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# Effect of Physical State of Fatty Acids on the Physical Properties of PGP-Based Emulsified Edible Film

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**Abstract:** Many factors influence physical properties of edible composite films fabricated from hydrocolloids and lipids. Novel edible emulsified films were made using PGP, glycerol and stearic (SA) or oleic (OA) fatty acids by Tween-80 addition and emulsification of filmogenic solutions. In this study, the effect of physical state of fatty acids on the pistachio globulin protein (PGP) edible film properties was investigated. There were significant differences ($p < 0.05$) in the water vapor permeability (WVP), tensile strength (TS) and opacity of emulsified films containing SA and OA. SA was more effective than OA in WVP diminishing. The decrease values of 42 and 26.5% were observed for films with SA and OA, respectively. TS and elongation at break values of films containing OA were lower than SA ones. Oxygen permeability (OP) was evaluated indirectly applying peroxide value measurement. No significant difference was observed in the OP of the films. Water solubility was not affected by the physical state of fatty acids either. The solid state of SA at the room temperature caused the resultant films to be more opaque than OA containing films. Our findings indicated that PGP films modified with SA had better physical properties special in WVP reduction than OA ones and could be potentially used for primary packaging of foods with high water loss problem.

**Key words:** Pistachio globulin protein % Edible emulsified film % Water vapor permeability % Stearic acid % Oleic acid

## INTRODUCTION

The application of petrochemical-based materials in food packaging has several disadvantages. One of the most important disadvantages is unrestricted mass transfer specially moisture among components of heterogeneous foods (Donhowe and Fennema, 1993). The decrease in moisture transfer among food components is feasible using edible films and coatings (Debeaufort, *et al*. 1993; Kamper and Fennema, 1984; McHugh and Krochta, 1994). The edible films and coatings are developed from biodegradable and natural substances with plant, animal and microbial origins. They present many advantages such as high biodegradability and great carrying capacity for functional components due to both natural origin and direct contact with food (Gontard, *et al*. 1992; Peroval, *et al*. 2002; Srinivasa, *et al*. 2007). Edible films are usually made from proteins, polysaccharides and lipids. The proteins and polysaccharides have polymerized structure and edible films obtained from them show good mechanical properties, whereas the lipids produce brittle films owing to low molecular weight and weak intermolecular interactions. On the other hand, proteins and polysaccharides present poor barrier against water vapor because of hydrophilic nature, whereas lipid films have excellent water vapor barrier (Bertan, *et al*. 2005; Callegarin, *et al*. 1997; Canhadas Bertan, *et al*. 2005; Khwaldia, *et al*. 2004; Rhim, *et al*. 1999; Yang and Paulson, 2000). Blending of protein or polysaccharide with lipid lead to production of a composite film having both good mechanical properties and low water vapor permeability (WVP).

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The *Pistacia* has several species but the fruits of *Pistacia vera L.* are often consumed as edible nuts. Two main compositions of *Pistacia vera L.* nuts are oil (57%) and protein (20.8%) (Shokraii, 1977). Pistachio nut attract oil industries attention because of high oil content. The oil cake is rich in mineral and protein (about 40%) and is commonly used as an animal feed. This by-product is a new source of plant protein and can be used for film making. Pistachio protein consists of four fractions: globulin (66%), albumin (25%), glutelin (7.3%) and prolamin (2%) (Shokraii and Esen, 1988). Ayranci and Cetin (1995) added the pistachio protein isolate to cellulose-based edible film. This resulted in increase of WVP of the edible film due to both hydrophilic nature of the protein and having high amounts of hydrophilic amino acids. Aghaei (2008) succeeded to make an edible film from the pistachio globulin protein (PGP). The resulting film showed high WVP. Modification of PGP film via addition of fatty acids led to remarkable decrease of WVP (Zahedi, *et al*. 2010).

