# Effects of Different Plasticizer Concentration on Characteristics of Biofilms Made from Semi-Refined Carrageenan (*Kappaphycus alvarezii*)

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**ABSTRACT** *Kappaphycus alvarezii* is a carrageenan-rich seaweed, which has good potential as a substitute for biodegradable biofilms. Due to brittleness of seaweed biofilms, plasticizer agent(s) is added to improve their elasticity. This study investigates the effects of various concentrations (10 – 30%, w/w) of glycerol and sorbitol as combined plasticizers on the physio-chemical properties of biofilms made from semi-refined carrageenan (SRC) extracted from seaweed (*Kappaphycus alvarezii*) obtained from Semporna, Sabah. The results showed that FTIR spectra showed no significant difference in all the biofilms. Biofilm with combined glycerol and sorbitol at 1:1 ratio has the highest tensile strength at 10.9 ± 1.8 MPa, but with lower elongation at break of 4.5 ± 1.1%. Increasing the concentration of the combined plasticizers caused anti-plasticization effects. The SEM results showed morphology of the biofilms with combined plasticizers were smoother and structurally better arranged. The concentration of the combined plasticizers did not significantly affect the swell ability and biodegradability of the biofilms as they are hydrophilic polymers in nature. All biofilms were completely degraded after one day of burial tests. Although the tensile strength of the SRC biofilms was still lower for heavy duty like carrier plastic, nevertheless they show promising potential as "green" food wrapping due to its high biodegradability.

**KEYWORDS**: Seaweed, *Kappaphycus alvarezii*; semi-refined carrageenan, biodegradable polymer; plasticizer agent
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## INTRODUCTION

Today, petroleum-based plastics have become a serious issue and it brings negative impacts to our environment due to its poor biodegradability (Ili Balqis *et al*., 2017). Due to these reasons, many researchers try to find an alternative polymer to replace these non-biodegradable synthetic polymers. It can be degraded and catabolized to carbon dioxide and water by microorganisms (Abdul Khalil *et al*., 2017). Biopolymers such as polysaccharides, proteins, fats can be used to synthesize biodegradable films. Among all these biopolymers, polysaccharides such as starch, cellulose, chitosan as well as carrageenan are the most useful edible polymers that can be used as film-forming materials (Saiful *et al*., 2013) where they should have the properties of water and gas resistance.

Biofilms from semi-refined carrageenan (SRC) are naturally brittle due to force interactions such as cohesive force and adhesive force during film formation (Sothornvit & Krochta, 2005). To solve this problem, plasticizer such as monosaccharides, disaccharides or oligosaccharides need to be added to decrease the intermolecular force between the chains in the polymers. Generally, edible plasticizers such as glycerol, sorbitol, xylitol, fructose as well as sucrose can be used. The efficiency of the plasticizer depends on its molecular weight where the lower the molecular weight, the greater the plasticization effect of the plasticizers (Tong *et al*., 2013). Moreover, the concentration of the plasticizers will also affect the solubility, biodegradability, physical, mechanical as well as thermal properties of the biofilms (Sanyang *et al*., 2015). Most of the previous reports used only one plasticizer agent. Hence, this paper described the influence of combining these two plasticizers on the properties of SRC biofilms.

## METHODOLOGY

*Samples and Sample preparations*

The fresh and healthy red seaweed samples (*K. alvarezii*) collected from Semporna, Sabah were dried at 60°C for 24 hours in an oven. Exactly 20.0g of the dried seaweed (moisture content around 40-50%) undergone SRC extraction in 500 ml 6% (w/v) potassium hydroxide (KOH) solution at 80°C for 30 minutes as described by Ali *et al*. (2018). For the biofilm production, a series of 2.0 g of SRC extract was dissolved in 100 ml deionized water, heated to 80°C while continuously stirred for 15 minutes before added with plasticizers at glycerol-to-sorbitol concentration ratio (%, w/w) as follows: 3:0 (A), 1:0.5 (B), 1:1 (C), 1:1.5 (D), 1:2 (E), 1:2.5 (F), 0:3 (G), respectively. After heating for another 15 minutes, the solutions were filtered, poured into a petri dish (biofilm cast) and left to dry at room temperature for 48 hours. All experiments were carried out in triplicates.

