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*Full Length Research Paper*

# Effect of different concentrations of olive oil and oleic acid on the mechanical properties of albumen (egg white) edible films

**Amal Taqi<sup>1,2\*</sup>, Kasim Abass Askar<sup>3</sup>, Khaled Nagy<sup>4</sup>, Lucia Mutihac<sup>2</sup>, and Ioan Stamatin<sup>1</sup>**<sup>1</sup>Nano-SAE Research Centre, University of Bucharest, Bucharest, Romania.<sup>2</sup>Faculties of Chemistry, University of Bucharest, Bucharest, Romania.<sup>3</sup>School of Biomedical and Biological Sciences, University of Plymouth, Plymouth, PL4 8AA, United Kingdom.<sup>4</sup>Food Technology Research Institute - Agricultural Research Centre, Giza, Egypt.

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The main objective of this study was to investigate the effect of olive oil and oleic acid addition to albumin (egg white) through emulsification to produce films on mechanical properties. Plasticizer was necessary to maintain film and coating integrity and to avoid pores cracks. Edible composite films were prepared from albumin and lipid material at (1 and 1.5%), respectively. The effect of unsaturated oleic acid with glycerol and monounsaturated olive oil on tensile strength, elongation at break, water vapor permeability (WVP), opacity (OP), solubility, colour and atomic force microscopy (AFM) was investigated. In general, the incorporation of lipid materials resulted in the increase ( $P < 0.05$ ) of tensile strength and elongation at break, and the reduction of WVP with some exceptions. Overall, the effect of monounsaturated was greater than that of unsaturated. The surface microstructure of the films was analyzed using atomic force microscopy (AFM).

**Key words:** Albumin protein, edible film, olive oil, oleic acid, mechanical properties.

## INTRODUCTION

Active packaging is combined consumer demands for high-quality food products and reduced environmental consequences of packaging have generated an increased research interest on biodegradable films and coatings and classified to increase the shelf life of food products by controlling mass transfer between the packaged products and the surroundings. Edible films derived from polysaccharides, proteins or lipids have been under intense investigation in recent years. The promise for such materials in food-packaging applications arises from their capability to supplement and possibly to improve the performance of existing synthetic packaging polymers, with reduced environmental impacts (Guilbert, 1986; Vanessa et al., 2007; Krochta and Mulder-Johnston, 1997). Edible films are often made from biological hydrophilic materials such as proteins, starch,

pectin, cellulose, alginate, and carrageenan. These tend to have stronger textures which are not effective water vapour barriers so most work on the barrier characteristics of edible films has been concentrated in the area of improved water vapour barrier properties (Vargas et al., 2009). However, edible films, in general, have high oxygen barrier properties at low relative humidities. Since foods containing lipid materials as neutral lipids, fatty acids or waxes are susceptible to oxidation, oxygen barrier characteristics for protective films are important, and oxygen barrier properties for edible films have been investigated (Kester and Fennema, 1989a, 1989b, 1989c).

Composite protein-oil films can combine the effective mechanical structural and oxygen barrier properties of protein films with the high moisture barrier characteristics of oils. Oil addition was reported to reduce water vapor permeability of cast protein films from caseinates (Avena-Bustillos and Krochta, 1993), whey protein (McHugh et al., 1994; Shellhammer and Krochta, 1997), wheat gluten (Gontard et al., 1994, 1995), corn zein (Weller et al.,

\*Corresponding author. Email: [amal.taqi@ivv.fraunhofer.de](mailto:amal.taqi@ivv.fraunhofer.de) or [xbmw2004@yahoo.com](mailto:xbmw2004@yahoo.com).

1998), and soy protein (Gennadios et al., 1998). Likewise, egg white (EW) functionality has been altered through interactions with oils (Ball, 1987; Yuno-Ohta et al., 1996).

Gennadios et al. (1996) reported that the properties of egg albumen films were similar to other protein films. Egg albumen films were clearer and more transparent than wheat gluten, soy protein isolates and corn zein films. They indicated that albumen films could be used for water soluble packets (pouches) for ingredients in the food, chemical, and pharmaceutical industries. Hence, Albumen (egg white) is a complex protein system comprised of a series of high molecular weight proteins containing a significant number of sulfhydryl and disulfide bonds.

