Optimization and characterization of edible film composite of k-carrageenan Kappaphycus alvarezii and beeswax nanoemulsion

by Feny Mentang 4

Optimization and characterization of edible film composite of k-carrageenan *Kappaphycus alvarezii* and beeswax nanoemulsion

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Abstract. The objective of this study was to optimize and analyze the effect of homogenization rate on the physical characteristics of the edible film of k-carrageenan composite of *Kappaphycus alvarezii* and beeswax. The edible film was made from the composite of k-carrageenan flour of *K. alvarezii* 4%, 1.2% beeswax, and 2% glycerol (b/v), then homogenized at different rates, A1= control, A2 = 2250, A3 = 2500, A4 = 2750, and A5 = 3000 rpm for 10 min. The physical characteristics, such as thickness, percent solubility, tensile strength, elongation, and water vapor tranmission rate, were analyzed for each sample. Results showed that the homogenization rate significantly influenced the change in the physical characteristics of the edible film of *K. alvarezii* carrageenan composite. It also highly significantly affected in reducing the water vapor transmission rate and increasing the thickness, solubility, tensile strength and elongation ($p < 0.01$). The best edible film was recorded in nannoemulsion of *K. alvarezii* carrageenan flour homogenized at 3000 rpm with mean value of 0.1539 mm thickness, 4.1494% solubility, 20.2 N mm^{-2} tensile strength, 33.3333% elongation, and 19.9775 g m^{-2} h^{-1} water vapor transmission rate.

Key words: edible film, homogenization, water vapor transmission rate, tensile strength, elongation.

Introduction. Seaweed is one of fisheries commodities potentially developed in Indonesia. Most seaweeds are exported in dry form and only about 20% is processed in Indonesia. *Kappaphycus alvarezii* commonly grow along the coastal area of Indonesia. To increase the added value, according to Desiana et al (2015), the seaweed quality can be developed to be carrageenan. Carrageenan has better price than that of dry seaweed. To increase the added value of *K. alvarezii* and to reduce the import of the processed product, carrageenan processing needs to be developed. Nowadays, k-carrageenan is obtained from seaweed *K. alvarezii* well-known as *Euchema cottonii* (van de Velde et al 2002). Carrageenan can be used as one of the materials usable to solve problems of plastic package utilization.

The dependence of Indonesian people upon the plastic package is very high, even though they have already known that plastic is highly dangerous for human health and environment. Therefore, people awareness is needed to restrict the plastic package utilization and encourage them to use safe alternative package for health and environment. In the last few years, many studies were conducted to make edible alternative package and increase food quality (Campos et al 2011; Işik et al 2013). One of the environmental friendly, safe, and edible packages is edible film. Its benefit is to protect the food product and the original product appearance can be maintained (Kinzel 1992).

Edible film can be defined as thin layer for main package of food and belongs to edible component (Hassan et al 2018). Edible film can be made from 3 composing materials, hydrokoloid, lipid, and composite of both (Donhowe & Fennema 1994). It is biodegradable and can be used to inhibit the shift of oxygen, carbondioxide, humidity, lipid, aroma, and develop the food features (Bourtoom 2008). Edible film of carrageenan has good inhibitory character to gas but has high vapor permeability due to its hydrophilic feature. These features restrict its usage in food packaging (Alves et al 2011). Nevertheless, improvement effort can be done through nano-technological application.

The development of edible film from carrageenan flour of *K. alvarezii* using nano-technological approach is an innovation in industrial development since it can give an added value of the carrageenan as biomaterial. The presence of nanotechnology gives the opportunity in science and technology development, in which the material features and types in nanoscale can become more effective, efficient, and beneficial. The nano-sized colloidal particles of the edible film will be efficiently able to penetrate the pores of the fisheries product. Besides, the nanotechnology application can exhibit the pseudoplastic, viscoelastic, biocompatible, and biodegradable functional characteristics that could easily react with organic molecules, being also safe for human body (Rumengan et al 2018).

North Sulawesi is one of the seaweed producers in Indonesia, especially *K. alvarezii*. The presence of nanotechnology makes the seaweed *K. alvarezii* be able to be developed and utilized to yield safe and environmental friendly packaging; *K. alvarezii* contains kappa carrageenan. According to Sun et al (2015), kappa carrageenan possesses very low solubility because it has large molecular weight and limits its application in the product.

