

The effect of plasticizers and chitosan concentration on the structure and properties of *Gracilaria* sp.-based thin films for food packaging purpose

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Abstract: *Gracilaria* sp. is well known as one kind of species of red algae. The major component of polysaccharide in this alga is agar that mostly used for making thin film. In this study, the *Gracilaria* sp.-based thin film had been prepared using two plasticizers (glycerol and sorbitol, 0.1, 0.2, and 0.3 wt %), and chitosan (1, 2, and 3 wt %). The FT-IR analysis confirmed the interaction that happened among the component of the mixture of *Gracilaria* sp., plasticizers, and chitosan was based on hydrogen bonding due to the presence of -OH and -NH₂ groups. The plasticizers and chitosan concentration have significant role to the mechanical properties of *Gracilaria* sp.-based thin film. The optimum concentration of plasticizers and chitosan based on mechanical testing result was found at 0.2 and 3.0 wt %, respectively. At those concentrations, the thin film that prepared with sorbitol showed the highest mechanical properties. Other characterizations, *i.e.* TGA (Thermogravimetric Analysis), SEM (Scanning Electron Microscopy), and WVP (Water Vapor Permeability) also brought the same result. The antimicrobial properties of the as prepared thin film in the presence of chitosan on agar medium and as a packaging on selected bread showed the *Gracilaria* sp.-based thin films was able to inhibit the growth of microbes. This antimicrobial activity can be used to declare the potential of *Gracilaria* sp.-based thin film as a new active food packaging.

Keyword: *Gracilaria* sp., chitosan, sorbitol, glycerol, active packaging.

Wpływ zawartości plastyfikatorów i chitozanu na strukturę i właściwości cienkich folii do pakowania żywności na bazie *Gracilaria* sp.

Streszczenie: *Gracilaria* sp. to dobrze znany gatunek krasnorostów. Głównym składnikiem tych alg jest agar (polisacharyd), najczęściej używany do wytwarzania cienkich folii. Na bazie *Gracilaria* sp. z dodatkiem dwóch plastyfikatorów: glicerolu i sorbitolu (0,1; 0,2; 0,3% mas.) oraz chitozanu (1, 2 i 3% mas.) otrzymano mieszaniny, z których wytworzono cienkie folie. Na podstawie analizy FT-IR stwierdzono powstawanie wiązań wodorowych pomiędzy grupami -OH i -NH₂. Na właściwości mechaniczne folii miała wpływ zawartość zarówno plastyfikatora, jak i chitozanu. Najlepsze właściwości mechaniczne uzyskano z zastosowaniem 0,2% mas plastyfikatora i 3% mas. chitozanu. Badania metodami analizy termogravimetrycznej, skaningowej mikroskopii elektronowej i oznaczona wartość przepuszczalności pary wodnej (WVP) potwierdziły te ustalenia. Wykazano, że otrzymane folie hamowały rozwój drobnoustrojów, mogą więc być stosowane jako nowe aktywne opakowania do żywności.

Słowa kluczowe: *Gracilaria* sp., chitozan, sorbitol, glicerol, opakowania aktywne.

Nowadays, food packaging technology has a lot of improvement, especially in the development of new materials for reducing the use of glass, tin, aluminum,

plastic, *etc.* [1]. Those materials are not ecofriendly due to the inability of materials to degrade themselves in the presence of microbe or decomposer. A product which has

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the following properties, biodegradable, edible, bioactive, *etc.*, is being tried to be achieved by the researchers [2–6]. The utilization of biomass or biopolymer as the active packaging raw material is quiet potential to be developed due to the raw material is based on natural product resources and most of them can be degraded in the mild condition with and without the presence of decomposer.

Gracilaria sp. is one species of red algae that the chemical composition mostly dominated by agar on its composition [7–9]. Agar that also known as 1,4-linked-3,6-anhydro- α -L-galactopyranose is one kind of polymer that used to prepare a gel form or even a thin film [10, 11]. A bioactive component is one requirement that need to be added into the food packaging materials to enhance their ability to inhibit the growth of microbes. Chitosan has been known as a polysaccharide that has antimicrobial activity, and it has been used as the additive in any kind of polymer matrixes as antimicrobial agent [12–14]. In addition, chitosan is also a good material due to its biocompatibility, ecofriendly, *etc.* [15, 16]. But the utilization of chitosan only is not effective due to its plasticity issues.

