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PRODUCTION OF BIO-DEGRADABLE CARRAGEENAN-BASED FILMS FROM *SOLIERIA ROBUSTA* (RED BAMBOO) OF KARACHI COAST BY USING GLYCEROL AND SORBITOL

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ABSTRACT

This research reveals the development of biofilms prepared by carrageenan extracted from *S. robusta* present in coastal waters of Karachi, Pakistan. The obtained biofilms were examined for various variables like physio-mechanical properties, microstructural study and level of biodegradation through change in carrageenan concentration accompanied by the influence of two plasticizers glycerol and sorbitol. The progressive increase in polymer content increases thickness, tensile strength and elongation properties of films. Sorbitol blended carrageenan films exhibited more firm structure therefore provide resistance against deformation, solubility and soil-degradation. However, glycerol was found involved in enhancing the flexibility of carrageenan films and promoted faster water solubility and soil-degradation.

Key words: Biofilm, Carrageenan, *S. robusta*, Plasticizers, Glycerol, Sorbitol

INTRODUCTION

Biofilmsare prepared from entirely natural resources (Ismail *et al*., 2015) whichact as a substitute to the petro-chemicals based plastics in order to overcome the hazards caused by their formation and use and are therefore modeled to degenerate easily with the passage of time (Deni *et al*., 2015; Ismail *et al*., 2015; Hii *et al*., 2016).The biopolymers involved in the preparation of bioplastics are environment-sustainable, biodegradable and ecofriendly in nature (Giyatmi *et al*., 2017). Various polysaccharides extracted from different plants and seaweedssuch as starch, pectin, chitosan, cellulose, agar, alginate and carrageenan have been known to form biodegradable films (Bourtoom, 2008;Abdou and Sorour, 2014; Deni *et al*., 2015; Arham *et al*., 2016; Tabassum, 2016; Tavassoli-Kafrani *et al*., 2016; Gurram *et al*., 2018). Plasticizers are low molecular weight molecules that tend to have a vital influence on the formation and characterization of biofilms (Bourtoom, 2008; Farhan and Hani, 2017). They insert themselves within the polymeric web, which results in the distortion of intramolecular hydrogen bonding and spacing of polymer chains, eventually boosting the plasticity and flexibility of the biofilms (Bourtoom, 2008; Mali *et al*., 2008; Felix *et al*., 2016).Carrageenan is one such hydrocolloid having stabilizing and emulsifying abilities, existing intracellularly in the cell walls of red seaweed (members of Rhodophycota) (Abdou and Sorour, 2014; Deni *et al*., 2015). Carrageenans are soluble sulfated polysaccharide mainly comprising of alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3, 6-anhydro- α -D-galactopyranose (Campo *et al*., 2009; Tavassoli-Kafrani *et al*., 2016).Carrageenan, because of its stabilizing and thickening properties carries high demand in pharmaceutical sector, food and dairy industry and cosmetic products (Tavassoli-Kafrani *et al*., 2016). During the past few years, various studies related to the formation of carrageenan biofilms from different carrageenophytes such as *Eucheuma cottonii*, *Eucheuma spinosum*, *Solieria filiformis*, *Hypnea musciformis*, *Kappaphycus alvarezii* are documented, along with the characterizations of their properties (Pascalau*et al*., 2012; Abdou and Sorour, 2014; Deni *et al*., 2015; Paula *et al*., 2015; Tavassoli-Kafrani *et al*., 2016; Farhan and Hani, 2017; Giyatmi *et al*., 2017).In Pakistan, research work has been conducted earlier on the production of biodegradable plastics made from agarophytes from present area of study (Tabassum, 2016; Hira *et al*., 2018). However, no study has been reported till date about the fabrication of bioplastic by using carrageenan extracted from red seaweed *Solieria robusta* obtained from Karachi coast.

Therefore, the goal of our study was to analyze the potential role of carrageenan extracted from *Solieria robusta* (Red Bamboo) collected from sea shores of Karachi, Pakistan in the production of plastics. The prepared plastics were tested for mechanical properties and their scale of biodegradation in soil and water mediums. Moreoverplasticizers, glycerol and sorbitol were used to observe the effect of plasticizers in formation of bioplastics.