Incorporation of lipid components to the edible hydrocolloid-based (proteins or polysaccharides) films improves the WVP and affects other characterizations of them. However, value of WVP decrease depends on many factors including physical state of lipid (Martin-Polo, *et al*. 1992b), polymorphism (Kester and Fennema, 1989a), preparation technique of edible film (Debeaufort, *et al*. 1993; Martin-Polo, *et al*. 1992a), the lipid concentration (Hagenmaier and Baker, 1994; Shellhammer and Krochta, 1997) and both distribution and particle size of lipid (Debeaufort, *et al*. 1993; Peroval, *et al*. 2002) as well as chemical properties of the hydrophobic substances such as polarity (Donhowe and Fennema, 1993; Kester and Fennema, 1989b), chain length of lipid (Hagenmaier and Shaw, 1990; Koelsch and Labuza, 1992; McHugh and Krochta, 1994) and saturation degree of fatty acids (Fernández, *et al*. 2007; Hagenmaier and Baker, 1997; Kamper and Fennema, 1984; Tanaka, *et al*. 2001).

The WVP of edible composite films containing solid lipids is usually much lower than liquid ones. Martin-Polo *et al*. (1992b) indicated that when the paraffin oil content increased from 75 to 100%, the permeability of paraffin based film increased 100 times. The saturation degree of lipid substances can influence the physical state especially in fatty acids with more than 8 carbons. Kamper and Fennema (1984) showed that as the degree of saturation of the lipid increased, the water vapor barrier improved. The reason is the higher water solubility in the liquid lipid in comparison with the solid lipid and/or on the molecular arrangement of the lipid. It has been proved that among the carboxylic acids, stearic and palmitic acids have the highest water vapor barrier. However, films containing arachidonic or behenic acids showed higher WVP (Morillon, et al. 2002). For this reason, we preferred to use the most effective fatty acid in WVP reduction i.e. stearic acid as a representative of solid fatty acids and a fatty acid with different physical state i.e. oleic acid as a representative of liquid fatty acids to investigate the effect of physical state of lipids on the edible film properties.

In this study, novel emulsified films from pistachio globulin protein, stearic acid and oleic acid were produced and water vapor permeability, oxygen permeability, mechanical properties, opacity, water solubility and moisture content of them were then measured.

## MATERIALS AND METHODS

**Materials:** The pistachio nuts oil cake from *Pistacia vera* L. varieties was prepared from Manila oil extraction factory in Kerman (Iran). The globulin fraction was extracted as described by Shokraii and Esen (1988) and purity of globulin was 80%. Tween-80 (emulsifier), glycerol (purity 83%), pure stearic (SA) and oleic (OA) fatty acids were purchased from Merck (Germany).

**Film Preparation:** Pretreatments indicated that filmogenic solutions containing 10% stearic acid produced brittle and 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 oleic or stearic fatty acids at concentration of 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 $cm^2$. The frames were dried at ambient conditions for 40 h. The mixture was homogenized with an IKA T25 digital homogenizer (Ultra-Turrax, Germany) for 1 min at 9000 rpm.

**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 absorbed by the desiccant. The WVP was determined from the weight increase of the glass cells. The glass cells were 1.5 cm (i.d.) and 7.0 cm in height, with an exposed area of 1.76 $cm^2$. The inside of the cells were filled with 4g anhydrous calcium chloride (0% RH) covered with the films and then placed in a desiccator containing a saturated $K_2SO_4$ 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 equation 1 as follows:

$$WVP = \frac{Slope \,.\, x}{A \,.\, S\left(R_1 - R_2\right)} \quad (1)$$

Where A is the area of the exposed film surface ($m^2$), S is saturation vapor pressure at 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.

**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 10ml 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.

**Mechanical Properties:** The tensile strength (TS) and percent elongation (% E) of the films (140 × 20 $mm^2$) 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. TS 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.

**Opacity Measurement:** The opacity measurements were performed according to the method described by Gontard et al. (1992) based on a modified standard procedure. The film specimens were cut into rectangles and placed in the spectrophotometer cell. A spectrum of each film was recorded using a UV-vis spectrophotometer (Shimadzu UV-160A). The area under the absorbance curve from 400 to 800 nm was determined 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.