*Physico-chemical Properties of SRC and SRC Biofilms*

Tensile strength (TS) and elongation at break (EAB) of the biofilms were tested using Universal Tensile Machine (Geotech Testing Machine Inc., Taiwan) where ASTM Standard Method D638 was used (Pai *et al*., 2011); whereas surface morphology observation using Scanning Electron Microscopy (SEM) at 500x magnification. Functional group analyses of SRC and SRC biofilms were performed using FTIR (Perkin Elmer, USA). Meanwhile, biofilms swelling properties was analyzed based on calculated water gain (WG) using equation (1) where 2×2cm$^2$ biofilm samples were soaked into 40 ml distilled water and left for one hour as described by Farhan & Hani (2017).

$$\text{WG (\%)} = \frac{Weight\ of\ swollen\ film - Weight\ of\ dry\ film}{Weight\ of\ dry\ film} \text{ x } 100\ \% \quad (1)$$

For biodegradability test, soil and sand burial tests for were performed as described by Nguyen *et al*. (2016), where 20 × 70 mm of biofilm samples were buried beneath the surface of soil and sand and observed daily. The percentage weight loss (WL) of film was calculated using the following equation (2).

$$\text{WL (\%)} = \frac{Initial\ weight\ of\ film - Weight\ of\ the\ film\ after\ degradation}{Initial\ weight\ of\ film} \text{ x } 100\% \quad (2)$$

*Statistical Analysis*

All data collected were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) as the post-hoc analysis both with $p < 0.05$ to determine the relationship between different samples.

## RESULTS AND DISCUSSION

The alkali extraction has produced 74 ± 0.9% of SRC with gel viscosity of 517 ± 14 cp. The yield is considered high and is expected of the red seaweed (*K. alvarezii*) where some smaller molecules of carrageenan lost during the neutralization process (Vairappan *et al*., 2014). Similarly, the gel viscosity is in acceptable range for a 6% KOH extraction where the sulphate groups of the carrageenan were removed. The FTIR spectra of the SRC and biofilms (Figure 1) have similar features where the broad band at 3300cm$^{-1}$ indicates the stretch bonded hydroxyl group (-OH) which formed by the polysaccharide of carrageenan and water (Martins *et al*., 2012) and carbonyl groups (C=O) of D-galactose, a monomer of carrageenan, at 1630cm$^{-1}$. Besides, the four specific peaks of κ-carrageenan (Ili Balqis *et al*., 2017) i.e. 1220 cm$^{-1}$ for ester sulphate groups (O=S=O), 1030cm$^{-1}$ for glycosidic linkages, 920cm$^{-1}$ 3,6-anhydrogalactose rings (C-O-C) and 840cm$^{-1}$ for galactose-4-sulphate

(C=O-S) are all detected. It suggests that the introduction of plasticizer into the SRC biofilm did not alter the main functional groups.

