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Synthesis and characterization of water-soluble polyvinyl alcohol/pomegranate peel powder films

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ABSTRACT
Biodegradable plastics had gained much attention among the researchers due to the arising of sustainability and environmental issues caused by petrochemical-based plastics. In this study, PVA films incorporated with pomegranate peel powder (PPP) were synthesized by using solution casting method. The effect of PPP on the surface morphology, physical properties, barrier properties and antibacterial activity of PVA based films were studied through FE-SEM, water solubility test, water contact angle test and Kirby-Bauer test. The incorporation of PPP had significantly enhanced the water solubility and surface energy of PVA-PPP films with the increasing of PPP content that caused reduction in water contact angle of the films. However, the films do not show a significant effect on the antibacterial efficacy against *E.coli*. In conclusion, PVA-PPP films have great potential to be used as green packaging materials for cosmetics products provided that further study on antibacterial efficacy against gram positive bacteria should be conducted.
Keywords: *Water-soluble; polyvinyl alcohol; pomegranate peel powder; antibacterial.*

1. INTRODUCTION
Packaging plays an important role in preserving cosmetics products. Good packaging material can protect cosmetics products from microbial contamination, UV photo degradation and oxidation. Cosmetics are products that normally applied to human skin mainly for beautifying, cleansing and protecting purposes [1]. These products are basically non-sterile, therefore inappropriate preservation of these products can provide beneficial environments for the growth of microorganisms.

Most of the cosmetics packaging is made of plastics due to light weight, durable and attractive properties. However, not all plastics are environmentally friendly. For instance, the breakdown of petroleum-based plastics releases various kinds of toxic chemicals that pose a threat to the environment. In order to solve this global concern, development of biodegradable plastics is one big step to lessen the usage of non-biodegradable plastics.

Bioplastics can be synthesized using various types of natural resources based on their desired properties. Pomegranate peel powder which poses good antioxidant and antimicrobial properties is an alternative to chitosan in producing antimicrobial bioplastics [2, 3]. It is a by-product obtained during pomegranate juice processing. The pomegranate peel has an abundant amount of polyphenols, including a wide variety of tannins, which possesses unique biological activities, inhibits microbial growth and reduces the risk of cancer and cardiovascular diseases [4]. Current study focused on the synthesis of water-soluble polyvinyl alcohol (PVA) based films incorporated with 1% to 5% pomegranate peel powder (PPP). The physical, mechanical and barrier properties of the PVA based film were investigated and antibacterial activity of PVA-PPP films against *E.coli* was also examined in this study.

2. MATERIALS AND METHODS
2.1. Materials.
PVA and glycerol were purchased from R&M Chemicals. Pectin from citrus peel and PPP were purchased from Sigma Aldrich and Craftiviti, respectively. Nutrient agar powder was purchased from MERCK while *Escherichia coli* stock was purchased from Sigma Aldrich.

2.2. Solution casting of PVA/PPP films.
The film solutions were prepared by dissolving 0.25% citrus pectin in 100 ml preboiled distilled water under magnetic stirring (800 rpm). The pectin solution was cooled down until 90 °C before the 5% PVA crystals were dissolved under 200 rpm stirring for 30 mins. 1% glycerol was then added into the solution and continues heated at 90 °C under 200 rpm for another 30 mins. Then, pomegranate peel powders (PPP) with concentration of 1% to 5% were added into the film solution and continue stirred at 200 rpm for an additional 30 mins at room temperature. Another film solution without adding PPP was used as the control. The film solutions were left cool overnight on the bench to reduce air bubbles. The film solutions were filtered to remove insoluble PPP fiber from the polymer solution. After that, 10 g of film solutions was casted onto 17 cm × 6 cm glass plate and oven dry for 10 hrs at 40 °C. The thickness of the films was controlled by casting a constant amount of film solutions on the same dimension glass plates. The dried films were then peeled off from the glass plate and stored in zip-lock bag for further analysis.

