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Original Article

SYNTHESIS AND CHARACTERIZATION OF CHITOSAN FROM CRAB SHELL WASTE AND ITS APPLICATIONS AS EDIBLE COATING

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ABSTRACT

Objective: Strawberries are fruit that has high economic value, but strawberries are perishable fruit. Therefore, required proper postharvest handling, one of them is using edible coating from chitosan. Chitosan was synthesized from crab shells waste will be a solution to prevent environmental pollution because of the abundance of crab shell waste. The purpose of this research was the synthesis and characterization of chitosan from craft shell waste and its applications as edible coating to increase strawberries' fruit shelf life.

Methods: Synthesis synthesis of chitosan has been done using batch methods with sequential processes were demineralization, deproteination, decolorization, and deacetylation using the acid-based solution. Characterization has been done, including chitosan yield, water content, ash content, viscosity, pH, and degree of deacetylation. The synthesized chitosan was formulated as an edible coating and tested for its antimicrobial activity, then used for edible coating on strawberries with a deep coating method and visual and organoleptic analysis of shelf life.

Results: The results of synthesis obtained 72.76% of chitosan yield with characteristics of water content 6.31%, ash content 0.96%, viscosity 68.19 cps, pH 7.01, and degree of deacetylation 80.17%. The average of inhibitory zone diameter of chitosan edible coating formulated with concentrations of 0.5%, 1%, 1.5%, and 2% were 7.62 mm, 10 mm, 14.27 mm, and 19.47 mm respectively. Chitosan edible coating can extend the shelf life of strawberries 5 d longer at room temperature and 7 d longer in the refrigerator than control.

Conclusion: Chitosan from crab shell waste can be formulated into an edible coating and can increase the shelf life of strawberries.

Keywords: Chitosan, Crab shell waste, Edible coating, Strawberries

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INTRODUCTION

Strawberries are fruit commodity that is favored by the people of Indonesia. *Fragaria chilosensis* species spread in several countries, such as America, Europe, and Asia. Meanwhile, the *Fragaria vesca* species are more widely distributed and are the first strawberry species to enter Indonesia [1]. Based on data from the Central Statistics Agency of West Java Province, it is known that the number of strawberry productions in West Java province in 2018 was 62,944 quintals [2]. Strawberries can be used in the fields of food, health, and beauty. Strawberry-based foods in the form of jam, juice, ice cream, and others. Strawberries are diuretic, antirheumatic, and anti-anemic, so they are good for body health. In the field of beauty, strawberries can be used as a face mask to treat acne, and tighten the face and teeth whitening [3]. Besides the many benefits, strawberry is a perishable fruit. Post-harvest handling can improve fruit freshness. One of the efforts to improve the quality of strawberry freshness is to provide an edible coating. The edible coating is a thin layer that can be eaten, used to coat food (coating), and serves as a barrier against mass transfer (such as moisture, oxygen, light, lipids, solutes), as well as a carrier for additives to improve the quality of food. The edible coating has the benefit of maintaining fruit freshness by maintaining color, texture, and inhibiting oxygen transmission that can grow microorganisms that cause damage to strawberries [4]. Edible coatings are made from natural ingredients that are safe for consumption, such as polysaccharides (pectin, chitin, and chitosan, starch), lipids (wax, acetoglyceride, shellac resins), and protein-composite (gelatin, corn protein, wheat gluten, donkey protein, etc.) casein, composite).

Chitosan is a cationic polysaccharide that can be used as a coating material to preserve fruit, works by inhibiting the ripening process of fruit by increasing its antioxidant capacity [5]. Chitosan can coat the preserved product by inhibiting the growth of destructive microorganisms so that there is minimal interaction between the product and the environment. Chitosan can reduce weight loss and water content so that the fruit remains fresh. Chitosan as a preservative can significantly reduce the respiration rate which is indicated by a decrease in the production of ethylene and CO_2. Chitosan can be synthesis from crab shell waste [6, 7].