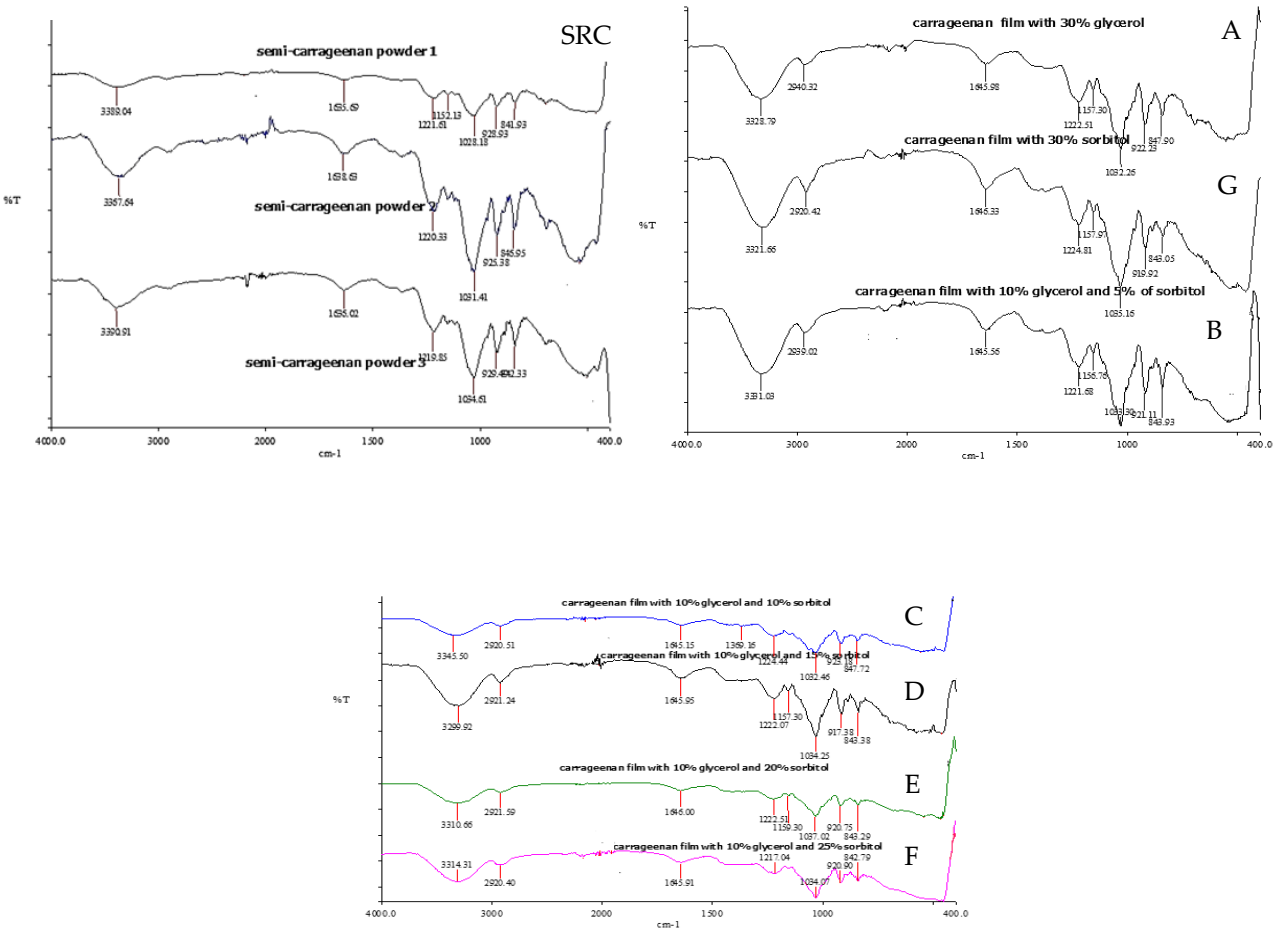



**Figure 1.** FTIR Spectra of SRC and SRC biofilms.

Table 1 shows the mechanical properties of the SRC biofilms with differing plasticizer concentration and ratio.

**Table 1.** Thickness and Mechanical Properties of Biofilms

| Biofilms (Glycerol : Sorbitol) | Thickness (mm) | Tensile Strength (MPa) | Elongation at break (%) |
|---|---|---|---|
| A (3:0) | 0.067 ± 0.015$^{a}$ | 5.95 ± 0.90$^{a}$ | 8.43 ± 1.15$^{b}$ |
| B (1:0.5) | 0.073 ± 0.018$^{a}$ | 7.73 ± 1.33$^{ab}$ | 5.89 ± 0.65$^{ab}$ |
| C (1:1) | 0.063 ± 0.018$^{a}$ | 10.91 ± 1.79$^{b}$ | 4.49 ± 1.10$^{a}$ |
| D (1:1.5) | 0.080 ± 0.010$^{a}$ | 7.32 ± 1.66$^{ab}$ | 8.01 ± 1.35$^{b}$ |
| E (1:2) | 0.063 ± 0.018$^{a}$ | 7.10 ± 1.18$^{ab}$ | 7.10 ± 0.92$^{ab}$ |
| F (1:2.5) | 0.067 ± 0.018$^{a}$ | 5.91 ± 1.16$^{a}$ | 8.58 ± 1.68$^{b}$ |
| G (0:3) | 0.067 ± 0.012$^{a}$ | 8.81 ± 3.28$^{ab}$ | 4.48 ± 1.33$^{a}$ |

Data reported are mean ± standard deviation and values of different letters$^{a-c}$ of the same column are significantly different ($p < 0.05$) to each other.