The molecular weights of these proteins range from 12,000 to 8,000,000 Daltons consisting of ovomucin fibres conalbumin, ovomucoid, lysozyme, globulins, and ovomucin in an aqueous solution of numerous globular proteins (Powrie and Nakai, 1986). Protein film materials are characterized by high moisture permeability, low oxygen and lipid permeability at lower relative humidity. At high relative humidity, protein films represent a compromise between the barrier function and its mechanical properties (Brody, 2005). Protein films are poor water vapor barriers and sensitive to water due to the inherent hydrophilic nature of proteins, which is due to the high cohesive energy density of protein films (Miller and Krochta, 1997). Olive oil as applied in this study is a natural juice complex compound, which preserves the taste, aroma, vitamins, volatile components, water soluble components and properties of the olive fruit. The beneficial health effects of olive oil are due to both its high content of monounsaturated fatty acids and its high content of the flavonoid polyphenols which are anti oxidative substances (Fiorino and Nizzi Griffi, 1992). Incorporation of hydrophobic compounds such as fatty acids, monoglycerides and waxes to a film solution to reduce the water vapour permeability of protein films has been studied. The aim was to investigate changes in physical characteristics of albumin films resulting from addition of different concentration of oils, the effect of adding olive oil as the hydrophobic phase and oleic acid to film-forming solution protein and glycerol as plasticizers on water vapor permeability (WVP), and solubility, tensile strength and elongation at break, opacity (OP), color and atomic force microscopy (AFM).

## MATERIALS AND METHODS

### Raw materials

Protein powder (egg white) albumin and oleic acid (99% pure) was purchased from Sigma-Aldrich Chemicals (Stein Hein, Germany). Olive oil was purchased from Sigma-Aldrich Chemie GmbH (Ec.No.2322770, product of Spain). Glycerol (plasticizer) and all the other chemicals used in this work were of reagent grade and were purchased from Reactively Bucharest (Romania).

### Film albumin- based preparation

Film was formed using casting process following previous work (Handa et al., 1999). Dried albumin [egg white powder (9 g/100 ml water)], polyethylene glycol (average M.W.400; 60% w/w of dried EW; plasticizer) was prepared. After pH adjustment ( $\pm 0.10$ ) to 11.25 with 2 N NaOH, solutions were heated in a water bath (45°C for 20 min) and cast on glass plates coated with Teflon overlays (Cole Parmer, Vernon Hills, Ill., U.S.A.). Castings were placed in an environmental chamber (25°C and 50% RH), peeled from plates, and specimens cut for property testing.

The different concentrations of lipids, [1 and 1.5% (v/v) olive oil and oleic acid] were added after pH adjustment ( $11.25 \pm 0.10$ ) into the solution and heated (45°C for 20 min). Mixtures were homogenized with an HG 30 homogenizer (Hitachi, Ltd, Tokyo) at (15,000 rpm) for 1 min. The prepared film-forming solutions were dried in a ventilated oven at 25°C for 20 h. After the water was evaporated, albumin films were manually peeled off.

## Characterizations methods

### Film thickness

The thicknesses of three replicates of each film formulation were measured with a micrometer (Mahr Millimar C1216, Germany).

### Water vapor permeability

WVP was measured using a modified ASTM method reported previously (Avena- Bustillos and Krochta, 1993). Films formed from solution 1, 2 and 3 were sealed in a glass permeation cup and kept in a cabinet desiccator with silica gel (0% RH; silica gel was heated at 180°C for at least 3 h prior to use for the determination). The cups were weighed at intervals of 1 h over a 12 h period and WVP ( $\text{g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$ ) of the film was calculated as follows:

$$\text{WVP} = (w \cdot x) / A \cdot t \cdot (P_2 - P_1) \quad (1)$$

The "w" is the weight gain of the cup (g), "x" is the film thickness (mm), "A" is the area of exposed films ( $\text{m}^2$ ), "t" is the time of gain (s), and " $(P_2 - P_1)$ " is the difference of vapor pressure across the film (Pa). This entire procedure was repeated twice for a total of nine tests on each film type.

