Edible Coating from Green Tea Extract and Chitosan to Preserve Strawberry (*Fragaria vesca L.*)

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Abstract. Food quality is an important factor in food sector. One way to increase the quality of food is by applying edible coating to slow down the degradation of foods quality. Combination of chitosan and green tea extract is one of the types of edible coating which many function as food preservative Green tea extract is chosen because of its high antioxidant contents. The goal of this research is to make a film from the composite of chitosan and green tea extract as a food coating and to observe the effect of chitosan concentration and the addition of green tea extract to the phenolic contents and antioxidant activity of the film. Moreover, this research also aims to apply chitosan film and green tea extract to foods, especially strawberries, and observing the effect of chitosan concentration and the addition of green tea extract to the shrinking of the fruit's weight as well as its antimicrobial activities. Strawberries were coated with five different coating formula including one set as blank (uncoated fruit). The result of this research showed that the increasing of chitosan concentration and the volume of green tea extract addition also increase the antioxidant activity on the film. The phenolic contents on the green tea extract addition will also increase its phenolic contents as well. However, the increasing number of chitosan concentration can reduce the phenolic content on the film. Meanwhile, during the application to strawberries, it is found that 3% chitosan concentration which is combined with the addition of 2.5% green tea extract will minimize the shrinking of the fruit's weight. Also 3% chitosan concentration will give a better antimicrobial activity. In the other side the addition of green tea extract will increase antioxidant activity, the addition of green tea extract also causing a decrease in antimicrobial activity.

INTRODUCTION

Edible film is a thin layer of a material that can be consumed and placed between products. Edible films can be either their own sheets or for coatings on food [4,17,21]. This layer serves to protect food products from mechanical damage by reducing the transmission of water vapor, smell, and fat. Edible coatings can be made from materials capable of forming a layer [4,23]. One of the materials that can be used as raw material for edible coating is chitosan

Chitosan can be used as a basic material for edible coating because it is one of the non-toxic and edible materials. The advantages compared to other materials are chitosan has a positive amine group that makes it able to form a good layer and its antimicrobial properties [4,17].

In edible coatings of chitosan can be added with other additives. One of them is by adding green tea extract. Green tea is known to contain many polyphenolic compounds. Polyphenol compounds are known to function as excellent antioxidants. Therefore, green tea extract will give its own value when added in the manufacture of edible coating.

The purpose of this research is (1) Making edible coating of combination of chitosan and green tea extract. (2) to observe the effect of the addition of green tea extract concentration to the content of phenolic compounds, antioxidant activity, weight loss, and antimicrobial activity. (3) observed the effect of adding chitosan concentration to phenolic content, antioxidant activity, heavy shrinkage, and antimicrobial activity.

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MATERIALS AND METHODS

Materials

Fresh strawberry and green tea leaves extract were obtained from local market in Semarang. Glycerol, CH3COOH and DPPH (2,2-dyphenil-1- pikrilhydrazil) were purchased from Merck, (Hohenbrunn,Germany). Commercial chitosan as reference (C6H11NO4)n, was purchased from Biotech Surendo, Cirebon, Indonesia. Distilled water for all experiments was produced from a home-made pure water unit.

Methods

Green tea leaves are heated in aquadest 90^0C for 10 minutes at ratio of 1gr to 5ml and filtered using Whatmann No.1 filter paper. Chitosan powders with different concentration (1-3%) were mire mixed with 1% acetic acid solution to dissolve it. Chitosan film-forming solution was prepared according to the procedure of *Siripatrawan, et al* [25]. Each of the prepared chitosan solution (1-3%) was added with glycerol 25% V/V and green tea extract solution according to the variables (0-10%V/V chitosan solution). The solution then stirred until homogeneous.

Fresh strawberries are immersed in the film solution for a while until the film solution coats the entire surface of strawberries evenly. Then the strawberries are removed from the film solution and let stand for a while until it dries. Coating is done on each sample variable.

