Pak. J. Bot., 53(4): 1525-1529, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-4(40)

PREPARATION AND ESTIMATION OF PHYSIO-MECHANICAL PROPERTIES OF ECO-FRIENDLY BIOPLASTICS OF *GRACILARIA CORTICATA* FROM KARACHI COAST

MARIUM ASIF[1*], ASMA TABASSUM[1], TAHIRA M. ALI[2] AND ALIYA RAHMAN[1]

[1]*Department of Botany, University of Karachi, Karachi-*75270*, Pakistan*
[2]*Department of Food Science & Technology, University of Karachi, Karachi-*75270*, Pakistan*
**Corresponding author's email: mariumasif143@yahoo.com*

Abstract

This study aims to prepare sustainable and eco-friendly bio-plastics of agar extracted from the red seaweed *Gracilaria corticata* collected from Karachi coast. The bioplastic samples were assessed against different parameters like physio-mechanical properties, solubility ratio and soil-biodegradation at different agar concentrations by using two different plasticizers glycerol & sorbitol as plasticizers. The Scanning Electron Microscopic images of bioplastic samples were also studied. The obtained results showed that the increasing amount of agar in bio-plastics directly affects the thickness and tensile strength of the films. It was also observed that plasticizer glycerol was found to be more efficient in maximizing the elasticity of the films whereas sorbitol contributed in enhancing the bio-film's tensile strength. Solubility tests and bio-degradation results of the bioplastic samples with lowest agar concentration, blended with glycerol revealed the highest solubility and decomposition rate.

Key words: Agar, *Gracilaria corticata,* Biodegradable plastic, Glycerol, Sorbitol.

Introduction

Plastic pollution now a days, is of great concern, affecting all kinds of living organisms when accumulated either on land or in waters (Moharam & Maqtari, 2014; Reddy *et al.*, 2014). Synthetic plastics are petro-chemical based polymers, which are being commercially utilized on a large scale due to their cheap costs and long lasting durability (Rahman, 2013; Reddy *et al.*, 2014). The disposal of abandoned plastic products has proven to be a big difficulty as their complete breakdown could take centuries (Rajendran *et al.*, 2012; Gade *et al.*, 2013; Moharam & Maqtari, 2014), whereas their incineration liberates harmful gases and chemicals which results in greenhouse effects (Gade *et al.*, 2013).

Considering the hazardous effects of synthetic plastic, there has been a growing interest for an alternative biocompatible and eco-friendly bioplastic from organic material that could degrde easily (Gill, 2014; Wang & Rhim, 2015). Several reports are documented regarding the formation of bioplastics made from organic polymers and plasticizers, extracted from plants such as pectin (Gurram *et al*., 2018), sugar palm starch (Sanyang *et al*., 2015), rice-starch chitosan films (Bourtoom, 2008) and proteins extracted from Soy-plant (Felix *et al*., 2016). Seaweeds are important autotrophic and photosynthetic plants, which are now often explored frequently due to the presence of some vital polysaccharides such as alginate, agar, floridean starch and carrageenan (Rajendran*, et al.*, 2012). These carbon containing polymers are found to be potentially active in the formation of bio-plastics (Rajendran *et al.*, 2012; Wang & Rhim, 2015; Farhan & Hani, 2017). Similarly agar, that is present intracellularly within the cell walls of red seaweed (Martins *et al.*, 2012; Gade *et al.*, 2013; Wang & Rhim 2015; Tabassum, 2016; Hii *et al.*, 2016) possess good gelling & emulsifying abilities and is reviewed in several studies as a promising agent for bioplastic synthesis (Wu *et al*., 2009; Hii *et al.*, 2016; Tabassum, 2016). Bioplastics prepared from agar are shown to have fine physical and mechanical properties besides good flexibility and tensile strength enabling their feasible use in commercial utilization (Arham *et al*., 2016). These plastics are also known to solubilize and decompose easily preventing their long residence time on the lands and as well as in waters (Hii *et al.*, 2016; Arham *et al*., 2016; Hira *et al.*, 2018).

