84 mg/100 g of vitamin C, while red cayenne pepper contains 181 mg/100 g of vitamin C.

According to Sapei (2014), vitamin C is generally easily oxidized and lost during processing and storage. Factors that affect the oxidation process include exposure to light, pH, solubility of oxygen, the presence of metal ions, sugar content, and storage temperature (Devi, 2017).

Respiration Test

Respiration rate is a parameter to determine the storability of agricultural products by measuring the rate of metabolism. The respiration rate of horticultural commodities including chili will continue to carry out the respiration process after harvesting. The respiration rate will be largely determined by temperature. For every 100C increase, the speed will double or triple (Van't Hoffs law). According to Winarno (2002), non-climacteric horticultural products including cayenne pepper in their respiration process will run slowly, so that the changes that occur in the cooking phase are not visible. This is to the data obtained, that the condition of green bird's eye chilies and red bird's eye chilies without chitosan coating treatment gave results with respiration rates that tended to increase from days 2, 4, 6, 8 to 10. Meanwhile, the 1% and 2% administration of chitosan to green and red bird's eye chilies shows an irregular graph of the chili's respiration rate (Figure 3) due to the slow respiration rate. This shows that the use of chitosan coating can function as a barrier to respiration rate which can maintain the freshness of cayenne pepper. Measurement of respiration rate is expressed in units of ml/g/minute.

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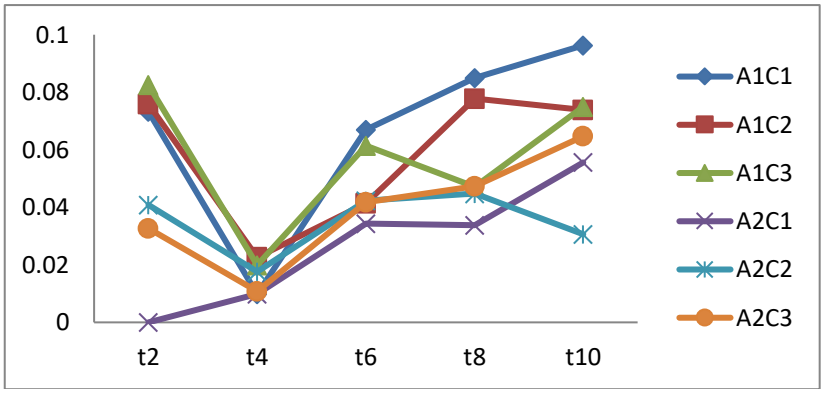

Figure 3. Graph of Respiration Rate of Green and Red Cayenne Pepper
Notes : A1 (green cayenne pepper); A2 (red cayenne pepper); C1 (chitosan 0%); C2 (chitosan 1%); and C3 (chitosan 2%).

Phenolic Test

The total phenolic content test was used to determine antioxidant activity. Research that has been conducted by Johari and Khong (2019), that the higher the total phenolic content, the higher the antioxidant activity. According to Noor., et.al (2014) the higher the phenol content, the higher the ability to suppress the growth of microorganisms. The results of measurements using a spectrophotometer show that the absorbance of gallic acid standard solutions with concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm made by the calibration curve has a relationship between concentration (C) and absorbance (A) which is obtained by the linear equation y = 0.173x + 0.126. Based on this, a linear regression equation is obtained with a correlation coefficient (R2) = 0.9817 which meets the eligibility requirements of the analytical method (Figure 4).

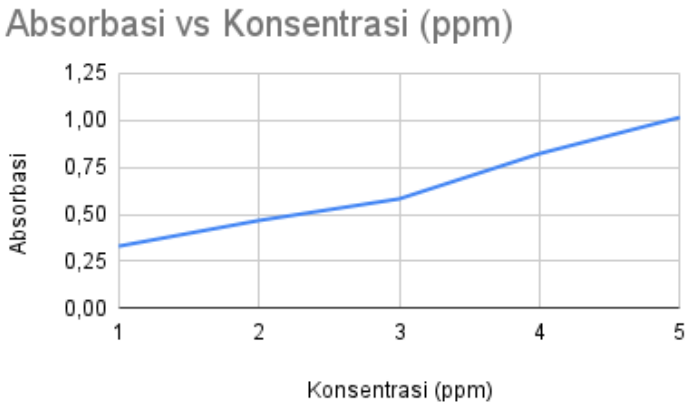

Figure 4. Calibration curve at a wavelength of 750 nm.

Table 4. Total phenol content of green and red cayenne pepper

Types of Cayenne Pepper (A)	Chitosan concentration		
	0%	1%	2%
Green (A1)	14,31 b B	13,03 a C	16,28 b A
Red (A2)	30.01 a A	9,49 b C	27,23 a B
Average	22.16	11.26	21.75

Note: The numbers followed by the same capital letters horizontally and lowercase letters vertically show no significant difference according to Duncan's multiple-distance test at a 5% significance level.

Based on the results of statistical tests on phenolic content measured on the 10th day of storage, shows that there is an interaction between the type of cayenne pepper and the concentration of chitosan. The concentration of chitosan showed significantly different results for the types of green and red cayenne pepper. The highest phenolic content for green cayenne pepper was the treatment with 2% chitosan concentration, which had a phenolic content of 16.28 mg GAE/ml sample. Meanwhile, the highest phenolic content in red

chili was shown in the treatment without chitosan. This is presumably because the chili conditions used are not uniform in the level of maturity, as a result, the phenolic concentration will be different at each level of maturity of the cayenne pepper.

Weight Loss Test

Table 5. Weight Loss Test of Green and Red Cayenne Pepper

Types of Cayenne Pepper (A)	Konsentrasi Chitosan		
	0%	1%	2%
Green (A1)	40,82 b C	54,02 b A	50,65 a B
Red (A2)	60,41 a A	55,84 a B	48,86 b C
Average	50,62	54,93	49,75

Note: The numbers followed by the same capital letters horizontally and lowercase letters vertically show no significant difference according to Duncan's multiple-distance test at a 5% significance level.

Based on the results of statistical analysis of the phenolic content measured on the 10th day of shelf life, it showed that there was an interaction between the type of cayenne pepper and the concentration of chitosan. Giving a concentration of 2% chitosan was able to reduce weight loss lower than the concentration of 0% and 1%. The best concentration that can be used as a barrier is a concentration of 2% which can reduce respiration rates as well as a barrier from CO2, O2, and water so that it can maintain the quality of chili fruit at room temperature. This shows that the higher the concentration of chitosan used, the thicker the coating that occurs. The thicker the coating, the more closed the pores will be so that it can slow down the respiration process so that weight loss can be suppressed (Pratiwi, 2009).

CONCLUSION

The chitosan powder produced from shrimp shell waste in this study met the Protan Laboratories standard criteria for water content, particle size, acetic acid solubility, acidity, color, aroma, and ninhydrin testing.

The results showed that there was an interaction between the type of cayenne pepper and the concentration of chitosan on vitamin C content, phenolic content, and fruit weight loss of cayenne pepper.

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