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Antibacterial Activity of Chitosan Monossacharides Complex against *Vibrio parahaemolyticus*

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ABSTRACT

The purpose of this research was to observe antibacterial activity of chitosan monosaccharides complex. All treatment are chitosan, chitosan glucose complex, chitosan galactose complex and chitosan fructose complex using a in vitro testing. The parameters were brown color analysis and antibacterial analysis of Vibrio parahaemolyticus. The result showed that all chitosan monosaccharides complex demonstrated better antibacterial than chitosan. The brown color analysis (0.031-0.224), chitosan glucose complex (C1) is the best to antibacterial from all treatments for Vibrio parahaemolyticus. All treatment showed chitosan monosaccharides complex were more than chitosan. Chitosan monosaccharides complex could be used as natural preservatives, edible packaging (film and couting) and food additive.

Keywords: Chitosan, Monosaccarides, Antibacterial

INTRODUCTION

Chitosan and its derivates are known to have antibacterial activity against some bacteria [1]. But chitosan as an antibacterial still relatively low. Therefore, it takes a combination of chitosan with other ingredients to enhance the antibacterial activity to food products.

Several studies have been developed to improve the characteristics of chitosan and chitosan modification by chemical or enzymatic. One way is by combining chitosan solution with other materials such as monosaccharides. Complex of chitosan-glucose resulting from the Maillard reaction has higher antibacterial activity in inhibiting *Escherichia coli* and *Staphylococcus aureus* compared with chitosan solution alone [2]. Kannat et al [3] reported the addition of chitosan-glucose complexes can increase the shelf life of the lamb chops for 2 weeks in cold storage compared with the addition of chitosan solution alone.

This study will be conducted testing of the bacteria *Vibrio parahaemolyticus*. The reason for using the bacterium *Vibrio parahaemolyticus*is causes gastrointestinal illness in human. Therefore, the manufacturing of chitosan monosaccharides complex are important and are expected to inhibit the bacterium of *Vibrio parahaemolyticus*is.

MATERIALS AND METHODS

Preparation of acetic acid solution
Glacial acetic acid taken as 1.02 ml (98% to 1%) and added with distilled water to 100 ml, then homogenized.

Preparation of chitosan with a variety of monosaccharides
Chitosan weighed as much as 1 g (1%) and inserted into a glass beaker, diluted with 1% acetic acid and added 50 ml aquades and distirer for ± 30 minutes (until homogeneous). After homogeneous added about 1 g (1%) monosaccharides (glucose, fructose and galactose), then the volume is adjusted to 100 ml using a volumetric flask. The solution that has been mixed and then sterilized by autoclaving at 121 ^{o}C for 15 min.

The treatments used in this study are as follows:
C0: 1% Chitosan
C1: 1% chitosan + 1% Glucose
C2: 1% Chitosan + 1% Galactose
C3: 1% chitosan + 1% Fructose

The brown color analysis
Maillard product was identified by spectrophotometer with 420 nm to brown color analysis [4].

Antibacterial test
Vibrio parahaemolyticus used in this study. Antibacterial activity was evaluated using diffusion method [5]. Actively growing lag phase cultures of bacteria were mixed in Nutrient agar (Nutrient broth with 1.5% agar) and plated. The various monosaccharides complex (C0: 1% chitosan, C1:chitosan + 1% Glucose, C2: 1% chitosan + 1% galactose and C3: 1% chitosan + 1% Fructose) were loaded onto different paper discs (Whatman no. 1 filter paper). The discs were placed on the agar medium containing cultures incubated for 24 h at 37 ^{0}C. Zone of inhibition was recorded in millimeters and mean values were reported.

RESULTS AND DISCUSSION

The brown color analysis
Analysis of brown color with a spectrophotometer is one simple to determine the level of browning or intensity of each sample. Absorbance values ranged from 0.031 to 0.224. The value of absorbance brown in this study can be seen in Figure 1.

