Antioxidant, antibacterial and antifungal activity of edible coating chitosan-galactose complex

by Rahim Husain

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Antioxidant, antibacterial and antifungal activity of edible coating chitosan-galactose complex

Rieny Sulistijowati[1], Rahim Husain[1], Muhammad Cakra Datau[2], Kusbidinandri[2]

[1]Faculty of Marine Science and Fisheries, Universitas Negeri Gorontalo, Indonesia
[2]Graduate Student, Faculty of Marine Science and Fisheries, Universitas Negeri Gorontalo, Indonesia

Email: rienysulistijowati@ung.ac.id

Abstract The purpose of this research was to observe the antioxidant, antibacterial and antifungal activity of an edible coating chitosan galactose complex. The treatments were: A Control chitosan; B Control commercial chitosan; C Chitosan with 0.5 g Galactose; D Chitosan with 1 g Galactose; and E Chitosan with 1.5 g Galactose. The coatings were evaluated using in-vitro testing. The parameters analysed were antioxidant activity (DPPH method), antibacterial and anti fungal activity (inhibition zone method). The result showed that the 1 g galactose-chitosan complex performed better as an antioxidant than chitosan. Antioxidant analysis with the DPPH method found IC_{50} values of 43.20 – 73.15 ppm, with chitosan galactose complex (D) as the best antioxidant among the tested treatments. Antibacterial activity of the chitosan galactose complex (D) against pathogen bacteria from tilapia fillets produced a 12 mm inhibition zone. Antifungal activity of the chitosan galactose complex (D) against pathogenic fungi from smoked fish produced an 18 mm inhibition zone. This chitosan galactose complex could be used as a natural food preservative.

1. Introduction

Most food packaging commonly used today is plastic. Plastics can endanger health and cause environmental pollution. To fulfil the demand for safe and environmentally friendly packaging materials, edible packaging based on chitosan has been developed. Edible coating is a type of packaging used as a semi-wet food coating for fruits and other fresh produce. Edible film is a thin and continuous layer formed through polymer chain interaction that produces a larger and more stable polymer aggregate. The chitosan packaging is both edible and biodegradable, and can be used as a coating or as an edible film.

Chitosan is commonly isolated from shrimp and crab waste, including the shells of these crustacea, through chitin deacetylation. Chitosan is a compound which has a positively charged free amino group that can bind to a negative charge on the microbial cell wall. This makes chitosan widely used in various fields, especially for preservation [1]. However, chitosan has several disadvantages, one of which is that it is not good at producing antioxidants; furthermore, in some applications, chitosan tends to be fragile and easily broken. These disadvantages can be overcome by adding materials to modify and enhance the properties of chitosan [2].

With the right modifications, the antioxidant and antimicrobial properties of chitosan-based compounds can be far superior to those of pure chitosan. The presence of amino groups and hydroxyl in chitosan means that chitosan can easily be chemically modified to produce chitosan derivatives.

These modifications can be produced through physical or chemical processes and are expected to improve the performance of chitosan and maintain its stability [3]. Some researchers have developed modification methods such as the addition of monosaccharides, glycerol and organic acids to improve the antimicrobial and antioxidant performance of chitosan. For example, a study by [1] concluded that a chitosan-galactose complex had the best antioxidant performance, with the intensity of brownish colour ranging from 0.031-0.224, while the antioxidant activity (measured with the DPPH method) was 92-131 ppm and the reduction power was 1,059-1,274. This study aimed to determine the antioxidant, antibacterial and antifungal activity of a range of chitosan-galactose complexes proposed for use as edible coatings.

2. Materials and Methods

This study was conducted at the Faculty of Marine Science and Fisheries, Universitas Negeri Gorontalo, in Gorontalo, Indonesia, and lasted for two months. The chitosan-galactose complex edible coatings were produced at the Faculty of Marine Science and Fisheries. The antibacterial, antifungal and antioxidant properties were tested at the Pharmaceutical Laboratory, Faculty of Sport and Health. The materials used to produce the edible coating complex were chitosan from vannamei shrimp shells, 1% acetic acid, galactose and 250 mL of distilled water. The materials used for antibacterial and antifungal activity tests were distilled water, Butterfield phosphate-buffer and Plate Count Agar (PCA) media, Potato dextrose Agar (PDA), sterile disc paper, physiological NaCl, sterile distilled water. The tools used were hot plate, magnetic stirrer, FTIR Spectroscopy (Bruker Tensor 37), oven (Yamato DV 40), incubator, vortex (Thermo Scientific), sterilization cabinet (Pathfinder).

The galactose was added to the chitosan in various concentrations. The treatments were: A Control chitosan; B Control commercial chitosan; C Chitosan with 0.5 g Galactose; D Chitosan with 1 g Galactose; and E Chitosan with 1.5 g Galactose. The coatings were evaluated using in-vitro testing. The parameters analysed were the antioxidant activity, tested with the DPPH method, and the antibacterial and antifungal activities both tested with an inhibition zone method using in-vitro testing. Antibacterial activity was tested on bacterial isolates from contaminated tilapia fillets, while antifungal activity was tested on pathogenic fungi isolates from smoked skipjack tuna. The data were analysed quantitatively.

2.1. Antioxidant Activity Test Procedure

Testing of antioxidant activity followed the protocols in [4] with modifications to the concentrations of the extracts of the sample mother liquor, the comparative antioxidants (Vitamins C and E) and the Diphenylpycrilhydrazil (DPPH) concentration. The sample extract solutions (pure chitosan, commercial chitosan, samples C, D, E and F) were prepared with a concentration of 1000 ppm, and then diluted to concentrations of 50, 75, and 100 ppm. Comparative antioxidants (Vitamin C and E) were made to a concentration of 1000 ppm and then diluted to concentrations of 50, 75, 100 ppm. The DPPH solution was made by dissolving DPPH crystals in ethanol as a solvent, with a concentration of 0.05 mM. The process of making the DPPH 0.05 mM solution was carried out in conditions with a low-temperature and protection from sunlight.

