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EFFECT OF DEEP EUTECTIC SOLVENT ON TENSILE PROPERTIES AND BIODEGRADATION OF PECTIN WITH EGGSHELL BIOPLASTIC

(Kesan Pelarut Eutektik kepada Sifat Tensil dan Biodegradasi Bioplastik Pektin dengan Cangkang Telur)

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Abstract

Pectin has excellent potential as a main source of bioplastic due to its biodegradability. However, the neat pectin-based film has poor chemo-physical properties and low mechanical performance. In this study, the pectin-based film is successfully modified by adding eggshell and deep eutectic solvent (DES), comprising choline chloride and malonic acid, as a filler and plasticiser to enhance the performance of bioplastic. Five pectin-based bioplastics were prepared: pectin/eggshell (without DES) and pectin/eggshell with each 1%, 2%, 3%, and 4% of DES. The bioplastics were characterised by the Fourier-transform infrared (FTIR) spectroscopy and inverted camera analysis. The presence of filler and the effect of different DES concentrations were studied based on mechanical properties, biodegradability, and water uptake. The results showed that the addition of different percentages of DES had decreased the tensile strength and increased the flexibility of the bioplastic. Biodegradability testing using compost soil demonstrated an increased degradation rate when a high concentration of DES (4%) was added. The DES of choline chloride and malonic acid shows high potential as a plasticiser in pectin/eggshell bioplastic.

Keywords: pectin, plasticiser, deep eutectic solvent, eggshell filler, bioplastic

Abstrak

Pektin berpotensi sebagai sumber utama bioplastik kerana sifat biodegradasinya. Walau bagaimanapun, bioplastik yang hanya berasaskan pektin sahaja mempunyai sifat kimia-fizikal yang lemah dan prestasi mekanikal yang rendah. Dalam kajian ini, filem bioplastik berdasarkan pektin telah berjaya diubah suai dengan menambahkan cangkang telur dan pelarut eutektik (DES), yang mengandungi kolin klorida dan asid malonik, sebagai pengisi dan pemplastik yang bertujuan untuk meningkatkan prestasi bioplastik. Lima bioplastik berasaskan pektin disediakan: pektin/cangkang telur (tanpa DES) dan pektin/cangkang telur dengan masing-masing 1%, 2%, 3%, dan 4% DES. Bioplastik yand dihasilkan dicirikan secara fizik menggunakan spektroskopi Fourier-penukaran inframerah (FTIR) dan analisis kamera terbalik. Kehadiran pengisi dan kesan kepekatan DES yang berbeza dikaji berdasarkan sifat mekanik, kebolehbiodegradasian, dan pengambilan air. Hasilnya menunjukkan bahawa penambahan peratus DES yang berbeza telah menurunkan kekuatan dan meningkatkan fleksibiliti bioplastik. Ujian biodegradasi menggunakan tanah kompos menunjukkan peningkatan kadar degradasi apabila peratus DES yang tinggi (4%) digunakan. DES bagi kolin klorida dan asid malonik menunjukkan potensi yang tinggi sebagai pemplastik di dalam bioplastik pektin/cangkang telur.

Kata kunci: pektin, pemplastik, pelarut eutektik, pengisi cangkang telur, bioplastik

