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AGAR FROM MALAYSIAN RED SEAWEED AS POTENTIAL MATERIAL FOR SYNTHESIS OF BIOPLASTIC FILM

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Abstract

The main aim of this study was to identify the potential use of agar extracted from red seaweed, *Gracilaria salicornia*, collected from the coastal area of Malaysia as the raw material for synthesis of bioplastic film. Agar was extracted via two extraction methods: (1) alkali extraction method and (2) photo bleaching extraction method. The yields of agar by both of the methods were 9 to 11 %. The alkali extracted agar (AEA) and photo bleached agar (PBA) were incorporated as the raw materials for the formation of bioplastic films while sago starch and glycerol were added to increase workability. Physicochemical properties of the two bioplastic films were characterised. FTIR analysis confirmed the presence of agar in both plastic films with the presence of 3,6-anhydrogalactose residues and further indicated that the interactions of agar and sago starch were strong in both PBA and AEA films. The results showed that tensile strength and percent elongation of PBA film (3.067 MPa, 3.270 %) was higher than AEA film (2.431 MPa, 2.476 %). Thermogravimetric analysis (TGA; % residual weight) revealed that AEA film has higher thermal stability (14.80 %) than PBA film (10.27 %) while rheological results proved that both films exhibited non-Newtonian behaviors. The AEA film was completely decomposed after 30 days in the soil burial test. Results of current study show a wide range of future possibilities and commercial applications of AEA and PBA bioplastic films.

Keywords: Agar, *Gracilaria salicornia*, Alkali extraction method, Photo bleaching extraction method, Bioplastic film.

Abbreviations

AEA	Alkali Extracted Agar
Au/Pd	Gold/Platinum
FTIR	Fourier Transform Infrared
NaOH	Sodium Hydroxide
PBA	Photo Bleached Agar
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis

1. Introduction

Nowadays, due to the depletion of petroleum sources and environmental effects caused by the conventional plastics, bioplastics films are being studied extensively as an alternative to conventional plastics because of their excellent biodegradability, biocompatibility and edibility. There are a few criteria for the formation of such films whereby at least one film forming agent and plasticizer are to be added to a solvent to form a film forming solution [1]. Some of the popular films forming agents are starch and cellulose derivatives from plant sources, as well as alginate from seaweed sources [2-5]. Other polysaccharides from seaweed (macroalgae) sources such as agar and carrageenan are getting more attentions due to their ability to form edible films as well as the ability of macroalgae to grow in harsh conditions [6] and to be renewed annually.

Agar is a polysaccharide which can be easily found in some families of red seaweed (Rhodophyceae), generally Gracilariaceae and Gelidiaceae. It consists of two major components which are agarose and agaropectin [7]. It is also a hydrophilic colloid which has the capability of forming reversible gels by cooling it from a hot aqueous solution [8]. Agar is first used in the fields of food, biotechnology and pharmaceutical applications before being introduced as a raw material for production of bioplastic film formation. According to Phan et al. [9], agar-based films possess transparent, strong and flexible characteristics at low moisture content. In addition, it is heat-sealable which makes it a good application for food packaging industry.

Conventionally, native agar was extracted by leaching the red seaweed in hot water, filtering the extract, and followed by concentrating the extract using freezing and thawing processes to eliminate water. This extraction method can be easily used to extract agar from *Gelidium* species but not for agar with high gel strength like *Gracilaria* species [7]. Pretreatment procedures are thus needed to effectively extract the agar with higher gel strength with the formation of 3,6-anhydrogalactose bridge [10]. Bleaching process is also incorporated during agar extraction process in order to reduce the colour of agar. Current industrial practice uses chemical (sodium hypochlorite) for bleaching process and it gives off hazardous chlorine gas. Therefore, an eco-friendly technique that is known as photo bleaching process was introduced in the study of Li et al. [11]. Through this process, the coloured organic matters inside seaweed were subjected to photochemical degradation [12].