Analysis

1. Antioxidant activity by DPPH analysis

The antioxidant activity of the chitosan film was evaluated according method from Blois [25]. Each sample solution put into the test tube. Each of these solutions added with 2 ml of 0.1 M DPPH solution. Meanwhile, a control solution was prepared by mixing 1 ml of solvent solution (acetic acid 1%) with 2 ml of 0.1 M DPPH solution. It is also necessary to make a blank solution, using 3 ml of solvent solution (1% acetic acid solution). Each solution that has been made is dissolved left until 30 minutes. After 30 minutes, each solution measured its absorbance using a spectrophotometer at a 517nm wave. The antioxidant activity can be calculated by following equation

$$Aktivitas\ antioksidan\ (\%) = \frac{Abs_{DPPH} - Abs_{sampel}}{Abs_{DPPH}} \times 100\%$$

2. Phenolic content

Total phenolic content in chitosan solution was determined according to the Folin-Ciocalteu method as described bu Singleton,Orthofer, and Lamuela-Raventos [25]. Each 1 ml sample extract was put into the test tube. Then added with 2 mL of10% folin compound and 2.5 ml of 7.5% Na2CO3 solution. The mixed solution is then allowed to stand for 30 minutes. After that, each solution measured its absorbance using a spectrophotometer with 765 nm wavelength.

3. Strawberry weight loss analysis

Strawberries without coating as controls and strawberries that have been coated with the film solution were weighed initially based on method by Kopkhar (1990). All strawberries are again weighed every day for a week. Then compared the value of heavy shrinkage strawberries are dicoating every day with strawberries without coating.

4. Antimicrobial activity *Siripatrawan, et al* [25].

Test microbial activity using total plate count method (TPC). The test stages were performed by weighing each strawberry sample as much as 1 gram which then diluted by dissolving into 9 ml of sterilized aquadest. Each aquadest that has been given strawberries mixed using a vortex. After mixing, the second dilution is done in the same way. After that, a third dilution is done in the same way. Then each diluted sample is taken 1 ml and fed into the petridish. After that the media poured into each petridish that has been filled samples and media made flat and then petridish dittup meeting. In all these processes are done in a sterile state. All petridish that already contains media and samples, are incubated for 24 hours in reverse position to prevent moisture from dripping into the media. After 24 hours, the number of bacteria is calculated.

RESULTS AND DISCUSSION

Phenolic Contents

The Foline-Ciocalteu phenol reagent is used to obtain an estimate of the amount of phenolic compounds contained in the chitosan film. Phenolic compound undergo complex redox reactions with phosphotungstat and phosphomolybdic acids contained in Foline-Ciocalteu reactants. Phenolic compounds have an effect on antioxidant activity [25]. Phenolics consist of one or more aromatic rings containing hydroxyl groups and potentially capable of removing free radicals by forming stable phenoxyl resonance radicals

The results showed that the addition of green tea extract on the base material of chitosan film caused an increase in the content of phenolic compounds. Increased concentrations of green tea extract (GTE) can significantly increase phenolic compound (Figure 1). The highest increase occurred in the addition of 10% GTE of ten times that of chitosan film without the addition of GTE.

Meanwhile, based on the results of the study also known that there is the influence of chitosan concentration on phenolic content. Phenolic content in film with chitosan concentration 3% less compared with phenolic content in film with 2% chitosan concentration. This is due to the increased concentration of chitosan, capable of causing a decrease in phenolic content [2]. Therefore, the phenolic content in the film at 3% chitosan concentration is lower than that of phenolic content in films with chitosan 2 %.

The DPPH test is used to show the antioxidant activity of the film. Chitosan film without GTE has little activity on DPPH (Fig.2). The mechanism of DPPH activity from chitosan is related to the fact that free radicals can react with free amino group residues (NH2) to form stable macromolecular radicals, and the NH 2 group can form ammonium (NH3 +) groups through the absorption of hydrogen ions from the solution [25]. In chitosan films with higher GTE content, the antioxidant activity becomes increasingly increasing. The highest antioxidant activity is shown in movies with a 10% GTE content. The antioxidant activity on chitosan film without GTE addition with chitosan film with GTE addition looks very significant. Differences in antioxidant activity in chitosan films with 10% increase in GTE can reach 10 times greater than the antioxidant activity in chitosan films without GTE addition. Antioxidant activity is largely derived from the content of polyphenolic compounds present in green tea extracts.