MATERIALS AND METHODS

Raw materials

The red seaweed *Solieria robusta* was collected from Karachi coast and brought to lab for carrageenan extraction. Prior to the extractions seaweeds were carefully washed and cleaned to get rid of all impurities. The dried seaweed was then subjected to extraction. Two plasticizers glycerol and sorbitol (99% purity) and distilled water (100%) were used in the blend formulations.

Carrageenan Hot-Water Extraction (HWE)

For carrageenan extraction, nativehot-water extraction method was applied (Aziza *et al*., 2008; Tavassoli-Kafrani *et al*., 2016) with few changes. A pre-determined amount of seaweed was boiled in hot distilled water with the ratio of 1:5 (w/v) for two to three hours at 95 ºC. The extraction was conducted repeatedly until complete release of carrageenan from seaweed. The left over thick solution was sieved through Muslin cloth, following the freezing of filtrate for 24 h at very low temperature. The frozen gel was then oven dried at 70 ºC for 24 h. The dried hydrocolloid was finally converted intofine powder for its utilization in preparation of biofilm solutions.

Evaluation of total carrageenan yield

The complete yield of carrageenan extract was based upon the total dry weight of seaweeds and calculated by applying equation that is earlier reported by Hii *et al.*, (2016) for the determination of agar yield.

$$Yield\,(\%) = \frac{Drywt.\,of\,Carrageenan}{Drywt.\,of\,seaweed} \times 100$$

Development of Biofilms

To prepare the biofilm samples, film casting method was used (Farhan and Hani, 2017). Two variables were monitored in this study i.e. the change in carrageenan concentration besides the influence of different plasticizers, glycerol & sorbitol in the production of biofilms. Four contrasting blends wereprepared based on thecompositions mentioned. Sample (1) C-2/G-30: 2% Carrageenan(w/v) + 30% Glycerol (w/w); Sample (2) C-3/G-30: 3% Carrageenan (w/v) + 30% Glycerol (w/w); Sample (3) C-2/S-30: 2% Carrageenan (w/v) + 30% Sorbitol (w/w); Sample (4) C-3/S-30: 3% Carrageenan (w/v) + 30 % Sorbitol (w/w). Firstly, carrageenan was dissolved into the distilled water in hot water bath at 90 ºC for half an hour with constant magnetic stirring. This was followed by the incorporation of plasticizers in the biofilm solutions while still remaining in water bath for another 20 minutes. The prepared hot emulsions were poured into the propylene casting plates and kept in oven for 24 hours at 50 ºC. After complete drying, films were removed from the molds andsamples were placed in desiccators under *55% RH* for three days to condition the films properly, before proceeding towards any testing.

Thickness of the carrageenan films

Digital Micrometer Screw Gauge (INSIZE 0-25mm/0-1) was used to measure the thickness of carrageenan-based bioplastics made from *S. robusta*. The rectangular strips were prepared of the bioplastic samples and around three readings were measured from the strips randomly. The mean values of all the readings were estimated.

Microstructural analyses of carrageenan films

To study the microstructural features possessed by different biofilm compositions, scanning electron microscopic images were observed at the magnification of 1000x by using Scanning Electron Microscope (JSM-6380, Japan). Gold was used to coat the samples up to 300 ºA.

Mechanical characterization of biofilms

To analyze the Tensile strength (TS) and Elongation at Break (EAB), rectangular strips of biofilm samples (10cm× 3cm) were prepared. The tensile testing machine (Zwick/Roell, GmbH & Co, D-890 79 ULM) was used to examine the mechanical properties having a load cell of 1 KN and at a speed of50 mm/min. The strips were clamped in between the grips of the machine with the initial distance of 10 cm. For each biofilm blend three values were taken.

Solubility range of carrageenan films

The water solubility rates of seaweed biofilms were estimated by using the method reported by Tapia-Blacido *et al*. (2011) with small adjustments. Three square pieces (3cm × 3cm) from each biofilm sample were dried in electric oven at 103ºC for 90 minutes and initially weighed (W1). The dried pieces were then immersed in 50 mL

centrifuging tubes containing 30 mL distilled water and placed in water bath for 24 h with slow stirring at room temperature. The undissolved pieces were recovered from the solutions via filtration followed by the oven drying of filtrate at 103ºC for 90 minutes. The dried residue collected from filter paper was then reweighed (W2) and the solubility ratios were calculated by the given equation:

$$Solubility\,(\%) = \frac{W1 - W2}{W1} \times 100\ \%$$

Soil-degradation of biofilms

To determine the degradation rates of biofilms, three pieces of each bioplastic sample of size 2 cm ×2 cm were prepared. The initial weight of all biofilms was measured (B1) and thenplaced into pots containing fertile garden soil that was rich in moisture and nutrients. After the post-burial period of 30 days, samples were weighed again (B2) and loss of dry matter was quantified by the following formula (Hii *et al*., 2016; Hira *et al*., 2018).