Agar can be extracted mainly from red seaweed families Gracilariaceae and Gelidiaceae. *Gracilaria* is the second largest genus of red seaweed and it is

comprised of more than 150 species distributed worldwide [13]. There are a total of 20 *Gracilaria* species in Malaysia and one of the most common species is *Gracilaria changii*, which grow abundantly in mangrove areas fringing the west coast of Peninsular Malaysia [14,15]. The demand for *Gracilaria changii* is high because of their high gel strength, proteins, fatty acids and bioactive compounds which have wide applications for cosmetic, pharmaceutical and food industries [16]. However, as a result of pollution, introduction of alien species, climate change as well as over-harvesting, the supply of *Gracilaria changii* become limited. Therefore, it is necessary to explore the potential market of other *Gracilaria* species in Malaysia. In view of this, the objective of this study is to investigate the agar quality from indigenous red seaweed, *Gracilaria salicornia*, using both alkali extraction and photo bleaching extraction methods. The characteristics of bioplastic film produced using the extracted agar were determined.

2. Materials and Methods

2.1. Seaweed sampling and preliminary treatment

Specimens of red seaweed *G. salicornia* were collected at the beach of Port Dickson, Malaysia. The collected seaweed was cleaned with tap water for several times and dried at 60 ºC in an oven. The dried seaweed specimens were subjected to size reduction before stored in air-tight bags with silica gels.

2.2. Seaweed extraction method

2.2.1. Alkali treatment extraction

Alkali treatment extraction method was carried out according to the method of Chirapart et al. [17] with minor modifications. Dried seaweeds (10 g) were treated with alkaline solution (500 mL of 5 %w/v NaOH) for 2 hours at 80 ºC. Following this, the alkali-treated sample was rinsed properly and placed in deionized water (room temperature). The pH of sample was adjusted to a range of 6.5 to 7.5. The sample was then heated at 120 ºC for 2 hours. The filtrate of sample was left to cool to room temperature and frozen overnight to concentrate the agar gel. The frozen solidified agar was thawed and dried at 50 ºC for 24 hours.

2.2.2. Photo bleaching extraction

Photo bleaching extraction method was carried out according to the method of Li et al. [11] with minor modifications. The initial extraction method is similar with alkali treatment extraction method with additional photo bleaching process. After pH of the treated seaweed was adjusted to a range of 6.5 to 7.5, the seaweed samples were soaked in distilled water and left overnight under fluorescent lamp prior to photo bleaching process for 8 hours. Following this, the seaweed were rinsed and concentrated according to the process as described in the alkali treatment extraction method (Section 2.2.1).

2.2.3. Determination of agar yield

The yield of agar was determined based on the initial dry weight of the seaweed and the final dry weight of the agar extracted, as shown in Equation 1.

$$\text{Yield (\% w/w)} = \frac{\text{dry weight of agar}}{\text{initial dry weight of seaweed}} \times 100\ \% \quad (1)$$

2.3. Preparation of bioplastic film

Bioplastic films were prepared by film casting method. The alkali extracted agar (AEA) and photo bleached agar (PBA) were used as raw material for the formation of bioplastic film. Sago starch and plasticizer (glycerol) were incorporated into film-forming solution to increase workability. Sago starch (6.8 g) was first gelatinized and homogenized in 240 mL distilled water using overhead stirrer in water bath (± 90 ºC). Agar powder was then added to the homogenized starch solution. Glycerol was mixed to the film-forming solution and stirred for 5 minutes. The amount of dissolved components were added based on the formulation from Wu et al. [18] to ensure the surface of bioplastic film produced is clear, smooth, flexible and without any phase separation. Then, the film forming solution was casted on petri dish and dried at 50 ºC overnight.

2.4. Characterisation of bioplastic film

2.4.1. FTIR spectroscopy

The spectra of each casted film sample were recorded using FTIR spectrometer (Thermo Scientific Nicolet iS10). Each spectrum was the average of 32 scans acquired at 2 cm^{-1} resolution. Transmittance mode was used to determine peak base lines and heights. The peak heights were calculated and converted to absorbance.

