Physical properties of edible emulsified films based on pistachio globulin protein and fatty acids

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Novel edible composite films were prepared from pistachio globulin protein (PGP), saturated fatty acids, and an emulsifier using the emulsification technique. The water vapor permeability (WVP) of the emulsified films was reduced by approximately 37–43% by fatty acid addition. The effect of fatty acid on the oxygen permeability (OP) of PGP films was indirectly determined as the oil peroxide value. The OPs of the emulsified films were lower than those of a PGP film without fatty acid, but the differences were not significant (P < 0.05). The mechanical properties of PGP films were also affected by fatty acid addition; the ultimate tensile strength (UTS) was diminished, and elongation at breaking (E) decreased considerably (35–70%). Furthermore, the incorporation of fatty acid increased the opacity of the films. Finally, differential scanning calorimetry showed that the glass transition temperature (T g) of the PGP film was ~127 °C and was not considerably affected by fatty acid addition.

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1. Introduction

The use of plastic for packaging has increased extensively in recent years, and there is growing interest in the use of biodegradable films for environmental and human health reasons. Edible films are non-polluting packaging materials because they are made from natural and biodegradable biopolymers (Debeaufort et al., 1998). Materials that can be used to make edible or biodegradable films include polysaccharides, proteins, lipids and polyesters or combinations of these materials. Edible, biodegradable films and coatings can act as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavor components and thus maintain the quality and increase the shelf life of food products. However, their water vapor barrier and mechanical properties are generally poorer than those of synthetic plastic films (Kester and Fennema, 1989a). Due to the hydrophobic nature of proteins, edible films prepared from protein do not act as an efficient water vapor barrier. However, protein films have a relatively good oxygen barrier and mechanical properties at low and intermediate relative humidity (RH) due to their large number of polar groups and extensive polymer interchain interactions (which in turn create a rigid protein network) (Park et al., 2002). In contrast, lipid films have low water vapor permeability (WVP) to their hydrophobic nature but are very brittle because of their monomeric structure (Callegarin et al., 1997). Furthermore, lipids generally produce opaque films or coatings and are often sensitive to oxidation. These properties may influence the organoleptic attributes of food and lower its marketability. Composite films produced from proteins and lipids have the potential to provide both the relatively high water vapor barrier of lipid films and the desirable mechanical properties of protein films. Up to now, edible protein films have been made from sources including gelatin, soy protein, corn zein, peanut protein, lupin protein, wheat gluten, casein, whey protein, wing bean protein, fish protein, cotton seed protein, pea protein, egg white protein, keratin, sorghum kafrin, rice protein, pickle brine proteins (Park et al., 2002), and sunflower protein (Ayllon-Meixueiro et al., 2000).

Many authors have studied the influence of lipid addition on the properties of edible films. Anker et al. (2002) reported that the addition of acetylated monoglyceride to a whey protein film reduced the WVP by about 0.5 and 70 times in the emulsified and bi-layer films (in comparison to a control whey film), respectively. Quezada-Gallo et al. (2000) showed that the nature of the lipid phase had a significant effect on the WVP but little influence on the mechanical properties of emulsified films composed of methylcellulose and paraffin oil plus paraffin wax. The addition of stearic acid to whey protein films also decreased the WVP and solubility of emulsified films but weakened their mechanical properties (Yoshida and Antunes, 2004). Pistachios are one of the most popular nuts worldwide; Iran is the largest pistachio nut producer in the world, and most of its crop is exported to other countries. Furthermore, the high oil content of the pistachio nut (about 50–60%) has

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been exploited for some industrial applications. Pistachio oil cake is rich in minerals and protein and is most commonly used as an animal feed. Pistachio protein consists of four fractions: globulin (66%), albumin (25%), glutelin (7.3%), and prolamin (2%) (Shokraii and Esen, 1988). The globulin is soluble in 0.5 M NaCl, and its extraction is economically favorable. A few groups have reported the use of pistachio isolate protein to produce edible films. In one case study, researchers investigated the effects of adding pistachio isolate protein to a cellulose-based edible film. The resulting composite edible film had an increased WVP due to the hydrophilic nature of protein, which contains many hydrophilic amino acids such as aspartic acid, glutamic acid, and arginine (Ayranci and Çetin, 1995).