As we know, if the mixture only consisted of chitosan and agar, the obtained film would have brittle characteristic due to the crystallinity aspect of polysaccharide [17–19]. Normally, the addition of plasticizer is needed to be added into the mixture to enhance the plasticity, flexibility, and elasticity. Glycerol and sorbitol are plasticizers that suitable to use for polysaccharide matrix [20, 21]. Previous study declared that the addition of those plasticizers can enhance the mechanical properties of polymers, *i.e.* higher strain and Young's modulus properties of starch film was obtained after the addition plasticizer at the certain amount [21]. The similar progress was confirmed in the preparation of sugar palm starch-based thin film, the increase of plasticizer reduced the tensile strength value of starch film, but it can enhance the elongation at break of film [22].

Based on literature reviews, the study on the effect of concentration plasticizers and chitosan into the properties of *Gracilaria* sp.-based thin film has not been evaluated. Due to that reason, the mechanical properties and antimicrobial properties of the as prepared thin film

will be discussed in the support of other characterization, *i.e.* FT-IR, SEM, TGA, and water vapor permeability (WVP).

EXPERIMENTAL PART

Materials

Gracilaria sp. (SW) was obtained from Cilegon, Banten, Indonesia. Chitosan (CH) with deacetylation degree 87.5% (determined using titration method) was purchased from Palas Jaya, Lampung Selatan, Indonesia. Acetic acid glacial, sorbitol (S), glycerol (G) were provided by Merck.

Preparation of *Gracilaria* sp.-based thin film

Gracilaria sp. was cleaned with water for three times and dried under sunlight. About 2.5 g of dried SW was soaked in 50 cm³ of distillate water for 6–7 h. The mixture was then placed on hotplate and stirred at 600 rpm at 70–80°C for 30 min. The hot suspension was then filtrated, and the plasticizer was added into the filtrated suspension (Table 1). The mixture of *Gracilaria* sp. with the plasticizer was heated and stirred using the same condition as previous. The obtained mixture was then casted into a rectangular mold that has size 20 × 12 × 0.5 (cm³) and placed in oven at 40–50°C for 24 h. The addition of chitosan into the mixture of SW was performed after obtaining the best thin film formulation which has the highest mechanical properties. In practice, the chitosan solution should be added after the addition of plasticizer (Table 2), and the film preparation was performed in the same manner as previous.

Methods of testing

The functional group of *Gracilaria* sp.-based thin film was determined using Cary 630 FTIR Spectrometer, and it was performed in the wave number range of 650–4000 cm⁻¹.

The mechanical testing of *Gracilaria* sp.-based thin film was performed using UTM RTF 1350 with 1000 N load cell. The thin film was cut in size of 2 × 5 (cm²) (the thick-

Table 1. Thin film formulation in the presence of plasticizer

<i>Gracilaria</i> sp., wt %	Plasticizer	Concentration of plasticizer, wt %	Code of sample
5	Sorbitol	0.2	SW 5% + S 0.2%
		0.4	SW 5% + S 0.4%
		0.6	SW 5% + S 0.6%
5	Glycerol	0.2	SW 5% + G 0.2%
		0.4	SW 5% + G 0.4%
		0.6	SW 5% + G 0.6%

Table 2. Optimization of thin film in the presence of chitosan

<i>Gracilaria</i> sp., wt %	Plasticizer	Concentration of chitosan, wt %	Code of sample
5	Sorbitol	1	SW 5% + S 0.2% + CH 1%
		2	SW 5% + S 0.4% + CH 2%
		3	SW 5% + S 0.6% + CH 3%
5	Glycerol	1	SW 5% + G 0.2% + CH 1%
		2	SW 5% + G 0.4% + CH 2%
		3	SW 5% + G 0.6% + CH 3%

ness of *Gracilaria* sp. film was about 1–1.2 mm) and put in the sample holder, the test speed of measurement was set at 5 mm/min.

The morphological property of the film of *Gracilaria* sp. was observed through scanning electron microscope FlexSEM 1000 at 5 kV of accelerating voltage, the film was previously coated with gold-platinum to enhance the image quality of samples.

Thermal stability of *Gracilaria* sp.-based thin film was determined using STA TG DTA 7300. The measurement was set at temperature of 30–450°C and the heat rate was 5°C/min.