$$Weightloss(\%) = \frac{B1 - B2}{B1} \times 100\ \%$$

Statistical Analysis

Values of different parameters were taken in triplets of each biofilm blend. The mean value ± Standard deviation (SD) were statistically evaluated using ANOVA One-way analyses of variance by using SPSS (Version 14, Inc, USA). The mean difference ($p<0.05$) between the comparable treatments were determined by applying Duncan's Multiple Range test (DMRT) at a confidence level of 95%.

RESULTS AND DISCUSSION

Optimization of carrageenan production

The long-accepted aqueous extraction method was applied for the estimation of carrageenan from red seaweed *S. robusta*. The resultant carrageenan was a crude thick and gummy extract, dark brown in color with translucent and opaque-textured. The total polymer yield ranged from 31.58% - 34.84% which was fairly similar with the carrageenan yield of 37.3% extracted from *K. alvarezii* and 34.05% from *E. spinosum* by freeze-thaw method documented by Istini *et al*. (1994) and Jayasinghe *et al*. (2016). The results of the current study were also in agreement with the carrageenan yield of 33.54%that was extractedfrom *S. filiformis* by HWE technique (Araujo *et al*., 2012). The synthesis and extraction of carrageenan is evidently known to get controlled by some factors, such as the variation in growth periods affecting the formation of carrageenan within the seaweed (Aziza *et al*., 2008). The impact of different extraction processesalso influences the carrageenan yield as Rafiquzzaman *et al*. (2015) noticed the high carrageenan extraction by ultrasonicator extraction method over conventional method. However, in this respective study, the presence of other cellular material along with the Floridian starch with carrageenan could be the reason responsible for such maximum yield of polymer by the application ofnative carrageenan extraction method as explained by Murano *et al*. (1997) and Araujo *et al*. (2012).

Table 1. Thickness and Mechanical Properties of biofilms.

Carrageenan-based Biofilms	Thickness (mm)	Tensile Strength (MPa)	Elongation at break (%)
C-2/G-30	0.072 ± 0.003^{a}	2.8 ± 0.98^{a}	20.56 ± 1.18^{b}
C-3/G-30	0.0930 ± 0.004^{bc}	9.5 ± 1.28^{b}	30.93 ± 0.32^{c}
C-2/S-30	0.0850 ± 0.00^{b}	4.1 ± 0.74^{a}	5.34 ± 0.56^{a}
C-3/S-30	0.1005 ± 0.008^{c}	11.7 ± 0.54^{b}	20.54 ± 1.95^{b}

Means ± Standard deviation are reported; values with different letters mentioned in a column are significantly different at $p<0.05$

Appearance and thickness of carrageenan-based biofilms

It was observed that all the biofilms prepared from the carrageenan of *S. robusta* were malleable and smooth textured. They peeled easily from the casting trays without any hindrance. The plasticization effect of glycerol and sorbitol within the biofilm samples enabled the production of smoother films; as both the polyols due to low molecular weight, easily intercalate themselves into the polymer structure thus minimizing the intra-hydrogen bonding by inducing the flexibility (Mali *et al*., 2008). It could be observed in Table **1** that the thickness of the samples varied with the change in carrageenan concentration as well as by the change of plasticizer. Samples with larger amount of dissolved polymer were apparently thicker ($p<0.05$); similar effect of increasing polymer quantity resulting in the formation of thicker films has also been noticed in previous reports (Abdul Khalil *et al*., 2018; Arham *et al*., 2016). Whereas, the plasticizer type was simultaneously found playing a noteworthy role. Samples plasticized with sorbitol produced dense films as sorbitol's molar mass is relatively larger then glycerol's molar mass (Sanyang *et al*., 2016). However our results conflicted with the ones documented by other researchers (Farhan and Hani, 2017) who reported the significant effect of glycerol producing thicker semi refined kappa-carrageenan films which could be attributed due to the swelling of glycerol molecules after absorbing moisture resulting in the increased thickness.