The objective of this research was to study the physical properties of emulsified films prepared from pistachio globulin protein and two saturated fatty acids (palmitic and stearic acids).

2. Materials and methods

2.1. Materials

The pistachio nut oil cake was prepared from Pistacia vera L. varieties at the oil extraction factory in Manila, Kerman, Iran. The globulin fraction was extracted as described by Shokraii and Esen (1988) and final globulin purity was measured at 80%. Tween-80 (emulsifier), calcium nitrate, glycerol (83% purity), and pure palmitic (C16) and stearic (C18) acids were purchased from Merck (Germany). Anhydrous calcium chloride and potassium sulfate (emulsifier), calcium nitrate, glycerol (83% purity), and pure palmitic (C16) and stearic (C18) acids were purchased from Merck (Germany). Anhydrous calcium chloride and potassium sulfate were obtained from Fluka (France).

2.2. Film preparation

Pilot experiment indicated that filmogenic solutions containing 10% stearic acid produced brittle, thick films. Thus, we used lower concentrations of this fatty acid to achieve thinner, more flexible films. The protein (6% w/v) was dissolved in distilled water, and the pH of the solution was adjusted to 11 with 1 M NaOH. The protein solution was then heated to 80 °C on a hotplate with a magnetic stirrer with a heating rate of about 2.7 °C/min and a mixing speed of 1000 rpm. Next, glycerol (100% w/w protein) was added to the solution and heating was continued for 25 min. Emulsified films were prepared by adding palmitic or stearic fatty acids at concentrations of 2, 4, and 6% w/w protein and Tween-80 (10% w/w fatty acid). The solution was subsequently filtered to remove any undissolved material and then cast onto aluminum frames at a ratio of 1 ml/3.5 cm². The frames were dried at ambient conditions for 40 h. The abbreviations for the different samples and their compositions are listed in Table 1. For the emulsified films, fatty acids and emulsifier were added to the solutions after heating for 25 min. The mixture was homogenized with an IKA T25 digital homogenizer (Ultra-Turrax, Germany) for 1 min at 9000 rpm.

2.3. Water vapor permeability (WVP)

The WVP was determined gravimetrically according to the ASTM E 96-00 method (2000), also known as the “desiccant method”, at 22 ± 1 °C. The water vapor transferred through the film and was absorbed by the desiccant. The WVP was determined from the weight increase of the glass cells. The glass cells width were 1.5 cm (i.d.) and 7.0 cm in height, with an exposed area of 1.76 cm². The cells were filled with 4 g anhydrous calcium chloride (0% RH), covered with the films and then placed in a desiccator containing a saturated K₂SO₄ solution (97 ± 1% RH). The weight of the cells was recorded using an analytical balance (±0.0001 g) at 1-h intervals for a period of 12 h and then every 12 h for 72 h total. When the relationship between the weight loss and time was linear, the slope of the plots was calculated by linear regression. Regression coefficients were greater than 0.99. The WVP was calculated using Eq. (1) as follows:

\[ WVP = \frac{\text{Slope} \cdot x}{A \cdot S \left( R_1 - R_2 \right)} \]  

where \( A \) is the area of the exposed film surface (m²), \( S \) is the saturation vapor pressure at the test temperature (kPa), \( R_1 \) is the relative humidity inside the desiccator, \( R_2 \) is the relative humidity inside the cells, and \( x \) is the average film thickness (mm). All of the films were equilibrated inside an air conditioning cabinet at 55% RH, 25 °C for 72 h before the permeability tests.

2.4. Water solubility (WS)

WS was defined as the percentage of film dry matter that was solubilized after 24 h of immersion in distilled water (Gontard et al., 1994). The initial dry matter (%) was determined gravimetrically by drying 2 × 2 cm² film samples (about 16 g) at 103 ± 2 °C for 24 h. The same films (2 × 2 cm²) were then immersed in

### Table 1

<table>
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<tr>
<td>PGP–C₁₈</td>
<td>6</td>
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### Nomenclature

