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Research Article

Study of the effect of Adding Green Tea (*Camellia sinensis*) Extract on the Properties of Biodegradable Film from κ-Carrageenan

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Abstract: Carrageenan is a polysaccharide widely used as a material in the packaging industry in search of plastic replacement. κ-Carrageenan contains the lowest amount of sulfat group among other types of carrageenan which makes it the most hydrophobic one. Hence, the most suitable kind for this particular purpose. This research combined commercially available of both κ-Carrageenan and green tea extract (gte) to create a biodegradable film and then to study its characteristics. The concentration of gte was made as 0 µg/mL; 0.5 µg/mL; and 1.0 µg/mL while the concentration of κ-Carrageenan and glycerol remained constant. Incorporation of gte produces in darker, colorless film. While there is no effect of gte addition in water content observed, the higher concentration of gte will create a thinner film and lower level of water vapor permeability (WVP). An additional study of the mechanical properties of κ-Carrageenan/gte film indicated a direct proportion between the concentration of gte and tensile strength of the film. Meanwhile, there is no sign of correlation between gte concentration and elongation at break. SEM study displayed a more compact surface with smaller pores when the concentration of gte is increased.

Keywords: Biodegradable film, active packaging, κ-Carrageenan, green tea extract

Introduction

The need for food supply is rising rapidly due to the growth of the world's population. Food distribution nowadays is not only limited to certain areas where the food is produced but has spread across the globe. These demands have created a situation where reliable food packaging has become a necessity [1]. Moreover, there is a popular trend in the use of safe and environmentally friendly materials in everyday needs including in food packaging. This leads to an emergence in research and development of innovative food packaging especially film made from biomaterial [2], [3].

Polysaccharide such as carrageenan, starch [4], [5], [6], [7], chitosan [8], [9], konjac [10], carboxymethylcellulose [11], alginate [12] are believed as suitable candidates. Even lipid [13] and protein-based films [14] have also been developed.

Carrageenan is a polymer that can be isolated from red algae and consists of galactan repetition of 1,3-linked β-d-galactose and 1,4-linked α-d-galactose. This polymer also contains ester sulfate groups that can vary among different types of carrageenan. K-Carrageenan has the lowest amount of sulfate group which makes it able to form hard, strong, yet fragile transparent gel/film when potassium ions are present [15], [16], [17]. Together with konjac, carrageenan-based film has presented a potential for antibacterial activity when treated with *Salmonella* phages solution [10]. Numerous studies have also reported the usage of carrageenan as an alternative eco-friendly food packaging material [17], [18], [19].

Green tea (*Camellia sinensis*) has become part of human culture since its benefits have been discovered [20]. Green tea contains a high amount of polyphenols [21], [22]. Other compounds found in green tea

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include alkaloids, terpenoids, vitamins, and amino acids. More than half of the polyphenol consists of catechins and the remaining include flavonoids, anthocyanins, and phenolic acids [20], [23]. Due to its health benefits, green tea has been incorporated into many research. The food packaging industry has been utilizing green tea to enhance the aroma and color of the packaging [24], prolong the ripening process of fruit and vegetables [25], antioxidant [26], and boost the antimicrobial of the packaging [27].

Platstics as they come from processing of petroleum are hard to decayed biologically. Additionaly, from the whole platics that produced, less that 15% goes into recycling with only almost one third of it are recycled into high-quality goods [28]. For this reaseon, plastic waste after use could create a serious problem for environment including aquatic life. Hydrocarbon compounds that form the plastic also quicken climate change [28], [29]. Among industries that utilize plastic, packaging is one its main consumer [29].

The idea of biodegradable film comes from the fact that our nature produces materials that can be used as ingredients for plastic replacement. This type of plastic can be easily degraded naturally. Hence, makes it safe for the environment [30].

This study aims to investigate the effect of adding green tea extract into the κ-Carrageenan-glycerol mixture to produce a film that firther will be developed in biodegradable film. As an attempt to upgrade the physical and mechanical properties of the film, supplementary substances can be added to the film prescription [31].

Materials and Methods

Materials

Analytical balance, Scannin Electron Microscope (JOEL JCM 6000plus, Laboratorium Mikrostruktur Universitas Muslim Indonesia), mikrometer (Mitutoyo), colorimeter (AMTAST CS-10, Laboratorium Kimia, Analisa, dan Pengawasan Mutu Pangan Universitas Hasanuddin), hygrometer, desiccator, thermometer, Texture analyzer (Shimadzu, Laboratorium Metalurgi Fisik Universitas Hasanuddin), Plexiglas plates (12 cm × 12 cm), dehumidifier (Blue Sky), oven, centrifugal tube 50 mL, and coomon glassware according to the procedures.