In the preparation of such eco-friendly plastics, plasticizers play a significant role by improving the elasticity and ductility of plastics (Vieira *et al*., 2011). These non-volatile compounds with low molecular masses, accumulate within the polymer chains and reduce their intramolecular forces thus providing the resistance against breaking or deformation (Bourtoom, 2008; Mali *et al*., 2008; Vieira *et al*., 2011; Felix *et al*., 2016). In this research, two polyols i.e. sorbitol and glycerol are utilized to observe their effect in bioplastic formation.

The objective of this study was to analyze the commercial application of bioplastics made from agar of an agarophyte *Gracilaria corticata* from the Karachi coast. This report also focuses the physio-mechanical properties of biofilms along with their rate of biodegradation and solubility ratios.

Materials and Methodology

Red seaweed (*Gracilaria corticata*) was collected from the Bhulleji coast (French beach) of Karachi and was brought to the lab for further processing. Glycerol and sorbitol (99%, v/v purity) and deionized water (100% pure) were used for the preparation of bioplastic blends.

Collection and processing of seaweed: Prior to the agar extraction, collected seaweeds were thoroughly washed with distilled water and was shade dried for 3 to 4 days. After complete drying, algae were ground into small pieces to facilitate extraction process.

Hot-water extraction (HWE) of agar: Agar was extracted via native hot-water extraction method reported earlier (Pelegrin & Murano, 2005; Tabassum, 2016; Hira *et al*., 2018). Dried and chopped seaweed material was immersed in hot boiling deionized water in the ratio of 1:5 (w/v) and followed by boiling for 2 to 3 hours. The left behind hot emulsion was then sieved two to three times repeatedly using muslin cloth. The filtrate was cooled to form gel and kept in freezer overnight. The frozen agar strips were thawed to remove as much water as possible and were subsequently oven dried at 70ºC until complete dehydration. The dried agar strips were then converted into powdery mass for bio-plastic preparation.

Determination of agar yield: The total amount of extracted agar was evaluated depending upon the dry weight of seaweed and dried amount of obtained agar by the equation as follows; (Hii *et al.*, 2016).

$$Yield\ (\%) = \frac{Dry\ wt.of\ Agar}{Dry\ wt.of\ seaweed} \times 100\ \%$$

Preparation of bioplastic: The bioplastic samples were prepared by applying film casting method (Arham *et al.*, 2016; Tabassum, 2016; Hira *et al*., 2018). Two plasticizers glycerol and sorbitol were incorporated to examine the effect of plasticizer type on biofilms. Two blends of agar/glycerol (A2G-30: 2% agar w/v: 30% glycerol w/w & A3G-30: 3% agar w/v: 30% glycerol w/w) and two blends of agar/sorbitol (A2S-30: 2% agar w/v: 30% sorbitol w/w & A3S-30: 3% agar w/v: 30% sorbitol w/w) were formulated. A pre-calculated amount of agar was dissolved in 150 mL of deionized water with continuous stirring in water bath at 90ºC for 30 minutes followed by the addition of plasticizers at a concentration of 30% (w/w) for further 20 minutes. The hot solutions were spread uniformly onto the propylene molds (12" × 12") for drying in oven for 24 hours at 50ºC. The dried plastics were peeled off from the molds and were kept in desiccators at 55% *RH* for proper conditioning prior to any testing.

Thickness of bioplastic samples: The thickness of bioplastic films was measured using digital micrometer screw gauge (INSIZE 0-25mm/0-1). Three measurements were randomly taken from each agar biofilm and the mean value was calculated.

Mechanical properties: Tensile Strength (TS) and elongation at break (EAB %) were analyzed using Universal Testing machine (Zwick/Roell, GmbH & Co, D-890 79 ULM) with a load cell of 1 KN and a test speed of 50 mm/min. Rectangular shaped strips (10cm × 3cm) of bioplastic films were clamped to the grips, 10 cm apart from each other. Three values of each film sample were recorded.

