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Original Article

Modifying the properties of whey protein isolate edible film by incorporating palm oil and glycerol

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Abstract

This study aimed to improve the properties of whey protein isolate (WPI) films by incorporating palm oil (6, 7, and 8% w/w) and glycerol (40, 50 and 60% w/w). The lightness of the films increased as glycerol levels increased, but the redness increased with the increased amount of oil content. Increasing the amounts of palm oil and glycerol improved flexibility ($P<0.05$), but reduced the strength of the film ($P<0.05$). Films with higher levels of palm oil and lower amounts of glycerol were less permeable to water vapor and oxygen, but more thermally stable. The size of particles and air bubbles in the films reduced with increased palm oil content, regardless of glycerol level. Among all formulae, the film prepared with 8% palm oil and 40% glycerol showed the best overall results. Modifying WPI films with palm oil and glycerol offers a simple technique for producing packaging with better environmental barrier properties.

Keywords: palm oil, glycerol, whey protein isolate, edible film, biodegradable packaging

1. Introduction

Studies on the production of bio-based packaging materials with antimicrobial and antioxidant properties are increasing as the demand for environmentally-friendly and active packaging has increased. Most of these studies have used polysaccharides and proteins as raw materials (Javanmard, 2009). The films prepared with whey protein isolate (WPI) have been reported to effectively minimize the effects of oxygen, flavor, and lipids on different food products (Miller & Krochta, 1997). Furthermore, proteins isolated from whey, such as α-lactalbumin and β-lactoglobulin, have been reported to increase the transparency and improve the flexibility of films without affecting color or odor (Javanmard, 2009). Nevertheless, due to their hydrophilic characteristic, WPI-based films are relatively poor moisture barriers (Kim & Ustunol, 2001).

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Using plasticizers to prepare films helps increase their mechanical and moisture barrier properties. Natural plasticizers that are generally used include water, glycerol, sorbitol, and lipids (Suyatma *et al.,* 2005). Glycerol is used most often because it has small molecules and can efficiently disrupt the hydrogen bonds and disperse the polymeric chains. Reducing the intermolecular attractions between the chains improved the flexibility, water vapor permeability (WVP), and oxygen permeability (OP) of the films (Vasconez *et al.,* 2009).

Using oils reduced WVP and OP and improved the mechanical properties and appearance of the films (Fabra *et al.,* 2008). For example, adding corn oil decreased WVP of both polysaccharide and protein films (Cerqueira *et al.,* 2012; Ekthamasut & Akesowan, 2001). Palm oil is a rich source of saturated fatty acids and antioxidants, such as carotenoids and vitamin E. Recently, palm oil has been used as a plasticizer to prepare bio-based films from carrageenan (Saiful *et al.,* 2013) and cassava starch (Perazzo *et al.,* 2014). Saturated fats increased the melting points of the films, while antioxidants extended the shelf life of packaged foods. However, little is known about using palm oil for the preparation of WPI-based films.

This study aimed to investigate, on a comparative basis, the effects of adding palm oil and glycerol at various concentrations on the physical, mechanical, thermal, and microstructure properties of the WPI-based films. The optimal quantities of both compounds were those that provided the best balance of all desired properties. The results of this study show the potential for these films. The stability of food products packed in these films should be investigated next.

2. Materials and Methods

2.1 Materials

WPI with 90% protein was supplied by the Thai Food and Chemical Co., Ltd., Bangkok, Thailand. The purified palm oil was obtained from the OPG Tech Industry Company Co., Ltd., Samut Prakan, Thailand. Glycerol was purchased from Ajax Finechem, Australia. Chemicals used to produce the films were food grade, while those used to determine its properties were analytical grade (Merck, Germany).

2.2 Film preparation

A 5% (w/v) WPI solution was prepared at room temperature by dissolving WPI powder in distilled water, adjusting the pH to 11 with 1 M NaOH, heating the mixture at 80 °C for 15 min, adding glycerol (40, 50, or 60% w/w of WPI), palm oil (6, 7, or 8% w/w of WPI), and Tween-80 (10% w/w of palm oil). The mixtures were heated at 80 °C for another 25 min with constant stirring and filtered through Whatman No. 4 filter paper. Samples (85 ml) of the mixtures were spread on 15x25 cm acrylic plates and allowed to dry at ambient conditions for 24 h. The dried films were kept at 25±2 ºC in a desiccator until further analysis within a week.