Introduction

Petrochemical plastic materials are important in our daily lives. However, the vast amounts of plastic waste materials generated contribute to worldwide environmental concerns [1]. About 34 million tons of plastic trash is produced each year, with 93% ending in landfills and the seas. Furthermore, the disposal of non-biodegradable plastic leads to significant global warming, ozone depletion, eco-toxicity, eutrophication, and long-lasting decomposition [2]. Improper management of plastic causes a severe effect on the ecosystem. Thus, biodegradable plastic is the best alternative to reduce plastic waste, save the environment and sustain green global issues. The use of natural sources, such as cellulose, pectin, starch, chitosan, and alginate as food packaging materials has slowly replaced petrochemical plastics [3, 4, 5]. Pectin is a polysaccharide found abundantly in fruits like apple, orange, lemon, mango, grapes, and peach [1, 6]. It is very compatible as a biodegradable and edible biopolymer for food packaging. However, due to its poor chemo-physical properties and low mechanical performance, the pectin biofilm must be improvised by adding a plasticiser and organic filler accordingly to meet the food packaging requirements [1]. Like most polysaccharides, pectin is glassy at room temperature, causing defects such as cracking or curling in a film due to its shrinking, followed by water evaporation or rapid drying [7]. Gennadios et al. [8] asserted that these films are often fragile and rigid due to extensive interactions between the polymer molecules. Therefore, a filler is commonly used to improve the rigidity and strength of the biofilm [9, 10]. A natural bio filler such as eggshell has grown popular since they are environmentally friendly, renewable, cheap, and abundantly available [11, 12].

However, a biofilm made of pectin with eggshells is mostly brittle. A plasticiser is a useful compound in minimising film brittleness by reducing intermolecular forces among polymer chains, increasing their mobility, and improving mechanical properties [13] by holding the polymer bonds together [14]. Deep eutectic solvent (DES) is a new category of plasticiser that has been competently tested for polysaccharide biofilms in various methods [15, 16, 17, 18]. The two most common plasticisers in the polymer industry are *o*-hydroxyphenyl (HBP) and di-2-ethylhexyl phthalate (DEHP), known for their ability to disrupt the endocrine system, with carcinogenic and mutagenic properties [19]. Phthalate is not bonded to plastic; therefore, it will easily leak out into the human body, causing major health effects [20]. According to Mekonnen et al. [21], an ideal plasticiser significantly reduces the glass transition temperature (T_g), biodegradable, non-volatile, non-toxic, and have minimal leaching or migration properties. DES, comprising two self-associating components, met the requirements as a plasticiser in bioplastic [22, 23]. The ability of DES to swell inside biopolymers has enabled bioplastic plasticisation [19], as well as improved thermal stability and decreased the glass transition temperature (T_g) of bioplastics [24]. Previously, Shafie et al. [17] have successfully used DES as a plasticiser in pectin extracted from *Momordica charantia* or known as *peria*. According to their findings, DES caused different degrees of plasticisation via hydrogen bonds and ionic bonds with the polymer chains, which improved thermal stability, tensile properties, percentage of moisture absorption, and water vapour transmission rate of bioplastic. However, the strength of biofilm is reduced without fillers. Hence, this study investigates the compatibility of pectin/DES with eggshells as a natural filler. The pectin/eggshell bioplastic was developed with different concentrations of DES (choline chloride: malonic acid) to analyse bioplastic performance based on tensile strength, biodegradability, and water uptake.

Materials and Methods

Material and reagents

The commercial pectin from citrus was purchased from Sigma Aldrich, Malaysia. The choline chloride (ChCl), malonic acid, and sodium hypochlorite were purchased from Acros, Belgium, with 99% purity. All chemicals used are of analytical grade. The DES was prepared as

suggested by Abbot et al. [15], with a 1:1 ratio of ChCl and malonic acid. The mixture was magnetically stirred at 80 °C until the colourless liquid was formed and maintained as a liquid after 24 hours at room temperature. The eggshell powdered filler was prepared according to Kasmuri et al. [14]. The eggshells were crushed using a mortar and pestle and placed in a beaker. Sodium hypochlorite was added until all the eggshells were soaked and stirred using a glass rod until heat and bubble were released. The beaker was covered using aluminium foil and left for 24 hours before filtering and washing using distilled water. The dried eggshell sample was then dried in the oven and left to cool at room temperature. The eggshell sample was then ground into powder to obtain the size of 58 μm.

Preparation of pectin-based bioplastic

The pectin-based bioplastic was prepared according to Cataldo et al. [25] and Almeida et al. [26]. A volume of 3% (w/v) aqueous pectin was stirred at 70 °C until completely dissolved. About 1% to 4% wt of DES was added to the pectin solution and stirred to homogenise and form film-forming solutions. Approximately 3% wt of eggshell filler was added until completely dissolved. The well-dispersed aqueous dispersions were poured into Petri dishes and dried in the oven at 50 °C for 24 hours. The control was prepared without DES, i.e., 3% of aqueous pectin with 3% wt of eggshell as the filler.