Scanning electron microscopy: Scanning Electron Micrographs of bioplastic samples were obtained via Scanning Electron Microscope (JSM-6380, Japan) to study the surface morphological features of bioplastic samples. The samples were coated up to 300ºA with gold. The scanning images were observed under the magnification of 1500x.

Solubility ratio: The water solubility (WS) ratio was determined as per basic standard method reported earlier (Wang & Rhim, 2015; Arham *et al*., 2016; Sanyang *et al*., 2016). Total of three specimens (3cm × 3cm) from each sample were oven dried at 220ºF for 24 hours and weighed (W1) properly. The dried pieces were then immersed in centrifuge tubes containing 30 mL distilled water, kept in water bath at 25ºC with slow shaking overnight. The solutions were filtered and the remnants on filter paper were dried at 220°F for two hours and reweighed (W2). The undissolved dry matter was calculated by using the formula;

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

Rate of biodegradability: To determine the biodegradability rate of bioplastic samples, each film sample was pre-weighed (B1) and buried for a month into pots containing conditioned garden soil. The final weight (B2) of the samples were recorded and the weight loss ratio of the films was calculated by using the following equation (Hii *et al*., 2016):

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

Statistical experimental design: Mean values were statistically evaluated using One-way analyses of variance (ANOVA). To determine the significant difference ($p<0.05$) among variable treatments, Duncan's Multiple Range test (DMRT) was applied with a 95 % level of confidence via SPSS software (version 14).

Result and Discussion

Yield of Agar from *Gracilaria corticata*: In the current study, the estimated total yield of agar extracted from *Gracilaria corticata* was around 18.5 %. However, our polysaccharide amount appeared to be smaller, when compared with the maximum agar yields achieved in earlier findings by Villanueva *et al*., 2010 (33% by *G. vermiculophylla*) & Wang *et al*., 2017 (23% by *G. tenuistipitata*). The impact of cold weather and techniques for the agar production is known to influence agar yield among seaweeds and may vary specie to specie (Soriano & Bourret, 2003; Nil *et al*., 2016; Rasheed *et al*., 2019). Therefore, it is suggested that the pre-treatment of seaweeds and their collection during temperate season can increase the production of agar.

Thickness of bioplastic samples: It was observed that the thickness of bioplastic samples was correlated with the variation in agar concentration along with the type of plasticizer used in a specific blend. It was shown that plastic sample with higher agar concentration blended with sorbitol was significantly ($p<0.05$) thicker than the bioplastic sample having low agar concentration and

plasticized with glycerol (Table 1). However, the formulations with same agar concentration or with similar plasticizer revealed no pronounced effects. These results can be generally linked to the provision of amount of dissolved solids; besides this, the comparatively bigger molar mass of sorbitol then glycerol automatically contributed in thickening of films (Arham *et al*., 2016; Sanyang *et al*., 2016). Wang & Rhim, (2015); Arham, *et al*., (2016) & Giyatmi *et al.,* (2017) also reported the direct relation of increasing amount of soluble solid content resulting in the production of thicker films.

Table 1. Physio-Mechanical properties of agar-bioplastic samples.

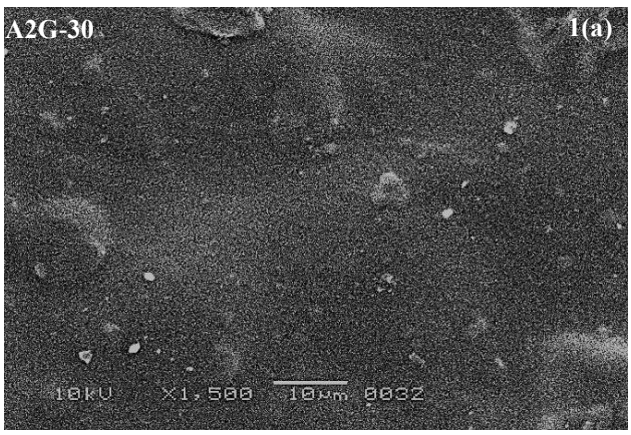
Bioplastic Samples	Thickness (mm)	Tensile strength (MPa)	Elongation at break (EAB %)
A2G-30	0.083 ± 0.001^{a}	1.36 ± 0.11^{a}	19.1 ± 0.18^{a}
A3G-30	0.100 ± 0.000^{ab}	2.75 ± 0.45^{b}	34.1 ± 0.24^{c}
A2S-30	0.089 ± 0.002^{ab}	2.17 ± 0.24^{b}	18.97 ± 0.80^{a}
A3S-30	0.105 ± 0.007^{b}	3.60 ± 0.23^{c}	30.99 ± 1.36^{b}