### Solubility in water

Film solubility in water was measured as a percentage of dry matter of the film solubilized in water for 24 h (Gontard et al., 1992; Rhim et al., 1997). The initial dry matter of each film was determined by drying at 100°C for 24 h. The films were cut into squares  $40 \times 40 \text{ mm}^2$ , weighed, immersed in 50 ml distilled water containing 0.02 g sodium azide / 100 ml to prevent growth of microorganisms, sealed using Para film and periodically agitated for 24 h at 25°C in a shaking incubator (Model VS-8480SL, Vision Scientific Co, Korea). The non-solubilized films in water were taken out and dried to determine the weight of dry matter. Tests were performed in triplicate and the solubility was calculated as follows:

$$\text{MS} (\%) = 100 \times (M_i - M_f) / M_i \quad (2)$$

Where, " $M_i$ " was the initial mass of the dried film and " $M_f$ " was the mass of the dried film insoluble in water after 24 h.

### Tensile strength

Film tensile strength (TS) and percent elongation at break (EL) were conditioned at  $23 \pm 2$  °C and  $50 \pm 5\%$  RH and a temperature for 24 h in an environmental chamber, using an Instron Universal Testing Instrument (DOLI GMBH Industriek Elektronik, Germany). The films were cut into strips 15 mm wide and 120 mm long using a scalpel. Self-alignment grips consist of one fixed end and one movable end to provide perfect alignment when load is applied. The grips are lined with thin rubber faces to prevent sample slippage and they are air-actuated to prevent sample breakage. The initial gauge separation was fixed at 50 mm and crosshead speeds were 100 mm/min. A minimum of eight strips prepared from each film type were analyzed for percent elongation at break (EL) tensile strength at break (TS) and elastic modulus (EM).

### Opacity measurement

The film samples were cut into rectangles and placed on the internal side of a spectrophotometer cell according to a modified standard procedure of the British Standards Institution (BSI, 1968). Film opacity was calculated with the area under the absorbance curve with respect to wavelength from (200 to 600 nm) and was taken as the opacity of the film. It was expressed as absorbance unit (AU)  $\times$  nm/unit thickness ( $\mu\text{m}$ ).

### Colour measurement

Color value of albumin protein films was measured with a Hunter Lab digital color difference meter Model Minolta (Hunter Associates Laboratory, Japan). Films were placed on a white standard plate ( $L = 97.71$ ,  $a = -0.17$ , and  $b = 2.40$ ). Values for L [lightness = 0 (black) - 100 (white)], a [redness = -60 (green)  $\pm$  60 (red)], and b [yellowness = -60 (blue)  $\pm$  60 (yellow)] were recorded. Total color difference ( $\Delta E$ ), yellowness index (YI), and whiteness index (WI) were calculated as described by Bolin and Huxsoll (1991):

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad (1)$$

$$YI = 142.86 \text{ b/L} \quad (2)$$

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (3)$$

Where,  $\Delta L = L_{\text{Standard}} - L^*_{\text{sample}}$ ;  $\Delta a = a_{\text{standard}} - a^*_{\text{sample}}$ ;  $\Delta b = b_{\text{Standard}} - b^*_{\text{sample}}$ .

Ten measurements were taken on each film.

### Atomic force microscopy

The surface topography of all types of films prepared in this work was analyzed by AFM (Explorer model, SPM – NTegra Prima). AFM images were acquired in semicontact mode using cantilevers type NSG10 with the characteristics as follows: length = 100  $\mu\text{m}$  ( $\pm$  5  $\mu\text{m}$ ), width = 35  $\mu\text{m}$  ( $\pm$  5  $\mu\text{m}$ ), thickness = 1.7 to 2.3  $\mu\text{m}$ , tip height = 10 to 15  $\mu\text{m}$ , 10 nm curvature radius of the tip, resonance frequency of 190 to 325 kHz and elastic constant of 5.5 to 22.5 N/m.

### Oxygen permeability measurements

At 23°C and  $50 \pm 1\%$  RH, films were placed in a stainless steel mask with an open testing area of 5 cm<sup>2</sup>. Masked films were placed into the test cell and exposed to 98% N<sub>2</sub>, and 12% H<sub>2</sub> flow

on one side and pure oxygen flow on the other. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness, measured at four random places. Three replicates of each film were evaluated.