The potential activity of *Gracilaria* sp.-based thin film as antibacterial against *E. coli* and *S. aureus* was determined by placing a thin film with diameter 5.5 mm on the MHA (Mueller Hinton Agar) medium that has been inoculated with the bacteria. The effectivity of *Gracilaria* sp.-based thin film was evaluated through the formation of zone of inhibition.

The water vapor permeability test was performed following a wet cup method of ASTM E 96-95. The observation was done for five hours, and every one hour the weight of petri dish was evaluated.

Thin film application as food packaging

In this study, one of commercial bread was used as the object to evaluate the antibacterial activity of *Gracilaria* sp.-based thin film as food packaging. The first group was only bread without any packaging; the second group was the bread that packaged using LDPE-based plastic; and the third group was packaged using *Gracilaria* sp.-based thin film. Those groups were store at temperature 30°C. After 48 h, about one gram of each bread was placed in 15 cm³ tube that has been filled with 9 cm³ of distillate water. This tube was then vortexed for 1–2 min and the dilution was performed until 10⁻⁸. About 0.1 cm³ of this solution was inoculated on the PCA (Plate Count Agar) medium and spread using glass spreader. The incubation was performed at 34°C for 24 h, and the growth of colonies was count using the Eq. (1):

$$\text{Number of bacteria} = \text{Number of colonies} \cdot \frac{1}{\text{dillution factor}} \quad (1)$$

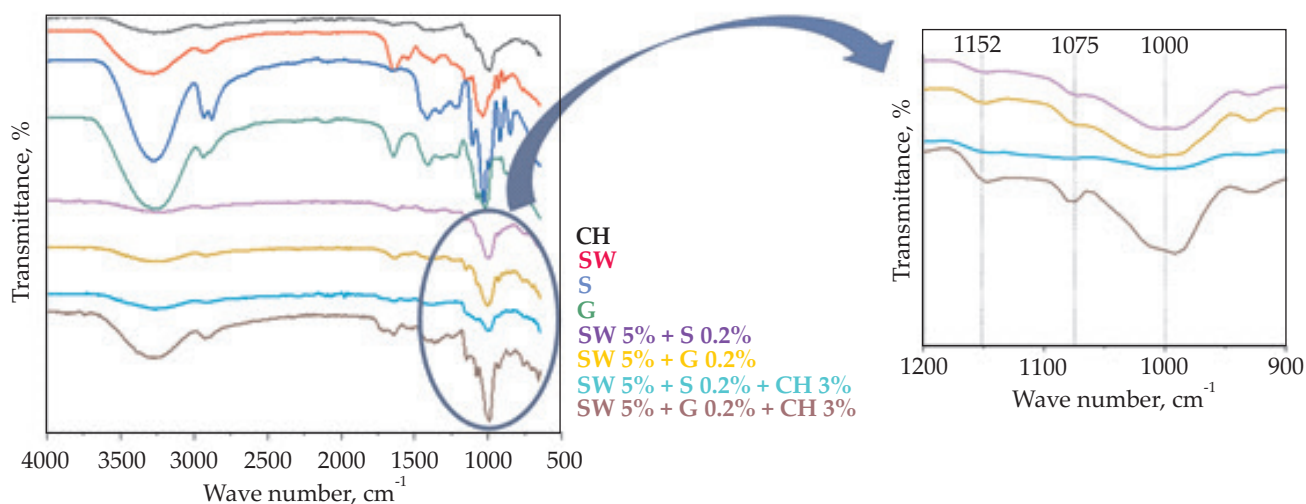


Fig. 1. FT-IR spectra of *Gracilaria* sp.-based thin films

RESULTS AND DISCUSSION

Functional group analysis

The physical/chemical interaction that occurred among the *Gracilaria* sp., plasticizer, and chitosan was evaluated using FT-IR analysis (Fig. 1). The typical peaks of each component (*Gracilaria* sp., plasticizer, and chitosan) can be found in the thin film samples, this indicated that only physical interaction (*i.e.* hydrogen bonding, van der Waals, *etc.*) that occurred among those materials during the film formation. The hydroxyl group of plasticizers and *Gracilaria* sp., and amine group of chitosan can be found as an overlapping peak around 3200–3600 cm^{-1} . The glycosidic bond (C-O-C) as the impact of the presence of 3,6-anhydro-L-galactopyranose in *Gracilaria* sp. can be found at 900–1200 cm^{-1} [23, 24]. The carbonyl of amide group from chitosan was appeared at 1639 cm^{-1} [25].