Characterisation of DES

The thickness of the bioplastic was measured using a Digimatic thickness gauge (Model 547-301, Mitutoyo). At least five measurements were performed at different positions for each bioplastic, and the average thickness was calculated. The mechanical properties, including tensile strength, Young's modulus, and elongation at break, were determined using the Instron Universal Testing Instrument (Instron 3365, Instron, USA) equipped with a 1 kN load cell. The sample was cut into a rectangular shape with the size of 7 cm × 1 cm. The initial grip separation was 50 mm, with a crosshead speed of 5 mm min^{-1}. At least five replicates were performed for each film formulation. The soil burial test was conducted according to the ASTM D6003-96 standard method to determine the ability of bioplastics to degrade in compost soil. The initial mass of each bioplastic was weighed and recorded. The bioplastic was buried in compost soil, and the mass was recorded daily until fully degraded. The W_o (g) is the average of the initial bioplastic weight before being buried in soil samples, and the W_t (g) is the average weight of bioplastic at the time of measurement.

The following equation was used to calculate the weight loss of the sample:

$$\text{Weight Loss}(\%) = \frac{W_o - W_t}{W_o} \times 100 \quad (1)$$

The synthetic seawater was prepared by dissolving 35 g of sodium chloride in a total mass of 1000 g distilled water in a beaker. The bioplastic with the size of 1.5 cm × 2 cm was weighed before being immersed in the synthetic seawater. The samples were dried and reweighed at three days intervals. The percentage weight loss of the bioplastic was calculated using the following formula, where M_0 is the average initial mass of the sample, and M_1 is the average residual mass of the bioplastic after the test.

$$\text{Weight Loss } (\%) = \frac{M_0 - M_1}{M_0} \times 100 \quad (2)$$

The water uptake method was conducted according to Zárate-Ramírez et al. [27]. A 1.5 cm × 2 cm sample was cut, dried in the oven at 50 °C for 24 hours, and weighed as W_o. The sample was then immersed in distilled water for 10 seconds at room temperature and reweighed as W_t. The experiment was conducted in triplicate. Water uptake was calculated using the following equation:

$$\text{Water Uptake } (\%) = \frac{W_t - W_o}{W_o} \times 100 \quad (3)$$

Results and Discussion

FTIR analysis was carried out to analyse the functional group present in pure pectin, eggshell, and pectin/eggshell (P-E) composites (Figure 1). The O-H stretching, C=O stretching, and C-O stretching at 3440 cm^{-1}, 1746 cm^{-1}, and 1018 cm^{-1} characterise the structure of pectin.

According to Bichara et al. [28], the main peaks observed at 3436 cm^{-1}, 1743-1640 cm^{-1}, and 1103-1146 cm^{-1} represented the O-H, C=O, and C-O glycosidic bonds in pectin. Meanwhile, the pure eggshell showed peaks at 712, 876, and 1425 cm^{-1}, as similarly reported by Li et al. [29], who characterised calcite by an in-plane bending, out-of-plane bending, and asymmetric CO_3^{2-} stretching at the peaks of 713, 875, and 1424 cm^{-1}. Similar findings were also reported by Siriprom et al. [30] and Pradhan et al. [31]. The combination of pectin with eggshell in the composite film shows all peaks in individual pectin and eggshell. However, a C-O peak from CO_3^{2-} at 1424 cm^{-1} disappeared in the composite as the formation of new bonding is expected between the calcite of eggshell with the glycosidic bond of pectin. The pectin/eggshell composite is enhanced with the addition of DES as a plasticiser. Figure 1(b) shows the FTIR analysis on the increasing concentration of DES from 1% to 4% on the pectin/eggshell composite. As observed, the intensity peaks at 3350 cm^{-1} and 1000 cm^{-1} are slightly decreased when the concentration of DES increases. The IR spectra of all films are dominated by vibration, related to the presence of the pectin compound. A similar result was also reported by Gouveia et al. [32], indicating the interaction between pectin and the additives.