In this study, chitosan was synthesized from crab shell waste from a crab processing industry in Lampung. Synthesis of chitosan has been done using the batch method with sequential processes were demineralization, deproteination, decolorization, and deacetylation using the acid-base solution. Characterization has been done, including chitosan yield, water content, ash content, viscosity, pH, and degree of deacetylation. The synthesized chitosan was formulated as an edible coating and tested for its antimicrobial activity, then used for edible coating on strawberries with a deep coating method and visual and organoleptic analysis of shelf life. From this research, was hoping that it can increase the value of crab shell waste into useful products, reduce waste in the environment, and be able to increase the shelf life of the fruit.

MATERIALS AND METHODS

Materials

Crab shell waste (*Portunus pelagicus*) obtained from PT Siger Jaya Abadi (Jalan Raya Tanjung Bintang No. 99, Serdang Village, Kec. Tanjung Bintang, South Lampung. Indonesia 35361.), Hydrochloric Acid (Merck, Germany), Sodium Hypochlorite (OneMed, Indonesia), Sodium Hydroxide (Merck, Germany), Glacial Acetic Acid (Merck, Germany), Strawberries from the Bandung plantation with the same maturity level harvested 2 w after flowering, aqua dest, 1% acetic acid, phosphate buffer, Nutrient agar (NA) media, Potato Dextrose Agar (PDA) media, paper discs, paper discs containing the antibiotic ciprofloxacin, McFarland's solution, and cultures of *Escherichia coli* bacteria.

Methods

Synthesis and characterization of chitosan

The crab shells that have passed the mesh sieve no. 80 as much as 100 g, was put into a 2000 ml glass beaker, then the

demineralization process was carried out by adding 1 M HCl solution into the crab shells with a ratio of 1:10 (w/v) between the crab shells and HCl. The mixture was stirred with a magnetic stirrer at room temperature for 3 h. The mixture was then filtered through Whatman filter paper, and the residue on the filter paper was neutralized. Furthermore, the residue then pH was neutral was dried in an oven at a temperature of 70 °C to dry at a constant weight. After the residue was dry, proceed to the protein removal process (deproteination), where the residue was put into a 2000 ml glass beaker and added with 1 M NaOH solution with a ratio of 1:10 (w/v) between the crab shells and NaOH. The mixture was heated at 60 °C on a hotplate while stirring for 1 hour. Then the mixture was filtered with Whatman filter paper and the residue on the filter paper was washed with distilled water until the pH was neutral, then dried in an oven at 70 °C until dry with constant weight. The decolorization process was carried out by adding 1% NaOCl solution with a ratio of 1:10 (w/v), the mixture was heated at 40 °C on a hotplate for 1 hour, then filtered with 41 Whatman paper, neutralized, and dried in an oven at 80 °C to constant weight. Chitosan is produced by deacetylation, where the dried deproteinized residue is put into a glass beaker and a 50% NaOH solution is added with a ratio of 1:10 (w/v) between the crab shells and NaOH. The mixture was heated at 90 °C on a hotplate for 2 h. The mixture was filtered with Whatman filter paper and the residue which was chitosan was washed with distilled water until the pH was neutral. Chitosan was then dried in an oven at a temperature of 70 °C to dry at a constant weight. The chitosan obtained will then be compared with the crab shells before treatment and characterized by an FT-IR spectrophotometer to see the functional groups contained in the chitosan, and determine the degree of deacetylation of chitosan-based on the baseline method [8]. Characterization of chitosan has been done including many parameters such as organoleptic, water contents, ash content, pH, viscosity, and deacetylation degree.

Formulation and antimicrobial testing of chitosan edible coating

Weighed some chitosan powder and put it in a beaker glass filled with 1% acetic acid, made formula of edible coating in 0.5%, 1%, 1.5%, and 2% of chitosan, then homogenized with a homogenizer until the chitosan dissolved. Antimicrobial testing was carried out by adding 15-20 ml of Nutrient Agar (NA) media (45±1 °C) into a petri dish, homogenized by rotating the cup to form a fig. 8, and allowed to solidify. Then, the bacterial suspension was scratched on the media and inserted sterile disc paper that had been soaked in each concentration of edible coating, ciprofloxacin antibiotic as a positive control, and 1% acetic acid as a negative control on the media using tweezers. Then incubated at 37 °C for 24 h with the cup upside down. The test was carried out three times (triple). Determined the diameter of the inhibition area.