Mechanical properties of *Gracilaria* sp.-based thin film

The mechanical properties, *i.e.* tensile strength and elongation at break, of the obtained film of *Gracilaria* sp.-based thin film can be seen in Fig. 2. Figures 2a and 2b show the type of plasticizer has a significance role to

the tensile strength and elongation at break properties of *Gracilaria* sp.-based thin film. The number of hydroxyl group in sorbitol is higher than in glycerol might have important role, this led to the increase of the number of hydrogen bonding formation in this thin film. In both cases of plasticizers, with the increase of plasticizer amount, the tensile strength was slightly decrease, that caused by the plasticization effect of sorbitol and glycerol. As the impact of this phenomenon, the increase of elongation at break can be observed. The same result also had been reported in several studies that used glycerol as plasticizer [26]. In Figs. 2c and 2d show the addition of chitosan at all of concentration increase the tensile strength and elongation at break of thin film. This result was supported with the previous study that prepared blend of polysaccharide, *i.e.* starch and chitosan [5]. Based on this result, two formulations of thin film were chosen for further analysis, *i.e.* *Gracilaria* sp. + 0.2% glycerol/sorbitol + 3% chitosan.

Morphology of *Gracilaria* sp.-based thin film

Figure 3 shows the surface morphology of *Gracilaria* sp.-based thin film before and after the addition of chitosan. Before the addition of chitosan into the *Gracilaria* sp.-based