2.4.2. Scanning electron microscopy (SEM)

Scanning electron microscope (Hitachi S3400N, Japan) with energy dispersive X-ray analysis (EDX) at 20 kV was used to obtain scanning electron micrographs and elemental composition of the bioplastic film samples. The bioplastic film samples were coated with Au/Pd up to 7 nm thick with high resolution sputtering instrument at a sputtering rate of 1.5 kV per minute. Then, cross sectional image of the samples were observed using x1000 and x5000 magnification.

2.4.3. Mechanical properties

Tensile strength (TS, MPa) and elongation at break (%) of the bioplastic film samples were determined using tensile machine (Tinius Olsen H10KS Universal, USA). The samples were cut into dumb-bell shape and fixed to the tensile machine at both ends prior to the mechanical testing.

2.4.4. Thermogravimetric analysis (TGA)

TGA was carried out using a thermogravimetric analyser (Mettler Toledo TGA-SD815a, Switzerland). The bioplastic film samples were cut into pieces (2.5 to 5 mg) and transferred to the sample holder. The testing temperature was from 30 to 800 ºC, with a ramp temperature of 20 ºC and nitrogen gas flow rate at 30 mL/min. Curves of weight loss of bioplastic film samples against temperature were plotted.

2.4.5. Rheological study

Constant shear rate test and dynamic oscillation test were conducted on AEA and PBA films using rheometer (Anton Paar GmBH-MCR 301, Austria) equipped with a parallel plate with diameter of 0.5 mm and gap of 1 mm. Each sample of bioplastic film was placed onto the bottom plate which equilibrated to 25 ºC and pressed by the top plate. Constant shear rate test was carried out with shear rate at 0.01 s^{-1} for duration of 477 s. Dynamic oscillation test was conducted in strain sweeping mode where strain amplitude was changed from 0.01 % to 100 % with the shear frequency of 1 Hz. G' (measure of elastic response) and G" (measure of viscous response) were monitored along the sweep amplitude test.

2.4.6. Soil burial test

Biodegradability of the bioplastic films were examined with soil burial test. The samples were buried in locations with different type of soils and left for 30 days. The initial weight (M_o) and the final weight (M_1) were recorded. The percentage of weight loss after 30 days is calculated by using Equation 3.

$$\text{Weight loss } (\%) = \frac{M_o - M_1}{M_o} \times 100\ \% \quad (2)$$

3. Results and Discussions

3.1. Physical appearance and yield of extracted agar

In this study, the alkali extracted agar (AEA) was in watery-soft gel-like jelly which clumped together in the solution. Generally, agar from *Gracilaria* species is with lower quality because of their high sulphate concentrations [19]. Alkali treatment was used to improve the gel properties by desulphate the native agar and allow the L-galactose-6-sulfate moieties in agar backbone to convert to 3,6-anhydro-L-galactose and hence increase the gel strength of the extract [20]. The gelling behaviour of AEA in this study indicated that pre-treatment of seaweed using alkaline solution help to remove the sulphate groups such as 4-O-methyl-α-L-galactose and D-galactose-6-sulphate residues, thus improving the gelling ability [17].

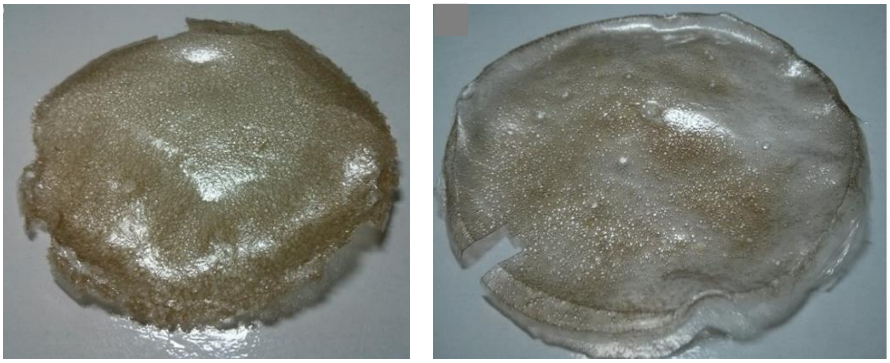
As for the photo bleached agar, the colour of seaweed turned from yellow-green (after alkali pretreatment) to pale yellowish after photo-bleaching process. The coloured organic matters which are normally found in *Gracilaria* species are phycoerythrobilin, chlorophyll α and carotenoids. They are conjugated-double-