- **AV**: absorbance value (nanometers)
- **C₁₆**: palmitic acid
- **C₁₈**: stearic acid
- **DSC**: differential scanning calorimetry
- **E**: elongation at break
- **LDPE**: low-density polyethylene
- **LSD**: least significant difference
- **MC**: moisture content
- **OP**: oxygen permeability
- **PGP**: pistachio globulin protein
- **RH**: relative humidity
- **Tₛ**: glass transition temperature (°C)
- **UTS**: ultimate tensile strength
- **WVP**: water vapor permeability
- **WS**: water solubility

### Abbreviations and corresponding sample compositions.

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50 ml of distilled water that contained sodium azide (0.02% w/v) to prevent microbial growth. After 24 h of immersion at 20 °C with gentle mechanical agitation, the samples were removed from the solution and dried (103 ± 2 °C, 24 h) to determine the weight of dry matter not dispersed in the water. By subtracting this from the weight of the initial dry matter, the weight of dry matter dispersed in water after 24 h of immersion was obtained and expressed as a percentage of the initial dry matter content.

2.5. Oxygen permeability (OP)

The oxygen permeability (OP) of the films was determined indirectly according to the method described by Ou et al. (2005). Cells were filled with 10 ml fresh, refined, and free antioxidant sunflower oil, covered with the films, stored inside an incubator at 25 ± 1 °C and 55% RH, and supplied with a saturated calcium nitrate solution for 45 days. The peroxide values of the oil were measured according to the method described by Shantha and Decker (1994) and reported as the OP index. Prior to the experiment, the films were conditioned at 25 °C, 55% RH for 48 h.

2.6. Mechanical properties

The ultimate tensile strength (UTS) and percent elongation (%E) of the films (140 × 20 mm²) were determined using a QTS texture analyzer (CNS Farnell, Essex, UK) according to the ASTM D882-02 (2002) method. Initial grip separation and crosshead speed were 100 mm and 50 mm/min, respectively. UTS was calculated by dividing the maximum load for breaking the film by the original minimum cross-sectional area, and %E was calculated by dividing the film elongation at rupture by the initial gauge length. Before analysis, the samples were conditioned for 48 h at 55% RH, 25 °C.

2.7. Differential scanning calorimetry (DSC)

Measurements of the film’s glass transition temperature (Tg) were performed using a differential scanning calorimeter (Shimadzu, DSC 60). About 2 mg of film samples was placed in a hermetically sealed aluminum pan and scanned from −100 to 230 °C at 10 °C/min. An empty pan was used as a reference. Tg was identified as the midpoint temperature of the shift in the baseline due to the change in heat capacity upon glass transition. Before the test, samples were conditioned at 55% RH, 25 °C for 48 h.

2.8. Opacity measurement

Opacity measurements were performed according to the method described by Contard et al. (1992) based on a modified standard procedure. Film specimens were cut into rectangles and placed in the spectrophotometer cell. A spectrum of each film was recorded by an area meter (Li-3100C) and defined as the film opacity. Opacity was expressed as product of the absorbance value and wavelength (AV · nm). Films were conditioned at 25 °C, 55% RH for 48 h prior to the test.

2.9. Moisture content (MC)

The moisture content (MC) of the film was determined by drying in an oven at 103 ± 2 °C for 24 h. Small test specimens were cut and placed on glass Petri dishes, and their weights were recorded before and after oven-drying. MC was calculated as the percentage of weight loss based on the original weight, using Eq. (2), where Mi is the weight of the Petri dish and film specimen before drying, Md is the weight of the Petri dish, and Mw is the weight of the film specimen after drying.

\[
\%MC = \frac{M_i - M_d}{M_i - M_w} \times 100
\]

MC values for PGP and emulsified films are provided in Table 2. The PGP film had the highest moisture content of all of the films tested. Partial protein–water interactions replaced with protein–fatty acid interactions by the emulsifier and MC reduction occurred in emulsified films.

2.10. Thickness measurements

Film thickness was measured with an electronic micrometer (QLR digit-IP54, China). Measurements were taken at the center of the film and at four positions along the rectangular strips for the mechanical properties. The mean measurements were used to calculate the barrier properties of the film.