The images of bioplastic sample surfaces were analysed using an Olympus CKX53 inverted microscope (Figure 2). The bubbles trapped in the pectin/eggshell biofilm surfaces are due to the agitation process and the addition of the eggshell powder with pectin solution during sample preparation. The number of bubbles is reduced with the addition of a high percentage of DES, as shown in Figures 2(b) to 2(e). In general, it is also observed that the solubility of eggshell powder increases with the concentration of DES, especially at 3% to 4% of DES (Figure 2(d) and 2(e)). Kong et al. [33] stated that the addition of plasticiser might modify polymer chain intermolecular forces and reduce viscosity in the system. Thus, the DES enhances the dispersion of the added eggshells within the polymer matrices, which is expected to affect the mechanical performance of the bioplastics.

The thickness of the biofilms is an important characteristic that determines its purpose and function. A thin biofilm can lead to fragility and easily be torn, while a very thick biofilm has less flexibility. Table 1 shows the thickness of the pectin/eggshell biofilms. According to ASTM D885, the standard maximum thickness for plastic is 1 mm. Meanwhile, the pectin/eggshell bioplastic is the thinnest bioplastic with 0.0985 ± 0.01 mm. The thickness of bioplastic increases as the plasticiser is added from 1% to 4%, i.e., 0.0991 ± 0.01mm to 0.2687 ± 0.06 mm. The film tends to be thicker as the amount of DES increases. The result is congruent with Esposito et al. [34], which showed that the pectin film thickness increased as polyamines were added as a new cationic plasticiser for pectin-based edible (PEC) films. In this work, the thickness of the PEC films is doubled in the absence of glycerol.

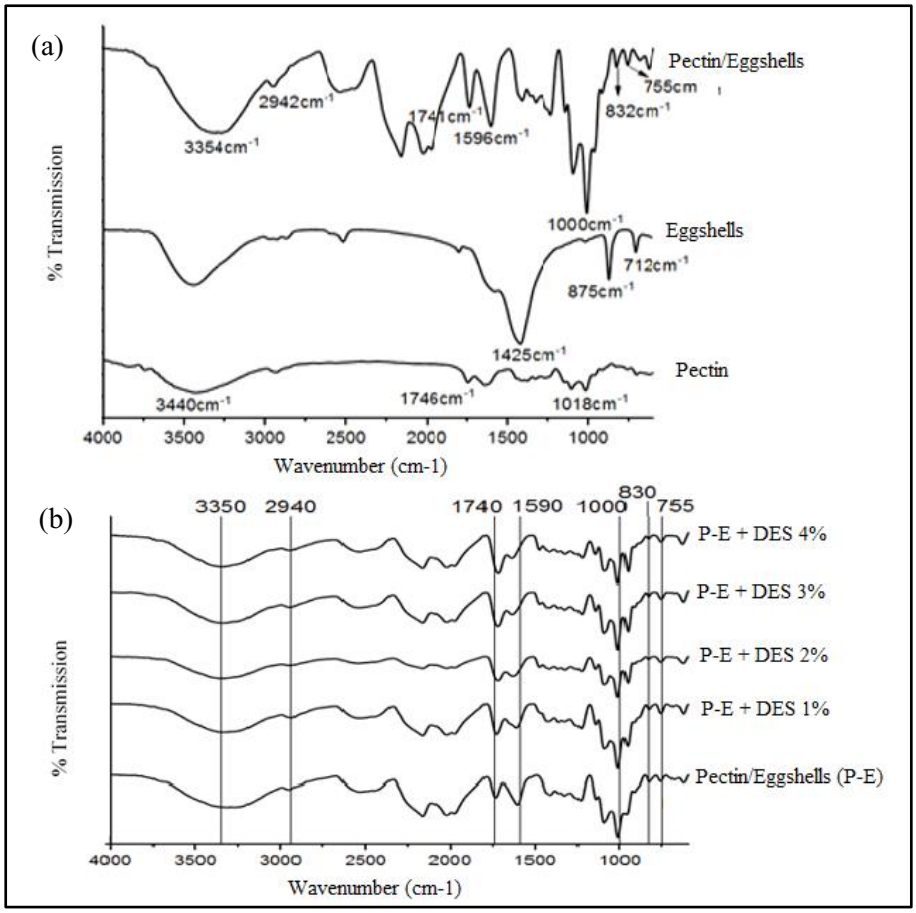
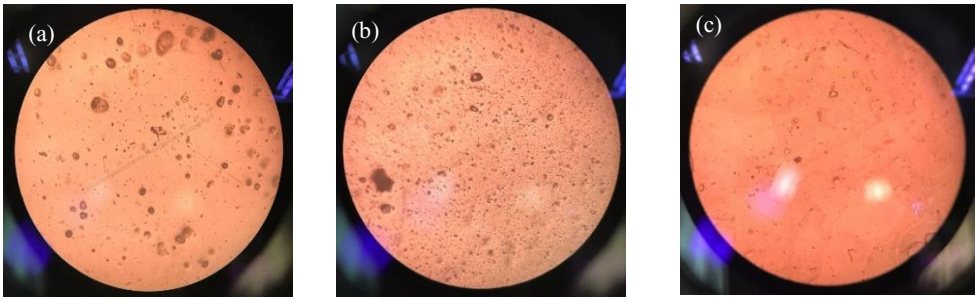