Meanwhile, in films with 3% chitosan intended to have greater antioxidant activity compared to fikm with 2% chitosan concentration. From Fig. 2 it is shown that antioxidant activity in film with 3% chitosan concentration is 67,59% bigger than film with 2% chitosan concentration. This is because, antioxidants are not only found in green tea extract. However, in chitosan itself there are antioxidant substances [25]. Therefore, with increasing chitosan concentration in the film can lead to increased antioxidant activity in the film.

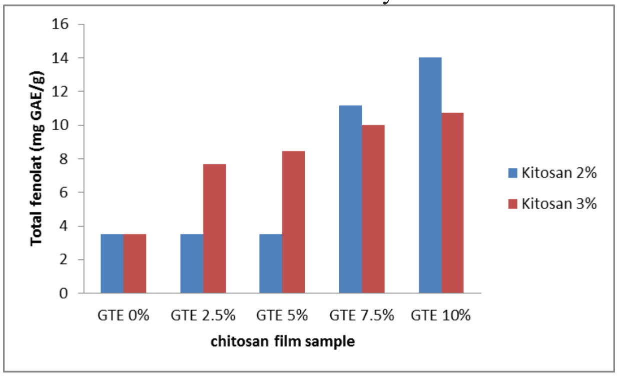

Fig 1. The effect of chitosan concentration on phenolic contain of green tea extract

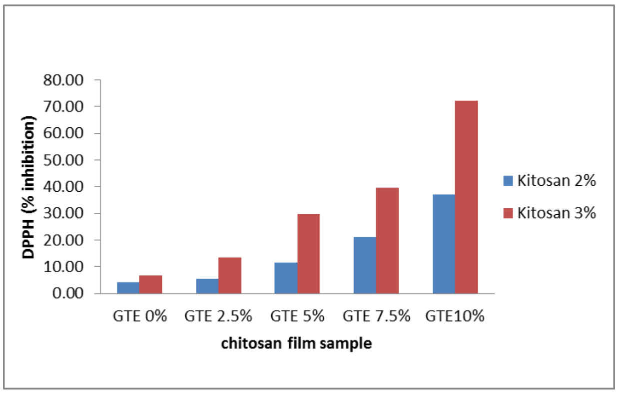

Fig 2. The effect of chitosan concentration on antioxidant activity of green tea extract

Weight Loss

Strowberry fruit is very susceptible to the speed of water loss resulting in shrinkage of fruit and weakening of tissue due to very thin skin of the fruit. Therefore, the weight reduction of fruit during the 4-day storage period was conducted to evaluate the effect of coating. All strawberries weighed heavily during the storage period (Fig. 3). However, after the second day of storage, the weight loss for all coated strawberries was significantly lower than that of strawberries without coating.

Chitosan acts as a physical barrier to loss of moisture, thus reducing dehydration and fruit shrinkage [30]. The addition of green tea extract on the chitosan film can reduce the rate of fruit shrinkage. The lowest shrinkage was obtained in strawberries with 3% incremental chitosan film of 2.5% GTE which lost weight 55% lower than fruit without coating.

In addition, when compared between strawberries with 2% chitosan film coating with 3% chitosan coating film there will be differences. In strawberries with 2% chitosan coating, the weight level of strawberries is greater than the depreciation of strawberries with 3% concentration of chitosan film coating. The amount of weight loss in strawberry fruit with 2% chitosan coating caused by the loss of water in strawberries. Meanwhile, in strawberries with 3% coat kitoasan there is a thicker layer of barrier that protects the fruit so it can suppress the evaporation of water content in strawberries [8]. This can be compared also with strawberry control (without coating) which experienced the greatest weight loss rate. This enormous weight loss is caused by the evaporation of the water content of the strawberries which causes the weight of the strawberries to decrease (shrink). This happens because the control strawberries contain no water barrier to evaporate as in the strawberry fruit that is coated.

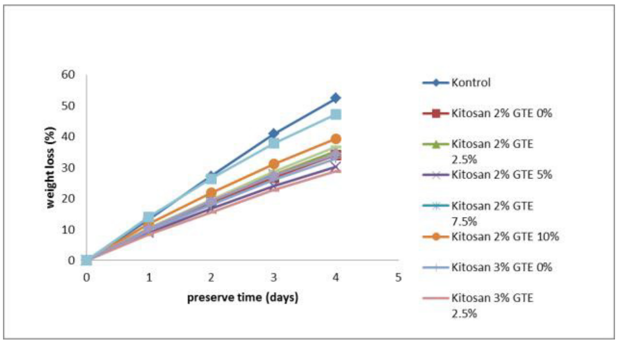

Fig. 3. The effect of chitosan concentration and green tea extract on strawberry weight loss.