The ingredients used in this research are κ-Carragernan (Indonesia Handmade), green tea extract (gte, Happy Green), gliserol p.a., silika gel, etanol 70%, dan aquadest.

Preparation of the κ-Carragernan/Green Tea Extract Film

κ-Carragernan/gte (*Camellia sinensis*) films were prepared using a method done previously by [32] with some modification on the drying process (in this study, it carried out using acrylic plate). The stock of gte solution (100 µg/mL) was made beforehand by mixing 1 mg of gte powder with 100 mL of aquadest then heated at 90 °C for about 30 minutes. Gte (100 µg/mL) then was put at room temperature to cool it down for at least 30 minutes. This concentration of gte later be diluted to achieve gte concentration of 0 µg/mL; 0.5 µg/mL; and 1.0 µg/mL. For each of these gte concentrations, this method follows: 3 grams of κ-Carragernan and 0,9 gram of glycerol were added into 150 mL gte solution. This mixture is then heated until it reaches 90 °C and stays for 30 minutes (or until the carrageenan dissolves thoroughly, evaporation of water can be disregarded). This mixture film solution was poured onto a square acrylic plate until it covered the entire surface. The film was then put in a room temperature for two days and then in a condition of 25 °C and 50% RH for at least two days before the characterization process took place. Each carrageenan/gte film was labeled as k_gte_0, k_gte_1, k_gte_2 for gte solution of 0 µg/mL; 0.5 µg/mL; and 1.0 µg/mL respectively.

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Characterization of Biodegradable Film

Color

Color of the film was observed using method by [33]. The color parameters was set as follows: L (luminosity), a (negative: green; positive: red) and b (negative: blue; positive: yellow). The color difference (ΔE) was calculated using formula 1:

$$\Delta E = \sqrt{(L* - L)^2} + (a* - a)^2 + (b* - b)^2 \quad (1)$$

where: L* (91.40), a* (-0,10), and b* (1.27) as calibration color parameter

Thickness

The thickness of the film was measured using micrometer. The measurement was done at four different locations on the film. Eeach measurement is done as triplo.

Water Content

The film was put in the oven at 105 °C until its weight is constant and calculated using formula 2:

$$water\ content\ (\%) = \frac{(M_i - M_t)}{M_i}\ x\ 100\% \quad (2)$$

where: Mi and Mt are initial and final weight of the film respectively.

Water Vapor Permeability (WVP)

WVP was measured using gravimetric method where film sample covered on the mouth of sentrifuge (50 mL) containing anhidrous silica gel. The tube was put in desiccator containing aquadest (100% RH, 25 °C). The centrifuge tube weight was measured every 24 hours for five days. WVP was calculated using formula 3:

$$WVP = \frac{W\ x\ X}{t\ x\ A\ x\ \Delta P} \quad (3)$$

where:

W = weight increase of centrifuge tube (gram), X = film thickness (m), t = time neede for tube weight to increase (s), A = permeation area of sample (m^2), ΔP = saturated vapor pressure at 25 °C.

Mechanical Properties

The mechanical properties of the formed film then be determined. There are two properties that will be studied which are tensile strength and elongation at break. Each concentration will be measured duplo and calculated using formula 4 and 5 [33]:

$$Tensile\ strength\ (MPa) = \frac{F}{x\ .W} \quad (4)$$

$$Elongation\ at\ break = \frac{\Delta L}{Lo}\ x\ 100 \quad (5)$$

Scanning Electron Microscopy (SEM)

The property of film surface will be observed using SEM with magnification of x8000

Results and Discussions

Color

At first sight, color holds a significant influence way more than other parameters such as shape that will become equal after some time of observation. The color itself is multidimensional where aspects like hue, saturation, lightness, etc. could define one color [33], [34]. This gives us an understanding that similar-looking colors could be actually different from each other. Color arguably is one factor that may shape

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consumer opinion over the healthiness of food [35]. In this study, the effect of the addition of gte to the color of the film will be examined as revealed in Table 1.