Figure 1. FTIR spectrum for (a) Pectin/Eggshell composite, pure eggshell, and pure pectin (b) Pectin/Eggshell composite with different DES concentrations



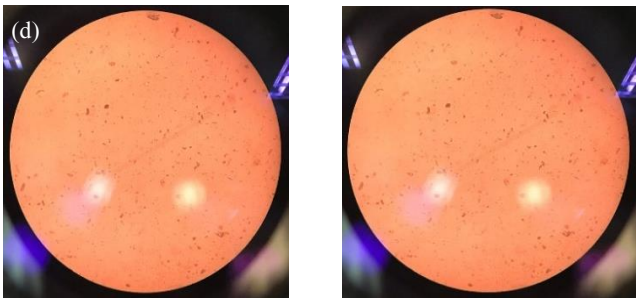


Figure 2. Bioplastic surface images in 4× magnification of (a) Pectin/Eggshell; (b) Pectin/Eggshell in DES 1%; (c) Pectin/Eggshell DES 2%; (d) Pectin/Eggshell DES 3%; and (e) Pectin/Eggshell DES 4%

Table 1. Thickness of pectin-based bioplastic

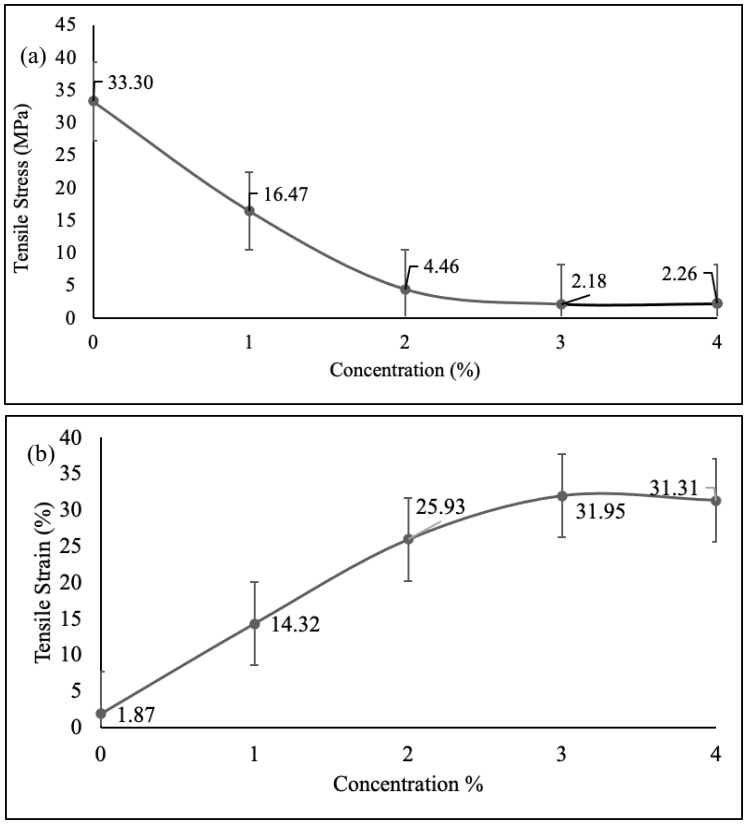
Sample Bioplastic	Thickness (mm)
Pectin/Eggshell (P-E)	0.0985 ± 0.01
P-E + DES 1%	0.0991 ± 0.01
P-E + DES 2%	0.1448 ± 0.02
P-E + DES 3%	0.2146 ± 0.02
P-E + DES 4%	0.2687 ± 0.06

The mechanical properties of the prepared bioplastic in 1% to 4% DES concentration were determined via tensile testing analysis. Figures 3(a-c) show the trend of tensile stress, strain, and modulus of the bioplastic at different DES concentrations. The results indicated that the pectin/eggshell bioplastic is the most rigid with high tensile stress at 33.30 MPa. As a filler, eggshells fill the space in the pectin polymer and increase the stiffness of bioplastic. The tensile strain of pectin/eggshell bioplastic is observed at 1.87%, with the modulus reaching 2754.00 MPa. A study by Kong et al. [33] stated that the addition of eggshell powder to polylactic acid (PLA) yielded a more rigid plastic than PLA only. The PLA/eggshell had superior tensile stress with 200% strain at break and a high modulus. Hence, a similar effect is observed in this study, where the addition of eggshells resulted in substantial improvement in the tensile strength and modulus of the pectin/eggshell bioplastic.