In the last few years, nanotechnology application has developed quickly in all fields, including food processing (Bouwmeester et al 2009). The uniqueness of nano-particles in the field of materials lies on their different function and feature in the similar macro-materials (Abdullah et al 2008). Since product manufacture using high quality and stable nano-technology necessitates difficult method, more simple methods and techniques are required in order to ease the edible film processing. Testing of nano characteristics can be done in different ways, one of which is size reduction. It can be carried out through homogenizer method (Suptijah et al 2011). Small particle size in nano-emulsion can increase the physical feature of the edible film by adding the surface per unit area (Galus & Kadzinka 2015).

Material and Method

Materials and equipment. Research materials include k-carrageenan flour of seaweed *K. alvarezii*, sago flour (Nusa Utara cap djago), beeswax, aquadest, glycerol (Merck), and 1 M NaOH (Merk).

Preparation of k-carrageenan and beeswax composite edible film. Edible film processing from carrageenan flour followed the method of Fabra et al (2009). Carrageenan flour of 4% (b/v) was dissolved in 1,000 mL of distilled water while heated at 65°C and homogenized at 600 rpm using WiseStir HS-100D homogenizer and Favorit HS0707V2 hot plate stirrer, then added with 2% plasticizer glycerol (v/v), 1.25% beeswax (b/v) and 0.5% sago flour (b/v). The pH was set to be neutral by adding 1 M NaOH. The solution was then homogenized at 0 (A0), 2,250 (A1), 2,500 (A2), 2,750 (A3), and 3,000 rpm (A4) for 10 min and poured into a 20 cm x 40 cm x 2 mm acrylic mold, cooled, and dried in the oven at 60°C for 6 hours, cooled for 20 min, and then removed from the mold.

Physical characteristics of edible film

Film thickness. The thickness of the edible film followed Aleman et al (2016), using a 0.0001 mm-micrometer. The measurement was done on 6 different sites to obtain the mean value.

Solubility. The measurement followed the modified method of Gontard et al (1993). The edible film sample was cut into 2 cm x 2 cm size, then dried on the filter paper at 105°C for 24 hours. The sample and filter paper were separately weighed, and the weight was determined as initial weight (W1). The sample was then put in 50 mL distilled water and soaked for 6 hours while stirred and filtered through Whatman No. 1 filter paper. The insoluble filter paper and edible film were dried at 105°C for 24 h. The sample was weighed as final weight (W2). The solubility was calculated using the following equation:

$$\text{Solubility} = \frac{W1 - W2}{W1} \times 100$$

where: W_1 = initial weight (g), and W_2 = final weight (g).

Tensile strength and elongation. Tensile strength and elongation were measured at the testing machine in which the value could be read on the monitor. The tensile strength was determined based on the maximum load at the time the edible film was disconnected, while the elongation was obtained at the range the edible film was disconnected. These were calculated as follows:

$$\text{Tensile strength} = \frac{F}{A}$$

where: F = tensile strength force (N), and A = surface area of the edible film (mm^2).

$$\text{Elongation} = \frac{B - A}{A} \times 100$$

where: A = initial length, and B = length at the disconnection.

Water vapor transmission rate. Water vapor transmission rate (WVTR) estimation followed Gontard et al (1992). The edible sample was placed on the cup until covering the cup surface that had contained silica gel (0% relative humidity), then tightened with hand rubber up to airtight. The cup was then weighed and put into a desiccator containing distilled water (100% relative humidity, 20°C). The cup was weighed every one hour for 8 hours. The water vapor transmission rate was estimated as follows:

$$\text{WVTR} = \frac{W}{A \times T}$$

where: W = weight increment of silica gel (g), A = surface area of the edible film, and T = storage time interval (hour).

Experimental design and data analysis. The experiment was set using a non-factorial Complete Randomized Design with 5 applications and 3 replications. Data analysis used ANOVA and continued with Duncan Multiple Range Test (Tapehe 2014).

Results and Discussion

Thickness. The measurements showed that mean thickness of *K. alvarezii* edible film ranged from 0.1350 to 0.1750 mm. ANOVA indicated that homogenization rate had highly significantly different effect on the edible film thickness ($p < 0.01$). The highest was recorded in control treatment (0 rpm), 0.1750 mm and the lowest at the homogenization rate of 2,750 rpm, 0.1350 mm. Duncan test (DMRT) also found significantly different effect between treatments ($p < 0.05$). Based on Duncan test, the effect of the homogenization rate application of 2,500 and 2,750 rpm is not significantly different, while the homogenization rates of 2,250 rpm, 3,000 rpm, and the control treatment yield significantly different thickness.