2.3. Characterization.
2.3.1. Field emission scanning electron microscopy.
The surface microstructure of the PVA control film and 5% PVA-PPP film surface was investigated by JSM-7800F Field Emission Scanning Electron Microscopy (FE-SEM). The films were coated with platinum and adhered to sample holder by using carbon tape to allow the image to be captured without distortion

due to the charging effect. Photomicrographs were taken at ×2000 magnifications using an accelerating voltage of 5 kV.

2.3.2. Water solubility.

The water solubility testing was conducted in triplicate in accordance to the method by Ahmad et al. [5] with a slight modification. The films of 1 cm × 3 cm in size were dried at 110 °C for 24 hrs and weighed. The dried films were then immersed into beakers containing 50 ml distilled water and stirred slowly for 24 hrs at room temperature. The solution was filtered and the undissolved films were oven dried at 110 °C for 24 hrs. The dried films were weighed to determine the dry matter soluble in water. The water solubility was calculated by Equation 1:

$$\text{Water solubility} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

where W_1 is the initial weight of the pre-dried film and W_2 is the final weight of the dried undissolved film.

2.3.3. Mechanical properties.

Tensile test following ASTM D882 was carried out using SHIMADZU AGS-X series universal tensile test machine to investigate the effect of incorporating different concentration of PPP in the PVA films on their tensile strength and elongation at break. The testing was performed with a crosshead speed of 25 mm/min, the distance between jaws of 50 mm and load force of 100 N at room temperature. The film samples were cut in 100 mm long, 5 mm width and thickness below 1.0 mm. Samples containing any defect such as air bubbles, holes, and tears or showing average thickness variation superior to 5% were inspected and rejected. The testing was conducted for 5 replicates and the average was reported.

Tensile strength of the film samples was calculated by dividing the load at break by the sample cross-sectional area (Equation 2). The result is expressed in megapascals (MPa) and reported to three significant figures.

$$\text{Tensile strength (TS)} = \frac{\text{Load at break (N)}}{\text{Width (mm)} \times \text{Thickness (mm)}} \quad (2)$$

The percentage of elongation was calculated by dividing the displacement of the films by the initial gauge length and multiplying by 100 (Equation 3).

$$\text{Elongation at break (\%)} = \frac{\text{Displacement (mm)}}{\text{Initial gauge length (mm)}} \times 100 \quad (3)$$

Elastic modulus of the film samples was calculated by dividing the tensile stress by the tensile strain (Equation 4).

$$\text{Elastic modulus (E)} = \frac{\text{Tensile stress (MPa)}}{\text{Tensile strain}} \quad (4)$$

2.3.4. Light absorption and opacity.

The light absorption and opacity test were carried out using Agilent Cary Series UV-Vis NIR Spectrophotometer in accordance with Kavoosi et al. [6] with some modifications to investigate the effect of incorporating different concentration of PPP into the PVA films on their opacity. The film samples were cut into 1 cm × 1 cm square and directly place onto the sample holder. The UV-visible absorption spectrum of the film cuts was measured over a wavelength range from 200 to 700 nm. The opacity of the films was calculated by dividing the light absorbance value at 280 nm (maximum absorbance of PVA film) with film thickness (Equation 5). The testing was conducted for 4 replicates and the averages were reported.

$$\text{Opacity} = \frac{\text{Light absorbance at 285 nm (A)}}{\text{Thickness of film (mm)}} \quad (5)$$

2.3.5. Water contact angle.

The contact angle of water on the film surface was measured using the DataPhysics (Germany) Optical Contact Angle OCA 25 following the method by Islam et al. [7] with a slight modification. The film samples were cut to 3 cm × 3 cm in size and placed on the sample stage of the instrument. 3 µL of deionized water was deposited on the film samples using drop method. The contact angle was measured for a specific time interval from 0 to 40 mins until the drop disappeared completely. Each measurement was performed for 3 replicates, and the mean value was calculated and reported.