2.3 Determination of physical properties

Ten different areas in each sample were determined for thickness using a digital micrometer (Gotech, Model GT-313-A, Gotech Testing Machines Inc, Taiwan) and color parameters using a digital color difference meter (Hunter Lab, Model CR-310, Japan).

2.4 Determination of mechanical properties

The tensile strength (TS) and elongation at break (EB) were measured according to the ASTM D882-95 method (American Society for Testing and Materials [ASTM], 2000) by an Instron Universal Testing Machine (Model 1000, H1K-S, UK). Ten samples from each treatment were incubated in saturated salt solution chambers at 27±2 °C for 24 h to obtain a relative humidity of 65±2%. They were cut into 150x25 mm strips. TS was calculated according to Equation 1, where F = maximum load and A_c = cross-sectional area of the sample.

$$TS = \frac{F}{A_c} \quad (1)$$

EB was calculated according to Equation 2, where ΔL = elongation at the moment of rupture of the sample and L_i = the initial gage length of the sample at the moment of rupture.

$$EB\ (\%) = \frac{\Delta L}{L_i} x100 \quad (2)$$

2.5 Determination of barrier properties

Film solubility was measured according to Shittu and Lawal (2007). The solubility was calculated using Eq. 3, where w = the initial dry weight of the 1x1 cm film sample, a_1 = suspension weight, and a_2 = the final dry weight (Rhim *et al.,* 1999).

$$Solubility\ (\%) = \frac{w(a_1 - a_2)}{w} x\ 100 \quad (3)$$

The WVP of the samples was determined following the ASTM D882-00 standard method (ASTM, 2000). WVP was calculated according to Eq. 4, where $\Delta W/\Delta t$ was the amount of water gain (g/day), A was the test area (m^2), L was the film thickness (mm), and ΔP was the partial vapor pressure difference between both sides of the film (mmHg) (Aydinli & Tutas, 2000).

$$WVP = \frac{\Delta W x A x L}{\Delta t x \Delta P}\ (g.mm/day.m^2.mmHg) \quad (4)$$

ΔP of the film sample was calculated using Eq.5 (Selke *et al.,* 2004), where Ps was the saturated water vapor pressure, RH_{out} was the relative humidity outside the cup, and RH_{in} was the relative humidity inside the glass permeation cell.

$$\Delta P = Ps\frac{(RH_{0ut} - RH_{in})}{100} \quad (5)$$

The OP of the film was determined following the method of Ou *et al.* (2005). The peroxide value (PV) of the oil was measured by the titration method (Zahedi *et al.,* 2011) and described as the OP index.

2.6 Determination of thermal property

Thermal property was measured using differential scanning calorimetry (DSC) (Model DSCQ100, TA Instrument, UK). The heating rate was set at 10 °C/min to heat the 10 mg sample pan to 250 °C under flowing N_2 at a flow rate of 20 ml/min. The glass transition temperature (Tg) was determined from the thermograms (Kim & Ustunol, 2001).

2.7 Determination of microstructure

Microstructure of the film was analyzed by scanning electron microscopy (SEM) (JSM-5910LV, JEOL, Japan), using an accelerating beam at a voltage of 15 kV.

2.8 Statistical analysis

The statistical significance of the data was determined by analysis of variance (ANOVA). The confidence limits were based on 95% ($P \leq 0.05$) and the significant differences among the means were determined by the Duncan's Multiple Range Test.

3. Results and Discussion

3.1 Effects of palm oil and glycerol on physical properties

All film samples visually appeared as transparent shiny sheets with a creamy yellow color. Palm oil contains the orange color of carotenoids, which most likely contributed to the yellow color of the films, as glycerol itself appeared to be colorless. Different palm oil concentrations caused slightly different shades of the color (Figures 1 and 2). As expected, the higher the palm oil concentration in the film, the more intense its yellow color. However, the lightness of the films was mainly associated with the glycerol content. According to Loiko *et al.* (2013), glycerol decreases the density of the protein–protein interactions by enhancing polymer chain movements, thereby increasing the free volume and lowering the Tg, which together allows more light to pass through the film matrix.