Table 1. Color parameter (L, a, b, dan ΔE) of κ-Carrageenan/gte (*Camellia sinensis*) film

Sample	L	a	b	ΔE
k_gte_0	93.11 ± 0.12	1,67 ± 0,07	3,34 ± 0,19	3,22 ± 0,09
k_gte_1	93,24 ± 0,04	1,68 ± 0,01	3,01 ± 0,16	3,10 ± 0,07
k_gte_2	92,93 ± 0,10	1,54 ± 0,03	3,27 ± 0,12	3,01 ± 0,02

Values are given as mean ± SD (n=3)

According to an article from hunterlab.com, L indicates lightness whereas lower L means darker film. Therefore, it can be concluded that the more concentration of gte is added, the darker the film gets. Value a and b represent red/green and yellow/blue respectively. An increase in gte concentration will result in an increase in the red level and reduce in the yellow level or more toward blue. A similar result was found in an article by [26] where the film gets darker and toward redness as the concentration of gte when added to rice starch-pectin film increases. ΔE describes the total color difference where higher ΔE indicates a greater color difference (hunterlab.com). Gte concentration in the film is inversely proportional to ΔE.

Thickness and Water Content

Film with no additional gte has thicker film compared to film with gte where a higher gte concentration yields in a thinner film (Table 2). While this pattern needs further study, it is suggested that the thickness of the film depends on carrageenan concentration, and the presence of gte will substitute carrageenan. Several studies revealed that the film thickness did not necessarily increase as additive compounds were added to the original film recipe [33]. The low level of gte concentration added to the mixture is arguably the reason why it does not affect water content (Table 2). An alternative method was suggested for better results. Instead of using acrylic plates, [7] used petri dishes. The reason is that all mixture will form film without any waste in order for a thicker film and an improvement in water content.

Water Vapor Permeability (WVP)

The amount of water that gets through the film pore per unit (g/m.s.Pa) area is the definition of water vapor permeability. Pores and cracks are the morphology that determines the ability of vapor or gas to pass the membrane film. Additionally, Flick's law and Henry's law are two laws that influence the solubility-diffusion of vapor which also affects how it goes through the film [36]. The result of wvp is illustrated in Table 2.

In terms of functionality, film made from natural polysaccharide tends to have a poor wvp performance. In order to improve it, wvp value need to be pushed down [37], [38]. Films that contain gte exhibit lower wvp. This evidence suggests that adding gte can restrain the movement of water vapor to pass through the film barrier [39].

The fact that gte contains a large quantity of polyphenol may contribute to the cohesiveness of the film surface due to the polyphenol structure where aromatic rings are abundant [32]. Hydrogen and covalent bonds that formed by phenolic compounds and polysaccharide can reduce the hydrogen binds between water molecules, thus reduce the flow of water [40]. Reports by [7], [32] illustrated a same pattern. Result by [41] where it studied the effect of film thickness to wvp value also observed falling value of wvp was linear to the thickness of the film.

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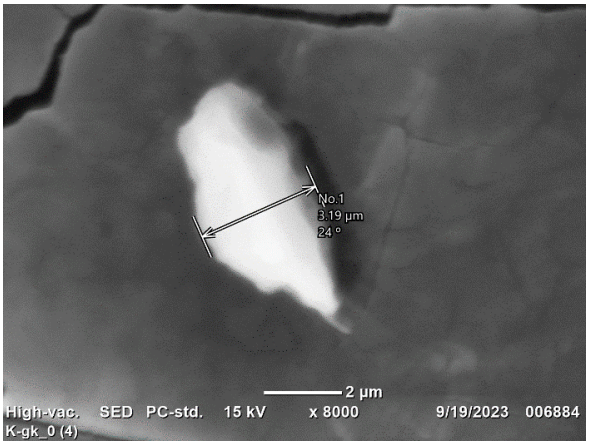
Table 2. Thickness, % water content, and WVP of κ-*C*ar*r*ag*e*enan/*gte* (*Camellia sinensis*) film

Sample	Thickness (mm)	% water content	WVP (10^{-11} g m^{-1} s^{-1} Pa^{-1})
k_gte_0	0,098 ± 0,062	19,0893 ± 1,755	1,6803 ± 0,042
k_gte_1	0,077 ± 0,015	19,2736 ± 1,907	1,3909 ± 0,024
k_gte_2	0,062 ± 0,026	18,5246 ± 0,692	1,0976 ± 0,0379

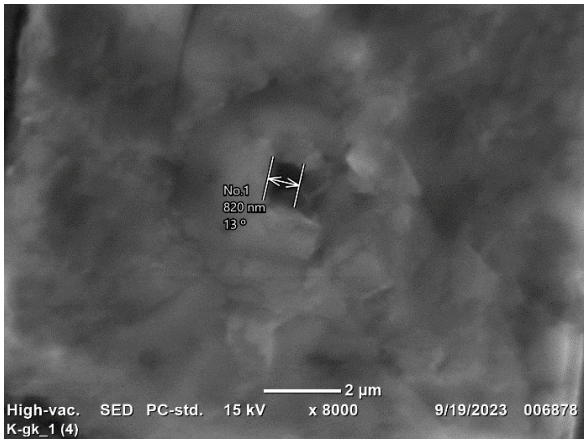
Values are given as mean ± SD (n=3)