Mean values ± standard deviation are reported, values with repeated alphabets in the same columns are not significantly different ($p<0.05$) by Duncan's Multiple Range Test

Tensile strength (MPa) and EAB (%) of agar films: After the conditioning of samples for around 72 hours at *RH 55%*, the mechanical properties of the bioplastic specimens were recorded. The fluctuation in polymer amount and plasticizer's effects on the tensile strength (MPa) and EAB (%) were noteworthy. Evaluations revealed the highest Fmax ($p<0.05$) attained by the blending of 3 % agar with sorbitol but showed lower flexibility of the biofilm. On the contrary, sample with similar agar concentration plasticized with glycerol were less resistant to rupturing while acquiring maximum ($p<0.05$) elongation. The tensile strength and Elongation improved progressively with the increase in agar content which could possibly be due to the presence of large amount of dissolved agar, possessing the strong three-dimensional structure providing firmness, besides the provision of larger space for the accumulation of water within the polymer structure enhancing the ductility of films (Wu *et al*., 2009; Arham *et al*., 2016). The effect of glycerol dominated over sorbitol enhancing the elongation, as glycerol is relatively more hydrophilic compound than sorbitol and therefore immensely attracts water molecules; also because of its low molecular weight than sorbitol, it readily accumulates within the polymer chains; these two factors simultaneously cause the expansion in the polymer's chemical structure thus magnifying flexibility and reducing strength (Mali *et al*., 2008; Hii *et al*., 2016; Farhan & Hani, 2017; Othman, *et al*., 2018). Similar conclusions are documented by Sanyang *et al*., (2015), witnessed the anti-plasticization effect of sorbitol over glycerol in Palm-Starch biofilms. Laohakunjit & Noomhorm, (2004), also observed the improvement in tensile properties when films were formulated using sorbitol while glycerol ensured good flexibility of films while preparing rice-starch biofilms.

Scanning electron microscopy of agar films: The scanning electron microscopic images were obtained at the magnification of 1500x to study the morphological surface features of the pre-conditioned bioplastic samples. The images of the agar bioplastics with the plasticizer glycerol & sorbitol (A2G-30; A3G-30; A2S-30 & A3S-30) illustrated dense surfaces without any phase separation (Figs. 1 and 2). However, the bioplastic sample (A3G-30) plasticized with glycerol, had clearly visible numerous open pores on the surface indicating the phase separation. The occurrence of these pores can be due to the leaching of glycerol from the sample while it's mounting in the SCM chamber under high vacuum as explained by Othman *et al*., (2018); this leaching caused the over drying ultimately leading to phase separation in bioplastic. Moreover, some irregular sized particles were noticed on the surface of the films, which could be interpreted as portion of undissolved polymer (spotted in white color). It was observed that the samples blended with sorbitol (A2S-30 & A3S-30) demonstrated smoother and homogenized surfaces than glycerol indicating the suitable compatibility of polymer with sorbitol. Tapia-Blacido *et al*., (2011) & Sanyang *et al*., (2016) also confirmed the production of fine and uniform films by using sorbitol.

