Characteristics and Properties of Biofilms Made from Pure Carrageenan Powder and Whole Seaweed (*Kappaphycus* sp.)

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**ABSTRACT**

Petroleum-based plastics are mass produced to meet customers’ demand due to their low cost and versatility. However, plastic waste has become a serious environmental problem. Hence, degradable plastics from renewable sources (e.g. biomass) are now trending for their “green” properties. In this paper, properties of biofilms made from whole seaweed (WS), *Kappaphycus* sp. and pure kappa-carrageenan powder (PC) were compared. Glycerol, as plasticizer, was added at differing amounts (1%, 2%, 3%, 4% and 5%, v/v) and their appearance, physical and mechanical properties, solubility, and biodegradability were studied. As results, for colour difference and transparency, WS-1% showed higher ΔE at 17.09 ± 0.85 with highest opacity at 13.73 mm⁻¹ and least ΔE was at 2.73 ± 0.13 for PC-5% with opacity at 0.49 mm⁻¹. For mechanical properties, PC-1% has the highest tensile strength and elastic modulus at 26.63 ± 2.18 MPa and 253.53 ± 19.43 MPa, respectively, whereas WS-5% has the lowest at 0.71 ± 0.15 MPa and 2.47 ± 0.44 MPa, respectively. As for biodegradability, by the first week, WS-5% lost 80% of its weight and PC-1% only lost 3%. Overall, PC biofilms showed better quality in terms of mechanical and physical properties but WS biofilms were faster to degrade and dissolve in water. Glycerol concentration affects most of the properties except for mechanical properties for WS and solubility of both. This study suggests that PC may be a better base material for stronger biofilms but WS are a better choice from environmental and cost aspects.

**Keywords:** Biodegradable; biopolymer; ecofriendly; kappa carrageenan; red algae

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1. Introduction

Algae or seaweed have been cultivated for their valuable gelatinous substance known as hydrocolloids, namely, alginate, agar and carrageenan. These are used as source of food, feed, fertilizor and even medicine because it contains protein, iodine, bromine, vitamin and substance of stimulatory and antibiotic nature, other than being used as gelling, stabilizing and thickening agents in industries [1,2]. Seaweed is the common name for countless species of marine plants and algae that grow in the oceans, rivers, lakes, and other water bodies [3]. The chemical composition of
seaweed is very versatile, varying with species and the harvesting season [4]. Generally, they consist of an organic fraction, mostly fiber and ashes, and inorganic fraction such as macro- and micro-minerals and nutrients [5]. Unlike terrestrial plants, some seaweed contain cellulose and anionic sulphated polysaccharides (carrageenan, agar and alginates) as the main component in their cell wall [6].

Plastics, mainly those of one time use, are thrown out as garbage which accumulate in the environment becoming rubbish where it has created a monumental problem on our health, ecosystems and wildlife due to its non-biodegradable properties. Furthermore, recycling them cost more and incinerating them produce harmful chemicals to the environment contributing to global warming [7]. According to an article in national geographic magazine by Parker [8], 8.8 million tonnes of plastic waste winds up in our ocean each year which is enough to cover every foot of the coastline around the world. Hence, an emerging solution which allows decomposition of the plastic waste was proposed by substituting plastic bag make-up material with biodegradable materials [9].

In recent years, biofilm from seaweed hydrocolloids, especially alginate and carrageenan, shows promising potential due to the good gas barrier and mechanical properties [10]. Few previous studies, as summarized by Tavassoli-Kafreni and others [11], seaweed-based bioplastic were focusing on blends with other materials, such as, chitosan, cellulose, antimicrobial compounds, essential oil and others, mainly for coating and packaging food. The development of bioplastic requires the polysaccharides, which acts as the main polymer chain, to form the bioplastic, where plasticizer is incorporated for its ability to soften and make bioplastic more flexible [12]. The more commonly used plasticizer in studies are glycerol especially in incorporating with seaweed-based polysaccharides [2, 13-16]. In this study, two types of biofilms were prepared namely from pure kappa-carrageenan (PC) and whole Kappaphycus sp. Seaweed (WS). The aim is to investigate if biofilms from WS are comparable to PC so that the long carrageenan extraction procedures and biomass waste could possibly be avoided. Both types of biofilms were added with glycerol at 1, 2, 3, 4 and 5 % (v/v), respectively, to reduce brittleness. Their characteristics were tested using standard methods.

2. Materials and Methods

2.1 Sample Preparation

Pure carrageenan powder (C1013) was purchased from Sigma-Aldrich (Germany). Meanwhile, fresh and healthy red seaweed, Kappaphycus sp., was obtained from Semporna, Sabah. Kappaphycus sp. was washed under running water and dried at 60°C overnight. It was then blended into powder form before being used in development of biofilms.