Microstructural analyses of carrageenan biofilms

Fig. **2** & **3** represents the microstructural images of carrageenan films (C-2/G-30; C-3/G-30; C-2/S-30; C-3/S-30) plasticized with glycerol and sorbitol. All the samples are shown to have slightly bumpy and granulated surfaces. The presence of such granules could be illustrated as the unsuccessful dissolution of carrageenan within the biofilm blend which has also been observed previously in other studies (Rhim, 2012; Paula *et al*. 2015). Moreover, the plasticizers are known to enhance the smoothness and reduce the brittle nature of films (Laohakunjit and Noomhorm, 2004; Thomazine *et al*., 2005); therefore in our study, both the plasticizers formed continuous and flexible films with no phase-separation. Besides this, the role of sorbitol plasticizer producing relatively smoother and compact carrageenan films than those plasticized with glycerol was observed, manifesting its better interaction with the polymer. Similar kind dominating effect of sorbitol enabling the formation of even and unwrinkled films is also reported by Tapia-Blacido, (2011); Sanyang *et al*., 2016 & Farhan and Hani, (2017) thus supporting our results.

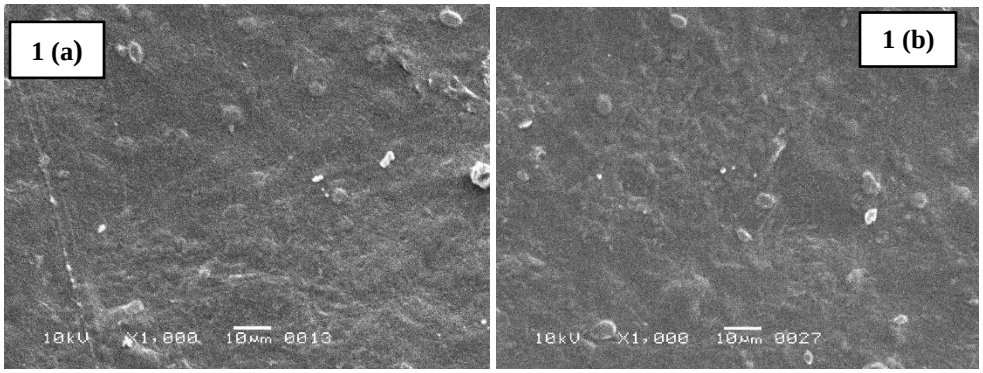

Fig. 1 (a) & 1 (b). SCM images of 2% *S. robusta* films plasticized with glycerol and sorbitol.

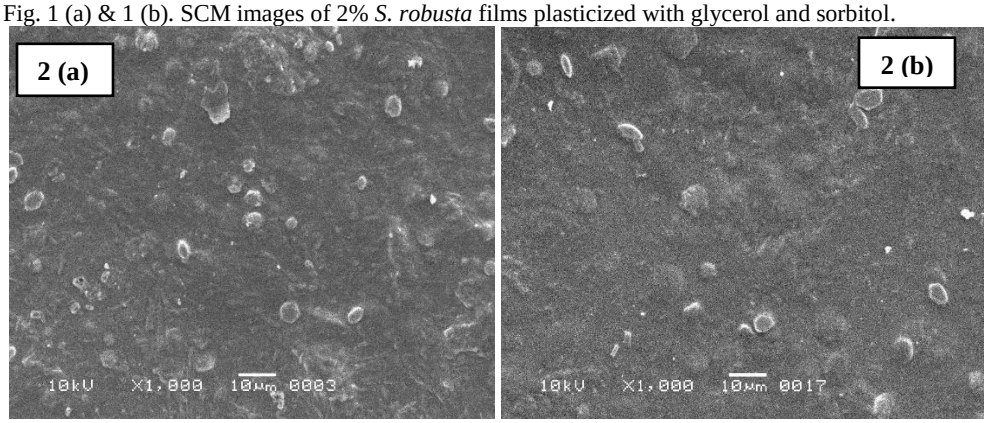

Fig. 2 (a) & 2 (b): SCM images of 3 % *S. robusta* films plasticized with glycerol and sorbitol.