The thickness of the carrageenan flour-based edible film of *K. alvarezii* tended to rise with homogenization rate. The present study found that the higher the homogenization rate is, the thicker the edible film is obtained. It could result from the hydrophilic features of the carrageenan and the homogenization rate yielding nano-particle, in which the nano-particle will trap water in the matrix and the edible film becomes thicker when dried. This finding is in agreement with Anandito et al (2012) that addition of hydrophilic material could increase the edible film viscosity due to high total solid so that when dried the thickness will rise. In this study, the edible film in no homogenization treatment (control) is thicker than that with homogenization treatment. It could result from that without homogenization, the edible film solution is more viscous, larger particle size, and more total solid so that the edible film produced becomes thicker. According to Park et al (2001), solution volume, mold size, and number of total solids in the solution affect the thickness of the edible film. Besides, decreased lipid particle size in the emulsion is influenced by homogenization rate (Togas et al 2017).

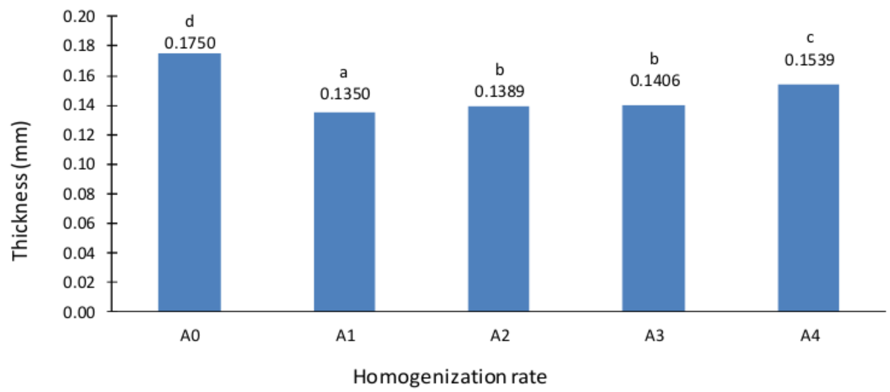

Figure 1. Mean thickness of edible film of *Kappaphycus alvarezii* carrageenan flour composite at different homogenization rate.

According to McClements (2004), the higher the homogenization rate and the longer the homogenization time are, the higher the energy will be used, which could make the edible film produced become thicker. Besides, since the edible film is a mixture of several material compositions, its solution is very viscous and has higher thickness than that of other composition (Prasetyaningrum et al 2010). In this carrageenan flour-based composite edible film, beeswax, glycerol, and sago flour were added. The compounds of beeswax and oil can make the edible film become thicker (Taqi et al 2011). The best thickness in the present study was 0.1539 mm. This thickness range is not much different from other previous studies. The thickness of the edible film reported Setyarini & Nurcahyani (2016) is 0.083-0.1660mm. It is usually less than 0.25 mm (Skurtys et al 2011).

The presence of plasticizer also influences the thickness of the edible film, in which the interstitial distance between polymer chains in the edible film matrix could increase due to the presence of plasticizer molecule distribution in the edible film (Jongjareonrak et al 2006; Tong et al 2013), that impacts to the increased thickness of it. Besides, the hydrophobic compound, such as beeswax, added into the edible film could make it be thicker (Taqi et al 2011).

Solubility. Mean solubility of the edible film ranged from 71.204 to 74.1494%. ANOVA showed that homogenization rate highly significantly influenced the solubility ($p < 0.01$), with the highest at 3,000 rpm and the lowest at 0 rpm (control treatment). Duncan test indicated that only control treatment had significantly different solubility than all other treatments, but there was not significantly different solubility $p < 0.05$) among higher homogenization rates (Figure 2). Through homogenization, lipid particle size in the emulsion declines so that the solubility rises. The solubility is an indicator to measure the film integrity, water resistance, and biodegradability of material as packing material (Cerqueira et al 2012).