Aliquots of 4.5 ml were taken from the test solution or comparator and were reacted with 0.5 ml of DPPH 0.05 mM solution in a test tube. The mixture was incubated at 37°C for 30 minutes, and then the absorbance was measured using Uv-Vis spectrophotometry at a wavelength of 519 nm. The antioxidant activity of each sample and comparative antioxidant vitamins C and E was expressed as percentage (%) inhibition, calculated using the following formula:

$$\%\ \text{inhibition} = \frac{\text{absorbance blanko} - \text{absorbance}}{\text{absorbance sample blanko}} x\ 100\ \%$$

Linear regression of sample inhibitory percentages versus concentration values (extracts or comparative antioxidants of Vitamin C and E) was performed with inhibitory percentages plotted on the x axis and concentration values on the y axis. The linear regression equation obtained in the form

of an equation of the form y = a + bx was used to determine the IC50 of each sample, where the IC50 value is the concentration of the sample (extract or comparative antioxidant Vitamins C and E) which reduced DPPH radicals to 50% of the initial concentration by taking the 'y' value of 50 and the corresponding value of x as the IC50 [4].

2.2. Antibacterial and antifungal activity test procedures

Antibacterial activity tests were conducted on bacterial isolates from contaminated tilapia fillet and antifungal activity tests were conducted on fungal isolates from smoked skipjack tuna. The inhibition zone method of in-vitro testing was implemented on agar plates. The diameter of the inhibition zone produced by each treatment (chitosan or chitosan-galactose compound) was measured.

3. Results

The test results are shown in Tables 1 to 3.

Table 1. Antioxidant properties of edible coating compounds measures by reduction on DPPH.

Edible coating formulation	IC_{50} (ppm)	Effectiveness
A Control chitosan	112.06	Average
B Control commercial chitosan	358.36	Low
C Chitosan-Galactose 0.5 g	56.82	Strong
D Chitosan-Galactose 1 g	43.20	Very strong
E Chitosan-Galactose 1.5 g	73.15	Strong
F Chitosan-Galactose 2 g	85.30	Very strong
G Vitamin C	16.00	Very strong
H Vitamin E	475.70	Low

Table 2. Edible coating antibacterial activity test results.

Edible Coating formulation	Inhibition Zone (mm)	Effectiveness
A Control chitosan	8	Average
B Control commercial chitosan	9	Average
C Chitosan-Galactose 0.5 g	10	Strong
D Chitosan-Galactose 1 g	12	Strong
E Chitosan-Galactose 1.5 g	10	Strong
F Chitosan-Galactose 2 g	11	Strong

Table 3. Antifungal Activity Test Results.

Edible Coating formulation	Inhibition Zone (mm)	Effectiveness
A Control chitosan	8	Average
B Control commercial chitosan	12	Strong
C Chitosan- Galactose 0.5 g	11	Strong
D Chitosan- Galactose 1 g	18	Very strong
E Chitosan- Galactose 1.5 g	14	Strong
F Chitosan- Galactose 2 g	15	Strong

4. Discussion

Table 1 shows than the antioxidant activity differed between the chitosan control solution and the chitosan galactose complex formulations. The IC50 values ranged from 43.20 ppm to 73.15 ppm. Treatment D produced the lowest IC_{50} of 43.20 ppm while treatment B produced the highest IC_{50} value of 358.36 ppm. The lower the IC_{50}, the more active the sample tested as an antioxidant compound [5];

in the test used, the smaller the IC_{50} value, the greater the ability of the sample to soak up free radicals (50% of the DPPH compounds). This shows that the 1g galactose chitosan complex (treatment D) had the highest antioxidant activity. The treatments with the second and third highest antioxidant activities were C (56.82 ppm) and (E 73.15) ppm while the control (pure chitosan) treatment had a much higher IC_{50} value of 112 ppm.

Table 2 shows that the antibacterial activity of all the chitosan-galactose compounds (0.5 - 2g galactose) effectively inhibited the bacterial pathogens from the tilapia fillet. While several physical and chemical modifications of chitosan can be expected to improve its performance and can maintain its stability [3], these results show that the addition of galactose can produce antioxidant and antimicrobial activity that is more effective than that of chitosan alone. The presence of amino and hydroxyl groups in chitosan enables chitosan to be easily chemically modified; for example, the addition of 1% glucose in chitosan 1% and sterilised acetic acid 1% can produce a chitosan glucose complex which is proven to be able to fight food-destroying bacteria, fungi and pathogenic bacteria, and also contains antioxidants. While the addition of various sugars (glucose, fructose, lactose, arabinose and galactose) can inhibit the bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* [6]. This study showed that a range of chitosan-galactose complex formulae could enhance the food preservative properties of chitosan; however the formulation with 1 g galactose (formula or treatment D) produced the best results in terms of antioxidant, antibacterial and antifungal properties.

5. Conclusion

Edible coating chitosan galactose complex with 1 g galactose (formula or treatment D in this study) could be used as a natural preservative and packaging material. Antioxidant analysis with DPPH method gave an IC_{50} of 43.20 ppm, with a 12 mm inhibition zone showing strong inhibition of pathogenic bacteria from tilapia fillets. Antifungal activity of the chitosan galactose complex (D) was very strong against pathogenic fungi from smoked fish, with an 18 mm inhibition zone.

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