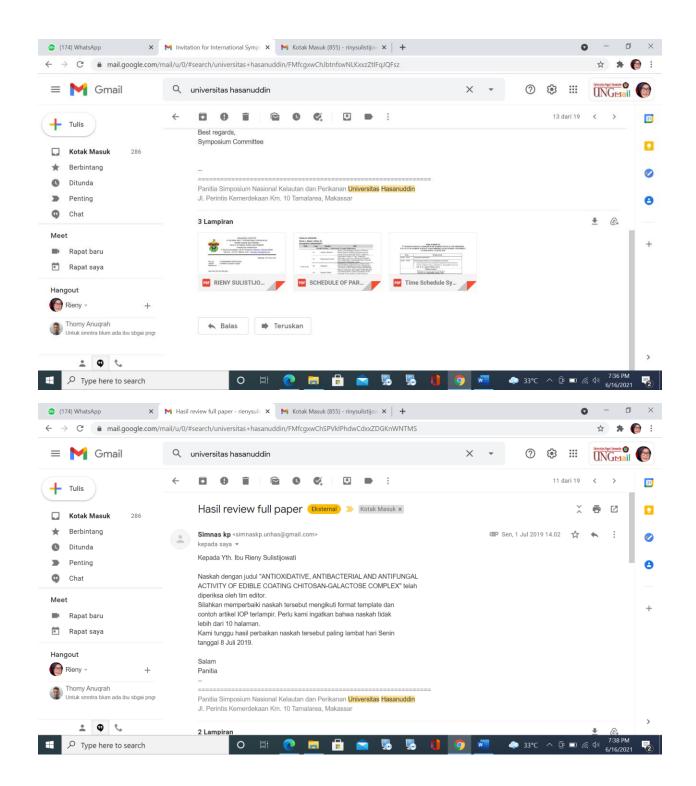
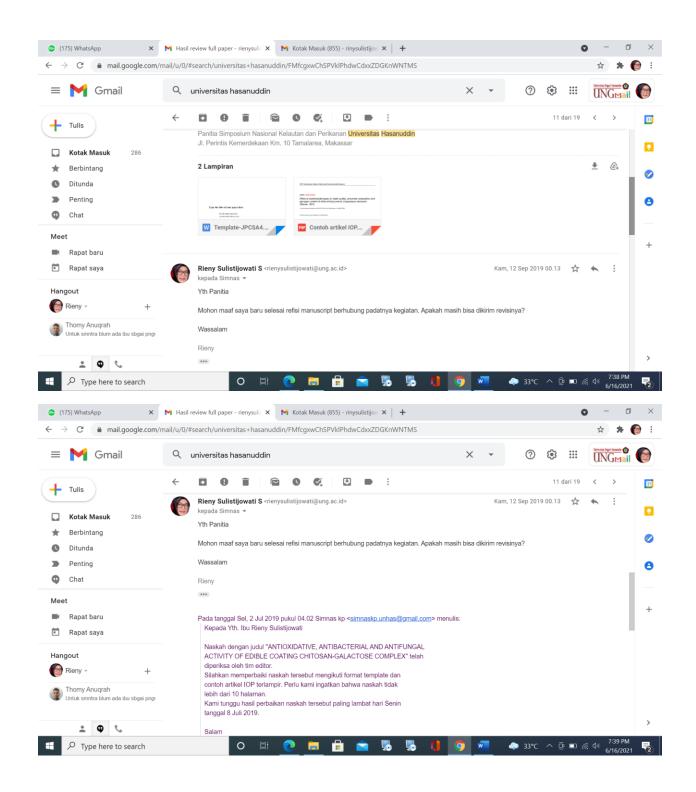
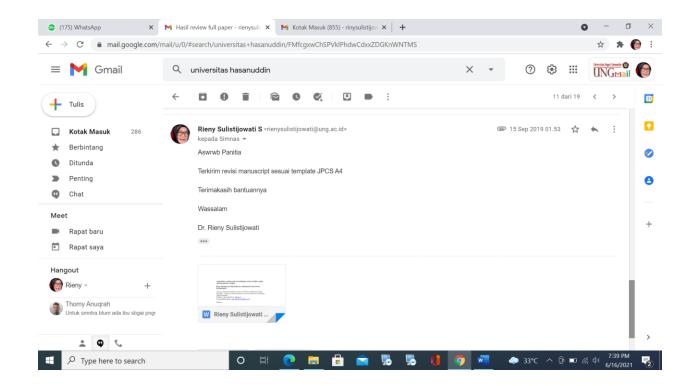


Int Symposium on Marine Fisheries 2019 (Antioxidative, Antibacteri and Antifungi Activity...)