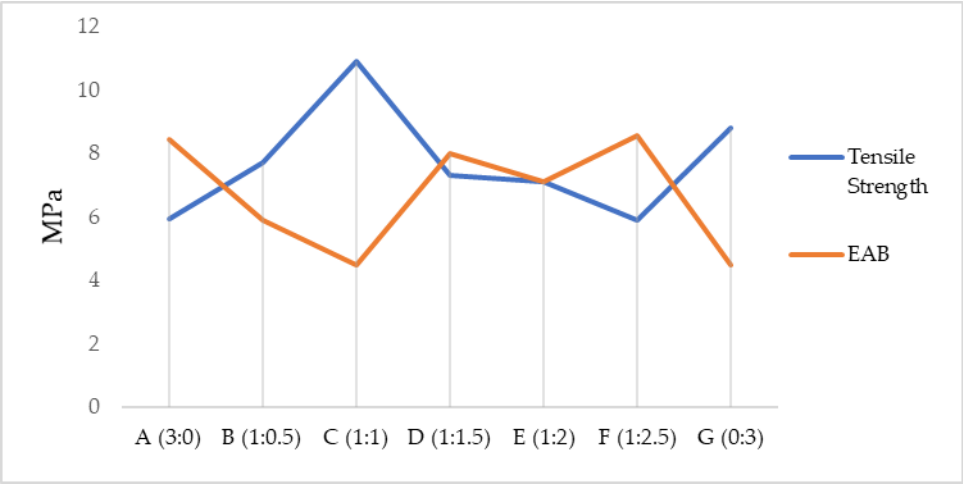
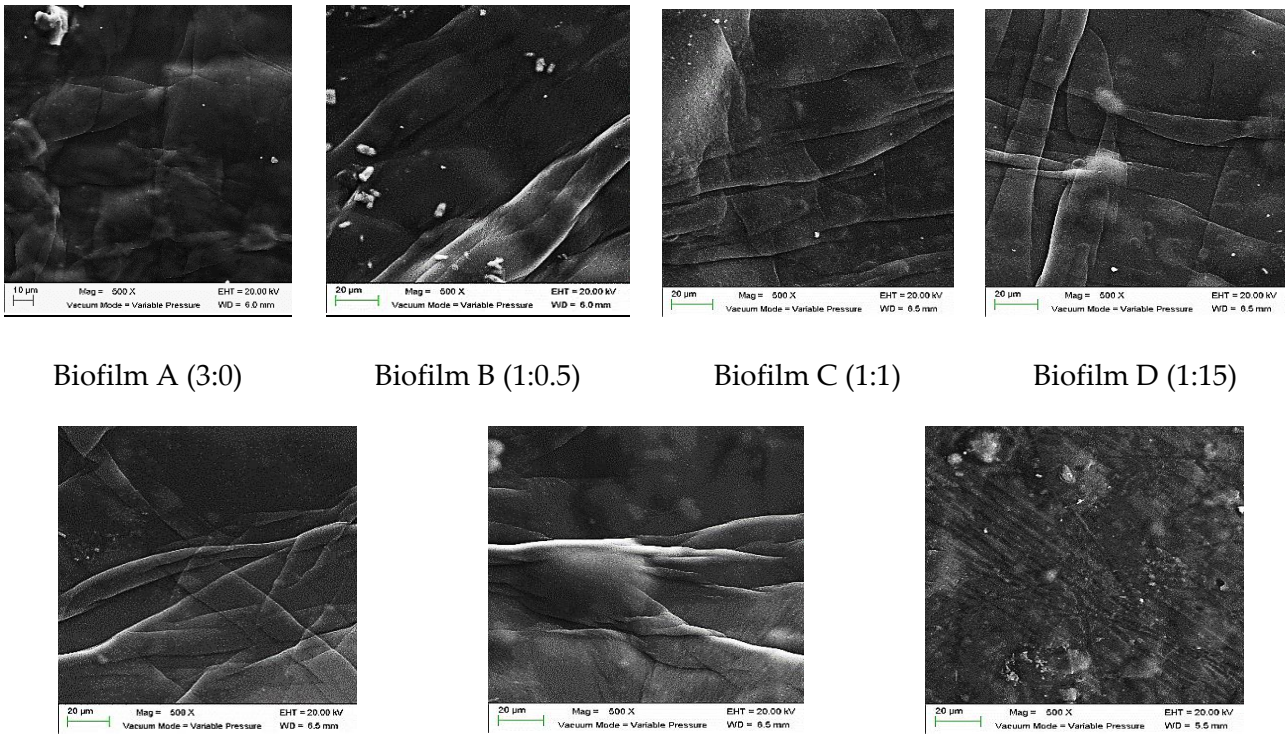