bond chromogenic group and vulnerable to photochemical degradation. By exposing the seaweed to solar radiation, coloured organic matters are mineralised into carbon monoxide, carbon dioxide ammonium and other forms of dissolved organic carbon [21]. According to Li et al. [11], photo bleaching process helps to increase the total organic carbon of seaweed. In addition, the authors discovered that sulphate in the seaweed can be further eliminated by photolysis process. The sulphate content of seaweed decreased with the increase of gel strength which may be due to the reaction of sulphate with free radicals.

The yield of AEA (10.68 % ± 0.08) was slightly higher than the yield of PBA (9.83 % ± 0.03). The agar yield from *G. salicornia* from both extraction methods in the present study are lower than those reported from various *Gracilaria* species [20, 22, 23]. Extraction temperature is one of the most important factors affecting the agar yield. Souza et al. [24] reported that higher yield of agar can be obtained with higher temperature. Other factors affecting the final agar yield are extraction time, environmental conditions, seasonal variations and physiological factors [25]. The low agar yield from present study might be caused by leaching of agar during the thawing-recovery process. Some agar might also lost during the washing and pre-treatment process [26].

3.2. Morphological properties of bioplastic film

Massive amount of air bubbles trapped inside the bioplastic film formed using AEA (Fig. 1a) as compared to PBA (Fig. 1b). The surface of the AEA film is dry and uneven. Although the films are with air bubbles, but in general they are still rigid, soft and flexible. In an attempt to eliminate the air bubble trapped inside both of the bioplastic films, the hot film forming solution was degassed using a sonicator unit.

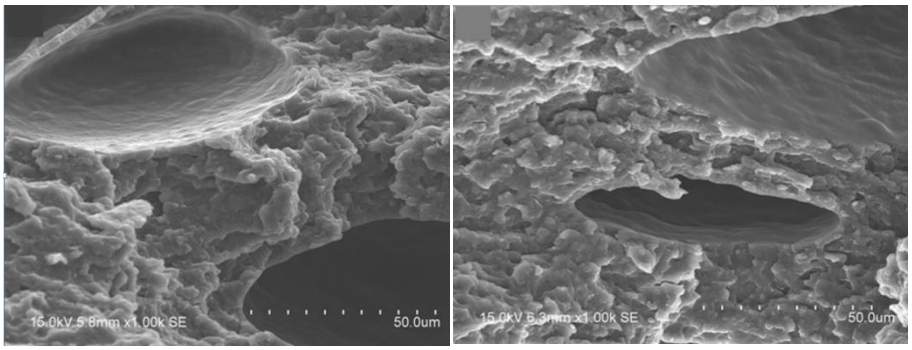


(a) AEA bioplastic film **(b) PBA bioplastic film**

Fig. 1. Physical appearance of bioplastic film.

SEM images of AEA and PBA bioplastic films were shown in Fig. 2. Both AEA and PBA bioplastic films were with air bubbles trapped in the film matrix, causing the structures for both of the films less dense and not homogeneous. Nonetheless, there was presence of continuous phase structure found in both

films, indicating that the added glycerol was successfully incorporated into the polysaccharide chain interior and disrupted the intramolecular hydrogen bonds [27]. It was noticeable that more air bubbles were present in the AEA bioplastic film than PBA bioplastic film which will weaken the mechanical properties of AEA bioplastic film.



(a) AEA bioplastic film **(b) PBA bioplastic film**

Fig. 2. SEM images of bioplastic films.