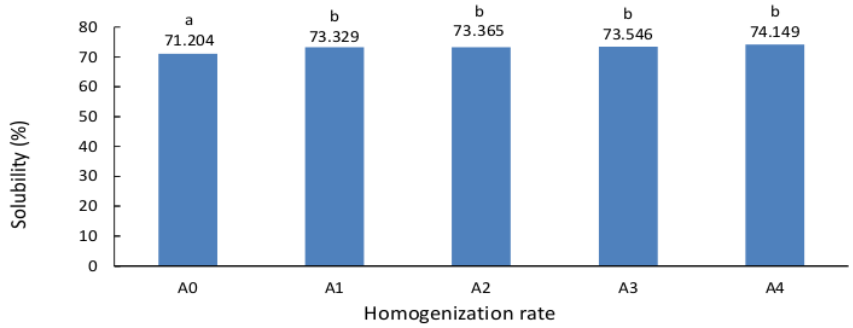

Figure 2. Mean solubility of the edible film of *Kappaphycus alvarezii* carrageenan flour composite at different homogenization rate.

Figure 2 demonstrates that the higher the homogenization rate is, the higher the solubility of the edible film will be. Also, the smaller the particle size is, the higher the solubility will be. It is in line with Weiss et al (2008) that small particle will increase the surface area causing high solubility. Besides, the intensity of homogenization affects the declined lipid particle size in the emulsion so that it could help increase the solubility in the water. The edible film processed at the homogenization rate of 3,000 rpm has higher solubility than that at 1,000 rpm (Togas et al 2017). The solubility of the best treatment was 74.1494%. This value is higher than that reported by Bourbon et al (2011), 42.05-47.11%, and almost similar to that of Rusli et al (2017), 60.51-74.20%.

Tensile strength. Mean tensile strength of the edible film ranged from 6.9 to 30.5333 N mm^{-2} (Figure 3). ANOVA showed that homogenization rate highly significantly influenced the tensile strength. Duncan test (DMRT) found the homogenization rate of 2,750 rpm had significantly different effect from that of other treatments. The highest was recorded at the homogenization rate of 2,750 rpm, 30.5333 N mm^{-2} and the lowest in control treatment (0 rpm), 6.9 N mm^{-2}.

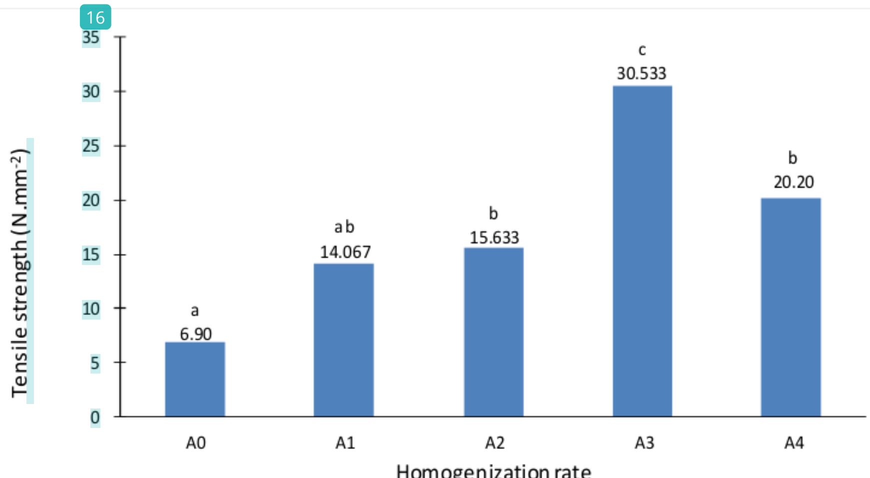

Figure 3. Mean tensile strength of the edible film of *Kappaphycus alvarezii* carrageenan flour composite at different homogenization rate.

Figure 3 demonstrates that mean tensile strength tends to increase with homogenization rate. According to Togas et al (2017), nanoemulsion application can increase the physical characteristics of the edible film, in which higher homogenization rate yields better tensile strength. Other factor affecting the tensile strength and elongation is carrageenan concentration (Irianto et al 2006; Putri & Fitrianto 2020). could result from that carrageenan is hygroscopic yielding a gel matrix that is able to increase the tensile strength and the elongation of the edible film (Abdou & Sorour 2014). The present study utilized 40% carrageenan. Higher carrageenan concentration will raise the ability to absorb water (Irianto et al 2006).