Shelf-life testing of strawberries

Strawberries that have been cleaned and sorted are immersed in the edible coating solution for 5 min at room temperature, then drained and dried until the edible coating sticks perfectly to the strawberries [9]. The quality testing of strawberries during storage was carried out by observing the fruit visually by looking at the color and shape of the fruit by comparing the fruit with the edible coating and the uncoated (control). Observations are made until the fruit is rotten or has been overgrown with microorganisms. The hedonic test was carried out with 20 panelists, each of which tested one fruit sample that was coated with an edible coating and one that was not coated with an edible coating (control). The assessment criteria will be converted into numbers, namely; 5: like very much, 4: like 3: normal, 2: don't like it, and 1: don't like it very much. This test is to determine consumers' acceptance of the color and appearance of the fruit. The panelists involved are semi-trained, namely panelists who are not experts and are not laypeople who do not understand the characteristics of organoleptic. The panelists involved are also strawberry lovers.

RESULTS

Synthesis and characterization of chitosan

Table 1: Yields of chitosan

Replicate	Crab shell powder (g)	Yields (g)			
		Demineralization	Deproteination	Decolorization	Deacetylation
1	100.5072	39.1891	34.4996	25.3437	18.4532
2	100.4063	38.7383	32.7452	24.7631	18.2398
3	100.2324	38.8657	32.1895	24.5658	17.6385
mean+SD		38.9310+0.232	33.1448+1.206	24.8909+0.404	18.1105+0.422

Values represent mean±SD, n=3.

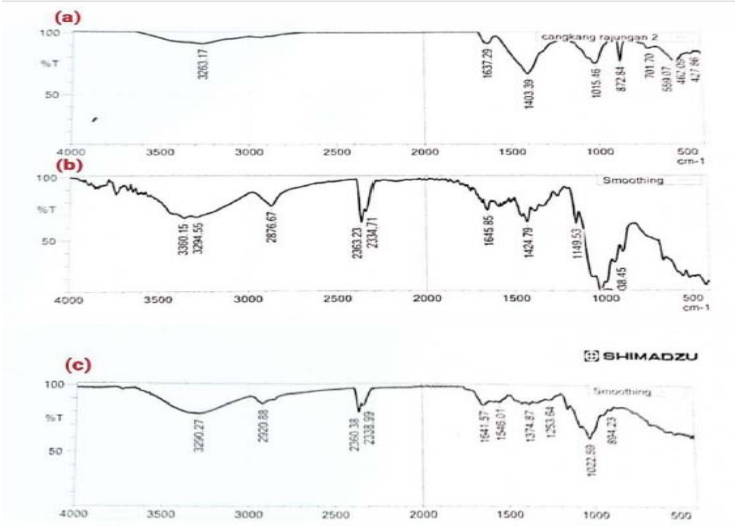

Fig. 1: **Spectrum FTIR (a) crab shell (b) chitin (c) chitosan**

Table 2: Characteristic of chitosan

Parameter	Characteristic
Organoleptic	Powder, yellow dish white, no smell
Water content (%)	6.31
Ash content (%)	0.96
pH	6.85
Viscosity (cP)	68.19
Deacetylation degree	80.17

Table 3: Antimicrobial testing of edible coating

Concentration of chitosan	Inhibitory zone (mm)			*Mean (mm)
	I	II	III	
0.5%	8.00	6.70	8.15	7.62
1%	9. 45	10.55	9.90	10.00
1.5%	9.70	15.75	17.35	14.27
2%	18.25	18.75	21.40	19.47
Positive Control	35.28	38.08	37.80	36.99
Negative Control	6.75	9.50	8.90	8.38