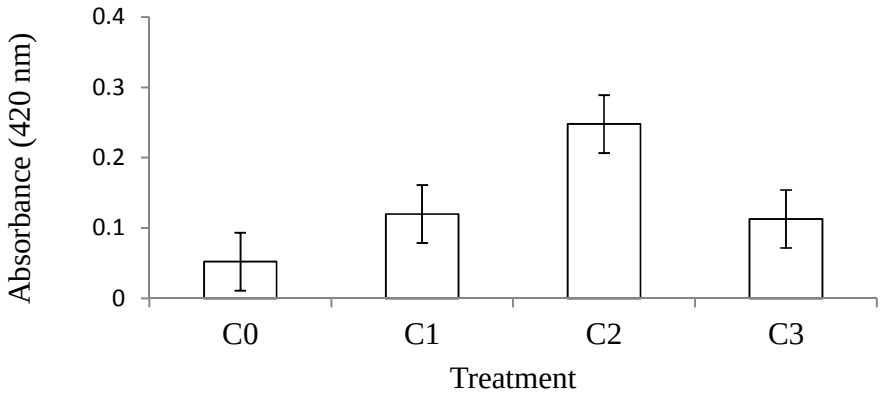

Figure 1. Absorbance brown color of chitosan monosaccharides complex (C0: 1% Chitosan, C1:chitosan + 1% Glucose, C2: 1% chitosan + 1% galactose and C3: 1% chitosan + 1% Fructose)

The brown color was formed due to amino acids and reducing sugars in a process, but it is also affected by the heating conditions [6]. Figure 1 showed chitosan galactose complex (C2) had highest brown color compared with other treatments. This was due to galactose had aldehyde functional groups on different C atoms whereas glucose had aldehyde functional groups and fructose had keton functional groups at the ends of the first C atoms [7]. Galactose also have more reducing sugars thus producing the highest absorbance for melanoidin more other sugar. Monosaccharides was third difference lies in the arrangement of atoms that caused differences in the level of sweetness, solubility and other properties of monosaccharides that makes the Maillard reaction of different colors.

Analysis of antibacterials against *Vibrio parahaemolyticus*

Vibrio parahaemolyticus, a gram-negative marine bacterium, is a worldwide cause of food-borne gastroenteritis. Therefore, the antibacterial testing against these bacteria is very important. Chitosan has antibacterial activity against some bacteria, one of which is *Vibrio parahaemolyticus*. Inhibition zone of chitosan monosaccharide complex are depicted in Fig. 1.

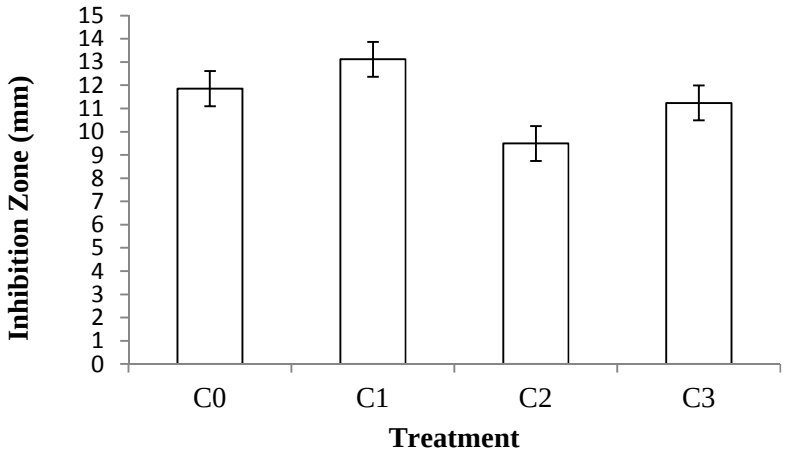

Figure 2. Chitosan monosaccharides complex against *Vibrio parahaemolyticus* bacterium (C0: 1% chitosan, C1:chitosan + 1% Glucose, C2: 1% chitosan + 1% galactose and C3: 1% chitosan + 1% Fructose)

As seen as Figure 1 showed that all treatments have antibacterial activity. Inhibition zone of chitosan monosaccharides complex against *Vibrio parahaemolyticus*is equal to 10 to 12 mm. The results showed the inhibition zone of C1 (chitosan glucose complex) was highest other treatments. Chitosan glucose complex showed strong antibacterial activity (12 mm). According Elgayyar et al.[5] inhibition zone > 11 mm the relatively strong while the inhibition zone of 6-11 mm relatively weak.

Antibacterial activity against *Vibrio parahaemolyticus* bacterium because of their positive charge on the chitosan molecule that binds to the negative charge on the microbial cell membranes, causing changes in permeability. Moreover, in the process of Maillard reactions produce furfural compound, maltol and the structure of 5-hydroxy methyl furfural can inhibited bacterial growth [8] and monosaccharides differences can affect the formation of compounds. It is also one of the causes of chitosan monosaccharides complex inhibit bacterial growth of *Vibrio parahaemolyticus* [9].

CONCLUSION

Differences addition of monosaccharides significant effect on the color brown. Analysis antibacterial test against *Vibrio parahaemolyticus*, chitosan monosaccharides complex proved to be better than chitosan. Inhibition zone of chitosan monosaccharides complex against *Vibrio parahaemolyticus*is equal to 10 to 12 mm.

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