All films appeared to be flexible, smooth, and uniform in texture with no visible oil droplets, pores or cracks (Figure 1). These characteristics were due to the presence of the glycerol. The use of glycerol, due to its plasticizing properties, produced a uniform and flexible texture of the film. Plasticizers improve the dispersion and elasticity of films by decreasing the frictional forces among the polymer chains in the film matrix and increasing the free volume and molecular movement of the films (Suyatma *et al.,* 2005). Adding glycerol into other edible films, such as galactomannan and chitosan films, had a similar effect of decreasing the cracks and holes and increasing the homogeneity and plasticity of the films (Cerqueira *et al.,* 2012). Femandez *et al.* (2007) studied the effects of unsaturation and fatty acids on the properties of WPI films and found that oil droplets formed on a film's surface when adding oil of 8.0% or more. This did not occur in our study when we used 8.0% palm oil. Unlike Femandez, we used Tween-80 as an emulsifier to promote dispersion of the palm oil in the film matrix which explains the different results. This result suggested that an emulsifier is an important component in making emulsion-based films with high concentrations of oil.

Thickness of the films indicates strength and barrier to gases and vapor. The thickness significantly increased ($P<0.05$) from 0.14 mm for the film with 6% palm oil and 40% glycerol to 0.18 mm for the film with 8% palm oil and 60% glycerol (Figure 3). The thicknesses attained were in agreement with other biodegradable films, which usually have thicknesses less than 0.30 mm (Dangaran *et al.,* 2009). It was confirmed that higher concentrations of solid materials used to prepare the films increased the solids content in the film matrix, and thus increased the thickness of the films. Perez-Mateos *et al.* (2009) reported from a structural study that high concentrations of oil changed the film conformation, and the conformational change resulted in an increase in the film thickness.

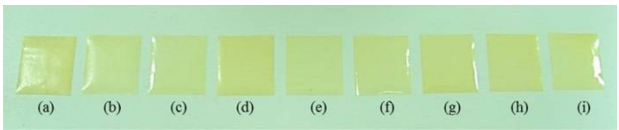

Figure 1. WPI films with different concentrations of glycerol and palm oil: (a) 40% glycerol and palm oil 6% palm oil, (b) 50% glycerol and 6% glycerol and 6% palm oil, (d) 40% glycerol and 7% palm oil, (e) 50% glycerol and 7% palm oil, (f) 60% glycerol and 7% palm oil, (g) 40% glycerol and 8% palm oil, (h) 50% glycerol and 8% palm oil, and (i) 60% glycerol and 8% palm oil.