Mechanical characterization of *S. robusta* films

In this study, the variation in carrageenan concentration and role of plasticizers were quite noticeable while determining the TS and Elongation at Break values of carrageenan-made biofilms. After the post-conditioning of films, the results obtained for the samples C2G-30 & C3G-30 and C2S-30 & C3S-30 were significantly different ($p<0.05$). As presented in Table1, the highest Fmax values were shown by the samples with the higher carrageenan concentration of 3 % that is C3S-30 (11.7 MPa) and C3G-30 (9.5 MPa). While, a dramatic reduction from 11.7 MPa to 4.1 MPa (sorbitol plasticized) and 9.5 MPa to 2.8 MPa (glycerol plasticized) was observed to be exhibited by the samples made from 2 % carrageenan concentration. This indicated the simultaneous effect of polymer concentration on the tensile strength of the specimens. Similarly, maximum elongation yields were also gained by the samples with 3% carrageenan concentration.

The improvement in mechanical properties as a result of a rise in carrageenan concentration has been also reported earlier by Abdou and Sorour (2014) and Abdul Khalil *et al*., (2018). Two factors were observed behind such results; primarily the existence of large quantity of polymer produced thicker films which encourages the utmost intramolecular hydrogen bonding within the biofilm structure boosting the strength (Wu *et al*., 2009; Arham *et al*., 2016). Furthermore, carrageenan being a hydrophilic compound facilitates more room for the accumulation of water molecules within the polymeric structure that resulted in the good flexibility of samples (Arham *et al*., 2016; Abdul Khalil *et al*., 2018).

Moreover, the contrasting effect of both plasticizers among the bioplastics was also observed. Samples blended with glycerol were more sensitive ($p<0.05$) towards deformation while showing considerable improvement in the flexibility and extension of biofilms achieving higher elongation (30.93 and 20.56) %. On the other hand, sorbitol films were more firm and compact however failed to gain good ductility (20.54. and 5.34) % as compared to the glycerol films. This could be due to the relatively low compatibility between sorbitol and water which resulted in non-successful incorporation of moisture within the polymer web and failed to produce good elongation values (Bourtoom, 2008). These results are comparable with the findings of Thomazine *et al*. (2005) who reported the efficiency of glycerol as a more suitable plasticizer enhancing the flexibility of gelatin-based films. Tapia-Blacido *et al*. (2011) also verified the anti-plasticization effect of sorbitol in the formation of amaranth flour films. Mali *et al*., (2008) also reported the opposite behavior of both the plasticizers in the formation of cassava starch films, notifying the superior plasticizing effect of glycerol over sorbitol.

Solubility range of carrageenan biofilms

The solubility range of bioplastics, is an important factor for their economical utilization and in determining the water-resistance capability of plastics (Wang and Rhim, 2015). Those biofilms that tend to easily dissociate in the presence of moisture substantially used as packaging material for numerous food products; whereas, biofilms with lesser solubility ratios opposing the effect of moisture are basically viewed for amplifyingthe shelf life of food items (Sanyang *et al*., 2016; Giyatmi *et al*., 2017). The solubility rates of carrageenan made biofilms in this studyevaluated by ANOVA ranged in between 65.5%-91.0% as shown in Fig. **3**. The highest ($p<0.05$) solubility percent (91%) was shown by the sample C-2/G-30 havinglowest carrageenan amount. The gradualincrease in the carrageenan concentration caused the reduction in the solubility strength of the films.This occurred most probably due to the mass availability of polymer that failed to dissolve properly, accompanied by the effect of plasticizer sorbitol (Arham *et al*., 2016). Similar results regarding the speedy dissociation of carrageenan films have also been studied earlier (Rhim, 2012; Giyatmi *et al*. 2017).Relativehydrophilic nature of glycerol over sorbitol seems to be responsiblefor the maximum solubility of the glycerol blended samples by disintegrating the internal molecular forces and ultimately leading to fast dissolution of polymer. Whereas, the use of the plasticizer sorbitol having lower affinity towards moisturelead to the lower solubility ratios. (Laohakunjit and Noomhorm, 2004; Tapia-Blacido, *et al*., 2011). Bourtoom (2008) and Farhan and Hani (2017) also supported such influence of both the plasticizers glycerol and sorbitol in the formation of biofilms.