3.3. FTIR spectroscopy of bioplastic film

Figures 3 show the FTIR analysis of the AEA (Fig. 3a) and PBA film samples (Fig. 3b). FTIR absorbance bands of AEA and PBA bioplastic films at 3264.56 cm^{-1} and 3274.23 cm^{-1} is contributed to the complex vibrational stretches associated with intra, inter and free molecular hydroxyl group. The FTIR absorbance bands at 2929.21 cm^{-1} and 2927.24 cm^{-1} indicated the hydrogen atom stretches with the carbon on the methane ring. Stretching molecules of conjugated peptide bond by acetone and amine groups in both the bioplastic film is indicated with the presence of absorbance bands at 1643.91 cm^{-1} and 1644.33 cm^{-1} [18]. The properties of ester sulphate in both AEA and PBA bioplastic films is shown in the FTIR absorbance bands at 1366.14 cm^{-1} and 1366.06 cm^{-1}. FTIR absorbance bands of AEA and PBA bioplastic film range from 926.75 cm^{-1} to 1150.15 cm^{-1}, and 926.80 cm^{-1} to 1149.65 cm^{-1}, indicates the C-O bond stretching on anhydro-galactose ring, which is common in the structure of agar.

Any movement of FTIR spectra absorbance bands due to the addition of different component indicates distinctive chemical interaction occurred. Ma et al. [28] mentioned that the FTIR spectra with lower absorbance bands of similar component might be due to the intense interaction in the material. More intense chemical interaction is feasible as this indicates a better entanglement between agar, sago starch and glycerol.

By analysing Fig. 3, the chemical interactions between agar, sago starch and glycerol were found stronger in PBA bioplastic film as compared to AEA bioplastic film. This is proven by lower absorbance bands for most of the peaks of PBA bioplastic film spectrum.

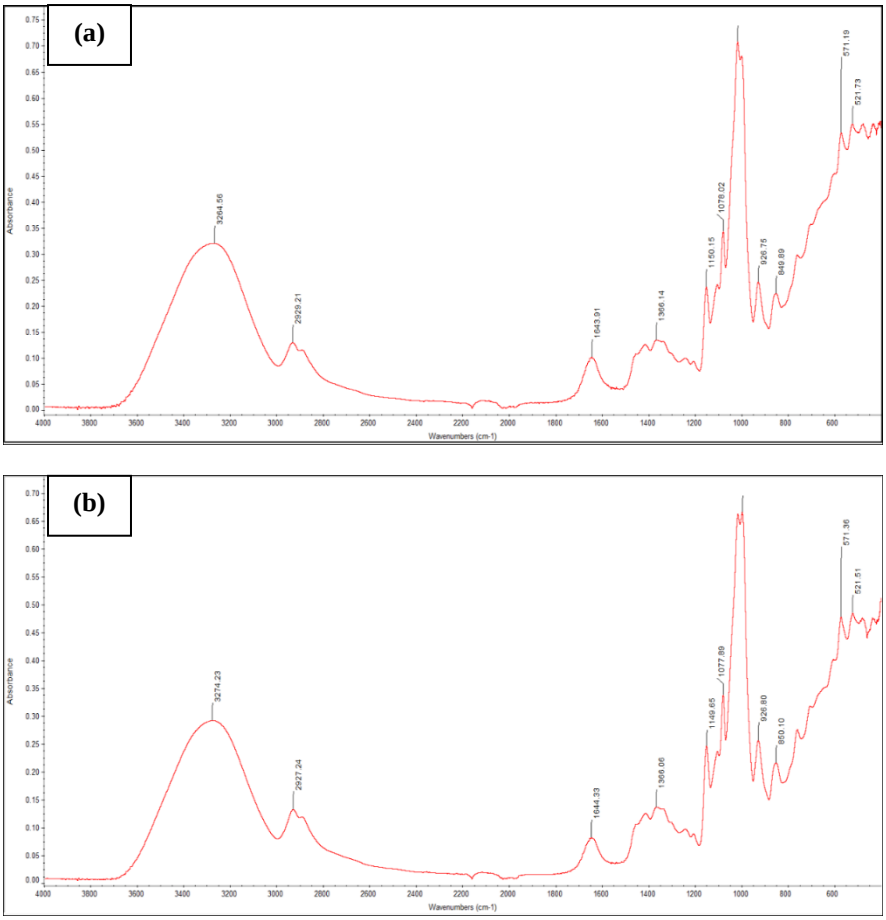


Fig. 3. FTIR spectrum of bioplastic film: (a) AEA bioplastic film, (b) PBA bioplastic film.