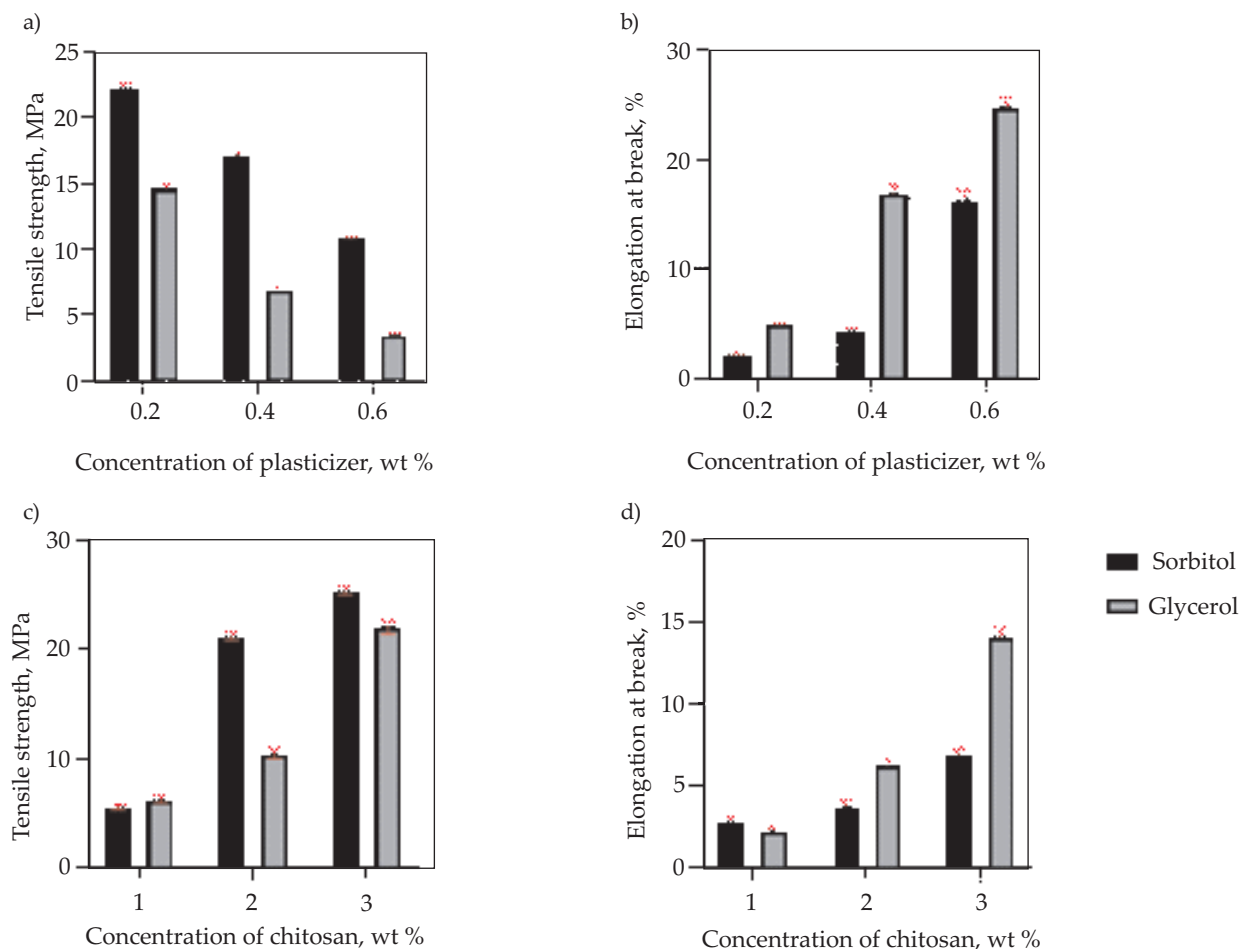


Fig. 2. a) Tensile strength, b) elongation at break of *Gracilaria* sp.-based thin film + plasticizer, c) tensile strength, d) elongation at break of *Gracilaria* sp.-based thin film + plasticizer + chitosan