Soil degradation of carrageenan films

The biodegradable plastics are composed of organic matter, therefore they get easily decomposed by a variety of soil-microbes which results in the production of energy rich end-products thus making it an eco-friendly plastic (Adhikari *et al*., 2016; Spaccini *et al*., 2016).In the current report, to examine the degradation rate of carrageenan biofilms, soil decomposition test was conducted. Fig. **4** displays the analytically evaluated results that revealed significantly different weight loss ratios indicating a considerable effect of different polymer concentration and kind of plasticizer. The sample plasticized with glycerol and minimum polymer amount (C-2/G-30) possessed maximum degradation of around 36%. However, the decaying rate was shown to be declined progressively with the increase in polymer concentration resulting in lower degradation rates i.e. C-3/G-30: 29.8%; C-2/S-30: 16.75%;C-3/S-30:

10.45%.Moreover the dominating effects of plasticizer glycerol were also noteworthy, because of water-loving nature of glycerol facilitated microbial growth (Wahyuningtiyas and Suryanto, 2017) which ultimately resulted in the rapid degradation of glycerol-biofilm samples as compared to the sorbitol-blended samples which degraded very slowly.

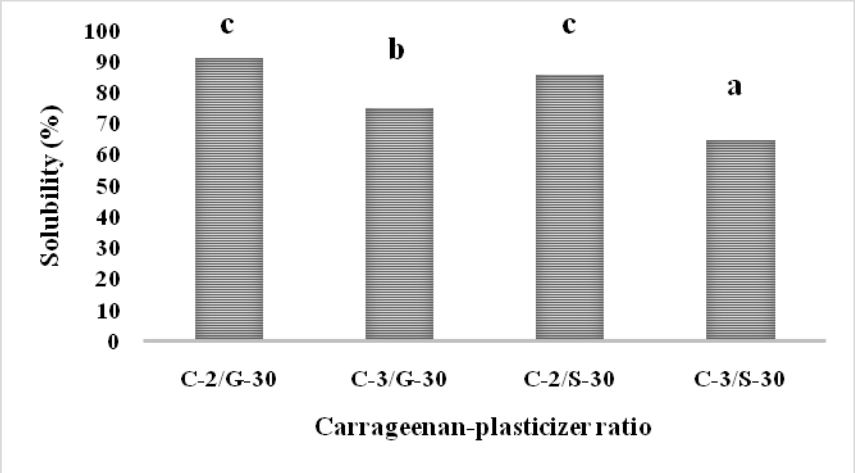

Fig. 3. Effect of carrageenan concentration and plasticizer kind on solubility ranges**.** Different alphabets above the bars indicate significant difference among means.

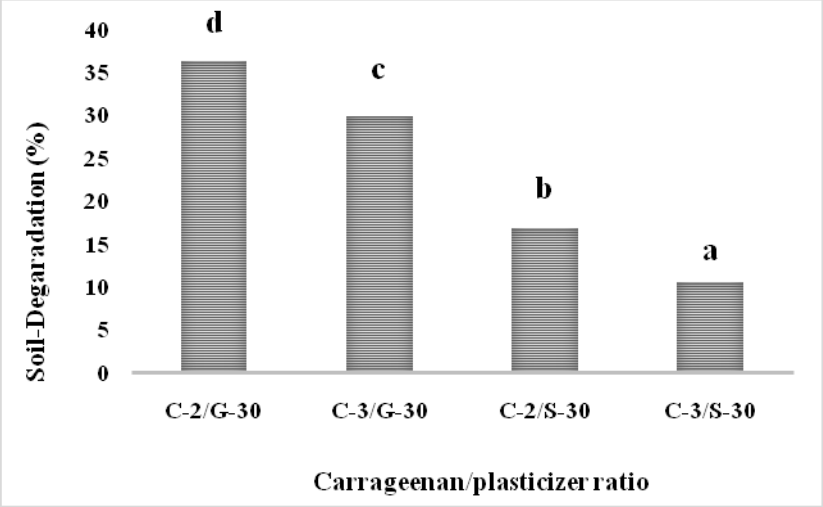

Fig. 4. Effect of carrageenan concentration and plasticizer kind on soil-decomposition. Different alphabets above the bars indicate significant difference among means.