### Statistical analysis

All experiments were repeated three times. Data were analyzed by ANOVA using the general linear model (GLM) of (SAS, 1990). Significant differences between means were determined by the least significant difference (LSD) test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Film thickness

The thickness for films incorporated with olive oil and oleic acid in different concentration (1 and 1.5%) (Table 1) were higher ( $P < 0.05$ ) than those of the films manufactured without lipids.

The results from our work were higher than those observed by Kester and Fennema (1986); and Greener and Fennema (1989). However these authors have reported that edible films containing hydrophobic substances such as waxes and oils can form thicker films. These factors may explain the apparent difference in olive oil and albumin films.

On the other hand, similar ageing conditions, and the amount of water evaporated from composite films were less compared with that evaporated from films manufactured without lipids. The films appeared stiff, glassy and extremely adherent to the plates where they were cast in contrast with others films which were easily removed from the plates. In particular, films obtained using olive oil has the easiest way to be removed without observing any damage even when they were folded. Thus, further studies were carried out using such a film (Cuq, et al., 1996).

### Color measurement

Color of albumin film with (1 and 1.5%) olive oil and oleic acid respectively can be an important factor in terms of consumer acceptance of edible films. The results of the measurements performed on the films are shown in Table 1. Accordance with the Hunter system, and the rectangular coordinates ( $L^*$ ,  $a^*$  and  $b^*$ ),  $\Delta E$ , YI and WI of films were defined. Control film was smooth and transparent having mean color values of L 95, 53, a - 0, 12, and b 4, 79. When oils were incorporated, films remained smooth and transparent at 1% oils incorporation, but with oils above 1%, the films became thick. The main differences in color values among albumin film with lipids were the occurrence of decreased L values and increased b values when increased concentrations of oils were added. The control sample

**Table 1.** The effect of different concentration of olive oil and oleic acid content on the Hunter color values of albumin films.

Edible film	Hunter color value			$\Delta E$	YI	WI	Thickness ( $\mu\text{m}$ )
	L*	a*	b*				
Albumin	95,53 <sup>a</sup>	-0,12 <sup>a</sup>	4,79 <sup>a</sup>	2,98 <sup>a</sup>	7,16 <sup>a</sup>	93,45 <sup>a</sup>	141,55 <sup>a</sup>
Albumin+1 %Olive oil	93,13 <sup>b</sup>	-0,51 <sup>b</sup>	7,84 <sup>b</sup>	7,97 <sup>b</sup>	12,03 <sup>b</sup>	89,56 <sup>b</sup>	162,84 <sup>b</sup>
Albumin +1,5% Olive oil	89,17 <sup>c</sup>	-0,85 <sup>c</sup>	10,99 <sup>c</sup>	9,88 <sup>c</sup>	15,83 <sup>c</sup>	84,54 <sup>c</sup>	184,02 <sup>c</sup>
Albumin +1 % Oleic acid	92,39 <sup>d</sup>	-0,39 <sup>d</sup>	3,71 <sup>a</sup>	3,75 <sup>a</sup>	5,74 <sup>a</sup>	91,53 <sup>ab</sup>	154,35 <sup>ab</sup>
Albumin +1,5% Oleic acid	90,98 <sup>c</sup>	-0,67 <sup>b</sup>	6,32 <sup>b</sup>	6,71 <sup>b</sup>	9,92 <sup>d</sup>	88,96 <sup>b</sup>	168,52 <sup>b</sup>

(L\*, a\* and b\*), hunter color value;  $\Delta E$ , total color difference; YI, yellowness index; WI, whiteness index. Means with different letters within a column indicate significant differences ( $P < 0.05$ ).

showed significantly higher L values of the films, which significantly decreased ( $P < 0.05$ ) with the addition of the oils. In contrast, b values increased with incorporation of oil content within the albumin films. This was somewhat expected because of the yellow color of these oils. Olive oil is relatively intense in color than oleic acid oil (Giese, 1996). Furthermore, the color of oils affected ( $P < 0.05$ ) the a-value of these films. The greenness (a) increased continuously as oils increased.

Color changes due to incorporation of different concentration oil can be more fully described using other color functions (Bolin and Huxsoll, 1991) such as  $\Delta E$  which indicates the degree of total color difference from the standard color plate, YI which indicates degree of yellowness, and WI which indicates degree of whiteness. The addition of lipids resulted in an increase ( $P < 0.05$ ) in  $\Delta E$  and YI, but decreased ( $P < 0.05$ ) WI. The  $\Delta E$  showed the same trend as YI, indicating that color difference of albumin films containing different concentration of lipids were mainly due to changes in yellowness (Kamontip and Adisak, 2001).