2.2 Development of Biofilms

The method used is as described by Rahmawati et al., [17] with slight modification. Exactly 2.0 g of the biomass materials (PC and WS, respectively) was dissolved in 100 mL of deionized water at 60°C and left to settle at room temperature for 30 minutes. This is followed by addition of glycerol (99% purity) to the solution at 1%, 2%, 3%, 4% and 5% (v/v), respectively, and heated to 80°C and maintained for 1 minute. By using a casting method, the solution was poured into a petri dish and left to dry at 50°C overnight in a ventilated dryer at 49 ± 4% RH before being peeled off.
2.3 Moisture Content

Adapted from a study by Ili Balqis et al., [18] with slight modification, the initial weight of sample was recorded before being heated in an oven at 105°C for 24 hours. The final weight was recorded and moisture content was determined as in Eq. (1).

\[
\text{Moisture Content (\%) } = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%
\]  

(1)

2.4 Colour and Transparency

The method used is as described by Tessaro et al., [19], where colour difference were determined using Colour Reader (Konika Minolta) and subtract the background reading (L* = 87.2, a* = +5.8 and b* = -3.3) from the sample readings (L, a, and b) as in Eq. (2).

\[
\text{Colour Difference (\Delta E)} = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2}
\]

(2)

Transparency was determined using the method adapted from Moey et al., [20], where biofilm sample was placed into the sample holder of UV-Vis Spectrophotometer (Agilent Cary 60) and read for absorbance at wavelength 660nm. Transparency (T) was determined from Eq. (3), where thickness was measure using digital caliper (Mitutoyo) before being placed in the UV-Vis spectrophotometer.

\[
\text{Transparency (T)} = \frac{\text{Absorbance}}{\text{Film Thickness}}
\]

(3)

2.5 Mechanical Properties

For mechanical properties biofilm samples were cut into strips of 15mm x 90mm and thickness were measured before being fitted onto the sample holder of a Universal Tensile Machine (Gotech Instruments) [20].

2.6 Solubility

Method adapted from Moey and others [20] were modified to determine solubility rate. Solubility of biofilms was determined using the rate of solubility as in Eq. (4), by recording the time taken to dissolve 25 x 30 mm² sample in 125 ml of water in different condition; tap, distilled, and hot (90°C), while continuously stirred at 200rpm.

\[
\text{Solubility} = \frac{25 \times 30 \times \text{thickness (mm}^3\text{)}}{\text{time (s)}}
\]

(4)

2.7 Surface Morphology

The surface morphology of the biofilm was determined by using Scanning Electron Microscopy (Hitachi S-3400N) to obtain the scanning electron micrographs at 500x magnification.
2.8 Biodegradability

Biodegradability were tested using soil burial test as suggested by Ismail et al., [21] with slight modification, where biofilm samples were buried both indoor (room temperature, no outer stimuli, 49 ± 4% RH) and outdoor (soil MC 38.2 ± 3.2%, 28 ± 2°C, 52 ± 3% RH). Percentage of degradation were determined using Eq. (5).

\[
\text{Percentage of degradation (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%}
\]  

(5)

2.9 Statistical Analysis

All data collected were analyzed using Analysis of Variance (ANOVA) and Duncan’s Multiple Range Test (DMRT) as the post-hoc analysis to determine the relationship between PC and WS biofilms, also in the changes affected by glycerol content.

3. Results and Discussion

3.1 Moisture Content

Moisture content was found to be higher in percentage with increase of glycerol even though drying condition were the same as shown in Figure 1. Both PC and WS biofilms were highest at 5% at 75.5 ± 0.3% and 76.6 ± 0.2%, respectively, while with the lowest at 1% with readings of 30.3 ± 5.0% and 32.2 ± 3.0%, respectively. Thickness of the biofilms ranged from 0.05 ± 0.01 mm to 0.16 ± 0 mm and 0.04 ± 0 mm to 0.09 ± 0 mm for PC and WS biofilms, respectively, where both PC and WS biofilms were the thinnest at 1% while the thickest at 5%.