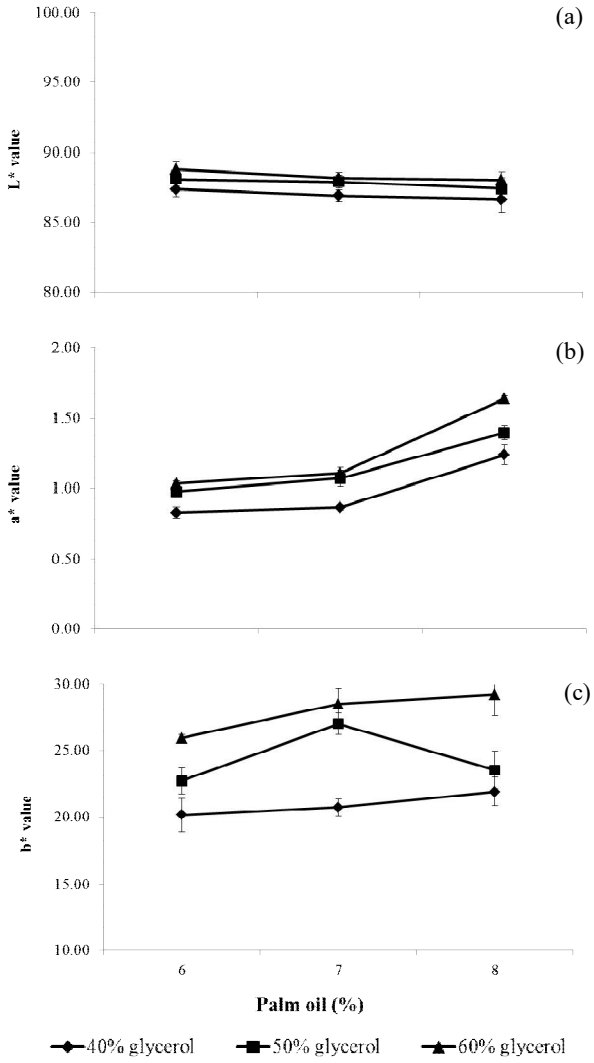

Figure 2. Color of the WPI films with different concentrations of glycerol and palm oil: (a) L*, (b) a*, and (c) b* values.

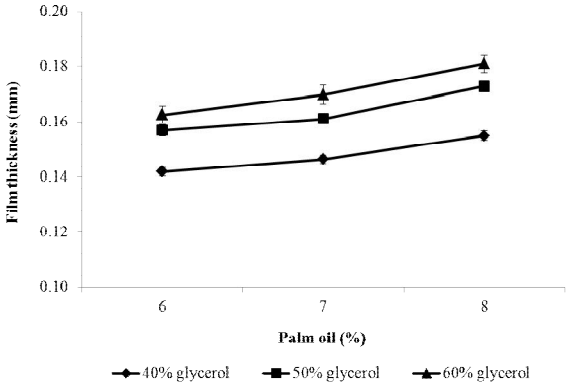

Figure 3. Thickness of the WPI films with different concentrations of glycerol and palm oil.

3.2 Effects of palm oil and glycerol on mechanical properties

TS reduced and EB rose (P>0.05) in the films with higher contents of glycerol and palm oil, respectively (Figures 4a and 4b). The strength of the films was highly dependent on glycerol concentration, while palm oil exerted a positive effect on EB. Increased oil concentrations did not result in differences (P>0.05) between TS values. The higher TS could be due to decreased interactions between WPI chains (Ziani *et al.,* 2008). Pranoto *et al.* (2005) also observed that the TS of chitosan film decreased with an increased amount of garlic oil.

The EB values were not significantly different (P>0.05) at low concentrations of glycerol and palm oil. However, at 8% oil content, a considerable increase of the EB value was observed. Different glycerol concentrations did not significantly affect EB (P>0.05). The increased EB in the film with high oil content suggested that a more stretchable matrix was formed when the hydrophobic portion increased in the emulsion-based films. Higher oil contents lead to a better elasticity of the film. In contrast, higher glycerol contents increased the moisture content in the films because glycerol is a hydrophilic compound. A previous study also reported that increasing soya oil concentration reduced TS and increased EB of WPI-soya oil composite films (Shaw *et al.,* 2002).

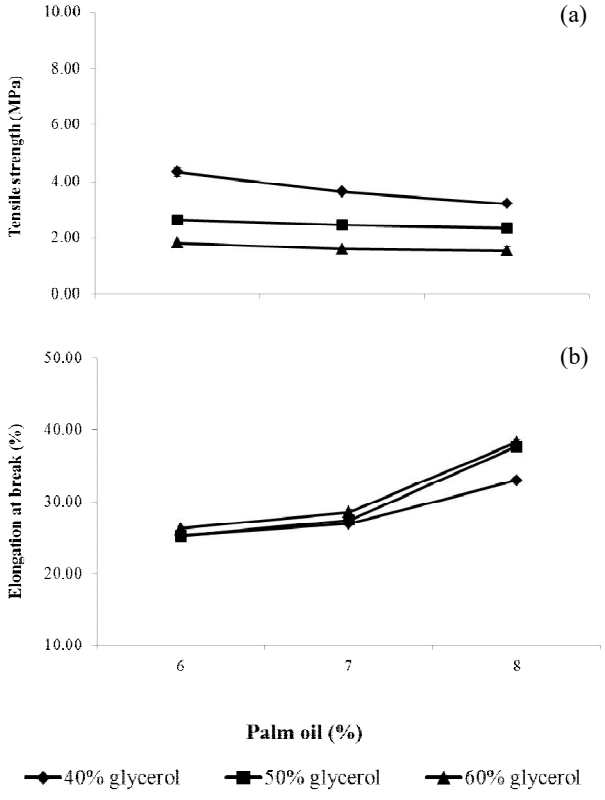

Figure 4. Tensile strength (a) and percentages of elongation at break (b) of the WPI films with different concentrations of glycerol and palm oil.