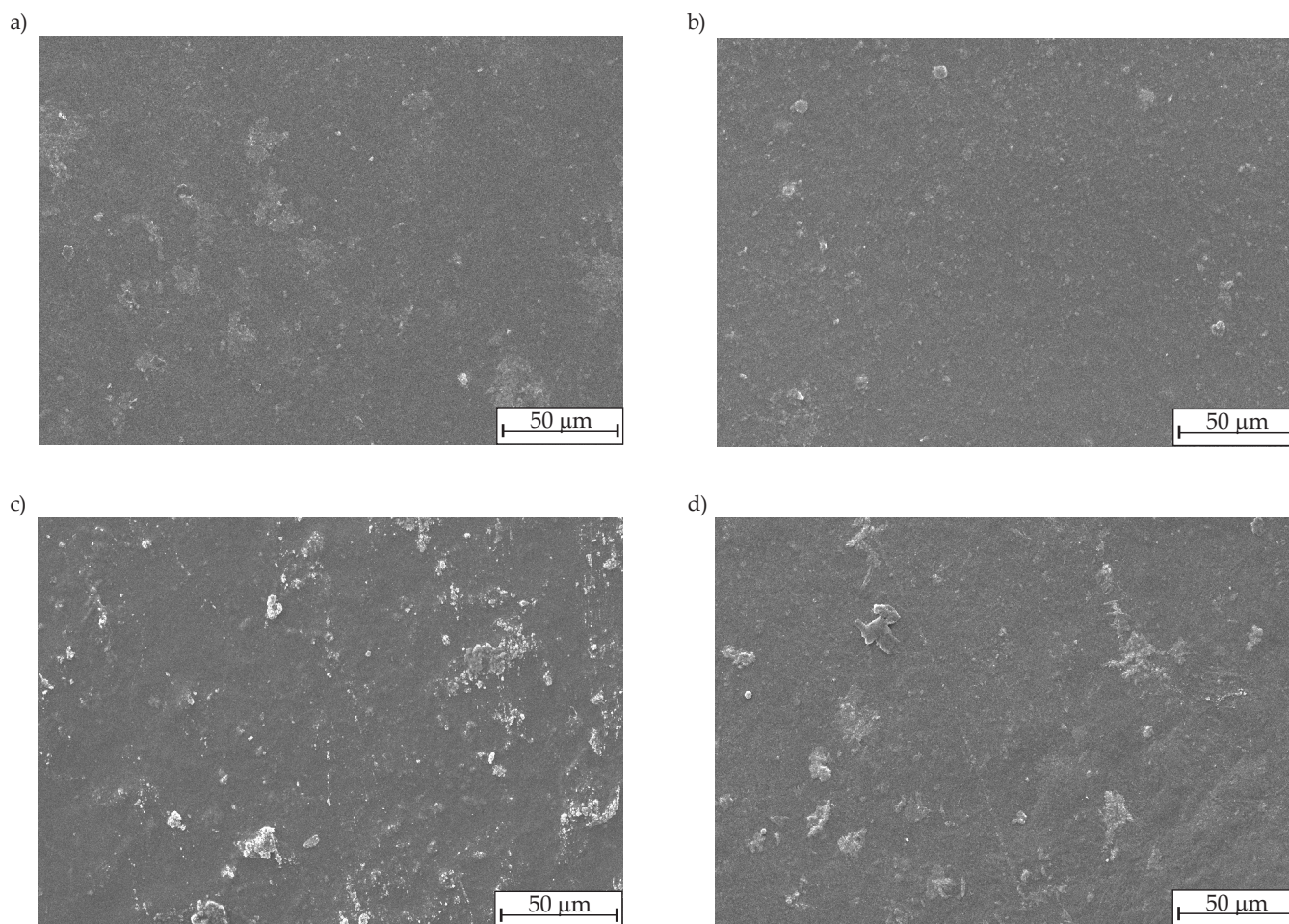


Fig. 3. Morphology of: a) *Gracilaria* sp. + sorbitol, b) *Gracilaria* sp. + sorbitol + chitosan, c) *Gracilaria* sp. + glycerol, d) *Gracilaria* sp. + glycerol + chitosan

thin film has a roughness surface (Figs 3a and 3c). But after the addition of chitosan, the surface of *Gracilaria* sp.-based thin film become smoother, indicating the homogeneity of thin film was increase. The different type of plasticizer also showed different homogeneity, this can be seen in Figs. 3a and 3c. The thin film that prepared with the addition of sorbitol has smoother surface than the film that prepared using glycerol. This homogeneity also can be used to explain the mechanical testing result. The thin film which has homogenous surface mostly showed the highest mechanical tensile strength due to better physical interaction through hydrogen bonding [5, 27].

Thermal stability of *Gracilaria* sp.-based thin film

Figure 4 shows the TGA graph of *Gracilaria* sp.-based thin film which has two steps of degradation. The first step was found at 35–105°C which affected in the mass reduction about 8–15% of each thin film (Table 3). The mass loss during this step can be assumed as the evaporation of water and any volatile compound [11, 28]. The second step was occurred between 175–340°C and reduce the mass of thin film by 47–55%. This step can be indicated as the carbonization of organic compound, including chitosan, polysaccharide in *Gracilaria* sp., sorbitol, and

Table 3. Mass loss of each *Gracilaria* sp.-based thin film

Code of sample	1 st step %	2 nd step %	Residue %
SW 5% + S 0.2%	10.42	52.14	15.97
SW 5% + G 0.2%	12.99	54.53	15.68
SW 5% + S 0.2% + CH 3%	7.10	46.91	46.14
SW 5% + G 0.2% + CH 3%	14.68	52.84	40.63

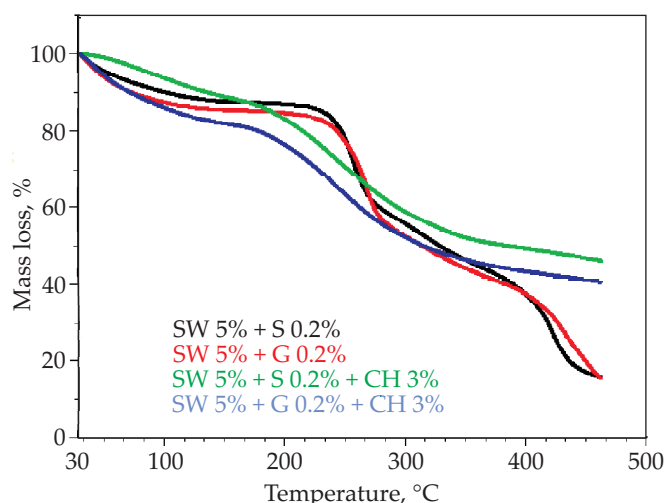


Fig. 4. TGA graph of *Gracilaria* sp.-based thin film

glycerol [22, 28]. Based on Table 3, we can conclude before the addition of chitosan, the thin film that prepared with the presence of sorbitol was more stable than the film that prepared using glycerol. This result can be assumed as the influence of hydrogen bonding that found in the thin film that prepared using sorbitol. After the addition of chitosan, the similar result also found, the presence of chitosan helped the thin film to be more stable at the high temperature. This can be seen from the increase of residue mass of these thin films after the addition of chitosan. The interaction between NH_2 group and hydroxyl group through hydrogen bonding can be a strong reason for the enhancement of thermal stability [11].