**Figure 2.** Comparison of TS and EAB for biofilms A-G.

The biofilms thickness was in the range of 0.063 – 0.080mm with average of 0.071mm. This was similar to those of commercial packaging films (polyethylene), which were between 0.025–0.075mm (Allahvaisi, 2012). There was no significant difference in the biofilm despite the differing plasticizers concentration. Tensile strength (TS) indicates the maximum tensile stress that the film can sustain. The TS of the biofilm improved significantly with combined glycerol and sorbitol at 1:1 ratio (biofilm A vs. biofilm C). Similar observations were reported by Krogars *et al*. (2003) and Adhikari *et al*. (2010) in starch biofilm. Multiple plasticizers had strong plasticizer-plasticizer interaction which further induced tightly bonded polymers resulting in stronger mechanical properties (Adhikari *et al*., 2010; Sanyang *et al*., 2015). Besides, because sorbitol consists more hydroxyl groups than glycerol, it able to increase the interaction between sorbitol and polymer chains and thus increase TS (Asria *et al*., 2015). However, adding more sorbitol in combination with glycerol did not improved the TS further (biofilms D – F) which may be due to anti-plasticization effect. According to Chang *et al*. (2006), this effect occurred when plasticization threshold was exceeded due to the plasticizer reveal a greater free volume of polymers because of an increase in hydroxyl groups. The TS of these biofilms (except biofilm C) are generally lower than the commercial films with TS in the range of 10–100 MPa (Han & Aristippos, 2005).

Elongation at break (EAB) is the maximum change in the length of a test specimen before breaking. From Table 1 and illustrated in Figure 2, EAB is inversely correlated to TS where the higher the TS, the lower the EAB. Previous researchers have reported similar observations (Srinivasa *et al*., 2007; Ili Balqis *et al*., 2017). According to Chang *et al*. (2006), increase in plasticizer concentration will change the fracture mechanism from brittle fracture to more elastoplastic fracture. It is interesting to note that EAB of biofilm C with equal concentration of plasticizers is similar to biofilm G with only sorbitol (30%) which is the least elastic of all the biofilms. Also, biofilm A (30% glycerol) has better stretch ability (flexibility) than biofilm G (30% sorbitol). This may due to the smaller molecular weight of glycerol which inhibited the interactions between biopolymers by increasing the molecular attraction (Sanyang *et al*., 2015). Furthermore, the previous researchers (Sanyang *et al*., 2015) reckoned that this reconstruction of the carrageenan molecular chains will reduce the rigidity and enhance the flexibility of the films. As in TS, these biofilms have lower elasticity than the commercial films which have > 10% EAB.

Biofilms A and G showed the roughest and heterogenous images as in Figure 3. This may due to the hydrophilicity of glycerol and sorbitol plasticizers absorb moisture hence forming voids on the surface of films (Fiori *et al*., 2015).