3.4. Mechanical properties of bioplastic film

Table 1 presents the tensile strength and percent elongation of both AEA and PBA bioplastic films. PBA bioplastic film exhibited higher tensile strength and percent elongation than AEA bioplastic film, which further confirmed the stronger chemical interactions between agar, sago starch and glycerol in PBA bioplastic film.

There are several other factors affecting the difference in tensile strength and percent elongation value of plastic film such as the molecular weight, conformations, chemical structures and hydration behaviour. Temperature and relative humidity greatly affect the tensile strength and percent elongation of bioplastic film [29].

In the present study, the production of bioplastic films was with the addition of glycerol in the composites, which allowed the glycerol to enter the interior polysaccharides chain of the starch and agar. By this, the inter- or intra-molecular

hydrogen bonds were disrupted and plasticized films can be created in a continuous phase structure [27].

Table 1. Tensile strength and percent elongation of bioplastic film.

Bioplastic Film	Tensile Strength (MPa)	Elongation (%)
AEA	2.431	2.476
PBA	3.067	3.270

3.5. Thermogravimetric analysis (TGA) of bioplastic film

Thermogravimetric analysis was conducted to determine the effects of thermal stability of bioplastic films produced in the present study (Table 2). Both of the bioplastic films experienced weight loss in two distinctive steps. The first weight-loss step occurred at temperature 187.91 ˚C and 191.76 ˚C for AEA bioplastic film (10.40 %) and PBA bioplastic film (9.19 %), respectively. The differences in temperature and weight loss were small. The first step of weight loss was mainly caused by the evaporation of moisture content of the bioplastic films and glycerol is known to be a hydrophilic substance that holds moisture [30].

Table 2. TGA of bioplastic film in nitrogen environment.

Bioplastic Film	Temperature Range (ºC)	Weight Loss (%)	Residual Weight (%)
AEA	26 to 188	10.40	89.61
	188 to 466	74.84	14.80
PBA	26 to 192	9.19	90.80
	192 to 506	80.52	10.27

At second distinctive step, major weight loss occurred at 465.24 ºC and 505.67 ºC for AEA and PBA bioplastic film, respectively. PBA bioplastic film contributed to a higher percentage of weight loss at 80.52 % compared to AEA bioplastic film with weight loss of 74.84 %, respectively at higher temperature. This phenomenon might be due to the thermal decomposition of components that made up the bioplastic film. Glycerol decomposed at temperature around 260 ºC whereas the thermal decomposition of agar and starch are at temperature around 300 ºC [31].

From the thermogravimetric curve for both bioplastic films (Fig. 4), the steep weight loss (degradation temperature) for AEA bioplastic film was around 300 ºC and for PBA bioplastic film, around 285 ˚C. The weight loss rate slowly reduced after 350 ºC which further reconfirmed that most of the components in the bioplastic film had been decomposed within the reasonable range (thermal decomposition, glycerol = 260 ºC, agar and starch = 300 ºC). AEA bioplastic film showed higher thermal stability (14.80 %) as compared to PBA bioplastic film (10.27 %). Although PBA bioplastic film has better tensile strength and elongation compared to AEA bioplastic film, it does not attribute to better thermal stability. Thermal stability of bioplastic film depends on many factors such as the ability of the molecule to discharge cation and network structure of molecules. For example, higher degree of cross-linking in a certain composite contributed to stronger thermal stability [32].