Glycerol, due to its hydrophilic and polar properties, tend to absorb and maintain the water in the body of the biofilm as a reaction of the hydroxyl group (OH) with water through hydrogen bonding [18]. Due to more moisture absorbed, naturally, higher glycerol content leads to thicker biofilms [22]. Carrageenan, which is the polysaccharide that supply the main polymeric chain to form bioplastic, react with more scattered glycerol in the mixture while creating more space in the film matrix, hence, directly increasing the thickness of biofilm made of commercial carrageenan [23]. The entrapment of moisture for both PC and WS biofilms were proven statistically to be not affected by source of base material (P>0.05) and more significantly affected by glycerol content (P<0.05).
3.2 Colour and Transparency

Colour difference of biofilms were obtained using a colour reader by using white A4 paper as a base, reading $\Delta E = 0$. Since the biofilms were placed on top of the white A4 paper, the transparency of biofilm affects the colour reading directly. So, biofilms of higher transparency gave a lower $\Delta E$ reading. The obvious difference in $\Delta E$ between PC and WS biofilms were because the commercial carrageenan powder was white, it produces milky colour biofilm and was transparent, whereas for WS biofilms, whole seaweed was used hence producing slightly transparent, light green colour biofilm as shown in Figure 2(a) and (b), respectively.

![Fig. 2. (a) PC-biofilm and (b) WS-biofilm](image)

Figure 3 illustrates the comparison of biofilms to a white A4 which lies at 0. The colour reader gives the direct reading of $L$, $a$ and $b$, where, $L$ shows black (0) to white (100), $a$ shows green (-) to red (+) and $b$ shows blue (-) to yellow (+) of the biofilm itself. However, the value of the A4 paper, $L^*$, $a^*$ and $b^*$, was subtracted from the biofilms to get $\Delta L$ difference in lightness (+) and darkness (-), $\Delta a$ greener (-) or redder (+) and $\Delta b$ bluer (-) or yellower (+), compared to the white A4 paper. As resulted, the most colour difference ($\Delta E$) for PC biofilms was at 1% and least at 5% by 6.16 ± 0.80 and 2.73 ± 0.13, respectively, with significant difference of $P<0.05$ or 1% with 2%. Whereas for WS biofilms, the highest $\Delta E$ was at 17.09 ± 0.85 and lowest at 11.53 ± 0.37 for 1% and 5% WS biofilms, respectively.

![Fig. 3. Comparison of colour difference between biofilms](image)

As for the colour comparison as shown in Table 1, biofilm becomes lighter as more glycerol was added where biofilms were lightest in colour at 5% and darker in colour at 1% for both PC and WS biofilms, respectively. To compare it more generally, WS biofilms were darker by -6.95 ± 1.08, greener by -1.14 ± 0.07, and more yellowish by 11.57 ± 1.87 than the white A4 paper, meanwhile, PC biofilms
were only slightly darker by \(-1.83 \pm 0.3\) greener by \(-0.42 \pm 0.03\) and slightly more yellow by \(3.76 \pm 1.42\).

Addition of glycerol is also known to decrease opacity of biofilm because elongated polymer chains can move more freely in the biofilm matrix due to the increase in intermolecular space, hence, allowing light to pass through easily [23]. Transparency of biofilm also shows the compatibility of the mixture, as inhomogeneous mixture tend to disperse light leading to less light transmitted through the biofilm resulting in a more opaque biofilm [24]. As shown in Figure 4, most transparent for PC-5% at 0.493 mm\(^{-1}\) whereas for WS biofilm was at 0.756 mm\(^{-1}\) for WS-5%, whereas most opaque for both PC and WS biofilms were at 1% glycerol content at 5.78 mm\(^{-1}\) and 13.725 mm\(^{-1}\), respectively. The sudden drop to 0.927 mm\(^{-1}\) at WS-2% indicates good ratio mix of base material with glycerol.

Table 1
Comparison of difference in colour by lightness/darkness (\(\Delta L\)), greenness/redness (\(\Delta a\)), blueness/yellowness (\(\Delta b\)) and total colour difference (\(\Delta E\)) for PC and WS biofilms