2.11. Statistical analysis

The experiments were factorial with a completely randomized design. The type and concentration of fatty acids were the independent variables. Data were analyzed by ANOVA using MSTAT-C software, version 1.42 (Michigan State University). The differences of means were detected by the LSD (least significant difference) at a probability level of P < 0.05. Five independent replicates were conducted for MC and opacity measurements, three for WVP, OP, mechanical properties, and WS measurements, and two for Tg measurements.

3. Results and discussion

3.1. Water vapor permeability

The WVP values of the PGP and emulsified films are presented in Table 2. Incorporation of fatty acids caused a significant difference (P < 0.05) between the WVP of the PGP film and emulsified films due to increased hydrophobicity, and WVP was reduced from 96.203 to 55.497 g mm/m² kPa d. Even at 2% fatty acid, the WVP of

<table>
<thead>
<tr>
<th>Film type</th>
<th>Moisture content (%)</th>
<th>WVP (g mm/m² kPa d)</th>
<th>Water solubility (%)</th>
<th>Peroxide value (meq O₂/kg oil)</th>
</tr>
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<tbody>
<tr>
<td>PGP</td>
<td>37.36 ± 2.355c</td>
<td>96.203 ± 7.33c</td>
<td>44.814 ± 5.010a</td>
<td>23.342 ± 0.433c</td>
</tr>
<tr>
<td>PGP–C16 (2%)</td>
<td>34.16 ± 3.798abc</td>
<td>60.706 ± 2.34a</td>
<td>41.481 ± 2.566a</td>
<td>21.788 ± 0.512a</td>
</tr>
<tr>
<td>PGP–C16 (4%)</td>
<td>36.20 ± 3.114ab</td>
<td>58.903 ± 1.51a</td>
<td>42.235 ± 1.986a</td>
<td>21.795 ± 0.815a</td>
</tr>
<tr>
<td>PGP–C16 (6%)</td>
<td>34.20 ± 3.049c</td>
<td>58.93 ± 1.52c</td>
<td>43.494 ± 2.08c</td>
<td>22.089 ± 0.155a</td>
</tr>
<tr>
<td>PGP–C16 (2%)</td>
<td>34.40 ± 2.701abc</td>
<td>59.1 ± 3.36d</td>
<td>41.708 ± 3.699a</td>
<td>22.973 ± 4.212a</td>
</tr>
<tr>
<td>PGP–C16 (4%)</td>
<td>34.76 ± 3.562bc</td>
<td>56.74 ± 1.32d</td>
<td>39.211 ± 1.365a</td>
<td>23.201 ± 2.791a</td>
</tr>
<tr>
<td>PGP–C16 (6%)</td>
<td>34.76 ± 3.562bc</td>
<td>55.497 ± 6.09c</td>
<td>42.504 ± 3.251a</td>
<td>23.210 ± 3.000a</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation. Different letters represent significant differences (P < 0.05) according to the LSD test.
the emulsified films was approximately two-thirds of that of the PGP film (59.9 g mm/m² kPa d). There were no significant differences (P > 0.05) in the WVP values among the emulsified films. However, as the amounts of fatty acids increased, WVP decreased by about 6%. Rhim et al. (1999) reported that the WVP of soy protein–fatty acid films decreased exponentially as the concentration of fatty acids increased, but WVP did not follow a specific trend as the chain length of the fatty acids increased. Our study showed that the fatty acid type had a minor effect on the WVP of the emulsified films; as the chain length of fatty acids increased, WVP decreased by about 3%. Similar results were obtained by other authors (Ayranci and Tunc, 2001; Hagenmaier and Shaw, 1990; McHugh and Krochta, 1994; Pearoval et al., 2002; Srinivasa et al., 2007). This behavior can be explained by the higher hydrophobicity and lower mobility of long chain fatty acids in comparison with short chain fatty acids. In contrast, Tanaka et al. (2001) did not observe any effect of saturated fatty acid chain length on WVP of fish protein–fatty acid emulsified films. Furthermore, short chain fatty acids exhibited a higher water vapor barrier than did long chain fatty acids in emulsified pectin films (Liu et al., 2005). These anti-thetical results indicate that each fatty acid may have a different degree of compatibility with the particular polysaccharide and/or protein utilized as a matrix (Rhim et al., 1999). Comparing the WVP of synthetic films such as polystyrene (0.414), LDPE (0.06–0.0838), polyvinyl chloride (0.0587), and polypropylene (0.0423) with those of biopolymeric films such as whey protein isolate plus palmitic acid (19.182), wheat gluten plus oleic acid (6.825), sodium caseinate plus lauric acid (9.507 g mm/m² kPa d) (Morillon et al., 2002), and our films show that biopolymeric films do not approach the WVP of synthetic films even when hydrophobic substances have been added. In general, the relative polarity of the support polymer and the type of lipid has the strongest influence on the water vapor barrier of emulsified films (Kester, 1988). Other parameters such as lipid particle size and distribution (Debeaufort et al., 1993; Pearoval et al., 2002), the physical state of the lipid (Martin-Polo et al., 1992), and polymorphism (Kester and Fennema, 1989b) also seem to play a role in WVP.