Water solubility of agar films: The rate of solubility of bioplastics is an important feature reflecting the ability of bioplastic being disintegrated in the presence of moisture, post-consumption, when utilized commercially (Arham *et al*., 2016). Bioplastics having low grade solubility are considered to be the best as they resist moisture for a longer period of time and helps to increase the shelf life of a products; whereas, some edible bioplastics used in the packaging of food material mostly degrade rapidly (Sanyang *et al*., 2015; Arham *et al*., 2016; Giyatmi *et al.*, 2017). In this study, the gradual increase in polymer content inversely affected the solubility rates which could be due to the maximum undissolved mass of agar and its nature of being insoluble at low temperature (Arham *et al*., 2016). Bioplastics blended with glycerol were shown to have highest solubility of 81.5% and 74% and was significantly different ($p<0.05$) than those of plasticized with sorbitol having values of 65.5% & 62.5% (Fig. 3). This indicated that sorbitol films were more resistant towards moisture degradation. The obtained solubility values are comparatively higher than the solubility rate of rice starch-chitosan films (Boutoom *et al*., 2008) and agar/alginate/collagen ternary blend (Wang & Rhim, 2015). However, such solubility trends are comparable to those reported in previous studies (Laohakunjit & Noomhorm, 2004; Sanyang *et al*., 2016; Arham *et al*., 2016) who all suggested the presence of strong attraction between glycerol and water molecules within the polymeric web which aids in rapid solubility of the sample.



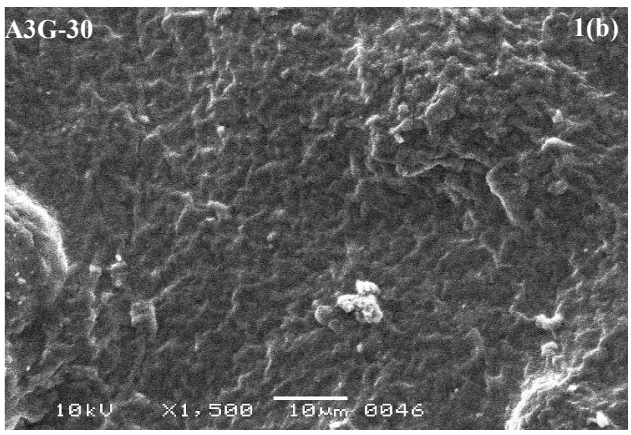
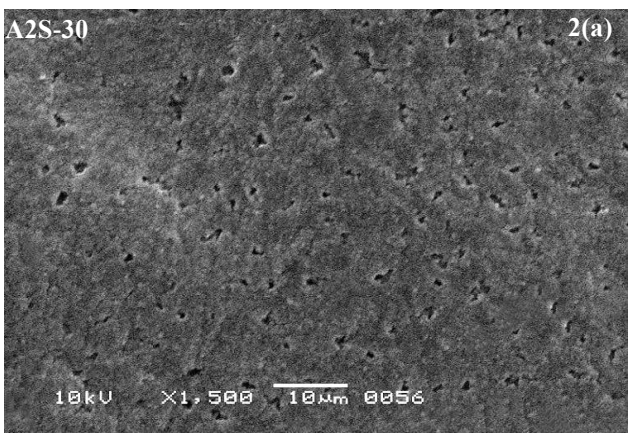


Fig. 1 (a) & 1 (b) SCM images of 2% & 3% *G. corticata* agar films plasticized with glycerol.



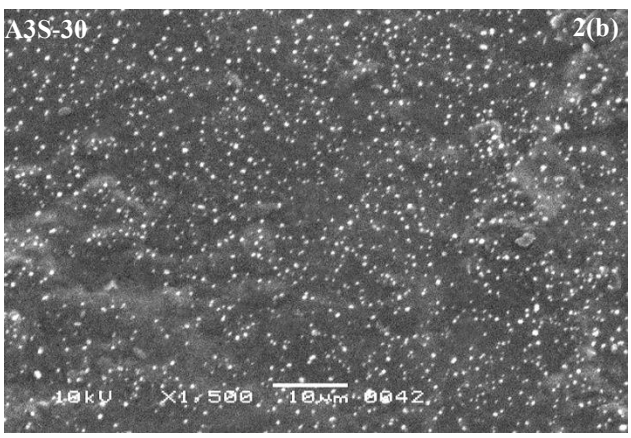


Fig. 2 (a) & 2 (b) SCM images of 2% & 3 % *G. corticata* agar films plasticized with sorbitol.