| Glycerol | PC | WS |
|----------|----|----|
|          | \(\Delta L\) | \(\Delta a\) | \(\Delta b\) | \(\Delta E\) | \(\Delta L\) | \(\Delta a\) | \(\Delta b\) | \(\Delta E\) |
| 1%       | -2.30 \(\pm\) 0.46\(^a\) | -0.40 \(\pm\) 0.09\(^a\) | 5.70 \(\pm\) 0.80\(^a\) | 6.16 \(\pm\) 0.56\(^a\) | -8.77 \(\pm\) 0.05\(^a\) | -1.10 \(\pm\) 0.02\(^a\) | 14.63 \(\pm\) 0.36\(^a\) | 17.09 \(\pm\) 0.40\(^a\) |
| 2%       | -1.87 \(\pm\) 0.03\(^a\) | -0.47 \(\pm\) 0.05\(^a\) | 3.10 \(\pm\) 0.50\(^a\) | 3.65 \(\pm\) 0.60\(^a\) | -7.00 \(\pm\) 0.02\(^a\) | -1.13 \(\pm\) 0.02\(^a\) | 11.90 \(\pm\) 0.36\(^a\) | 13.85 \(\pm\) 0.36\(^a\) |
| 3%       | -1.80 \(\pm\) 0.03\(^a\) | -0.43 \(\pm\) 0.05\(^a\) | 2.57 \(\pm\) 0.60\(^a\) | 3.19 \(\pm\) 0.50\(^a\) | -6.57 \(\pm\) 0.02\(^a\) | -1.20 \(\pm\) 0.02\(^a\) | 10.87 \(\pm\) 0.86\(^a\) | 12.76 \(\pm\) 0.86\(^a\) |
| 4%       | -1.73 \(\pm\) 0.03\(^a\) | -0.40 \(\pm\) 0.10\(^a\) | 2.47 \(\pm\) 0.10\(^a\) | 3.05 \(\pm\) 0.45\(^a\) | -6.40 \(\pm\) 0.02\(^a\) | -1.17 \(\pm\) 0.02\(^a\) | 10.70 \(\pm\) 0.53\(^a\) | 12.52 \(\pm\) 0.53\(^a\) |
| 5%       | -1.47 \(\pm\) 0.03\(^a\) | -0.40 \(\pm\) 0.05\(^a\) | 2.27 \(\pm\) 0.10\(^a\) | 2.73 \(\pm\) 0.45\(^a\) | -6.00 \(\pm\) 0.02\(^a\) | -1.20 \(\pm\) 0.02\(^a\) | 9.77 \(\pm\) 0.13\(^a\) | 11.53 \(\pm\) 0.13\(^a\) |
|          | 0.15 \(\pm\) 0.03\(^a\) | 0.06 \(\pm\) 0.10\(^a\) | 0.13 \(\pm\) 0.45\(^a\) |                  | 0.20 \(\pm\) 0.02\(^a\) | 0.10 \(\pm\) 0.02\(^a\) | 0.31 \(\pm\) 0.13\(^a\) | 0.37 \(\pm\) 0.13\(^a\) |

Data reported are mean \pm standard deviation and values of different letters \(^a-d\) of the same column are significantly different (P<0.05) to each other

Fig. 4. Comparison of transparency between biofilms

3.3 Surface Morphology

SEM images of PC and WS biofilms were taken at the magnification of 500x as shown in Figure 5, from lowest glycerol content to the highest. As observed in the micrographs, some small white spots are apparent which due to the exposure to dust and impurities during storage period. From the micrograph, PC-1% shows a smooth surface, however, there were cracks observed due to not enough
moisture. Overall, there were not much difference for PC biofilms 2% to 5% which may indicate that the biofilms were homogenous and smooth on the surface with slight impurities on top.

For WS biofilms, 1% glycerol showed slightly cracked surface with growth of mold since it was taken three months after the development of the biofilms. As for WS-2%, it can be observed that the image of the film was homogenous and smooth, however, mold was observed to start to grow when the micrographs were taken. Micrographs of WS-3% show slightly murky image, whereas WS-4% and WS-5% have murkier image, which show the inhomogeneity of the mixture at higher content of glycerol.

![Fig. 5. SEM Micrographs for each biofilm samples at 500x magnification](image)

### 3.4 Mechanical Properties

Mechanical properties tested include tensile strength, elastic modulus, and elongation at break. A previous study by Tong et al., [25] showed that addition of plasticizer decreases the tensile strength but increases elasticity of biofilms. Elasticity can also be represented by Young’s modulus or elastic modulus which is the measurement of stiffness, where lower value means higher elasticity [26].

Table 2 shows the comparison of mechanical properties of PC and WS biofilms at different concentrations of glycerol. As glycerol increased, the tensile strength decreased with the highest tensile strength at PC-1% and lowest at PC-5% at 26.63 ± 2.18 MPa and 2.93 ± 0.34 MPa, respectively. Lower tensile strength gives higher degree of elasticity which means lower elastic modulus, where the highest was at PC-1% and lowest at PC-5% at 253.53 ± 19.43 MPa and 10.89 ± 2.60 MPa, respectively. As for the maximum elongation, PC biofilm that elongated the most was at 47.3 ± 4.7% and the least at 27.0 ± 1.7% of their initial length at glycerol content of PC-2% and PC-1%, respectively.