Values represent mean, n=3. Microbial: Escherichia *coli*

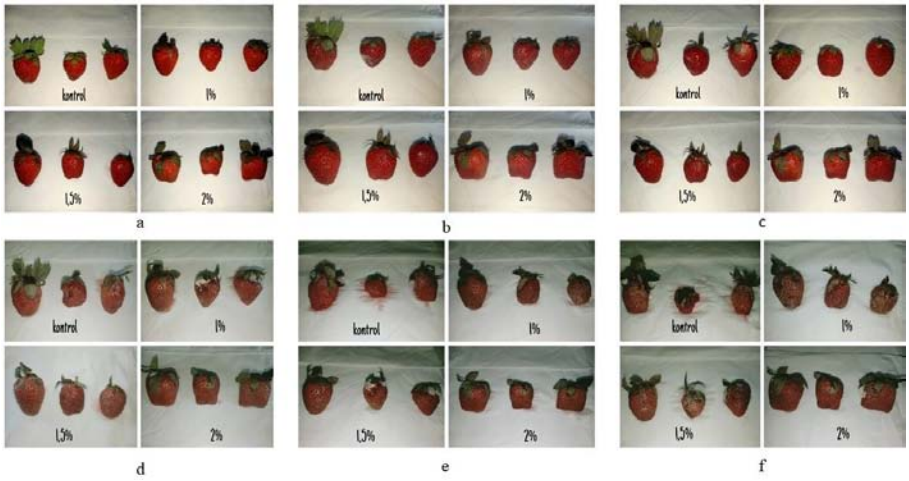

Fig. 2: Visualization of strawberries which storage in room temperature a) day 0, b) day 1st, c) day 2nd, d) day 3rd, e) day 4th, f) day 5th

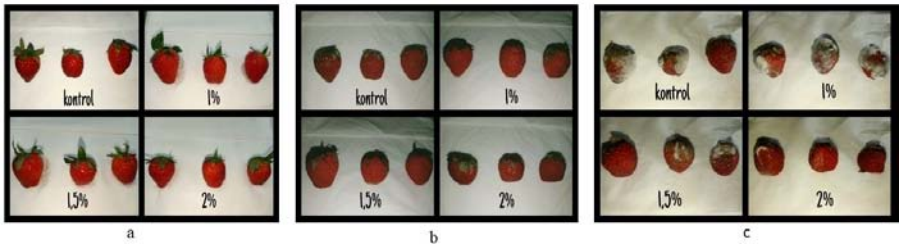

Fig. 3: Visualization of strawberries which storage in the refrigerator a) day 0, b) day 5th, c) day 14th

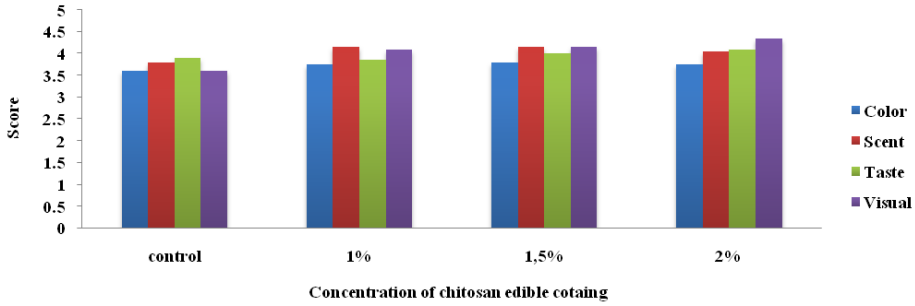

Fig. 4: Hedonic testing, Number of panelist: 20, Scoring 1: do not like; 2: do not like it much; 3: normal; 4: like; 5: really like