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

**Moisture Content (MC):** 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 Equation (2), where $M_i$ is the weight of Petri dish and film specimen before drying, $M_p$ is the weight of Petri dish and $M_d$ is the weight of Petri dish and film specimen after drying.

$$\%\, MC = \frac{M_i - M_d}{M_i - M_p} \times 100 \quad (2)$$

**Thickness Measurement:** The films thickness was measured with an electronic micrometer (QLR digit-IP54,

China). The measurements were taken at the center of the film and at four positions around the perimeter for the WVP and eight positions along the rectangular strips for mechanical properties. The mean measurements were used to calculate the WVP of the film.

**Statistical Analysis:** The results of measurements were analyzed by one-way analysis of variance using MSTAT-C software, version 1.42 (Michigan State University). The difference of means was 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.

## RESULTS AND DISCUSSION

**Water Vapor Permeability:** The results showed that incorporation of the fatty acids to the PGP film caused a significant ($p < 0.05$) decrease in WVP of the films (Fig. 1). SA was more effective than OA in WVP reduction. The similar results were obtained for other emulsified films containing $C_{18}$ based on whey protein isolate (WPI) (Fernández, *et al*. 2007), fish protein (Tanaka, *et al*. 2001) and soy protein (Rhim, *et al*. 1999). This result can be explained by three reasons: (1) existence of a double bond in OA leads to relative mobility which favors water vapor diffusion (Gontard, *et al*. 1994). (2) Volume of $CH_2$ groups in liquid form is greater than when they are crystallized. Therefore, solid fatty acids have dense structure that limits water diffusion (Kamper and Fennema, 1984). (3) As mentioned, relative water solubility in the liquid lipid is higher than the solid ones (Kamper and Fennema, 1984). Different behavior was observed by several authors for OA. Handa *et al*. (1999) reported WVP was not affected by addition of OA to egg white protein-based edible film. They stated that OA increases the negative charges along protein chains, thus the hydrophobic effect of fatty acid neutralized.

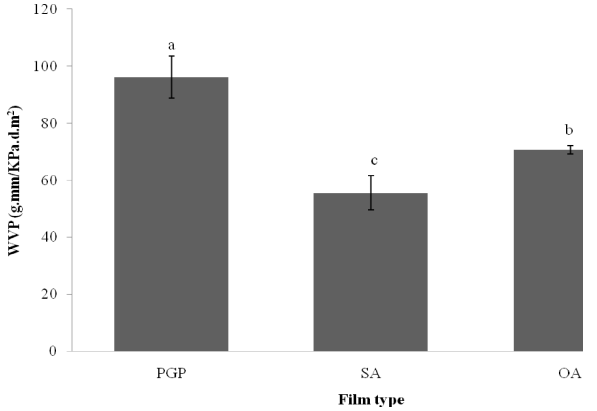


Fig. 1: Effect of stearic (SA) or oleic (OA) acids addition on the WVP of the PGP film.

**Oxygen Permeability:** The appropriate OP of an edible film or coating depends on application case. When the edible film is used on high fat foods, the OP must be very low. For living tissues such as fruit, a coating with high permeability is required to exchange respiratory gases. The OP of edible films is commonly measured by standard method described in ASTM (1988). Ayranci and Tunc (2003) modified this method and applied iodimetry method to determine amount of permeated $O_2$. These techniques present numeral values. Ou *et al*. (2005) used an indirect method based on the peroxide value measurement to comparative evaluation of OP in the edible films. We used this method to determine the effect of fatty acid addition on the OP of PGP film. The OP did not change significantly ($p > 0.05$) in resultant emulsified films (Table 1). It seems that low concentration of fatty acids prevents significant effect of physical state on the OP. A positive effect of SA on the OP of emulsified films made from *Amaranth cruentus* flour and chitosan was observed by Colla *et al*. (2006) and Srinivasa *et al*. (2007), respectively. In contrast, addition of $C_{14}$, $C_{16}$ and $C_{18}$ saturated fatty acids to gelatin film led to an increase of OP due to formation of micro pores in the films body (Bertan, *et al*. 2005; Canhadas Bertan, *et al*. 2005).