For WS biofilms, the highest tensile strength was at WS-1% and lowest at WS-5% at 2.07 ± 0.15 MPa and 0.71 ± 0.15 MPa, respectively. Elastic modulus was the highest at WS-1% and lowest at WS-5% at 8.05 ± 0.78 MPa and 2.47 ± 0.44 MPa, respectively, and showed only a significant difference statistically at P<0.05 for pairing of 1% with 2% and 2% with 5%. Although the result for maximum elongation was not significant (P>0.05), WS-1% shows the least elongated and most at WS-2% at 66.8 ± 1.8% and 79.2 ± 10.1 %, respectively.

The obvious difference between PC biofilms and WS biofilms in tensile strength and elastic modulus is due to the percentage of carrageenan content in the mixture. This is because, prior to the study, extraction of semi-refined carrageenan using alkaline solution (6% KOH) on *Kappaphycus* sp. which yielded about 31.8 ± 0.5% (w/w) semi-refined carrageenan, hence, the make-up material of WS biofilms have less binding carrageenan properties compared to pure carrageenan. As the binding intermolecular force was weaker, the polymeric chain was able to stretch out more, hence reducing the stiffness enabling WS biofilms to be more elastic but weaker than PC biofilms.
Table 2
Comparison of mechanical properties of PC and WS biofilms

| Glycerol | PC |   |   |   | WS |   |   |   |
|----------|----|---|---|---|----|---|---|---|
|          | Tensile Strength (MPa) | Elastic Modulus (MPa) | Maximum Elongation (%) |      | Tensile Strength (MPa) | Elastic Modulus (MPa) | Maximum Elongation (%) |      |
| 1%       | 26.63 ± 2.18<sup>a</sup> | 253.53 ± 19.43<sup>a</sup> | 27.0 ± 1.7<sup>a</sup> | 2.07 ± 0.15<sup>a</sup> | 8.05 ± 0.78<sup>a</sup> | 66.8 ± 1.8<sup>a</sup> | 1.8<sup>a</sup> |      |
| 2%       | 18.36 ± 1.00<sup>b</sup> | 69.52 ± 11.01<sup>b</sup> | 47.3 ± 4.7<sup>b</sup> | 1.95 ± 0.17<sup>a</sup> | 6.71 ± 0.26<sup>b</sup> | 79.2 ± 10.1<sup>b</sup> |      |      |
| 3%       | 7.53 ± 0.99<sup>c</sup> | 48.02 ± 8.49<sup>c</sup> | 36.9 ± 2.9<sup>c</sup> | 1.33 ± 0.23<sup>b</sup> | 3.98 ± 0.21<sup>c</sup> | 73.1 ± 2.6<sup>b</sup> |      |      |
| 4%       | 5.72 ± 1.09<sup>d</sup> | 18.60 ± 1.09<sup>d</sup> | 42.7 ± 3.6<sup>b</sup> | 1.05 ± 0.18<sup>b</sup> | 3.45 ± 0.44<sup>c</sup> | 70.7 ± 6.2<sup>b</sup> |      |      |
| 5%       | 2.93 ± 0.34<sup>e</sup> | 10.89 ± 2.60<sup>d</sup> | 42.1 ± 5.1<sup>c</sup> | 0.71 ± 0.15<sup>c</sup> | 2.47 ± 0.57<sup>c</sup> | 68.5 ± 6.2<sup>c</sup> |      |      |

Data reported are mean ± standard deviation and values of different letters <sup>a</sup>-<sup>d</sup> of the same column are significantly different (P<0.05) to each other.

3.5 Solubility

Solubility rate of biofilms in tap water, distilled water and hot water were as presented in Table 3 for both PC and WS biofilms. For PC biofilms in tap water and hot water, PC-1% had the lowest solubility rate at 2.30 ± 0.2 mm<sup>3</sup>/min and 48.45 ± 4.12 mm<sup>3</sup>/min, respectively. Meanwhile the fastest was at PC-5% with 8.81 ± 0.58 mm<sup>3</sup>/min and 148.04 ± 4.69 mm<sup>3</sup>/min for tap and hot, respectively. As for distilled water, the highest rate was shown by PC-4% and lowest at PC-1% at 7.54 ± 0.07 mm<sup>3</sup>/min and 2.39 ± 0.12 mm<sup>3</sup>/min, respectively.