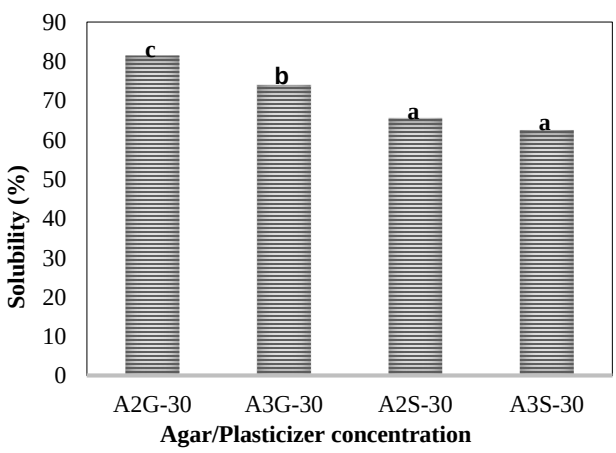


Fig. 3. Effect of different agar: plasticizer ratios on the solubility rates of agar films.

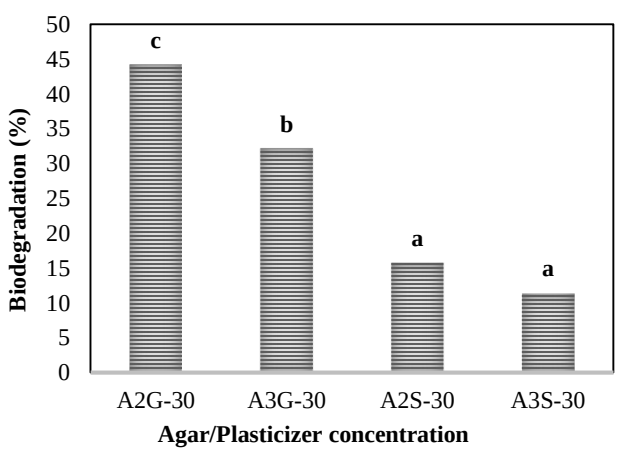


Fig. 4. Effect of different agar: plasticizer ratios on the biodegradation rates of agar films.

Biodegradation of agar films: Soil burial test was performed to analyze the level of deterioration caused to the bioplastics via microbial growth when discharged into the soil (Hii *et al*; 2016; Wahyuningtiyas & Suryanto, 2017). The values calculated by ANOVA of the samples being buried for 30 days were 44.2% for G2G-30; 32.15% for G3G-30; 15.78% for G2S-30 & 11.35% for G3S-30 (Fig. 4). As the base material and plasticizers used in this study, agar, glycerol & sorbitol are known to have hydrophilic properties (Vieira *et al*., 2011; Hii *et al*., 2016) therefore all the samples showed weight loss. However the type of plasticizer played a pronounced role in the decomposition test. Samples with glycerol plasticizer showed highest weight loss values ($p<0.05$) when compared to sorbitol. This aspect could possibly be explained by the maximum moisture retaining by glycerol from the surrounding habitat, enabling the microbial growth. The more the water activity, higher would be the growth of microorganisms which can accelerate the degradation of samples (Hii *et al*., 2016; Wahyuningtiyas & Suryanto, 2017).

Conclusion

In current study, Physio-Mechanical properties, accompanied with the water solubility and biodegradability rates of the bioplastics made from agar of *G. corticata* were examined with reference to the change in agar concentration and the effects of plasticizers (glycerol & sorbitol). The increase in agar concentration efficiently improved the film thickness, TS & E but lowered the solubility and soil degradation rates. The scanning micrographs verified the appropriate bonding of agar with sorbitol which also accounts for amplifying the thickness and TS of the films whereas glycerol being a hygroscopic material was shown to increase the flexibility, water solubility and Biodegradation ratios. These outcomes unveil the commercial utilization of agar plastics as biodegradable films for packaging different products.

References

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.