Table 1: Peroxide value, water solubility and moisture content values of PGP and emulsified films.

| Film type† | Water solubility†† (%) | Proxide value†† (meq $O_2$/kg oil) | Moisture content†† (% w.b) |
|---|---|---|---|
| PGP | 44.815 ± 5.01[a] | 23.342 ± 0.43[a] | 37.36 ± 2.36[a] |
| PGP+ 6% stearic acid | 42.504 ± 3.25[a] | 23.211 ± 3.00[a] | 34.76 ± 3.56[a] |
| PGP+ 6% oleic acid | 44.453 ± 2.76[a] | 24.962 ± 2.04[a] | 36.20 ± 2.95[a] |

†) PGP: Pistachio globulin protein.

††) The values are mean of replications ± standard deviation. The same letters represent insignificant difference ($p > 0.05$) according to LSD test.

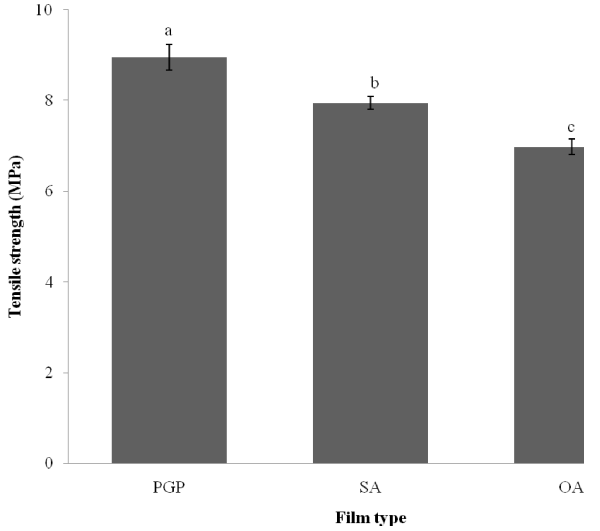


Fig. 2: Effect of stearic (SA) or oleic (OA) acids addition on the tensile strength of the PGP film.

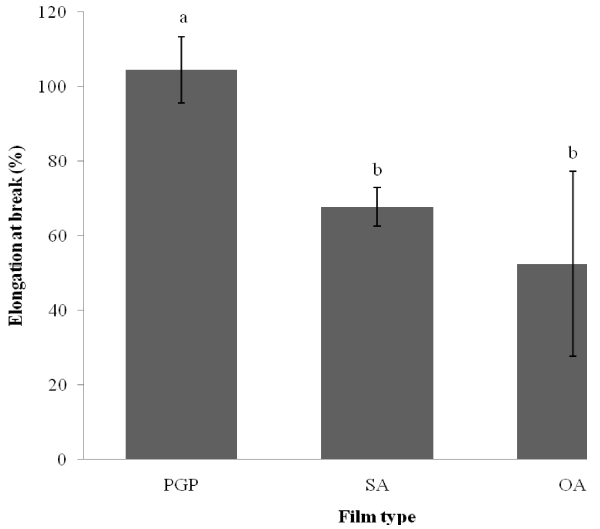


Fig. 3: Effect of stearic (SA) or oleic (OA) acids addition on the elongation at break of the PGP film.

**Mechanical Properties**: Addition of the fatty acids to the PGP film resulted in significant ($p < 0.05$) diminishment of both tensile strength (TS) and elongation (E) (Figs. 2 and 3). This was in agreement with findings of other researchers (Khwaldia, *et al*. 2004; Peroval, *et al*. 2002; Srinivasa, *et al*. 2007; Yang and Paulson, 2000). The PGP film indicated greater resistance during the test due to both polymeric structure and different interactions that occurred during the film formation. Presence of hydrophobic substance in the film matrix led to nonpolar-polar interactions between fatty acid molecules and protein chains which are weaker than polar-polar interactions. Therefore, polymer network in the emulsified