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

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#### Abstract.

The purpose of this research was to observe the antioxidative, antibacterial and antifungal activity of edible coating chitosan galactose complex. All concentration added galactose to edible coating are chitosan (A) and commercial chitosan (B) as control, galactose 0.5(C); 1(D); 1.5(E); 2 g (F) galactose-chitosan using in-vitro testing. The parameters were analysis antioxidant activity with DPPH method, antibacterial and anti fungi activity with inhibition zone methods. The result showed that 1 g galactose-chitosan complex better antioxidative than chitosan. Antioxidant analysis with DPPH methods of IC<sub>50</sub> (43.20 – 73.15 ppm) chitosan galactose complex (D) is the best to antioxidative from all treatment. Antibacterial activity is chitosan galactose complex (D) is 12 mm inhibition zone toward pathogen bacteria of Nila fish fillet. Antifungal activity is chitosan galactose complex (D) is 18 mm inhibition zone toward fungi pathogen of smoke fish. Chitosan galactose complex could be used as a natural preservative.

Keyword: *Edible coating*; *Chitosan – galactose complex*; DPPH; *antioxidant; antibacterial; antifungal.* 

# 1. Introduction

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 both the edible coating and edible film based on chitosan were developed as biodegradable the edible coating is a packaging used as a semi-wet food coating or fruits.

While the edible film is a thin and continuous layer of polymer chain interaction that produces a larger and more stable polymer aggregate. Chitosan is widely isolated from the waste of shrimp, crab or crab skin through chitin deacetylation. The nature of chitosan which has a positively charged free amino group can bind to a negative charge on the microbial cell wall. This makes chitosan widely used in various fields, especially for preservation [1],(Selly et al. 2013), but chitosan still has several disadvantages, namely not producing antioxidants optimally. Even in various applications, chitosan tends to be easily fragile and broken. This can be overcome by adding materials and modifying chitosan [2].(Rao, Chander and Sharma 2005).

The right modification of chitosan can produce good antioxidant and antimicrobial compounds compared to the use of chitosan alone. The presence of amino groups and hydroxyl in chitosan causes easily chemically modified chitosan to produce chitosan derivatives. Modifications can be done physically and chemically and are expected to improve the performance of chitosan and maintain its stability [3]. (Li, Hein and Wang, 2008). Some research has developed modification methods such as the addition of monosaccharides, glycerol and organic acids to improve the performance of chitosan as an antimicrobial and antioxidant, including the results of a study by [1] revealed that chitosan-galactose complex is the best antioxidant where the intensity of brownish colour ranges from 0.031-0.224 while the antioxidant activity with the DPPH method is 92-131 ppm and the reduction power is 1,059-1,274. This study aims to determine the antioxidant, antibacterial and mould activities of edible coating chitosan-galactose complexes.

# 2. Materials and Methods

The study was conducted at the Faculty of Marine Science and Fisheries Universitas Negeri Gorontalo and lasted for two months. Edible coating chitosan-galactose complex carried out at Faculty of Marine Science and Fisheries. Antibacterial, mould activities and antioxidant were carried out at the Farmasi Laboratory, Faculty of Sport and Health. The materials used in making edible coating complex were chitosan from vaname shrimp shells, 1% acetic acid, galactose and 250 mL distilled water. The materials used for antibacterial and antifungal activity tests are distilled water, Butterfield phosphate-buffered and Plate Count Agar (PCA) media, Potato dextrose Agar (PDA), sterile disc paper, physiological NaCl, sterile distilled water. The tools used are hot plate, magnetic stirrer, FTIR Spectroscopy (Bruker Tensor 37), oven (Yamato DV 40), incubator, vortex (Thermo Scientific), sterilization cabinet (Pathfinder), All concentration added to edible coating are chitosan (A) and commercial chitosan (B) as control, galactose 0.5 (C); 1 (D); 1.5 (E); 2 g (F) galactose-chitosan using in-vitro testing. The parameters were the analysis of antioxidant activity with DPPH method, antibacterial and antifungal activity with inhibition zone method using in-vitro testing. Antibacterial activity test on tilapia fillet contaminant bacterial isolates and antifungal activity test on smoked skipjack mushroom isolates. Data were analyzed quantitatively.