3.3 Effects of palm oil and glycerol on water solubility, WVP, and OP

Water solubility of edible film is related to water resistance. The water solubility of the tested films increased when glycerol content increased, whereas water solubility decreased when palm oil content increased (P<0.05) (Figure 5). Since glycerol is a hydrophilic compound, while palm oil is a lipophilic compound, higher concentrations of glycerol promotes retention of water molecules in the matrix and supports hydrogen bonding between glycerol and water (Cuq, 2002). On the other hand, adding more palm oil into the films decreases the number of hydrogen bonds in the matrix which creates less hydrophobicity and subsequently reduces water solubility. Previous studies observed a similar trend when adding corn oil into polysaccharide-based films (Cerqueira *et al.,* 2012) and adding sunflower oil into gelatin film (Peres-Mateos *et al.,* 2009).

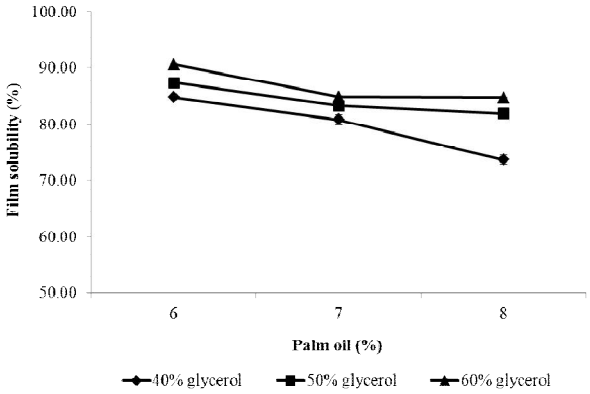

Figure 5. Water solubility of the WPI films with different concentrations of glycerol and palm oil.

The WVP of the films ranged from 9.36 to 11.69 g.mm/m^2.day.kPa (Figure 6). WVP increased with increasing glycerol content (P<0.05), and decreased with increasing palm oil content (P<0.05). As previously discussed, binding glycerol to other biopolymer molecules, such as whey protein in this study, lessens the protein-protein interaction, increases the movement of polypeptide chains, and consequently increases the WVP (Ziani *et al.,* 2008). The presence of glycerol also improved the diffusion rate of water molecules through the films by decreasing the occurrence of pores and cracks, whereas fatty acids in palm oil diminished the penetrability of water, by penetrating into the pores or coating on the films (McHugh *et al.,* 1994). A similar behavior on WPI-based edible film has been reported (Femandez *et al.,* 2007). However, a diverse trend was observed for sodium caseinate film (Fabra *et al.,* 2008) and chitosan film (Ziani *et al.,* 2008). This discrepancy is possibly related to the type and quantity of materials used. Each protein is known for its specific hydrophilic characteristic, while the lipophilic character of the film is associated with the fatty acids present in the oil.

The PV of the films tended to decrease as the concentration of palm oil increased and the concentration of glycerol decreased (Figure 7). The PV reached 2.22±0.02 meq O_2/kg oil when the palm oil content was 8% and the glycerol

concentration was 40%. Khwaldia *et al.* (2004) reported that OP reduced with the addition of anhydrous milk fat in a sodium caseinate film. In contrast, adding oils to gelatin/ triacetin film increased the OP (Bertan *et al.,* 2005). These results proved that the compounds used in developing films affect the configuration of the films dramatically, as previously mentioned in the study of WVP.