2.3.6. Antibacterial efficacy.

The antibacterial activity of the films was examined using the Kirby-Bauer method as described by Hanani et al. [8]. *E. coli* strains were seeded onto nutrient agar by using an autoclaved cotton swab and incubated at 37 °C for 24 hrs before the tests. The sample films were aseptically cut into 6 mm diameter disc and placed on the nutrient agar. Amikacin antimicrobial susceptibility disc was used as positive control for this testing. Cellulose disc dipped in 70% ethanol was used as negative control. The plate was then incubated at 37 °C for 24 hrs. The diameters of the inhibition zones were measured in triplicate and the averages were reported. The testing was repeated by using cellulose discs engrafted with PVA film solution and 1% to 5% PVA-PPP film solutions as testing samples to investigate the effect of diffusion rate on releasing of antibacterial agent. The zone of inhibition was measured by Equation 6:

Zone of inhibition (mm) = diameter of bacterial free zone (mm) – diameter of film disc (mm) (6)

3. RESULTS

3.1. Surface morphology.

Figure 1 revealed that incorporation of PPP had affected the neatness of microstructure of the PVA film. Figure 1(a) shows the smooth surface of the PVA control film deduced that the homogeneous dispersion of citrus pectin and PVA crystals. This is most likely due to the formation of hydrogen bonds between the hydroxyl groups of pectin and the hydroxyl groups of PVA. The high content of PPP caused agglomerates formed on the film surface. The agglomerates will increase stress field on their nearby regions and initiates cracks or dents which resulting in a weakening of the mechanical properties of the films [9]. Figure 1(b) shows the surface defects of 5% PVA-PPP film caused by the excess amount of fillers. The agglomerates, cracks and dents found on the film surface had supported the measured mechanical and physical properties of 5% PVA-PPP film.

3.2. Water solubility.

Water solubility is an important property of water-soluble films for green sustainable packaging. PVA control film showed a solubility value of 47% which lower than the value reported by previous study [6]. This differential solubility could be related to the presence of insoluble PPP fiber as cross-linker.

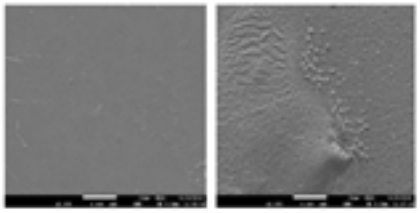

Figure 1. FESEM micrographs of (a) PVA control film and (b) 5% PVA/PPP film.

PVA resins are naturally water soluble while the solubility of polymeric PVA film reduced to some extent [10]. This water resistance was proven when film samples maintain their integrity in water after 24 hrs. This indicates that PVA network remained intact in water and only the PVA monomers and other nonbinding material were soluble and the scaffold of PVA network remained insoluble. However, the solubility of PVA films was found to increase gradually by increasing PPP content in the PVA matrix (Figure 2). This might be attributed to the formation of new hydrogen bonds between -OH group of PVA and -OH group of phenolic compounds which weaken the existing hydrogen bonding that stabilizes the PVA network. This effect hinders polymer chain-to-chain interactions, reduces the PVA matrix integrity which in turn increases the solubility of the films. Thus, it was proven that the incorporation of PPP had significantly increased the water solubility of the PVA film.

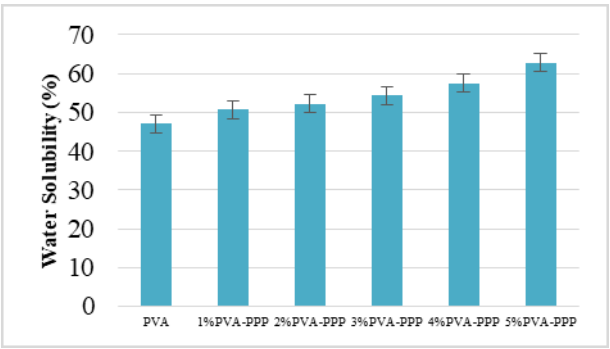

Figure 2. Water solubility of PVA-PPP films.