### WVP measurements

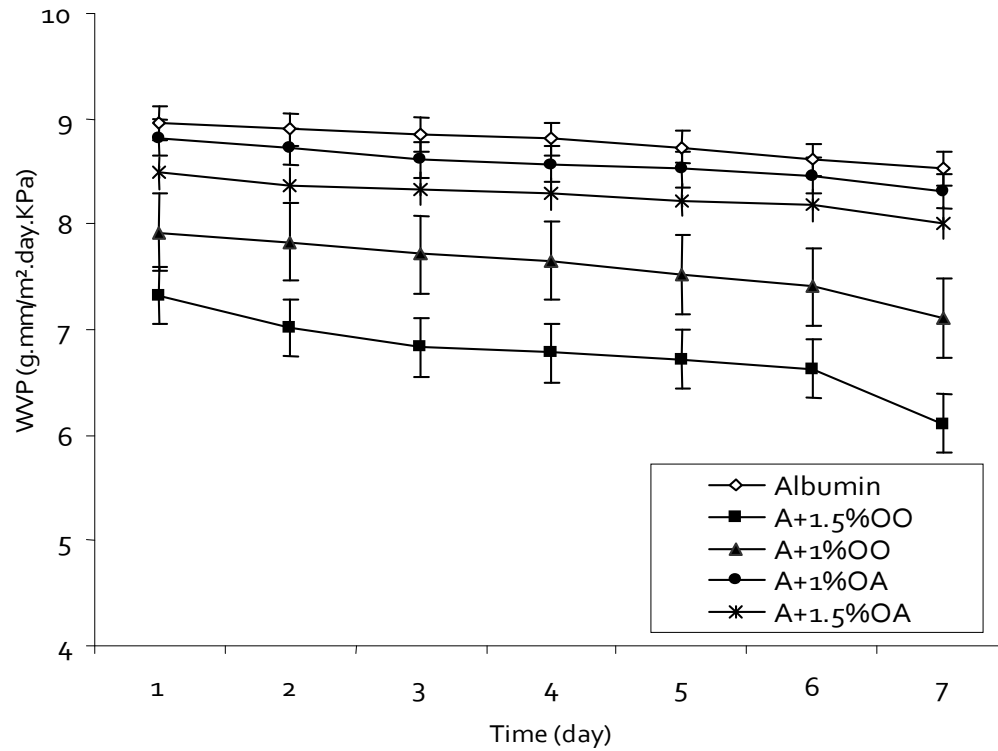
Film prepared from Albumin without oil had higher WVP (8, 83 g.mm/m<sup>2</sup>.d.KPa) compared with films prepared with oils, but the difference was not significant ( $P > 0.05$ ) with films prepared from (1 and 1.5%) oleic acid (Figure 1). WVP of composite films were expected to decrease with increasing length of lipid hydrocarbon chain, due to the hydrophobicity of the fatty acid alkali chain. By varying the number of C atoms in the alkali chain of the molecule, one can control the water vapor permeation (Fama et al., 2004). Oleic acid reduced WVP of wheat gluten (Gontard et al., 1994) and soy protein (Gennadios et al., 1998) films. However, oleic acid did not affect ( $P < 0.05$ ) the WVP of albumin films in our study. King et al. (1984) observed an increase of negative charge in albumin proteins treated with oleic acid. Oleic acid likely increased negative charges (and hydrophilicity) along albumin protein chains, thus negating the hydrophobic effect of fatty acid acyl chains. In general, moisture barrier ability of lipids decreases with increasing

unsaturation (Fennema et al., 1993). The use of olive oil lowered ( $P < 0.05$ ) WVP in albumin films, regardless of oil concentration (1 and 1.5%) used. The form of lipid added to edible films has been shown to have a pronounced effect on the WVP.