CONCLUSION

The potent role of carrageenan extracted from *S. robusta* in the production of bioplastics was investigated in the present research. The plasticization effect of two different plasticizers glycerol and sorbitol along with the alteration in polymer concentrations were also analyzed. The outcomes demonstrated that the bioplastic samples with high polymer content and plasticized with sorbitol gave the maximum thickness and mechanical strength values, however, this respective formulation revealed significant decrease in solubility and degradation rates. On the contrary, low polymer content and plasticizer glycerol enhanced the Elongation at break values and also amplified the solubility and soil-decomposition rates, owing to the glycerol's hydrophilic nature.

REFERENCES

Abdou, E. S. and M. A. Sorour (2014). Preparation and characterization of starch/carrageenan edible films. *Int. Food Res. J*., 21(1): 189-193.

Abdul Khalil, H. P. S., S. W. Yap, Y. Y.Tye, P. M. Tahir, S. Rizal and M. R. N. Fazita (2018). Effects of corn starch and *Kappaphycus alvarezii*, seaweed blend concentration on the optical mechanical and water vapor barrier properties of composite films. *Bioresources*, 13(1):1157-1173.

Adhikari, D., M. Mukai, K.. Kubota, T. Kai, N. Kaneko, K. S. Araki and M. Kubo (2016). Degradation of bioplastics in soil and their degradation effects on environmental microorganisms. *J. Agric. Chem. and Env*., 5(1):23-34.

Araujo, I. W. F., J. A. G.Rodrigues,E. S. O. Vanderlei, G. A. Paula,T. B. Lima and N. M. B., Benevides (2012).Iota-carrageenans from *Solieria filiformis* (Rhodophyta) and their effects in the inflammation and coagulation. *Acta Scientiarum. Technology, Maringá*, 34(2):127-135.

Arham, R., M.T. Mulyati, M. Metusalach and S. Salengke (2016). Physical and mechanical properties of agar based edible film with glycerol plasticizer. *Int. Food Res. J*., 23(4): 1669-1675.

Aziza, M., T. Givernaud, M. C. Khay and L. Bennasser (2008). Seasonal variation of the growth, chemical composition and carrageenan extracted from *Hypnea musciformis* (Wulfen) Lamouroux harvested along the Atlantic coast of Morocco. *Sci. Res. Essays*, 2(10):509-514.

Bourtoom, T. (2008). Plasticizer effect on the properties of biodegradable blend film from rice starch-chitosan. *Songklanakarin J. Sci. Technol*., 30(1): 149-165.

Campo, V. L., D. F., D.B. Kawano, Silva Jr. and I. Carvalho (2009). Carrageenans: Biological properties, chemical modifications and structural analysis-A review. *Carbohydr. Polym*., 77 (2): 167-180.

Deni, G. D., S. A. Dhaningtyas, I. Fajar and Sudarno (2015). Characterization and evaluation physical properties biodegradable plastic composite from seaweed (*Eucheuma cottonii*). *AIP Conference Proceedings*, 1699 (1): 042201.

Farhan, A. and N. M. Hani (2017). Characterization of edible packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol. *Food Hydrocolloids*, 64: 48-58.

Felix, M., V. Carpintero, A. Romero and A. Guerrero (2016).Influence of sorbitol on mechanical and physio-chemical properties of soy protein-based bioplastics processed by injection molding. *Polímeros*, 26(4):277-281.

Giyatmi, S., D, Melanie, M. Fransiska, Darmawan and H. E. Irianto (2017). Barrier and physical properties of Arrowroot starch-carrageenan based biofilms. *J. Biol. Sci*., 25: 45-56.

Gurram, R., P. F. S. Filho, M. J. Taherzadeh and A. Zamani (2018). A solvent-free approach for production of films from pectin and fungal biomass.*J. Polym. Environ*., 26: 4282–4292.

Hii, S.L., J. Y. Lim, W. T. Ong and C. L.Wong (2016). Agar from Malaysian red seaweed as potential material for synthesis of bioplastic Film. *J. Eng. Sci. Technol.*, 11: 1–15.

Hira, A., A. Tabassum, M. A. Tahira and R. Aliya (2018). Comparison in biodegradable films formulated from two red seaweeds of Karachi coast. *Pak. J. Mar. Sci*., 27(2):87-92.

Ismai, l. A., W. Hammami, F. Mensi and L. Ktari (2015). Bioplastic from agar: Hydrophilic and thermos-mechanical properties.*Bull. Inst. Natn.Scien. Tech. Mer de Salammbô*, 42:17-19.