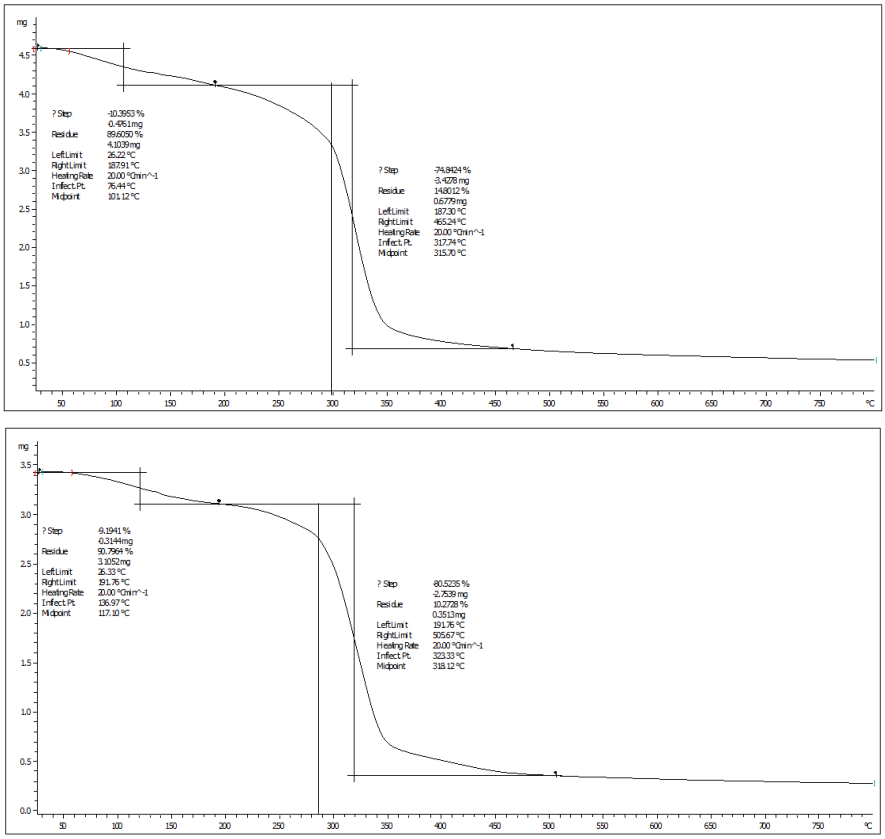


Fig. 4. Thermogravimetric curve of bioplastic film: (a) AEA bioplastic film, (b) PBA bioplastic film.

3.6. Rheological properties of bioplastic film

The constant shear rate tests indicated that both of the bioplastic films are with non-Newtonian and shear thickening behaviour. This is because the ratio of shear stress to shear rate of the films were not constant and the viscosity varies with different shear stress [33]. Moreover, with increasing of shear stress, the viscosity for both of the films raised tremendously from 145 Ps.s at 1.48 Pa to 7740 Ps.s at 77.4 Pa (AEA bioplastic film) and from 102 Ps.s at 1.03 Pa to 2550 Ps.s at 25.5 Pa (PBA bioplastic film).

Figure 5 shows the amplitude sweep test which was conducted by applying the amplitude of deformation or amplitude of shear stress in an increasing order while maintaining constant frequency. From the strain region of 0.1 to 1 %, the G' and G" for both of the films were in a rather constant position, indicating that structure breakdown rate for both of the bioplastic blends were same as the structure rebuilding rate.

When the magnitude of strain proceed into the region of non-linear viscosity (strain region of 10 to 100 %), the modulus G' start to decrease all the way till the end while the modulus G" start to increase before decrease towards the end

of the process. This is phenomena of strain overshoot when the structure re-arranges and thus provides resistance to large strain amplitude, which increase the value of G". The re-arranged structure is not able to withstand when the strain continues to increase. Thus, causing it to breakdown and the decrease of G" modulus at the end of the process. According to Hyun et al. [34], this type of shear flow behaviour is also known as the weak strain overshoot. Therefore, the bioplastic blends of AEA and PBA could be classified as weak gel system. Weak gels contain crosslink which is able to flow without fracture and have the ability to reform [35].