### 2.1 Antioxidant Activity Test Procedure

Testing of antioxidant activity was referenced from [4] by modifying sample extracts of the mother liquor, comparative antioxidants (Vitamins C and E) and the concentration of Diphenylpycrilhydrazil (DPPH). The sample extract solution (pure chitosan, commercial chitosan, samples C, D, E and F) was prepared with a concentration of 1000 ppm, then diluted to a concentration of 50, 75, 100 ppm. Comparative antioxidants (Vitamin C and E) were made with a concentration of 1000 ppm and then diluted to a concentration of 50, 75, 100 ppm. The DPPH solution to be used is made by dissolving DPPH crystals in ethanol solvents with a concentration of 0.05 mM. The process of making a DPPH 0.05 mM solution is carried out in

low-temperature conditions and protected from sunlight. A total of 4.5 ml of the test solution or comparator was reacted with 0.5 ml of a DPPH 0.05 mM solution in a test tube. The mixture was incubated at 37 ° C for 30 minutes, then measured its absorbance using Uv-Vis spectrophotometry at a wavelength of 519 nm. The antioxidant activity of each sample and comparative antioxidant Vitamins C and E is expressed as per cent inhibition, which is calculated by the following formula:

#### % inhibition= <u>absorbance blanko-absorbance</u> x 100 % <u>absorbance sample blanko</u>

Sample concentration values (extracts or comparative antioxidants of Vitamin C and E) and inhibitory percentages are plotted on the x and y- axes in the linear regression equation, respectively. The linear regression equation obtained in the form of an equation y = a + bx is used to determine the IC50 value of each sample. IC50 value is the concentration of the sample (extract or comparative antioxidant Vitamins C and E) which can reduce DPPH radicals by as much as 50% of the initial concentration by stating the 'y' value of 50 and the value of x to be obtained as IC50 (Tias, 2010).

# 2.2 Antibacterial and antifungal activity test procedures

Antibacterial activity test on tilapia fillet contaminant bacterial isolates and antifungal activity test on smoke skipjack mushroom isolates with inhibition zone method using in-vitro testing. Antibacterial activity test on tilapia fillet contaminant bacterial isolates and antifungal activity test on smoked skipjack mushroom isolates.

# 3. Results

EDIBLE COATING SAMPLE	IC 50	Effectiveness
	(ppm)	
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	-276.11	Very strong
G. Vitamin C	-187.46	Very strong
H. Vitamin E	475.70	Low

# Table 1. Results of Antioxidant Edible Coating Reduction on DPPH

### Table 2. Antibacterial Activity Test Results

Sample Edible Coating	Inhibition Zone	Efektivitas
	(mm)	
A Control chitosan	8	Average
B Control commercial	9	Average
chitosan		_
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

SAMPLE EIBLE COATING	Inhibition Zone (mm)	Efektivitas
A Control chitosan	8	Average
B Control commercial	12	Strong
chitosan		
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

**Table 3. Antifungal Activity Test Results** 

#### Discussion

Table 1 shows the chitosan control solution and chitosan galactose complex have antioxidant activity with varying values. IC50 values range from 43.20 ppm to 73.15 ppm. Treatment D produced the largest IC50 of 43.20 ppm while treatment B produced the smallest IC50 value of 358.36 ppm. This shows that the 1g galactose chitosan complex is the sample that has the highest antioxidant using this DPPH method because the smaller the IC50 value, the greater the ability of the sample to soak free radicals as much as 50% of DPPH compounds. The second and third highest antioxidant activity was C 56.82 ppm and E 73.15 ppm while A3 treatment had IC50 value of 117 ppm. The smaller the IC50 means the more active a sample is tested to be an antioxidant compound (Molyneux, 2004). [5]

Table 2 shows that the antibacterial activity of the chitosan galactose complex 0.5 - 2g complex solution effectively inhibits the bacterial pathogen of tilapia filet. Proper modification of chitosan can produce antioxidant and antimicrobial compounds that are better than the use of chitosan alone. The presence of amino and hydroxyl groups in chitosan causes chitosan to be easily chemically modified to produce chitosan derivatives. Modification can be done physically and chemically, it is expected to improve the performance of chitosan and can maintain its stability [6]. Chitosan modification can increase its role as an anti-microbial and antioxidant. The addition of 1% glucose in chitosan 1% and acetic acid 1% that has been sterilized is called chitosan glucose complex which is proven to be able to fight food-destroying bacteria, fungi and pathogenic bacteria and have 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 [7].

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### Conclusion

Edible coating chitosan galactose complex 1 g could be used as a natural preservative packaging materials. Antioxidant analysis with DPPH method of IC50 (43.20 - 73.15 ppm), 12 mm strong inhibition zone toward pathogen bacteria of tilapia fish fillet. Antifungal activity is chitosan galactose complex (D) is 18 mm strong inhibition zone toward pathogen fungi of smoke fish.

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