Antibacterial activity

The objective of this study is to produce a food packaging that has bioactive property, *i.e.* antibacterial. Within this kind property, the food will have a longer storage time and also can enhance the food quality [16]. Two bacteria were used in this study to determine the antibacterial activity of *Gracilaria* sp.-based thin film, *i.e.* *E. coli* and *S. aureus*. Those two bacteria were chosen due to its population that can be easily found in the stale food [29, 30]. The *Gracilaria* sp.-based thin film without the addition of chitosan has no antibacterial activity against *E. coli* and *S. aureus* (Table 4). The antibacterial activity was appeared after the addition of chitosan into the thin films. This can be induced by the presence of amine group of chitosan. This amine group of chitosan can be activated only when the hydrogen transport occurred in the system. The activated amine group has known as a cationic amine charge (NH_4^+). This positive charge of amine group can easily interact with negative charge of phospholipid group in the surface of cell wall of bacteria [15, 31, 32]. The obtained result in Table 4 is relevance with the theoretical mentioned above. Another reason is due to the thickness of gram-negative cell wall, these

Table 4. Antibacterial activity of thin films

Code of sample	Zone of inhibition, mm	
	<i>S. aureus</i>	<i>E. coli</i>
SW 5% + S 0.2%	0	0
SW 5% + G 0.2%	0	0
SW 5% + S 0.2% + CH 3%	6.75 ± 0.14	7.35 ± 0.25
SW 5% + G 0.2% + CH 3%	6.43 ± 1.10	7.20 ± 0.13

bacteria has thinner cell wall compare to gram positive bacteria, as the result the cell wall of *E. coli* can be easily disturbed [32].

Water vapor permeability (WVP)

In this study, the WVP test revealed the thin film that prepared using sorbitol and glycerol has the following WVP value, $9.7 \cdot 10^{-7}$ and $2.1 \cdot 10^{-6}$ g/(s · m · Pa). Those values indicated the thin film that prepared using sorbitol has good WVP property compare to thin film that prepared with glycerol [33]. Hydrophilicity of thin film has an important role in this property, the more hydrophilic of the thin film, the more water that can interact with the surface of thin film. This can induce the water to evaporate more through the hydrophilic surface [34]. As the result, *Gracilaria* sp.-based thin film that added with sorbitol has the higher water vapor barriers properties than with glycerol.

The application of thin film as food packaging

To determine the effectivity of *Gracilaria* sp.-based thin film as food packaging, in this study we also performed a simple test regarding that objectivity. In this study, the bread that was chosen has the 3–4 days expired date. Figure 5 shows the image of bread and its bacteria that isolated from the treated bread after several days' observation. The number of bacteria that found in the bread was quite variative that depended on the treatment. The bacteria number in the bread without any packaging was designated too many to count. The second high of bacteria was found in the bread that covered with LDPE-based plastic with value of $4.13 \cdot 10^{10}$ CFU/g, that following by *Gracilaria* sp.-based thin film with the addition of glycerol with value of $2.0 \cdot 10^{10}$ CFU/g. In other hand, the *Gracilaria* sp.-based thin film has the lowest bacteria number ($1.0 \cdot 10^8$ CFU/g), this value agrees with the zone of inhibition of *Gracilaria* sp.-based thin film (Table 4). The addition of chitosan was proven to be effective as the antibacterial agent in the preparation of *Gracilaria* sp.-based thin film. The effectivity of chitosan as antibacterial agent also can be found in several previous study, *i.e.* the combination of starch-chitosan has been proved to be effective as packaging for cheese that able to inhibit the growth of fungal and bacteria [27].