Table 3
Comparison of solubility rate of PC and WS biofilms in water of different conditions

| Glycerol | PC |   |   |   | WS |   |   |   |
|----------|----|---|---|---|----|---|---|---|
|          | Tap | Distilled | Hot |      | Tap | Distilled | Hot |      |
| 1%       | 2.30 ± 0.20<sup>c</sup> | 2.39 ± 0.12<sup>b</sup> | 48.45 ± 4.12<sup>d</sup> | 5.13 ± 0.54<sup>b</sup> | 11.64 ± 2.73<sup>b</sup> | 72.24 ± 5.21<sup>c</sup> |      |      |
| 2%       | 3.67 ± 0.54<sup>b</sup> | 3.27 ± 0.05<sup>c</sup> | 90.05 ± 2.57<sup>d</sup> | 5.48 ± 0.88<sup>b</sup> | 12.56 ± 1.28<sup>a</sup> | 122.92 ± 28.18<sup>b</sup> |      |      |
| 3%       | 4.52 ± 0.76<sup>b</sup> | 4.18 ± 0.19<sup>b</sup> | 120.36 ± 8.04<sup>b</sup> | 7.36 ± 1.49<sup>a</sup> | 12.67 ± 2.42<sup>b</sup> | 137.07 ± 20.79<sup>ab</sup> |      |      |
| 4%       | 8.13 ± 0.63<sup>a</sup> | 7.54 ± 0.07<sup>a</sup> | 140.42 ± 5.80<sup>ab</sup> | 7.91 ± 1.96<sup>a</sup> | 13.62 ± 1.55<sup>a</sup> | 148.64 ± 15.17<sup>a</sup> |      |      |
| 5%       | 8.81 ± 0.58<sup>a</sup> | 7.50 ± 0.15<sup>a</sup> | 148.04 ± 4.69<sup>a</sup> | 8.87 ± 0.61<sup>a</sup> | 19.84 ± 2.13<sup>a</sup> | 160.62 ± 13.70<sup>a</sup> |      |      |

Data reported are mean ± standard deviation and values of different letters <sup>a</sup>-<sup>d</sup> of the same column are significantly different (P<0.05) to each other.

As for WS biofilms, solubility rate for tap, distilled and hot water were all highest at WS-5% at 8.87 ± 0.61 mm<sup>3</sup>/min, 19.84 ± 2.13 mm<sup>3</sup>/min and 160.62 ± 13.70 mm<sup>3</sup>/min, respectively. Meanwhile, the lowest was WS-1% at 5.13 ± 0.54 mm<sup>3</sup>/min, 11.64 ± 2.73 mm<sup>3</sup>/min and 72.24 ± 5.21 mm<sup>3</sup>/min, respectively. As shown in Figure 6, there are significant difference (P<0.05) in the solubility rates in all the three conditions, which is most likely due to the carrageenan content in the biofilms. Carrageenan is known to form a very stable structure in biofilm formation which directly lower the gap available for water movement and in turn slows down the time to dissolve the biofilm [2]. This may explain the higher solubility property of the WS biofilms compared to PC biofilms. As a general comparison, PC and WS biofilms showed no significant difference (P>0.05) compared to each other.
for tap and distilled water. However, there was significant difference (P<0.05) for hot water when compared.

![Fig. 6. Comparison of Solubility between biofilms made of PC and WS](image)

### 3.6 Biodegradability

Biodegradability test were conducted both indoors and outdoors, however, outdoors data were unable to be retrieved due to the tropical weather of unexpected rain. Figure 7 shows the degradation rate of biofilms buried indoors for four weeks. Since glycerol encourages water retention in biofilms, microorganism from the soil react to the water within the biofilm and starts decomposing due to breakdown of polymer chains in the biofilms, naturally, higher moisture retained by biofilm tend to encourage more microorganism activity hence, enhancing the biodegradation process [9, 21, 27]. As in Figure 7, reaction was slow for PC biofilms after the first week but a sudden rise in the second week due to the biofilm matrix in which PC biofilms were held together more strongly than WS biofilms.

![Fig. 7. Comparison of biodegradability buried indoors with timeframe of 4 weeks for PC- and WS-biofilms](image)

Since no water was continuously spread on soil after it was dug up, the soil dried up and less activity was observed after one week. Conditions of the bioplastic showed only slight change between week 1 and 2, then the condition remained constant after week 2 for both PC and WS biofilms as shown in Table 4. After one month, water was sprayed on the soil daily to heighten the moisture in soil for a week, it was found that all the biofilms were completely decomposed at the end of the week.
### Table 4
Comparison of biodegradability at after 1 and 2 week of indoor soil burial test for PC and WS biofilms

|       | Week 1 (%) |       | Week 2 (%) |       |
|-------|------------|-------|------------|-------|
| PC    | 1%         | 2%    | 3%         | 4%    | 5%    |
|       | [Image]    | [Image] | [Image]    | [Image] | [Image] |
|       | [Image]    | [Image] | [Image]    | [Image] | [Image] |
| WS    | Week 1     |       | Week 2     |       |
|       | [Image]    | [Image] | [Image]    | [Image] | [Image] |

3.7 Applications

The biofilms produced show potential to replace petroleum-based plastic and non-degradable packaging especially those that do not require great mechanical properties, for instance the single-use packaging for instant noodle, flavouring, coffee mix, candy wrapper and others. Application of biofilms produced can be modified according to their needs. Table 5 shows examples of packaged items using PC and WS biofilms to show the potential of incorporating them as an alternative for food packaging. Studies on the properties of these applications such as shelf-life, odour and other properties are still ongoing.