The DES was later added to pectin/eggshell as a plasticiser. The tensile stress and modulus are reduced from 33.30 to 16.47 MPa and 2754.00 to 774.00 MPa, with the addition of 1% DES to the pectin/eggshell. However, the addition of 1% DES caused the tensile strain to increase from 1.87% to 14.32%. The continual addition of DES from 1% to 3% has decreased the tensile stress from 16.47 to 2.18 MPa. A similar effect was observed on the modulus, which is reduced from 774.00 to 14.00 MPa with 1% to 3% DES. The opposite happened to tensile strain, which increased from 14.32% to 31.95% DES. The addition of plasticiser creates substantial intermolecular interaction between the polymer chains, preventing them from gathering close to each other, restricting the molecular chains to compact and cohesive strength in the sample. Thus, it can be concluded that DES has reduced the tensile stress of pectin/eggshell by subsequently weakening the hydrogen bonds between the pectin chains. Eggshells that caused the films to break after a certain force is applied. Muscat et al. [35], who previously used starch to make a film, stated that plasticisers diminish the strong intramolecular attraction between the starch chains. A similar

observation by Sanyang et al. [36] found that the tensile strength decreases with an increasing percentage of mixture glycerol/sorbitol/glycerol-sorbitol as the plasticiser increased from 15% to 45% on sugar palm starch films. However, the tensile strain value remains at the same range with 4% DES, which is 2.26 MPa. Similar results are observed for tensile strain and tensile modulus, i.e., constant at the addition of 4% DES. The addition of plasticiser did not significantly change the properties of bioplastic after 3%, indicating that at 3%, the liquid has swelled and filled up the free volume within polymer chains to prevent intermolecular interaction. Hence, the study only conducted only up to 4% of DES.