3.3. Tensile strength.

The effect of the additional PPP as natural fillers into PVA film on the tensile properties, including tensile strength (TS), percent elongation at break (ε) and Elastic modulus (E) as shown in Figure 3. The TS value for PVA control film is 16.3814 MPa and was found to be higher than the results reported by Asrofi et al. [11] due to the additional of citrus pectin as a thickening agent in the film matrix. Pectin is a group of heteropolysaccharides naturally found in the cell wall of vascular plants which act as a cementing matrix in the cellulosic fiber [12]. The natural role of pectin in providing mechanical strength to the plant cell wall has encouraged the use of pectin as matrix in biocomposite materials. Besides, pectin also acts as gelling agent, stabilizer and emulsifier which helps in the miscibility of the polymer solution and PPP [13].

PPP containing both soluble and insoluble fibers have contributed to a different function. There was no previous study on the different properties of insoluble and soluble PPP fiber. However, several studies were done on pomegranate peel extract and pomegranate peel powder where we can see the different contributions of the extract and powder towards the mechanical properties of polymer film [8, 14]. Insoluble fiber acts as reinforcing material in the polymer matrix. In contrast, a significant decrease in tensile strength of soluble PPP was obrserved due to the intermolecular interaction between functional group of PVA and PPP components. In this study, low tensile strength is required according to the intended film application. Therefore, film samples with soluble PPP fiber were used in this testing instead of the films with insoluble PPP fiber.

The incorporation of soluble PPP caused a significant decrease in tensile strength and elastic modulus and an increase in elongation at break, resulting in a flexible and stretchy film. According to Safaee-Ardakani et al. [15], PVA films were mainly stabilized by the weak bond including hydrogen bond between hydroxyl groups of monomers. The reduction of the film tensile strength by the addition of soluble PPP is likely related to the formation of intermolecular interaction between functional group of PVA and PPP components. Thus, the original hydrogen bonds between PVA chain that stabilized the film matrix could be replaced with new hydrogen bonds between -OH group of PVA and -OH group of phenolic compounds in PPP. This effect hinders polymer chain-to-chain interactions, reduces the PVA matrix integrity which in turn decreases the tensile strength. Elastic modulus is a measure of the stiffness of film. A stiff material has high elastic modulus and changes its shape only slightly under elastic loads. By the incorporation of soluble PPP, the elastic modulus of the PVA films decreased significantly. This shows that addition of PPP can reduce the film stiffness which in other words the films turn to be more flexible. This result is in accordance with the values of elongation at break.

Elongation at break is an indication of flexibility and extensibility of film prior to breakage. The parameters TS and ε represent a pair of relative performance indicators, such that ε decreases as TS increases. In this study, the flexibility of the PVA based films increased might be because of the increase in pore sizes and porosity of the films by adding soluble PPP to the polymer matrix. Not only this, the increasing flexibility of the films by the addition of PPP could also be related to formation of new hydrogen bonds between PPP and PVA matrix which caused an increase in the segmental mobility of PVA chain and more sliding effect of PVA chains against each other [16]. However, PVA film with 5% PPP shows shorter elongation as compared to that of the 4% PVA-PPP film. This is due to the excess amount of PPP gathered and formed agglomerates on the film surface. These agglomerates will increase stress field of the affected regions and results in cracks and dents formation [9]. This statement was supported by FE-SEM testing which discussed in section 3.1.