3.2. Water solubility

The WS of edible films is an important consideration when choosing a film for specific applications. If the WS of a film is high, it cannot protect food from moisture or from water loss. In addition, when coated foods are processed in water, as during osmotic dehydration, high WS can lead to the disintegration of the coating (Gontard et al., 1994). All PGP-based films tested here maintained their integrity in water over 24 h. The incorporation of fatty acid into the PGP film did not significantly alter (P > 0.05) the WS (Table 2). However, the PGP film (with no fatty acid) had the highest WS, and the addition of hydrophobic substances is probably responsible for a small decrease in WS. The WS of cod gelatin films containing different levels of sunflower oil decreased as the amount of the oil increased (Perez-Mateos et al., 2009). In contrast, Gontard et al. (1994) found that the addition of lipids to a gluten film caused an increase in WS. They suggested that the breakage of strong inter-molecular bonds in the protein network and the formation of brittle bonds with lipids resulted in structural instability and that emulsified films were therefore more susceptible to dissolution. An increase in WS as a function of lipid concentration was also reported for gelatin–lipid films (Bertan et al., 2005b) and gelatin–Brazilian elemi films (Bertan et al., 2005a).

3.3. Oxygen permeability

The gas permeability of a biopolymeric film depends on several factors, such as the nature of the gas, the structure of the material, the relative humidity and the temperature (Gontard et al., 1996). The peroxide value (PV) of sunflower oil is directly related to OP, therefore, it was used as an indicator of OP. The results of PV measurements of the PGP-based films are given in Table 2. The addition of fatty acids did not significantly affect (P > 0.05) the OP of emulsified films. The PV values of emulsified films were slightly lower than that of the PGP-only film. Khwaldia et al. (2004) reported that the addition of anhydrous milk fat to a soybean oil emulsion reduced its OP. Similarly, the addition of lipid components to filmogenic solutions decreased the OP of wheat gluten composite films because the hydrophobic characteristics of these components reduce the water content of the films and thus their oxygen solubility (Gontard et al., 1996). In contrast, the addition of lipids to gelatin/triacetin film increased their OP (Bertan et al., 2005b). Those authors suggested that this behavior was caused by the creation of microscopic holes in the film body.

3.4. Mechanical properties

To effectively protect food quality, edible films must maintain their integrity against external stress from the time the food is wrapped until it is consumed. The mechanical properties of the PGP-based films are shown in Fig. 1A and B. Both the concentration and fatty acid type significantly affected the UTS and %E of the emulsified films (P < 0.05). The TS of the PGP film decreased when fatty acid was incorporated into the protein matrix; an exception was the PGP–C16 film containing 4% fatty acid, which showed the highest UTS among all films. Many previous studies have reported similar behavior (Khwaldia et al., 2004; Pearoval et al., 2002; Shellhammer and Krochta, 1997; Srinivasa et al., 2007; Yang and Paulson, 2000). The heterogeneity of the film matrix created by fatty acid incorporation probably led to weakened intermolecular interactions and a consequent decrease in the TS of the emulsified films. No significant difference (P > 0.05) in UTS was observed between PGP and PGP–C16 films at concentrations of 2% and 4%, whereas the UTS of all PGP–C18 films were significantly (P < 0.05) lower than that of the PGP film. Interestingly, increasing C18 concentration had a positive effect on UTS, which increased with the addition of more C18. Consistent with this observation, Rhim et al. (1999) reported that the UTS of soy protein–fatty acid films decreased at low concentrations of fatty acids because of poor interactions between protein and fatty acids, but increased at fatty acid concentrations >20%. They argued that the increase in the ratio of fatty acid/protein beyond this point strengthened films because fatty acids are solid at room temperature.