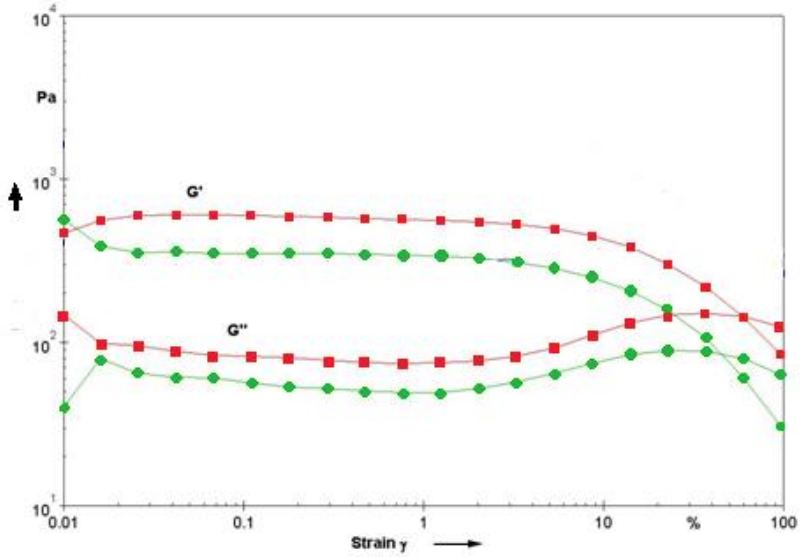


Fig. 5. Graph of G' and G" of AEA bioplastic blend (■) and PBA bioplastic blend (●) against the strain.

3.7. Soil burial test

Soil burial test was carried out to determine the biodegradability of AEA and PBA bioplastic films in different type of soil condition. Biodegradability is known as the ability of polymer undergoes structural alteration and the scission of polymeric chains that affects by environmental factors and microorganism activities [36]. The percentage of weight loss of PBA bioplastic film was lower than AEA bioplastic film (Table 3). This might be due to the packed structure of PBA bioplastic film compared to AEA bioplastic film. More air bubbles were trapped in AEA bioplastic film as seen in the SEM image. Hence, the decreased in the dense structure of poly matrix of AEA bioplastic film and increased in the water absorption capability that promotes the growth of microorganisms.

Based on the results from the present study (Table 3), AEA bioplastic film was able to fully decompose after 30 days while for PBA bioplastic film, it was half decomposed. It was found that highest weight loss for bioplastic film was achieved at the burial location that was damp and shaded from sunlight although all the burial locations were having similar pH range of 6.9 to 8.3.

Agar is known as sulphated polysaccharide which contains charged groups in their extended chains with properties of high hydrophilicity. Therefore, the presence of different extracted agar increased the hygroscopic characteristics of the bioplastic films and promotes the growth of microorganisms and hence the weight loss of the films. Besides, composite bioplastic films that contained starch speed up the degradation where it improved the porosity and also provided food source for the growth of microorganism. The promotion of the microorganism growth caused the bioplastic films to lose its structural integrity and facilitate the attack of polymeric matrix [37].

Addition of hydrophilic starch and glycerol in the composition film also improves the water absorption capability and hence increases the water activity of films and promotes the microorganism growth. Glycerol from the composite film passes through the cell membrane and metabolises by the microorganisms also enhanced the weight loss of bioplastic films [38].

Table 3. Percentage of weight loss of bioplastic film

Location	Weight loss (%)	
	AEA bioplastic film	PBA bioplastic film
Location 1 (Dry; exposed to sun, near to building)	75.05	25.78
Location 2 (Shaded by building; soil is damp)	99.29	43.27
Location 3 (Dry; exposed to sun; near to shrubs)	61.51	36.84

4. Conclusions

In the present study, agar extracted from different extraction technique were rigid, brittle with uneven surface and have an average yield of 9 to 11 %. FTIR spectra of AEA and PBA bioplastic films confirmed that there are chemical interactions between agar, sago starch and glycerol with PBA having the stronger interaction effects. The SEM images and mechanical tests further revealed the PBA bioplastic film is with better mechanical properties (higher tensile strength and percent elongation) which have denser and packed structure. The AEA bioplastic film exhibited excellent biodegrability with the highest weight loss of 99.29 % in comparison to PBA bioplastic films with 43.27 % weight loss within 30 days of soil burial test. TGA results indicated that thermal stability of AEA bioplastic film is better than PBA bioplastic film. Rheological analysis of the biopolymer blends showed that all the bioplastic blends produced are non-Newtonian fluid and thus performed like a weak gel system.

As conclusion, there are a lot of explorations and optimizations to be made to both of the bioplastic films but there is no doubt that agar extracted from *G. salicornia* are able to bring future potential to a wide range of bioplastic applications in the industry.

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