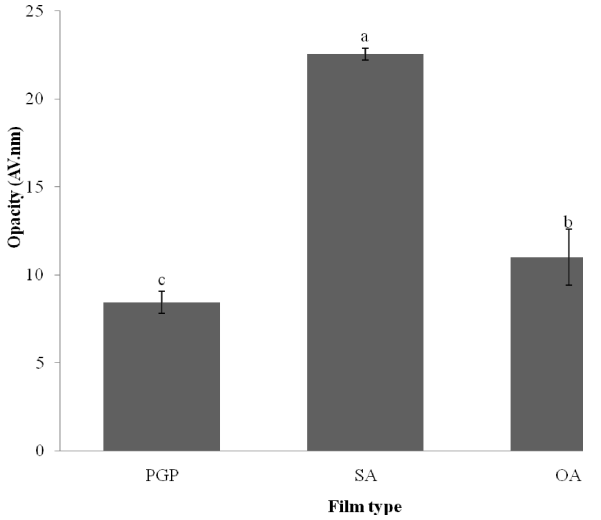


Fig. 4: Effect of stearic (SA) or oleic (OA) acids addition on the opacity of the PGP film

films was weak and negligible. The physical state could affect mechanical properties of the emulsified films. Both E and TS of films containing SA were higher than OA ones, but only difference of the TS was significant ($p < 0.05$). Solid state of SA at the room temperature probably caused the resultant emulsified films became stiffer than OA containing films. Tanaka *et al*. (2001) reported that SA and OA have different influences on mechanical properties of fish protein film. SA decreased both TS and E, whereas OA increased both of them. Addition of SA to the WPI film increased TS and decreased E, while OA induced various behavior in the films. E increased up to 10% by OA addition and thereafter decreased but TS decreased continuously (Fernández, *et al*. 2007).

**Opacity:** The surface appearance (color, glossiness and shininess) of edible films and coatings is important to increase consumer acceptability. Incorporation of the fatty acid to the PGP film produced opaque films. The similar results were reported by other researchers (Bertan, *et al*. 2005; Canhadas Bertan, *et al*. 2005; Gontard, *et al*. 1994; Rhim, *et al*. 1999). Opacity of the emulsified films had significant differences ($p < 0.05$) with the PGP film (Fig. 4). The opacity of the film containing SA was two orders of magnitude greater than the OA incorporated film. The major reason for this difference was the physical state of fatty acids at the room temperature. Light scattering effect of the emulsion stabilized by the emulsifier also intensified the opacity. Fernandez *et al*. (2007) observed SA was more effective than OA toward the opacity increase because of both a high melting

temperature and the emulsion instability. Transparency of egg white protein-based emulsified film wasn't remarkably influenced as a consequence of 10% OA addition (Handa, *et al*. 1999).

**Water Solubility:** The WS of biopackaging favors their degradability, however, it also limits its application (Yu, *et al*. 2006). Edible films having low WS are necessary to protect foods with high or intermediate water activity as well as to process coated foods in water such as osmotic dehydration. The small decrease in WS of the PGP film occurred with addition of the fatty acids but differences were not significant ($p > 0.05$) (Table 1). Although the films were immersed in water for 24 h, but they didn't collapse and retained structural integrity. Addition of 10% OA to egg white protein-based film decreased WS (Handa, *et al*. 1999). Rhim *et al*. (1999) observed the WS of emulsified soy protein film increased as a function of OA concentration, whereas this property did not affect the saturated fatty acids specially SA. They concluded that the WS was mainly influenced by chemical nature of the films. This view was supported by observations of Bertan *et al*. (2005) and Yoshida and Antunes (2004) for gelatin and WPI-based edible films because SA enhanced the WS of the gelatin film, while it decreased the WS of the WPI films. The WS of the PGP film (44.8%) was higher than the gluten film (32%), the soy protein film (27%) and the gelatin film (30.5%) at the similar test conditions. This may be attributed to both high amounts of the hydrophilic amino acids and a high concentration of glycerol.

**Moisture Content:** The MC of PGP-based films is given in Table 1. The MC of the emulsified films was smaller than the PGP-based film. There was no significant difference ($p > 0.05$) in MC values of film samples.

## CONCLUSION

The physical state of the fatty acids was an efficacious factor to modify some physical properties of the PGP-based films. The films having SA indicated good water vapor barrier in comparison with OA ones. Relative mobility, higher volume of $CH_2$ groups and relative water solubility caused an enhancement in WVP of OA containing films. Although using of solid lipids such as SA in hydrocolloid-based films formulation usually produces opaque and also weak films, we prefer these lipids to liquid ones because of high resistance of resulted films against water permeation special in foods with high water content.

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