### Table 5
Instant noodle, coffee mix, and flavouring repackaged in PC- and WS-based biofilms

|                          | Current Packaging | PC-based Biofilm | WS-based Biofilm |
|--------------------------|-------------------|------------------|------------------|
| **Coffee Mix**           | ![Image]          | ![Image]         | ![Image]         |
| **Flavours**             | ![Image]          | ![Image]         | ![Image]         |
| **Instant Noodles**      | ![Image]          | ![Image]         | ![Image]         |
4. Conclusion

It is shown here that using PC as base material gives a tougher and smoother bioplastic product, however, it takes longer to dissolve or degrade compared to WS biofilms. This is an important factor to consider in promoting it as an edible biofilm. Besides much cheaper in price than PC, WS-based biofilms have a natural colour which can be promoted as natural colour packaging and more cost-effective industrially. Biofilms made of whole seaweed promotes chemical- and waste-free packaging and modification can be made on the recipe to suit the biofilms’ application in any industry. Although the mechanical properties may be in shortfall compared to conventional plastic, but this is an initiative to tackle single use plastic packaging. Furthermore, research on improving the mechanical properties of WS biofilms via blending with fillers such as cellulose from agriculture waste are on-going in our lab.

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References

[1] El-Din, Soad M. Mohy, and Amani MD El-Ahwany. “Bioactivity and phytochemical constituents of marine red seaweeds (Jania rubens, Corallina mediterranea and Pterocladia capillacea).” *Journal of Taibah University for Science* 10, no. 4 (2016): 471-484. https://doi.org/10.1016/j.jtusci.2015.06.004

[2] Paula, Gabriela A., Norma MB Benevides, Arcelina P. Cunha, Ana Vitória de Oliveira, Aláides MB Pinto, João Paulo S. Morais, and Henriette MC Azeredo. "Development and characterization of edible films from mixtures of κ-carrageenan, ι-carrageenan, and alginate." *Food Hydrocolloids* 47 (2015): 140-145.

[3] Qin, Yimin, ed. *Bioactive Seaweeds for Food Applications: Natural Ingredients for Healthy Diets*. Academic Press, 2018.

[4] Yong, Yoong Soon, Wilson Thau Lym Yong, Su En Ng, Ann Anton, and Suhaimi Yassir. "Chemical composition of farmed and micropropagated Kappaphycus alvarezi (Rhodophyta, Gigartinales), a commercially important seaweed in Malaysia." *Journal of Applied Phycology* 27, no. 3 (2015): 1271-1275. https://doi.org/10.1007/s10811-014-0398-z

[5] Fayaz, Mohamed, K. K. Namitha, KN Chidambara Murthy, M. Mahadeva Swamy, R. Sarada, Salma Khanam, P. V. Subbarao, and G. A. Ravishankar. "Chemical composition, iron bioavailability, and antioxidant activity of Kappaphycus alvarezi (Doty)." *Journal of agricultural and food chemistry* 53, no. 3 (2005): 792-797.

[6] Holdt, Susan Løvstad, and Stefan Kraan. "Bioactive compounds in seaweed: functional food applications and legislation." *Journal of applied phycology* 23, no. 3 (2011): 543-597.

[7] Rajendran, N., Sharanya Puppala, M. Sneha Raj, B. Ruth Angeeleena, and C. Rajam. "Seaweeds can be a new source for bioplastics." *Journal of Pharmacy Research* 5, no. 3 (2012): 1476-1479.

[8] Parker, Laura. "We made plastic. We depend on it. Now we’re drowning in it." *National Geographic* 16 (2018). https://doi.org/10.7312/asme19001-009

[9] Emadian, S. Mehdi, Turgut T. Onay, and Burak Demirel. "Biodegradation of bioplastics in natural environments." *Waste management* 59 (2017): 526-536. https://doi.org/10.1016/j.wasman.2016.10.006

[10] Praseptiangga, D. "Development of seaweed-based biopolymers for edible films and lectins." In *International Conference On Food Science and Engineering 2016*, pp. 012003-1. 2017. https://doi.org/10.1088/1757-899X/193/1/012003

[11] Tavassoli-Kafrawi, Elham, Hajar Shekarchizadeh, and Mahdieh Masoudpour- Behabadi. "Development of edible films and coatings from alginates and carrageenans." *Carbohydrate polymers* 137 (2016): 360-374. https://doi.org/10.1016/j.carbpol.2015.10.074