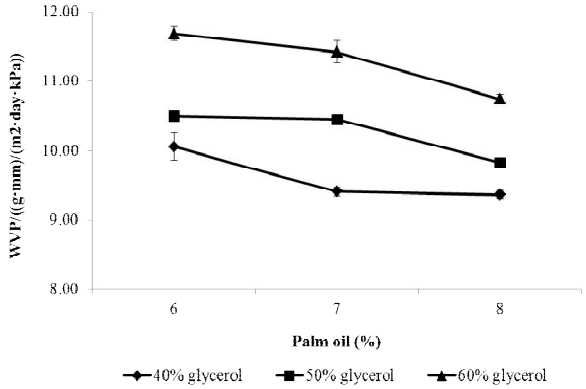

Figure 6. Water vapor permeability (WVP) of the WPI films with different concentrations of glycerol and palm oil.

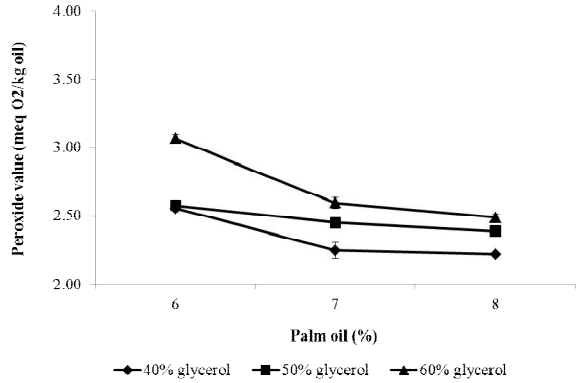

Figure 7. Peroxide values of the WPI films with different concentrations of glycerol and palm oil.

3.4 Effects of palm oil and glycerol on glass transition temperatures (Tg)

Figure 8 shows the Tg of the film samples with each sample displaying only one Tg. Tg increased with as the palm oil content increased ($P<0.05$) due to the decreased moisture content of the films that reduced movement of the film matrix. Similar trends have been noted when adding corn oil to chitosan film (Cerqueira *et al.,* 2012), and soya oil to WPI film (Shaw *et al.,* 2002). In contrast, a reduced effect of glycerol on Tg was reported in muscle protein-based films (Sobral *et al.,* 2005) and gelatin-based films (Vanin *et al.,* 2005). These results confirmed two findings. First, glycerol can reduce the denaturation of protein during heating by dropping the Tg through an increase of the free volume of the protein complex (Lee *et al.,* 2012). Second, glycerol alters the interactions between the macromolecules resulting in an increase in chain movements and subsequently supporting water gathering and reducing the Tg temperatures (Cerqueira *et al.,* 2012; Sobral *et al.,* 2002).

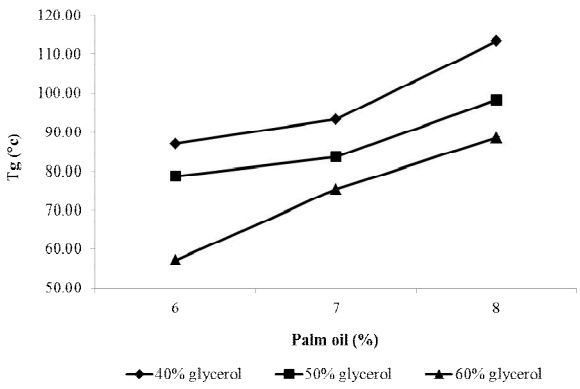

Figure 8. Glass transition temperatures of the WPI films with different concentrations of glycerol and palm oil.

3.5 Effects of palm oil and glycerol on microstructure

SEM revealed the particle appearance and air bubble size differences between the formulas (Figure 9). Particle and air bubble sizes of the films of all glycerol levels were similar and slightly decreased with increasing palm oil content. The film surface incorporated with 6% palm oil appeared as coarsely porous, filled with large air bubbles, and concentrated with oil droplets (Figure 9a-9c). However, a significantly uniform surface was achieved with 8% palm oil (Figure 9g-9i). This heterogeneous matrix of the film surface with lower palm oil content related to the greater moisture content of the films. The more water the film contained, the more air bubbles evaporated during casting which yielded a more porous film surface. As a result, the film matrix did not retain oil droplets well, thereby enabling them to appear on the film's surface. Both the size and number of voids affect the barrier properties of the films. The high porosity of the films with low palm oil content allowed moisture and gas to pass through the films. As such, the films with low palm oil content showed poor water vapor permeability (Figure 6) and gas barrier properties (Figure 7).