The best tensile strength in the present study was 20.20 N mm^{-2} at the homogenization rate of 3,000 rpm. High homogenization rate results in smaller particle size. According to Kohls & Beaucage (2002), the smaller particle size makes the interaction between the filler and the polymer matrix be higher so that the tensile strength rises. Zhong & Xia (2008) reported that the edible film tensile strength in the tapioca flour-based chitosan solution is 49.4 MPa. Tasha et al (2016) who used sorgum flour found higher tensile strength (17.54 kgf cm^{-2}) than that of Warkoyo et al (2014) 0.59-1.00 kgf cm^{-2}. The present study found higher tensile strength than that reported by Tasha et al (2016) and Warkoyo et al (2014).

Elongation. Mean elongation of the edible film in the present study ranged from 18.6667 to 34.6667%. ANOVA indicated that the homogenization rate possessed high significantly different effect on the elongation ($p < 0.1$). Duncan test (DMRT) found that control treatment (A0) did not yield significantly different elongation from A1 (2,250 rpm). Treatment A3 (2,750 rpm) showed no significantly different effect from A4 (3,000 rpm) as well. However, treatment A3 and A4 had significantly different effect from A0, A1, and A2. Treatment A0 and A1 had significantly different effect from A2 (Figure 4). The highest elongation was 34.6667% obtained at the homogenization rate of 2,750 rpm and the lowest was 18.6667% in control treatment (0 rpm).

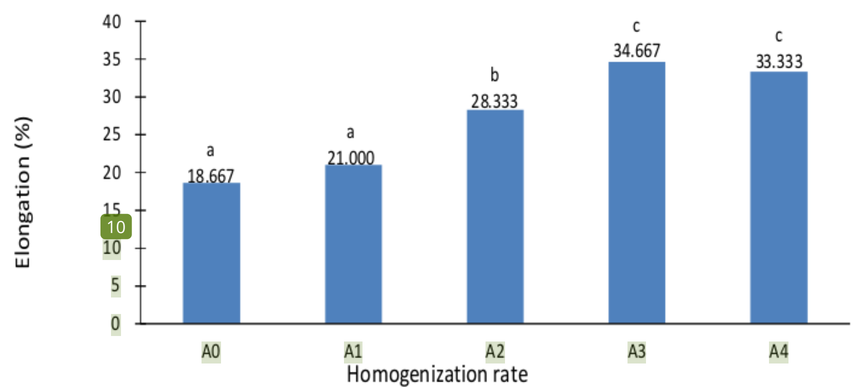

Figure 4. Mean elongation of the edible film of *Kappaphycus alvarezii* carrageenan flour composite at different homogenization rate.

Figure 4 shows that the higher the homogenization rate is, the longer the elongation will be. Edible film elongation is highly determined by the material composition. Present study utilized carrageenan flour, beeswax, glycerol, and tapioca flour in constant amount, so that the change in edible film elongation is highly possibly affected by homogenization rate. Silva et al (2012) stated that nanocrystal could strengthen plasticizer-containing edible film. Also, according to Marbun (2012), nano-particle can reduce the matrix plasticity. Edible film elongation is also influenced by glycerol addition as plasticizer (Rusli et al 2017). Like tensile strength, higher carrageenan concentration will be able to increase the elongation because carrageenan is hygroscopic (Abdou & Sorour 2014). The elongation of the best treatment in the present study was 33.3333% at the homogenization rate of 3,000 rpm. This finding is higher than that reported by Togas et al (2017), 22.5%.

Water vapor transmission rate. Mean water vapor transmission rate of the edible film ranged from 19.9775 to 27.8054 g m^{-2} h^{-1}. ANOVA showed that the homogenization rate had highly significantly different effect on the water vapor transmission rate ($p < 0.01$). Duncan multiple range test (DMRT) found that all treatments of homogenization rate resulted in significantly different water vapor transmission rate from the control treatment (A0), the homogenization rate of 2,250 rpm (A1) yielded significantly different water vapor transmission rate than that of 2,500 (A2), 2,750 (A3), and 3,000 rpm (A4), and the homogenization rate of 3,000 rpm had significantly different effect than that of 2,500 and 2,750 rpm, but treatment A2 and A3 had no significant different effect ($p < 0.05$). It means that the homogenization rate influences the water vapor transmission rate.