The addition of fatty acids caused a significant loss in %E (P < 0.05). This is probably because emulsified films have a low water content relative to protein films (Quezada-Gallo et al., 2000). Both water and fatty acids act as plasticizers in edible films, but plasticizers with low molecular weight are more effective due to their high mobility. In contrast, polar plasticizers mainly form strong bonds with proteins, whereas weak interactions between proteins and fatty acids lead to a weak molecular structure and decreased %E. The %E of PGP–C16 films increased when the fatty acid amount increased, while an irregular trend was observed for the PGP–C18 films. The increase in %E with increasing fatty acid content could be attributed to its plasticizing effect. Some researchers have reported that the addition of lipids to edible films enhanced %E (Bertan et al., 2005a,b; Colla et al., 2006). However, many studies have reported the opposite effect (Khwaldia et al., 2004; Pearoval et al., 2002; Srinivasa et al., 2007; Tanaka et al., 2001). The UTS values of PGP-based films were 5.282–9.74 MPa, which are lower than those of synthetic polymers such as LDPE (9–17 MPa) (Briston, 1986), polystyrene (35–55 MPa) (Houston, 1986), and cellophane (114 MPa) (Pearoval et al., 2002). In case of %E, PGP-based films were stretched 40–100 orders higher than cellophane (20%) and...
polystyrene (1%), but could not be stretched nearly as well as LDPE (500%).

3.5. Differential scanning calorimetry

The results of DSC measurements indicated only one glass transition temperature \( T_g \) for the PGP and emulsified films (Fig. 2).

Because of values for \( T_g \) measurements were very close for all emulsified films, we showed only the \( T_g \) of the 6% concentration films. The PGP film converted from glassy to rubbery state at 127.19 °C, and the addition of 6% C16 did not change this temperature, while \( T_g \) moved to 125.01 °C in the presence of 6% C18. The \( T_g \) of the film indicates the compatibility of these components with the biopolymer, so the detection of only one \( T_g \) for all films means

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**Fig. 1.** The mechanical properties of pistachio globulin protein (PGP) and emulsified films: (A) tensile strength (UTS), (B) elongation at break (E). Different letters represent significant differences \( (P < 0.05) \) according to the LSD test.
that all substances contributing to the film structure have been dispersed very well and are compatible with one another. Pommet et al. (2003) studied the plasticizing effect of fatty acids on gluten films. They observed that films plasticized with 6- to 10-carbon fatty acids exhibited one \( T_g \) point. In contrast, two \( T_g \) were obtained for films containing lauric, myristic, and palmitic acids because the heterogeneous distribution of fatty acid in polymer matrix induced regions in which the fatty acid phase was relatively crystalline.

3.6. Opacity

Visual characteristics of biodegradable films such as gloss, color, and transparency can affect consumer acceptability and even food quality. The impact of fatty acids on opacity is shown in Fig. 3. The color of the PGP film was yellow and the color intensity depended on film thickness. The addition of fatty acids increased the film opacity, and the opacity increased significantly \((P < 0.05)\) as fatty acid level increased. The same trend was observed by Bertan et al. (2005a). Perez-Mateos et al. (2009) and Quezada-Gallo (2000). This may be attributed to the physical state of the fatty acid (solid) at room temperature. Perez-Mateos et al. (2009) stated that sunflower oil caused whiteness in cod gelatin film due to the light scattering effect of the emulsion. The addition of \( \text{C}_{16} \) and \( \text{C}_{18} \) to a filmogenic solution of gelatin resulted in opaque films, whereas the presence of Brazilian elemi had little effect on opacity (Bertan et al., 2005a).

4. Conclusions

The addition of palmitic or stearic acid to aqueous PGP solutions improved the barrier properties and water solubility of the resulting emulsified films. The WVP was greatly decreased upon the addition of fatty acid, but the differences between the WVP values of various emulsified films were not significant. However, the emulsified films were mechanically weaker and less transparent than the control film.

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