Biofilm A (3:0) Biofilm B (1:0.5) Biofilm C (1:1) Biofilm D (1:15)

Biofilm E (1:2) Biofilm F (1:25) Biofilm G (0:3)

**Figure 3**. SEM images of biofilms' at 500x magnification.

The morphology of biofilm G was rougher compared to biofilm A was due to the larger molecular weight of sorbitol difficult to compatible with carrageenan molecules (Nguyen *et al.*, 2016). Biofilm C showed the most smooth and homogenous surface which means the combination of plasticizers of 10% glycerol and 10% sorbitol were totally miscible and compatible with carrageenan molecules in polymer matrix (Nguyen *et al.*, 2016).

After soaking in distilled for one hour, the swelling properties of biofilms are shown in Figure 4 where all the biofilms were very water-sensitive and highly water-soluble biofilms. The concentration of plasticizers did not significantly affect the swelling properties of biofilms except for biofilm G with sorbitol plasticizer has significantly more pronounced swelling. This is expected because sorbitol has more hydroxyl groups than glycerol hence increasing the solubility of the biofilm. Furthermore, the SRC biofilms itself were polysaccharide and so sensitive to water due to its hydrophilic properties. The addition of glycerol and sorbitol plasticizers which have hydrophilic groups into the biofilms also increased the water absorption properties of biofilms.

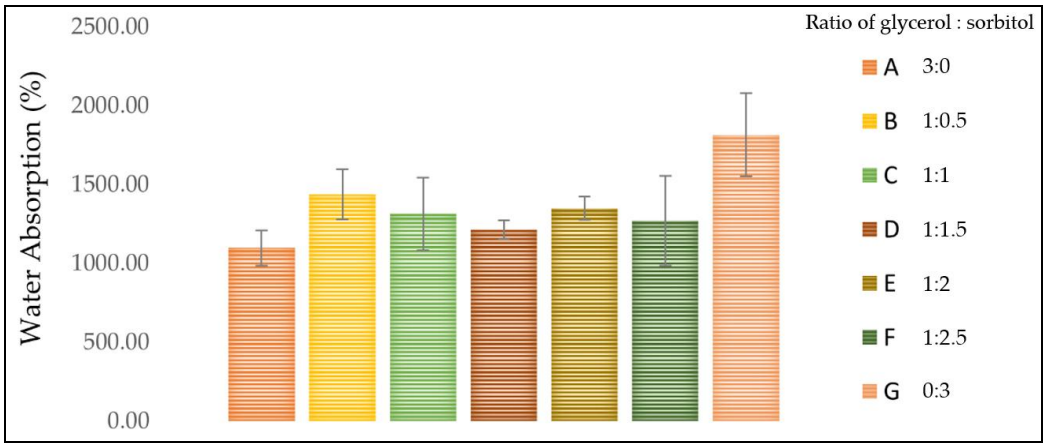



**Figure 4.** Swelling properties of Biofilms

As for the biodegradability test, all the biofilms were completely degraded after one day hence WL could not be calculated. This is not unexpected because the SRC biofilms were hydrophilic in nature and thus hydrolytic degradation is highly possible (Karbowiak *et al*., 2006). Plasticizers-made SRC biofilms dissolved and decomposed easily in the sand and soil due to hydrolytic degradation, where the biofilms will undergo chain scission-fragmentation of the polymer chains by chemical hydrolysis in soil also the microorganism will break down the biofilms into carbon dioxide, water and biomass (Kyrikou & Briassoulis, 2007).

## CONCLUSION

The production of biofilms using SRC powder, glycerol and sorbitol plasticizers were compatible with each other. Combined glycerol and sorbitol at equal concentration improved the mechanical property of the film with smoother textures. For higher elasticity, its either 30% glycerol or 15 – 25 % sorbitol combined into 10% glycerol. All the biofilms were highly soluble in water and highly degradable in soil and sand. There are still a lot more future work needed to improve the mechanical properties of these SRC biofilms; one of them is reinforcing with cross-linkage agents such as cellulose. Also, water vapour permeability, oxygen permeability, thermal stability and shelf life should be studied for more comprehensive results. Overall, this study promotes the potential of SRC biofilms as "green" alternative for industrial applications such as food and fruits wrapping film.

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