The biodegradability test was carried out to determine the weight loss of the pectin/eggshell after ten days in soil. The biodegradation process mainly depends on the moisture content and the presence of microorganisms in the ground. Figure 4 indicates the biodegradability behaviour in the weight loss of the bioplastic at a different DES percentage. All bioplastics show a continuous weight loss increase, with the bioplastic with 4% DES fully degraded (100%) on the third day and bioplastic with 1%, 2%, and 3% DES fully degraded on the fifth day. The pectin/eggshell composite without DES showed the highest percentage loss on the first day, but only 93% degraded on the fifth day and remained around 97% on the tenth day of the test. The higher percentage of DES concentration in bioplastic, led to a higher weight loss of the bioplastic. The bioplastic at 4% DES degrades faster than lower DES concentration. Kasmuri et al. [14] stated that the presence of the eggshell filler reduced the biodegradability rate since 95% of the eggshell is made up of calcium carbonate compounds, which mostly remained in the soil after the bioplastic had degraded. However, as proven by the tests, DES enhanced the biodegradability properties of bioplastic.



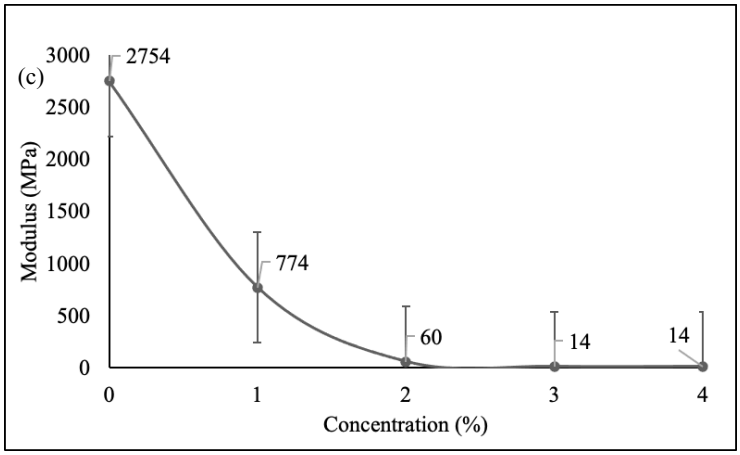


Figure 3. The properties of bioplastic in (a) tensile stress; (b) tensile strain and (c) modulus

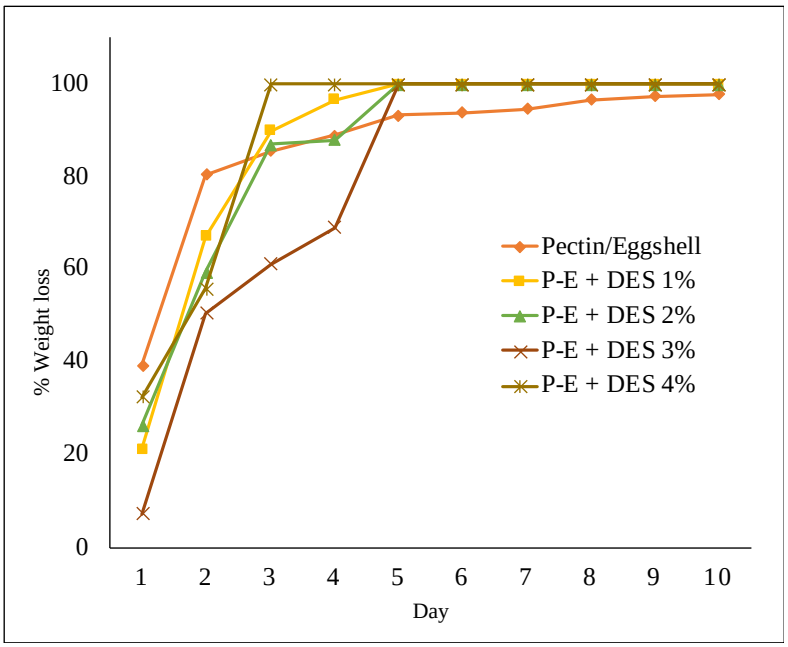


Figure 4. The percentage of weight loss of bioplastic in 10 days of biodegradability test in soil