DISCUSSION

Synthesis and characterization of chitosan

The chitin obtained after demineralization, deproteination and decolorization was 24.8909 g. The deacetylation process is the main process in the synthesis of chitosan. Deacetylation is the process of converting the acetyl group (-NHCOCH3) in chitin into an amine group (-NH2) with the addition of a high concentration of strong base NaOH. A chitin deacetylation reaction is an amide hydrolysis reaction of-(1-4)-2-acetamide2-deoxy-D-glucose. The concentration of OH-ions greatly affects the release of the acetyl group from the acetamide group of chitins. According to Azhar *et al.* stated; that the stronger a base the greater the concentration of OH-in the solution, which can increase the strength of the base in influencing the deacetylation process of the acetyl group from the acetamide group of chitins [10]. The better the deacetylation process, will better the quality of the chitosan formed. The quality of chitosan can be seen through the degree of deacetylation. In this process, the final average weight was 18.1105 g, which means the yield of chitosan was 18%. In characteristic testing showed that chitosan synthesized from crab shells meets the requirement in physical dan chemical properties (table 2).

Spectrum FTIR of synthesized compound absorption appears at wave number 3290.27 cm^{-1} shows the absorption of the–OH stretching vibration, which overlaps with the–NH stretching vibration [11]. The widening of the absorption peak at the wavenumber 3290.27 cm^{-1} indicates that the deacetylation process has occurred, the N-H group (-$NHCOCH_3$) has been reduced or lost and there is overlapping absorption due to the acetyl group, which has been deacetylated to form an amine (-NH_2) [12]. On wavenumber 2880 cm^{-1} shows the stretching vibration of C-H. on the wavenumber 1641.57 cm^{-1} indicates the presence of a C=O group (amide band) which indicates the presence of an acetyl group in the chitosan polymer chain but reduced by the deacetylation process. Wavenumber 1546.01 cm^{-1} is the bending vibration of–NH, which is the absorption band of the amine group as a characteristic of chitosan. At wavenumbers 1253.64 and 1022.59 cm^{-1} indicates the presence of the C-O-C functional group, and the wave number 894.23 cm^{-1} (very weak) indicates the presence of silica mineral content in small amounts, in contrast to the minerals in shells and chitin (fig. 1).

Formulation and antimicrobial testing of edible chitosan coating

Edible coatings with chitosan concentrations of 0.5%, 1%, 1.5%, and 2% were tested for antimicrobial activity against *Escherichia coli* bacteria by agar diffusion method using disc paper. The test microbes used in this study were in the form of direct ATCC (American Type Culture Collection). In this test, a positive control in the form of the antibiotic ciprofloxacin and negative control of 1% acetic acid was used. The presence of antimicrobial activity from edible coatings with concentrations of 0.5%, 1%, 1.5%, and 2% was indicated by the formation of an inhibition zone around the paper disc. Antimicrobial testing obtained that edible coating with chitosan concentrations of 0.5%, 1%, 1.5%, and 2% had inhibition zones of 7.62 mm, 10.00 mm, 14.27 mm, and 19.47 mm, with positive and negative control inhibition zones of 36.99 mm and 8.38 mm, respectively. This proves that the edible coating of chitosan from crab shells has antimicrobial activity because it has a visible diameter of the inhibition zone. The greater the concentration of the edible coating, the greater the inhibition zone produced, so the greater its ability as an antimicrobial.