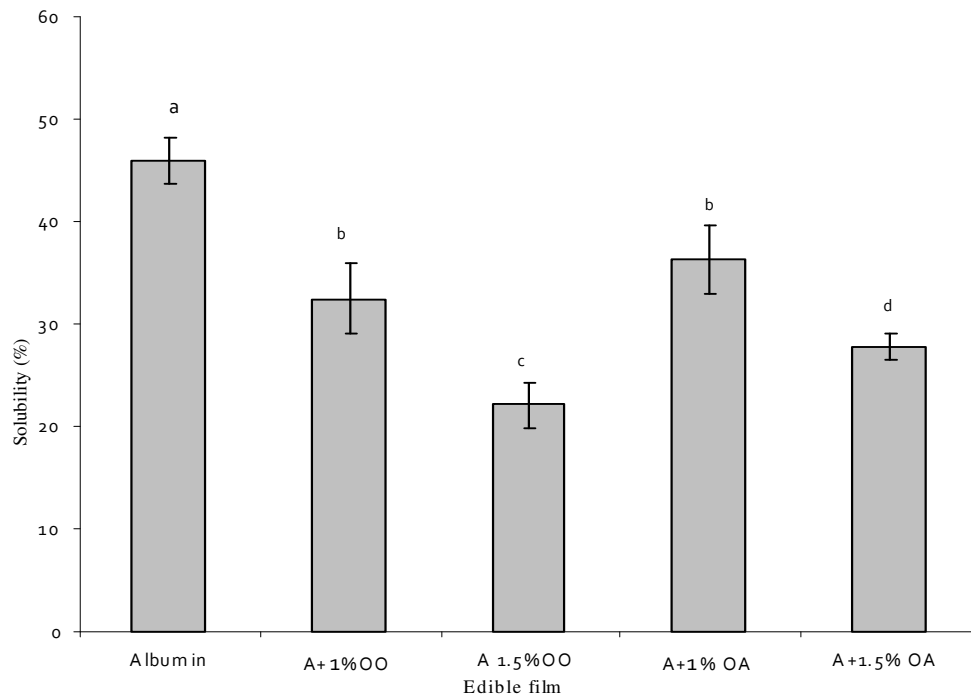
Olive oil had shorter fatty acid chains; it produced more positive effects on the WVP properties of extruded films. The reason for this may be because of the extrusion manufacturing process used. Besides the type of lipids used in the manufacture of edible/biodegradable films, the amount of lipid was also reported to be an important factor for the moisture barrier property of films; the greater the amount of lipid used, the lower the WVP (Shellhammer and Krochta, 1997). This is limited by the scope of the extrusion technology used.