Properties of WPI films could be effectively modified by using materials with low commercial value, like palm oil and glycerol. The film with the most optimal properties was obtained by adding 8% palm oil and 40% glycerol. The developed film should effectively fulfill the general requirements of industrial application as preservation packaging on various food products.

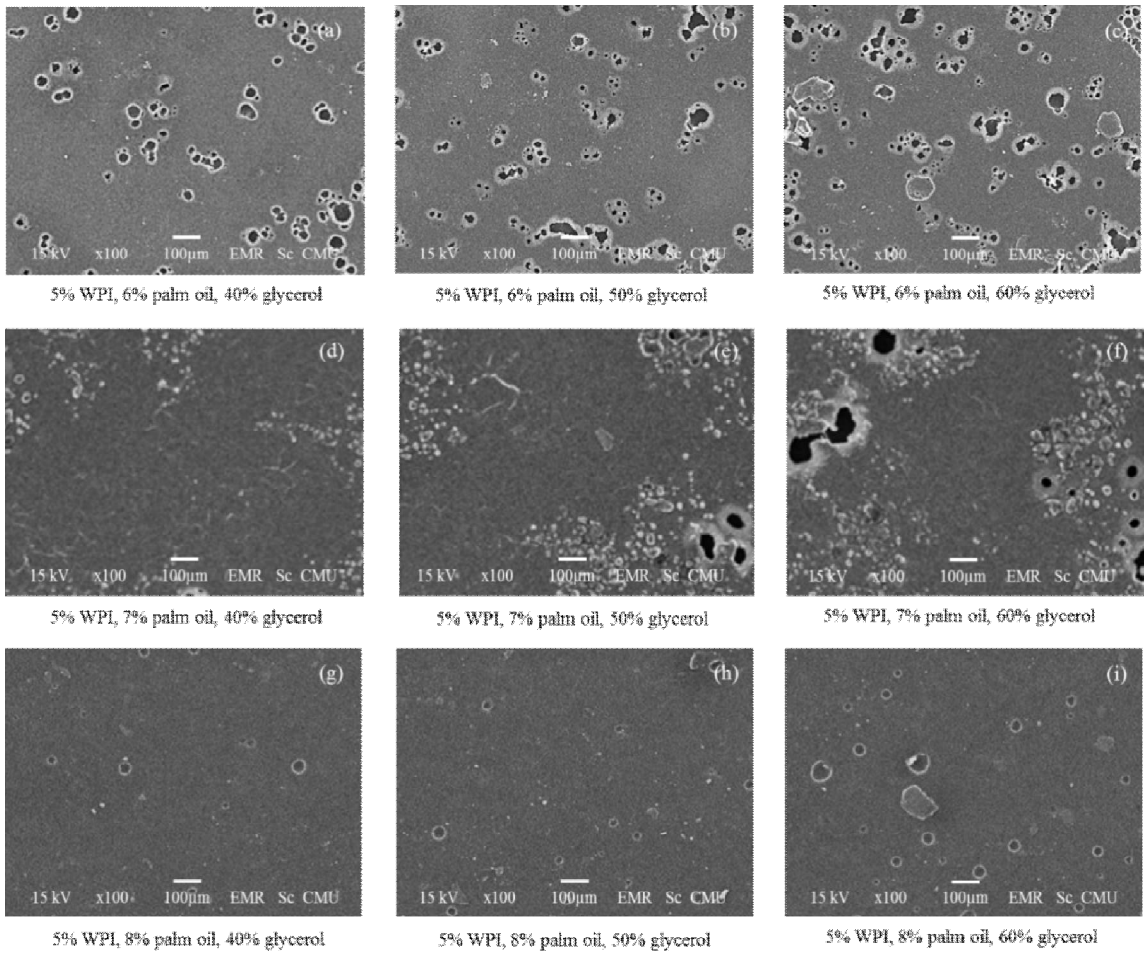

Figure 9. Surface morphology of the WPI films with different concentrations of glycerol and palm oil.

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