Shelf-life testing of strawberries

Testing the shelf life of strawberries is done by observing the condition of the fruit visually for 5d (until the fruit is rotten or overgrown with microorganisms). On the 0th or first day, fruits that were coated with edible coating and those that were not coated with an edible coating (control) both had a bright red color and were fresh. On the 1st day of observation, it was seen that the control fruit began to grow with microorganisms, on the 2nd day the control fruit changed its shape to become concave because the fruit had shrunk, and on the 3rd day the control fruit was released a lot of water so it could not be consumed. On the 3rdday of observation, it was seen that the fruit covered with 1% and 1.5% edible coating began to grow microorganisms, while the fruit with 2% edible coating treatment began to grow microorganisms on the 5th day. This proves that the edible coating treatment of 1% and 1.5% on strawberries stored at room temperature (25 °C) can increase the shelf life of strawberries 3 d longer than the control, and the 2% edible coating treatment can increase the shelf life of strawberries 5 d longer than control. In the refrigerator temperature, the edible coating can extend the shelf life of strawberries compared to storage at room temperature (25 °C). The control fruit began to grow with microorganisms on the 7th day, which showed that the edible chitosan coating was effective as a strawberry preservative. The fruit with 1% edible coating treatment began to grow with microorganisms on the 10th day, the 1.5% concentration of edible coating began to grow microorganisms on the 12th day, and the 2% edible coating concentration began to grow microorganisms on the 14th day. This shows that strawberries coated with 1%, 1.5%, and 2% chitosan edible coating and stored in a refrigerator (4 °C) have a shelf life of 10, 12, and 14 d, It's longer than the control. The higher the concentration of chitosan in the edible coating, the more effective in preventing the growth of microorganisms to extend the shelf life of strawberries. Strawberries stored at room temperature will spoil more quickly because of the humid and unstable environment, which will make the strawberries runny quickly, making it easier for microorganisms to grow. Organoleptic testing was done to evaluate the level of preference for color, scent, taste, and visual appearance of strawberries coated compared to uncoated, and the results showed there are no significant differences.

CONCLUSION

Chitosan from crab shell waste can be formulated into an edible coating and can increase the shelf life of strawberries.

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AUTHORS CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by [Esti Mulatsari], [Esti Mumpuni], [Rahmatul Qodriah], [Jimmy Gunawan], [Marcelyna], and [Diana Serlahwaty]. The first draft of the manuscript was written by [Esti Mulatsari] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Budiman S, Saraswati D. Berkebun stroberi secara komersial. Jakarta: Penebar Swadaya; 2005. p. 12-6.
2. Badan Pusat Statistik Provinsi Jawa Barat. Produksi tanaman sayuran menurut kabupaten kota dan jenis tanaman di Provinsi Jawa Barat. Badan Pusat Stat. 2018:95-101.
3. Rahmatia D, Pitriana P. Bercocok tanam stroberi. Jakarta: Sinar Wadja Lestari; 2007. p. 11.
4. Embuscado Milda E. KCH, editor. Edible films and coatings for food applications. Springer Dordrecht Heidelberg London New York; 2009. p. 212.
5. Rosida DF, Hapsari N, Dewati R. Edible coating dan film dari biopolimer bahan alami terbarukan. Ponorogo: Uwais Inspirasi Indonesia. Vols. 14 & 16 p; 2018.
6. Sugita P. Sahriza A. Kitosan: sumber biomaterial masa depan. Bogor: IPB Press; 2009.
7. Hardjito L. Chitosan sebagai bahan pengawet pengganti formalin; 2006.

8. Winardi RR, Harefa M. Karakter mutu strawberry (Fragaria virginiana) selama penyimpanan dengan perlakuan edible coating campuran sorbitol dan pati sagu; 2018. p. 169-78.
9. Suptijah P, Jacoeb AM, Rachmania D. Karakterisasi nano kitosan cangkang udang vannamei (litopenaeus vannamei) Dengan Metode Gelasi Ionik. Vol. XIV; 2011.
10. Azhar M, Efendi J, Syofyeni E, Lesi RM, Novalina S. Pengaruh konsentrasi NaOH dan KOH terhadap derajat deasetilasi kitin dari limbah kulit udang. FMIPA Universidad Negeri Padang; 2015. p. 3-4.
11. Nugroho A, Nurhayati ND, Utami B. Sintesis dan karakterisasi membran kitosan untuk sensor deteksi logam berat. Molekul. 2011:123-6.
12. Djaenudin D, Budianto E, Saepudin E, Nasir M. Ekstraksi kitosan dari cangkang rajungan pada lama dan pengulangan perendaman yang berbeda. J Teknol Perikanan Kelautan 2019;10(1):49-59. doi: 10.24319/jtpk.10.49-59.