Istini, S., M. Ohno and H. Kusunose (1994). Methods of analysis for agar, carrageenan and alginate in seaweed.*Bull. Mar. Sci. Fish. Kochi Univ*., 14:49-55.

Jayasinghe, P. S., V. Pahalawattaarachchi and K. K. D. S., Ranaweera (2016). Effect of extraction methods on the yield and physiochemical properties of polysaccharides extracted from seaweed available in Sri Lanka. *Poult. Fish. Wildi. Sci*., 4(1):1-6.

Laohakunjit, N. and A. Noomhorm (2004). Effect of plasticizers on mechanical and barrier properties of rice starch film. *Starch/Stäerke*, 56(8): 348–356.

Mali, S., M. V. E. Grossmann, M. A., Maria Alejandra Garcia, M. N. Martino and N. E. Zaritzky (2008). Antiplasticizing effect of glycerol and sorbitol on the properties of Cassava starch films. *Braz. J. Food Technol*., 11(3):194-200.

Murano, E., R. Toffanin, E. Cecere, R. Rizzoand S. H. Knutsen (1997). Investigation of the carrageenans extracted from *Solieria filiformis* and *Agardhiella subulata* from Mar Piccolo. Taranto, *Mar. Chem.*, 58(3-4): 319-325.

Pascalau, V., V. Popescu, G. L. Popescu, M. C., Dudescu, G. Borodi, A. Dinescu, I. Perhaita and M. Paul (2012).The alginate/k-carrageenan ratio's influence on the properties of the cross-linked composite films.*J. Alloys Compd*., 536(1): 418-423.

Paula, G. A., N. M. B. Benevides, A. P. Cunha, A. V.Oliveira, A. M. B.Pinto,J. P. S. Morais and H. M. C.Azeredo, (2015). Development and characterization of edible films from mixtures of k-carrageenan, l-carrageenan and alginate. *Food Hydrocolloids*, 47:140-145.

Rafiquzzaman, S. M., R. Ahmed, J. M. Lee, G. Noh, G. Jo and I. S.Kong (2015).Improved methods for isolation of carrageenan from *Hypnea musciformis* and its antioxidant activity.*J. Appl. Phycol.*, 28(2): 1265-1274.

Rhim, J. W. (2012). Physical-mechanical properties of agar/κ-Carrageenan blend Film and derived clay nano composite film. *J. Food Sci*., 77(12):66-73.

Sanyang, M. L., S. M. Sapuan, M. Jawaid, M. R. Ishak and J. Sahari (2016). Effect of plasticizer type and concentration on tensile thermal and barrier properties of biodegradable films based on Sugar Palm (*Arenga pinnata*) starch. *J. Food. Sci. Technol.*, 53(1):326–336.

Spaccini, R., D. Todisco, M. Drosos, A. Nebbioso and A. Piccolo (2016). Decomposition of bio-degradable plastic polymer in a real on-farm composting process. *Chem. Biol. Technol. Agric*., 3(4):1-12.

Tabassum, A. (2016). Biofilms from agar obtained from an agarophyte of Karachi coast. *Pak. J. Mar. Sci.,* 25(1):37-40.

Tapia-Blacido, D. R., P. J. A. Sobral and F. C. Menegalli (2011). Optimization of amaranth flour films plasticized with glycerol and sorbitol by multi-response analysis. *LWT -Food Sci.Technol*., 44(8):1731-1738.

Tavassoli-Kafrani, E., H. Shekarchizadeh and M. M. Behabadi (2016). Development of edible films and coatings from alginates and carrageenans. *Carbohydr. Polym.*, 137:360-374.

Thomazine, M., R. A. Carvalho and P. J. A. Sobbal (2005). Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. *J. Food Sci*., 70(3):172-176.

Wahyuningtiyas, N. E. and H. Suryanto (2017). Analysis of biodegradation of bioplastics made of Cassava Starch. *J. Mech. Eng. Sci*., 1(1): 41-54.

Wang, L. F. and J. W. Rhim (2015). Preparation and application of agar/alginate/collagen ternary blend functional food packaging films. *Int. J. Biol. Macromol*., 80: 460–468.

Wu, Y., F. Geng, P. R. Chang, J. Yu and X. Ma (2009). Effect of agar on the microstructure and performance of potato starch film. *Carbohydr. Polm*., 76(2): 299–304.

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