### Solubility in water

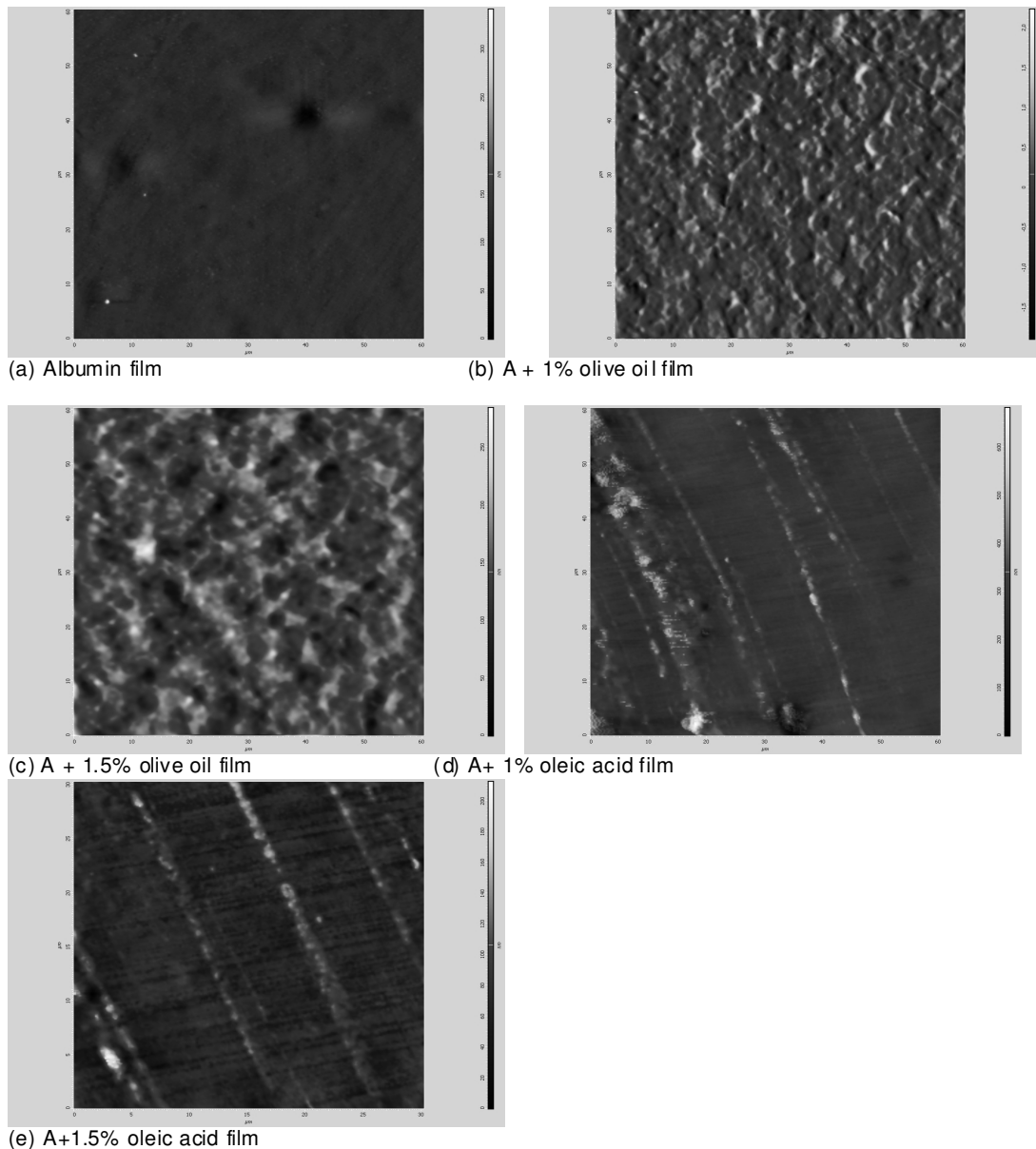
Solubility in water is an important property of albumin protein films. Potential applications may require water insolubility to enhance product integrity and water resistance. Film solubility can be viewed as a measure of the water resistance and the integrity of a film (Rhim, et al., 1999). According to the studies on soy protein isolates and egg white by Gennadios et al. (1998), the solubility of the films decreased with increased lipid addition due to the cross-linking treatments which can improve moisture resistance of protein films. The change (Figure 2) in water solubility of albumin films with (1 and 1.5%) olive oil and oleic acid, respectively showed a significant ( $P < 0.05$ ) decrease in albumin films incorporated with greater concentrations of olive oil. The water solubility of these films was decreased by adding lipids; this could be explained by the fact that oils, with the help of hydrophobic substances that dispersed in the films, changed the polarity of the components. The results prove that olive oil induces a remarkable decrease in the water solubility of the films. Water resistance is an important property of edible films for applications in food protection where water activity is high or when the film must be in contact with water during processing of the coated food to avoid exudation of fresh or frozen products (Ribeiro, et al., 2007).



**Figure 1.** Effect of different concentrations of olive oil and oleic acid content on WVP (g mm/m<sup>2</sup> day. KPa). Each point represents the average of at least three replicates. Error bars indicate standard deviations.



**Figure 2.** Effect of different concentrations of olive oil and oleic acid content on solubility in water (%) of albumin film, albumin olive (OO) oil film, and albumin oleic acid (OA) films. <sup>a,b,c,d</sup> Significant difference ( $P < 0.05$ ) between elongation in different edible films.