Another biodegradability test was conducted in synthetic seawater. All bioplastics immediately swelled and curved when immersed in seawater. After one day in seawater, the pectin/eggshell bioplastic was fully degraded, but not the pectin/eggshell/DES bioplastics. Figure 5 shows the degradation of bioplastics in seawater. The result is contrary to the biodegradability test conducted in soil. In seawater, the bioplastic with a high DES concentration takes longer to degrade than those without DES. The inclusion of electrolytes from NaCl in synthetic seawater influenced bioplastic breakdown. According to Trindade et al. [37], ionic liquid shows the effect of salting out in salt solution. The same effect might be seen in the biofilm

containing DES. The added salt (NaCl) might compete with the ions in DES, causing the migration of NaCl molecules from the DES ions, decreasing the hydration and solubility of bioplastic-containing DES.

The analysis of water uptake was performed to measure the ability of bioplastic to absorb water with the addition of different concentrations (%) of DES. Figure 6 shows that the highest percentage of water uptake is at 1% concentration of DES, with 85% of water uptake. According to Suppakul et al. [38], the presence of hydroxyl group in plasticiser is strongly interacted with water molecules by forming hydrogen bonds. DES (choline chloride: malonic acid) consists of O-H bonds that interact directly with water, increasing the volume of the water uptake. However, when the percentage concentration of DES is more than 1%, the water absorption is reduced since the holding ability is weakened.

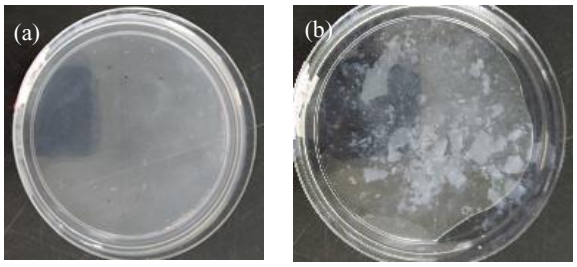


Figure 5. The images of (a) complete degradation of pectin/eggshell bioplastic and (b) incompleted degradation of pectin/eggshell/DES bioplastic in seawater

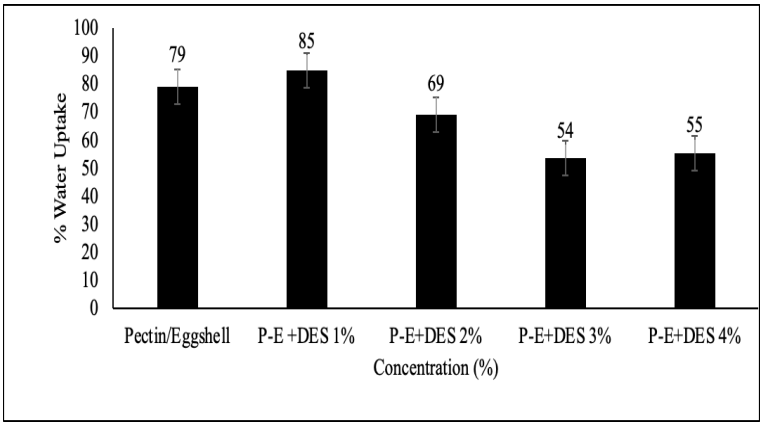


Figure 6. Percentage water uptake of bioplastics test in different concentration (%) of DES

Conclusion

The pectin/eggshell bioplastics with different concentrations of DES were successfully prepared. The eggshell and choline chloride: malonic acid are compatible with pectin, and no crystallisation occurred during the formation of biofilm. DES allowed the bioplastic to become more flexible by disrupting the polymer chains, causing an increase in elongation and a decrease in tensile strength of pectin/eggshell/DES. The higher concentration of DES leads to more flexibility and higher biodegradation in soil but with lower water uptake. These results indicated that

Choline chloride:malonic acid could be used as a potential plasticiser for pectin/eggshell bioplastic.

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