Mean water vapor transmission rate tends to decline with increased homogenization rate (Figure 5). It could result from higher tensile strength and thickness of the edible film at high homogenization rate. High tensile strength could inhibit the water vapor permeability, while high thickness could inhibit water diffusion into the film. The structure of the composing materials and plasticizer concentration highly influences the water vapor transmission rate as well. Plasticizer affects the flexibility and permeability (Ulusoy et al 2018). According to Galus & Kadzinska (2015), homogenization intensity can affect the lipid size in the emulsion in relation with declined water vapor permeability rate of the edible film dried. Moreover, Togas et al (2017) stated that the edible film processing under high homogenization rate had lower water vapor transmission rate than that under lower rate. The best water vapor transmission rate obtained in this study was 19.9775 g m^{-2} h^{-1}. This value is lower than that reported by Togas et al (2017), 25.3411 g m^{-2} h^{-1}. Tasha et al (2016) found higher water vapor transmission rate, 616.226 g m^{-2} 24 h^{-1}. The nano-particle-involving edible film has an optimum composition in nano-particle material usage to obtain the mechanic properties and better water vapor inhibitor (Rafieian & Simonsen 2014).

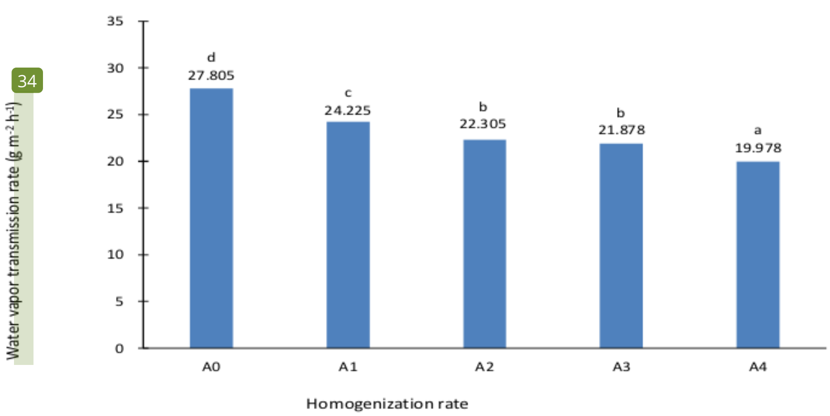

Figure 5. Mean water vapor transmission of the edible film of *Kappaphycus alvarezii* carrageenan flour composite at different homogenization rates.

The physical characteristics cover thickness, solubility, tensile strength, elongation, and water vapor transmission rate. The water vapor transmission rate is one of the determinants that can influence the permeability of the packaging. Standard edible film processing with a maximum WVTR of 10 g m^{-2} d^{-1} refers to the Japanese Industrial Standard (JIS) Z 1707: 1975 concerning plastic film for food packaging (Santoso et al 2007). Afifah et al (2018) reported that good carrageenan-based edible film has a WVTR of 25.38 g m^{-2} d^{-1}, while de Moura et al (2008) found that oxygen permeability and water vapor attraction can be developed through nanotechnology application. Moreover, Supeni (2012) stated that carrageenan can reduce the vapor water transmission rate of the edible film. Our previous study using 4.5% carrageenan and 0.8% beeswax composite homogenized at 3000 rpm for 5 min obtained good edible film quality for vapor water transmission rate, elongation, and solubility, but not for thickness and tensile strength. The film is easily torn and hardly glued (Togas et al 2017).

Conclusions. Homogenization rate affected the physical characteristics of *Kappaphycus alvarezii* edible film product, i.e. thickness, solubility, tensile strength, elongation, and water vapor transmission rate. The best treatment was recorded in the nano-emulsion of 4% *Kappaphycus alvarezii*, 1.2% beeswax, and 2% glycerol at the homogenization rate of 3000 rpm. The best physical characteristics were found at 0.1539 mm thickness, 74.1494% solubility, 20.20 N mm^{-2} tensile strength, 33.3333% elongation, 19.9775 g m^{-2} h^{-1} water vapor transmission. Edible film produced could be applied as plastic packaging substitute of fisheries processed product. The carrageenan edible film as food packaging material could reduce plastic waste issues and increase the seaweed development potency in North Sulawesi.

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