3.4. Light absorption and opacity.

The PVA film showed light absorbance in the range between 200 and 700 nm, while maximum absorbance was at 285 nm. The opacity values of PVA films are shown in Table 1. Incorporation of PPP into the PVA film caused a significant increase in the light absorbance and opacity. Thus, the PVA films lost their typical transparent and colourless appearance. However, the resulting PVA-PPP films gained in light barrier properties, which could be interesting in cosmetics packaging for preventing UV-induced photodegradation in photosensitive products and lengthen their shelf life [17]. This will be an advantage for cosmetics industry due to the increasing market demand for more

natural and non-additive products. The increase in the light absorbance more likely depends on the distribution of PPP in the PVA matrix as well as the interaction between PPP and PVA. This effect led to differences in film matrix morphology with different light absorbance. The opacity of the PVA film was increased with increasing concentration of PPP in the PVA matrix, more likely because of the light scattering effect of PPP. Chirdon et al. [18] reported that the orientation of fillers affects the absorption and scattering coefficient differences in fiber reinforced polymer matrix.

Besides, the colour of PVA-PPP films getting darker as the concentration of PPP incorporated into the PVA film increased. This statement can be supported by the research done by Mousavinasab SM in 2011 [19] which state that darker shades of the films contain darker pigments that absorb more light and result in lower light transmittance which result in better light barrier properties.

Table 1 Light absorbance, thickness and opacity and light transmittance of PVA based film incorporated with various concentration of PPP.

Sample	Light absorbance at 285nm (A)	Thickness (mm)	Opacity (A/mm)	Transmittance (%)
PVA	0.5872	0.135	4.3496	25.87
1%	1.6113	0.136	11.8478	2.45
2%	2.4815	0.138	17.9819	0.33
3%	2.7239	0.139	19.5964	0.19
4%	3.1889	0.141	22.6163	0.06
5%	3.3382	0.143	23.3441	0.05

3.5. Water contact angle.

Water contact angle is an important parameter to determine the surface wettability of the films for a wide range of applications such as water-soluble pods and food packaging. Figure 3 shows the incorporation of PPP into PVA matrix had significantly increased the film surface wettability.

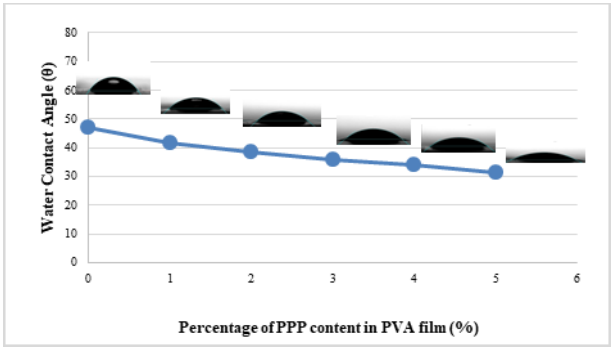

Figure 3. Water contact angle of PVA-PPP films.

PPP has hydrophilic nature and contains many hydroxyl groups mainly from phenolic compounds [20]. Incorporation of PPP into PVA matrix had affected the neatness of molecular organization of PVA [21]. The increasing content of PPP in PVA matrix had directly contributed to the high number of hydroxyl groups from phenolic compounds which formed new hydrogen bonding with the PVA hydroxyl group. However, these new hydrogen bonding was weaker than the original hydrogen bonding between PVA monomers.

The hydrophilicity of the films was increased with an increasing amount of PPP and therefore increased the film surface water absorption which in turn decreased the contact angle. Film samples with a higher content of PPP have higher surface energy that creates a strong attractive force to pull the water droplet down, causing it to spread out. This surface energy is stronger than the surface tension of the water molecules that would normally keep it in droplet form. Thus, it was proven that the incorporation of PPP had significantly increased the wettability of the PVA film.