Antimicrobial Activity

Chitosan has antimicrobial activity against bacteria, yeast, and fungi. Chitosan is considered a soluble chelating agent and activator because of the positive charge on C-2 of the glucosamine monomer. These characteristics provide good antimicrobial activity. The destruction of protein and intercellular components occurs due to the interaction between the amine groups in the positively charged chitosan molecule and the negatively charged microbial cell membrane [19].

In this study chitosan is used as a coating on strawberries. Analysis of bacterial growth was done by Total Plate Count (TPC) method. Fig. 4. shows the total bacteria of strawberry fruit coated with 2% and 3%

concentration chitosan film with the addition of GTE at various concentrations and has been stored for 6 days. Non-coating sroberries contain bacteria close to the maximum number of colonies that humans can consume 6 x 106 CFU / g. Strawberry fruit with chitosan coating without addition of GTE has the lowest total bacteria (± one tenth of fruit without coating). The addition of GTE causes an increase in total bacteria. The increased concentration of GTE causes the total bacteria to increase. This is caused by the increasing concentration of GTE the amine group content in chitosan becomes decreased so that antimicrobial activity also decreased.

Meanwhile, from Fig. 4. on strawberries with chitosan coating concentrations of 2% and 3% there were antimicrobial antiviral differences. For strawberries with 3% concentration chitosan coatings a higher antimicrobial activity was demonstrated with less total bacteria compared with strawberries with 2% concentration of chitosan coating. This antimicrobial activity difference occurs because antimicrobial activity in chitosan one of them influenced by chitosan concentration [19]. If the chitosan concentration is greater, the antimicrobial activity becomes larger so that fewer microbes will contaminate the strawberry fruit that is coated. Therefore, the number of microbes in strawberries with chitosan coating 3% less than the number of microbes in strawberries with 2% coating chitosan.

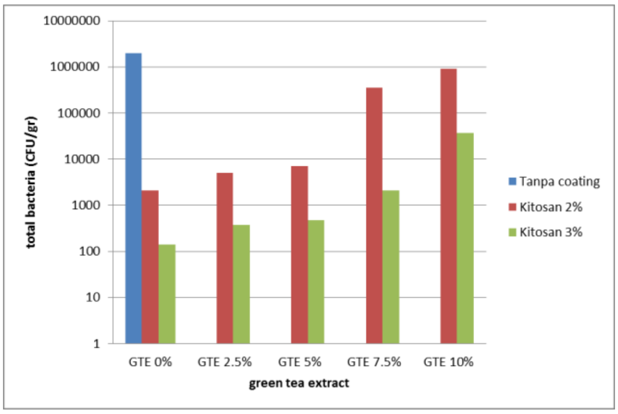

Fig. 4. The effect of blending chitosan concentration and green tea extract on antimicrobial activity.

CONCLUSION

The results showed that the addition of green tea extract can increase total phenolic content, antioxidant activity, and moisture loss barrier. In green tea extract there are antioxidant compounds one of them is a polyphenolic compound. Each addition of green tea extract in chitosan solution will increase total phenolic content and antioxidant activity. From antimicrobial test, it was found that green tea extract can decrease antimicrobial activity from chitosan film because with addition of green tea extract the amine group content in chitosan decreased so that antimicrobial activity also decreased.

Meanwhile, increasing chitosan concentration can cause total phenolic content reduction. However, in addition to decreasing total phenolic content, chitosan concentrations actually increase antioxidant activity, barrier to moisture loss and antimicrobial activity. This is because in chitosan also contain antioxidant substances so that the antioxidant activity increases along with the addition of chitosan concentration. While chitosan serves as a physical barrier to the loss of moisture. From antimicrobial test results obtained at greater chitosan concentrations then increased antimicrobial activity characterized by decreased bacteria that contaminate the test strawberries.

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