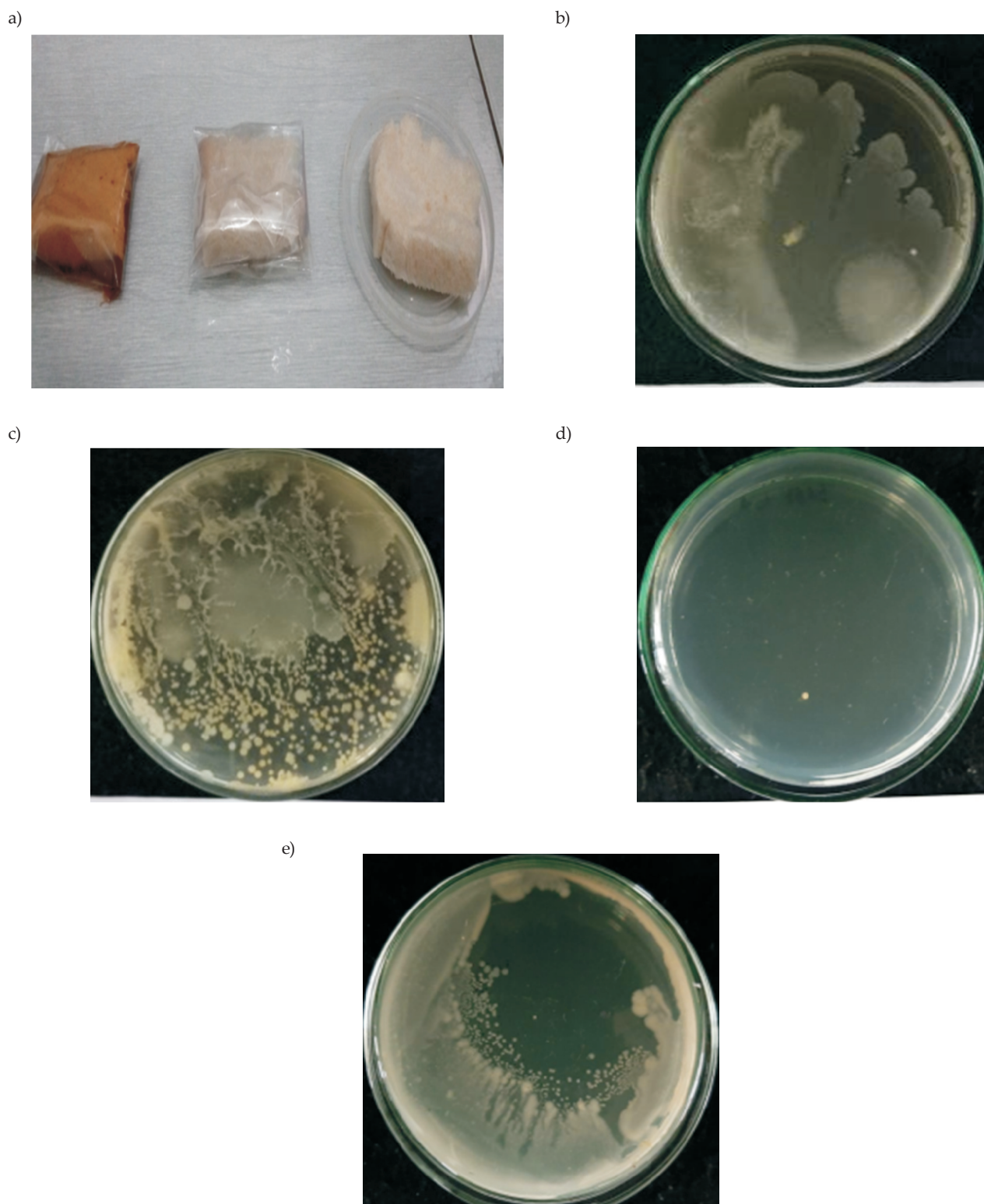


Fig. 5. a) Coated and uncoated bread samples, b) total plate count results of uncoated bread, c) coated by commercial plastic, d) coated by thin film of SW 5% + S 0.2% + CH 3%, e) of SW 5% + G 0.2% + CH 3%

CONCLUSIONS

The study revealed that plasticizer has important role to the mechanical properties of thin film, and the addition of sorbitol showed the highest mechanical properties compared to glycerol. The addition of chitosan also resulting in

improvement of mechanical properties, thermal stability, and antibacterial activity. Morphology analysis confirmed the presence of chitosan improve the homogeneity of thin film through the hydrogen bonding interaction. In addition, chitosan can be used as bioactive material in the preparation of biopackaging due to its antimicrobial activity.

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