[12] Onen Cinar, Senem, Zhi Kai Chong, Mehmet Ali Kucuker, Nils Wieczorek, Ugur Cengiz, and Kerstin Kuchta. "Bioplastic Production from Microalgae: A Review." *International Journal of Environmental Research and Public Health* 17, no. 11 (2020): 3842. https://doi.org/10.3390/ijerph17113842
[13] Gozan, Misri, and Citra Noviasari. "The effect of glycerol addition as plasticizer in Spirulina platensis based bioplastic." In *E3S Web of Conferences*, vol. 67, p. 03048. EDP Sciences, 2018.

[14] Dianursanti, Citra Noviasari, Larasati Windiani, and Misri Gozan. "Effect of compatibilizer addition in Spirulina platensis based bioplastic production." In *AIP Conference Proceedings*, vol. 2092, no. 1, p. 030012. AIP Publishing LLC, 2019.

[15] Machmud, M. Nizar, Reza Fahmi, Rohana Abdullah, and Coco Kokarkin. "Characteristics of red algae bioplastics/latex blends under tension." *International Journal of Science and Engineering* 5, no. 2 (2013): 81-88.

[16] Zarina, Siti, and Ishak Ahmad. "Biodegradable composite films based on κ-carrageenan reinforced by cellulose nanocristal from kenaf fibers." *BioResources* 10, no. 1 (2015): 256-271.

[17] Rahmawati, M., M. Arief, and W. H. Satyantini. "The Effect of Sorbitol Addition on the Characteristic of Carrageenan Edible Film." *Earth and Environmental Sciences* 236 (2019): 1-8.

[18] Balqis, AM Ili, MAR Nor Khaizura, A. R. Russly, and ZA Nur Hanani. "Effects of plasticizers on the physicochemical properties of kappa-carrageenan films extracted from Eucheuma cottonii." *International Journal of Biological Macromolecules* 103 (2017): 721-732.

[19] Luchese, Cláudia Leites, Tania Garrido, Jordana Corralo Spada, Isabel Cristina Tessaro, and Koro de la Caba. "Development and characterization of cassava starch films incorporated with blueberry pomace." *International Journal of Biological Macromolecules* 106 (2018): 834-839.

[20] Moey, Siah Watt, Aminah Abdullah, and Ishak Ahmad. "Development, characterization and potential applications of edible film from seaweed (Kappaphycus alvareziii)." In *AIP Conference Proceedings*, vol. 1614, no. 1, pp. 192-197. American Institute of Physics, 2014.

[21] Ismail, Nurul Aina, Syuhada Mohd Tahir, Norihan Yahya, Muhmad Firdaus Abdul Wahid, Nur Ezzati Khaireddin, Ibtihah Hashim, Nurfarhana Rosli, and Maryam Aqilah Abdullah. "Synthesis and characterization of biodegradable starch-based bioplastics." In *Materials Science Forum*, vol. 846, pp. 673-678. Trans Tech Publications Ltd, 2016.

[22] Müller, Carmen MO, Fabio Yamashita, and Joao Borges Laurindo. "Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach." *Carbohydrate Polymers* 72, no. 1 (2008): 82-87.

[23] Farhan, Abdulaal, and Norziah Mohd Hani. "Characterization of edible packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol." *Food Hydrocolloids* 64 (2017): 48-58.

[24] Ma, Wen, Chuan-He Tang, Shou-Wei Yin, Xiao-Quan Yang, Qin Wang, Fu Liu, and Zi-Hao Wei. "Characterization of gelatin-based edible films incorporated with olive oil." *Food Research International* 49, no. 1 (2012): 572-579.

[25] Tong, Qunyi, Qian Xiao, and Loong-Tak Lim. "Effects of glycerol, sorbitol, xylitol and fructose plasticisers on mechanical and moisture barrier properties of pullulan–alginate–carboxymethylcellulose blend films." *International Journal of Food Science & Technology* 48, no. 4 (2013): 870-878.

[26] Arham, R., S. Salengek, M. Metusalach, and M. T. Mulyati. "Optimization of agar and glycerol concentration in the manufacture of edible film." *International Food Research Journal* 25, no. 5 (2018).

[27] Thakur, Sourbh, Jyoti Chaudhary, Bhawna Sharma, Ankit Verma, Sigitas Tamulevicius, and Vijay Kumar Thakur. "Sustainability of bioplastics: Opportunities and challenges." *Current Opinion in Green and Sustainable Chemistry* 13 (2018): 68-75.