3.6. Antibacterial activity.

The antibacterial activities of different concentration of PVA-PPP solid films and its film solutions are presented in Table 2. The solid films of PVA control, 1% and 2% PVA-PPP do not show any inhibition zone (Figure 4(a)) while 3% to 5% PVA-PPP show weak inhibition zone surrounding the sample discs (Figure 4(b)). However, the diameter of the inhibition zones cannot be measured as the zones were not clear and some of the sample films shrunk and slightly detached from the agar medium due to its hydrophilic nature. For cellulose discs engrafted with the film solutions, the inhibitory effect against *E. coli* was found to increase with the increasing concentration of PPP in the PVA matrix. However, 4% PVA-PPP solution shows slightly smaller inhibition zone than that of the 3% PVA-PPP solution (Figure 4(d)). This might due to the human error while engrafting the polymer solution on cellulose disc. By comparing both solid films and film solutions, the latter shows better inhibitory effects towards *E. coli*. This was due to the difference in diffusion rates of solid polymer and polymer solutions. The phenolic compounds in PVA-PPP solution had a higher diffusion rate as compared to the cured PVA-PPP film. Recent studies reported that cured material does not release any antibacterial components on direct contact with agar medium [14].

Table 2. Antibacterial efficacy of PVA-PPP films and cellulose discs engrafted with PVA-PPP solutions against *E.coli.*

Sample	Existence of Inhibition Zone		Diameter of Inhibition Zone (mm)	
	Film	Solution	Film	Solution
PVA control	No	No	-	-
1% PVA-PPP	No	No	-	-
2% PVA-PPP	No	No	-	-
3% PVA-PPP	Yes	Yes	-	1
4% PVA-PPP	Yes	Yes	-	Less than 1
5% PVA-PPP	Yes	Yes	-	5

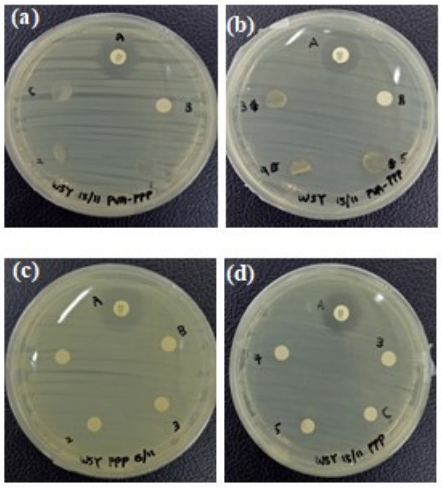

Figure 4. Antibacterial activity of (a,b) PVA-PPP films and (c,d) cellulose disc engrafted with PVA-PPP solutions against *E.coli*.

PPP is rich in polyphenols, including a wide variety of tannins, which exhibit antibacterial activities due to their ability to modify the morphology of microorganisms by precipitating protein, causing cell membrane leakage resulting in cell lysis [22]. However, in this testing, the antibacterial activities of both PVA-PPP films and PVA-PPP film solutions were not significant by observing the diameter and clearness of the inhibition zones. This is due to the small increment of PPP loadings in the PVA matrix. Moreover, this result can be explained by the previous study which reported that the antibacterial activity of phenolic compounds was stronger against Gram-positive bacteria than against Gram-negative bacteria [23].

4. CONCLUSIONS

In this study, the incorporation of pomegranate peel powder (PPP) had significantly affected the surface morphology, physical, mechanical and barrier properties of the PVA based films. Surface defects were seen on 5% PVA-PPP film surface due to the high loading of PPP which leads to formation of agglomerates. The films show an increasing trend in water solubility with increasing concentration of PPP. Besides, PVA based films with higher PPP content have lower tensile strength, higher elongation at break as well as lower elastic modulus which resulting in a flexible and stretchy film. Surface defects were seen on 5% PVA-PPP film surface due to the high loading of PPP which leads to formation of agglomerates. Higher PPP content contributes to better light barrier properties whereby the increasing of film opacity had significantly reduced light transmittance through the films. Furthermore, surface energy of the films increasing with the amount of PPP had caused reduction in water contact angle. However, in this study, the incorporation of PPP does not have a significant effect on the antibacterial activity of PVA based films against *E. coli* as phenolic compounds were known to be more effective towards gram positive bacteria.

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