Scanning Electron Microscope (SEM)

A scanning electron microscope (SEM) was employed to study the surface morphology of the film as displayed in Figure 1. The films have some pores and cracks. Aggregation of carrageenan and granule forms are detected. The higher the gte concentration, the smaller the pores on the film surface, which had a rougher and more irregular shape. This indicates there is strong interaction between phenolic compounds and polysaccharide takes place which created hydrogen and covalent bonds which affect the homogeneity of the film further alter the surface of the film [11], [40]. A similar result can be observed from [11] where the higher the amount of additive substance present in the film, the more irregular, rough, and granule the film is.



(a)



(b)

(c)

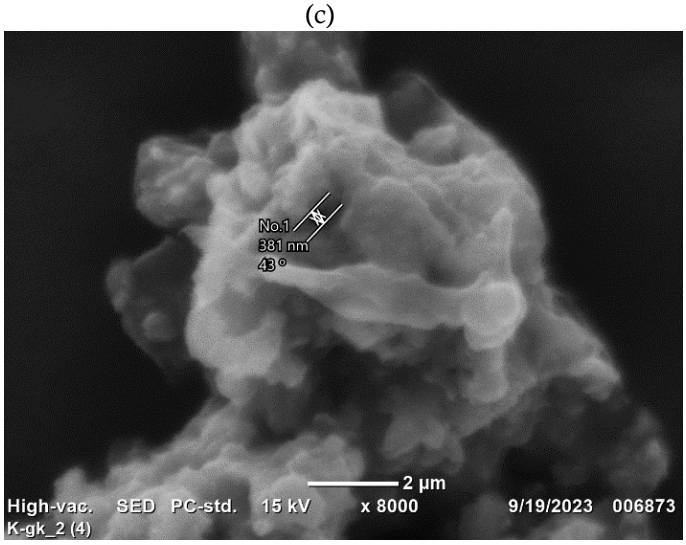


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Figure 1. Scanning electron microscope surface of κ-Carrageenan/gte (*Camellia sinensis*) film

Mechanical Properties

In search of biodegradable film whose quality mimics plastic film, mechanical properties such as tensile strength and elongation at break are vital points to be considered. It reflects intermolecular bonds and the durability of the film. How well the interaction between substances forming the film can be determined by studying their mechanical properties [7], [19]. Tensile strength describes how reliable a film is to prevent damage caused by external forces. On the other hand, elongation at break exhibits how far a film can be stretched before it starts to break [7].

The result for tensile strength and elongation at break can be seen in Table 3. Gte concentration has a direct proportion to tensile strength ranging from 0.616 ± 0.045 to 3.560±0.154 MPa. Tensile strength rises along with the concentration of gte. Polyphenol group contained in gte will create hydrogen bonds with carrageenan and this makes the film more compact and resistant [7], [32]+. Polysaccharide-based films naturally do not have good mechanical properties. Some methods have been developed to overcome this disadvantage, one of them is to add additional additives like plasticizers and other natural polymers [7].

Gte concentration performed a seemingly random effect to elongation at break (Table 3). As the gte is added (0.5 µg/mL), the elongation declines and rises when the concentration is at 1 µg/mL. Hydrogen bonds between polyphenols in gte and carrageenan lead to the formation of a stiffer film. Hence the more hydrogen bonds occur in the film, the more rigid it becomes. However, our result seems in a disagreement to this statement. Further research need to be completed in order to have a better understanding of this phenomena.

Table 3. Tensile strength and elongation at break of κ-*C*ar*r*ag*e*enan/*gte* (*Camellia sinensis*) film

Sample	Tensile Strength (MPa)	Elongation at break (%)
k_gte_0	0,616 ± 0,045	50,069 ± 1,749
k_gte_1	2,163 ± 0,103	23,181 ± 1,658
k_gte_2	3,560 ± 0,154	37,282 ± 6,233

Values are given as mean ± SD (n=2)

Conclusion

κ-Carrageenan/gte (*Camellia sinensis*) extract films had been successfully prepared using different concentrations of gte. The addition of gte has the effect of decreasing the thickness, water vapor permeability, and pores of the film while making the film more rigid. On the other hand, gte is observed to have no significant role in the water content and color change of the film. However, a film with a higher amount of gte tends to be darker, red, and bluish. While tensile strength increases as the gte level rises, elongation at break will go down when film solution is mixed with gte and rise as the more gte present in the film. This results give us a chance to develop active packaging where they are both safe and environmentally friendly and better mechanical properties even at low gte concentrations.

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