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

Farhan, A. and N.M. Hani. 2017. Characterization of edible packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol. *Food Hydrocoll.,* 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.

Freile-Pelegrin, Y. and E. Murano. 2005. Agars from three species of *Gracilaria* (Rhodophycota) from Yucatan Peninsula, *Bioresou. Technol*., 96: 295-302.

Gade, R., M.S. Tulasi and V.A. Bhai. 2013. Seaweeds: A novel biomaterial. *Int. J. Pharm. Sci*., 5(2): 40-44.

Gill, M. 2014. Bioplastic: A better alternative to plastics impact. *Int. J. Res. Appl., Natrl. Soc. Sci.*, 2(8): 115-120.

Giyatmi, S. Melanie, D. Fransiska, M. 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*., 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.

Rasheed, I., Tabassum, A., Khan, U. and R. Aliya. 2019. Fourier transform infrared (FT-IR) spectroscopy of agar from red seaweeds of Karachi Coast. *Int. J. Biol. Biotech*., 16(1): 59-63.

Laohakunjit, N. and A. Noomhorm. 2004. Effect of plasticizers on mechanical and barrier Properties of rice starch Film. *Starch/ Stäerke*, 56: 348-356.

Mali, S., M.V.E. Grossmann, M.A. 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.

Martins, J.T., M.A. Cerqueira, A.I. Bourbon, A.C. Pinheiro, W.S. Bartolomeu and A.A. Vicente. 2012. Synergistic effects between k-carrageenan and Locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocoll.*, 29: 280-289.

Moharam, R. and M.A. Al. Maqtari. 2014. The impact of plastic bags on the environment: A field survey of the city Of Sana'a and the surrounding areas,Yemen. *J. Eng. Res. Rev*., 2(4): 69-61.

Nil, S., S. Ali-Mehidi, A. Zellal and S.M.E.A. Abi-Ayad. 2016. Effects of season on the yield and quality of agar from *Gelidium sesquipedale* (Rhodophyta) from Mostaganem, Algeria. *Afr. J. Biotechnol.*, 15(10): 350-355.

Othman, M., N. Hasmida, Z. Halim, N.A. Jamal, K. Khalid, N. Zakaria and S.A.M. AL-Bat'hi. 2018. Mechanical properties of *Gracilaria lichenoides* reinforced bioplastic film. *Mater. Sci. Eng*., 290: 1-6.

Rahman, M.K. 2013. Using plastic Bags and its damaging impact on environment and agriculture: An alternative proposal. *Int. J. Learn. & Dev*., 3(4): 1-14.

Rajendran, N., S. Puppala, M.S. Raj, B.R. Angeeleena and C. Rajam. 2012. Seaweeds can be a new source for bioplastics. *J. Pharm. Res*., 5(3): 1476-1479.

Reddy, M.S., P.S. Reddy, G.V. Subbaiah and H.V. Subbaiah. 2014. Effect of plastic pollution on environment. *J. Chem. Pharm. Sci*., 28-29.

Sanyang, M.L., S.M. Sapuan, M. Jawaid, M.R. Ishak and J. Sahari. 2015. Effect of plasticizer type and concentration on tensile thermal and barrier properties of biodegradable films based on Sugar Palm (*Arenga pinnata*) starch. *Polymers*, 7: 1106-1124.

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.

Soriano, E.M. and E. Bourret. 2003. Effect of season on the yield and quality of agar from *Gracilaria* species (Gracilariaceae, Rhodophyta). *Bioresour. Technol*., 90(3): 329-333.

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

Tapia-Blácido, 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. *Food. Sci. Technol*., 44: 1731-1738.

Vieira, M.G.A., M.A.D. Silva, L.O.D. Santos and M.M. Beppu. 2011. Natural-based plasticizers and biopolymer films: A review, *Eur. Polym. J*., 47: 254-263.

Villanueva, R.D., A.M.M. Sousa, M.P. Gonçalves, M. Nilsson and L. Hilliou. 2010. Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). *J. Appl. Phycol*., 22: 211-220.

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: 299-304.

(Received for publication 3 May 2019)