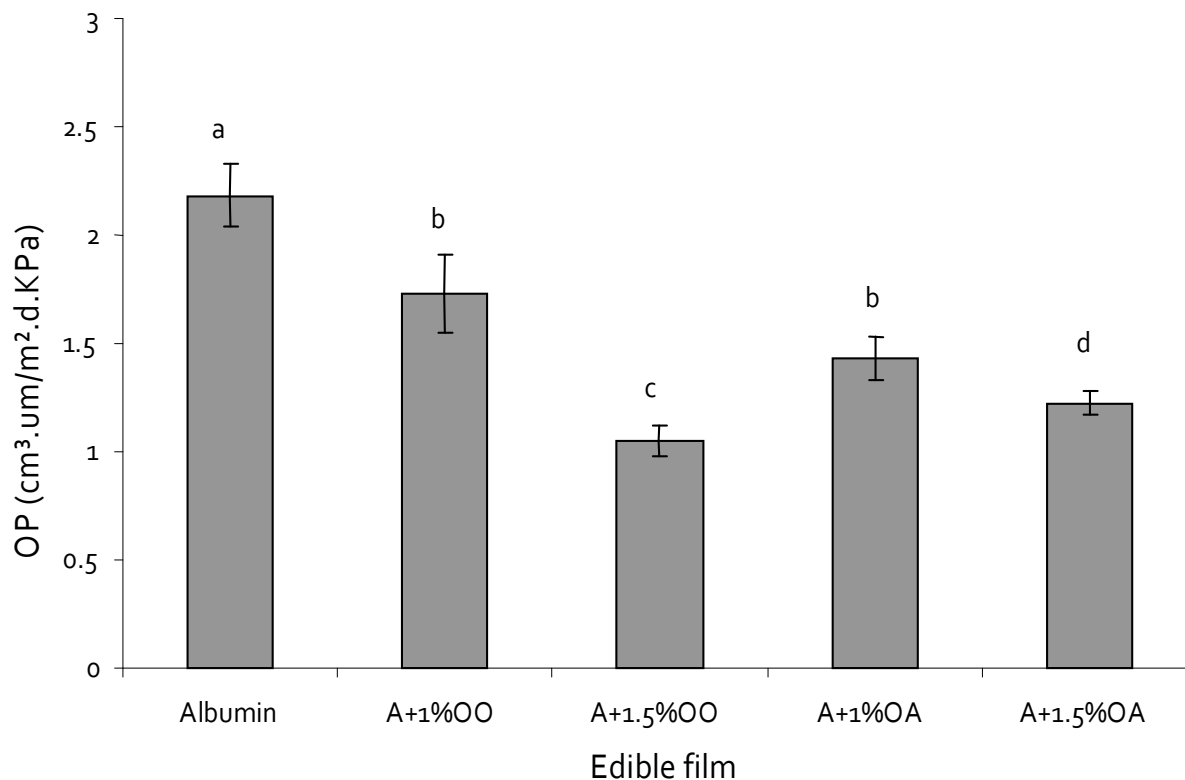


**Figure 3.** AFM images of the films' surface morphology at  $60 \times 60 \mu\text{m}$  scan size. (a) Albumin; (b) Albumin + 1% olive oil; (c) Albumin + 1.5% olive oil; (d) Albumin + 1% oleic acid; (e) Albumin + 1.5% oleic acid.

### Atomic force microscopy (AFM)

The use of AFM to observe the morphology and topography of the surface film proved to be a good technique for evaluating the properties of films (Parra et al., 2004) containing mixtures of albumin and olive oil and oleic acid at  $60 \times 60 \mu\text{m}$  scan size. The presence of both oils seemed to lead to some reduction in smoothness of the albumin film probably due to the manner of integration of the oils with the protein matrix surface. At both oil concentrations tested at (1 and 1.5%), a trend towards higher roughness values was obtained in films with olive

oil, as compared to those with oleic acid and control film. Such a trend is coherent with the different behavior of both oils as drying takes place. Oleic acid remains homogeneously integrated in the protein matrix, which results in relatively regular surfaces. In contrast, olive oil undergoes aggregation during the drying step, thus causing irregularities (Figure 3) on the films' surfaces, which is seen as a reduction in gloss and the presence of a thin layer with holes on top of the base layer. This may lead to the conclusion that, in the case of olive oil, the film suffers a phase separation and the phase of the thin film on top of the base film wets only partially the base phase.



**Figure 4.** Effect of different concentration of olive oil and oleic acid on oxygen permeability of albumin films. <sup>a,b,c,d</sup> Significantly different ( $P < 0.05$ ) between elongation in different edible film.

The mean thickness of the thin film on top was estimated to be of about 141, 44 to 184, and 02 µm.

On the other hand, Kester and Fennema (1989) and Park (1999) observed that the heterogeneity of cast films was plasticized with lipids. Distinct lipid layers were found in cast films due to the instability of the emulsions formed between the lipids and proteins or polysaccharides.

### Oxygen permeability

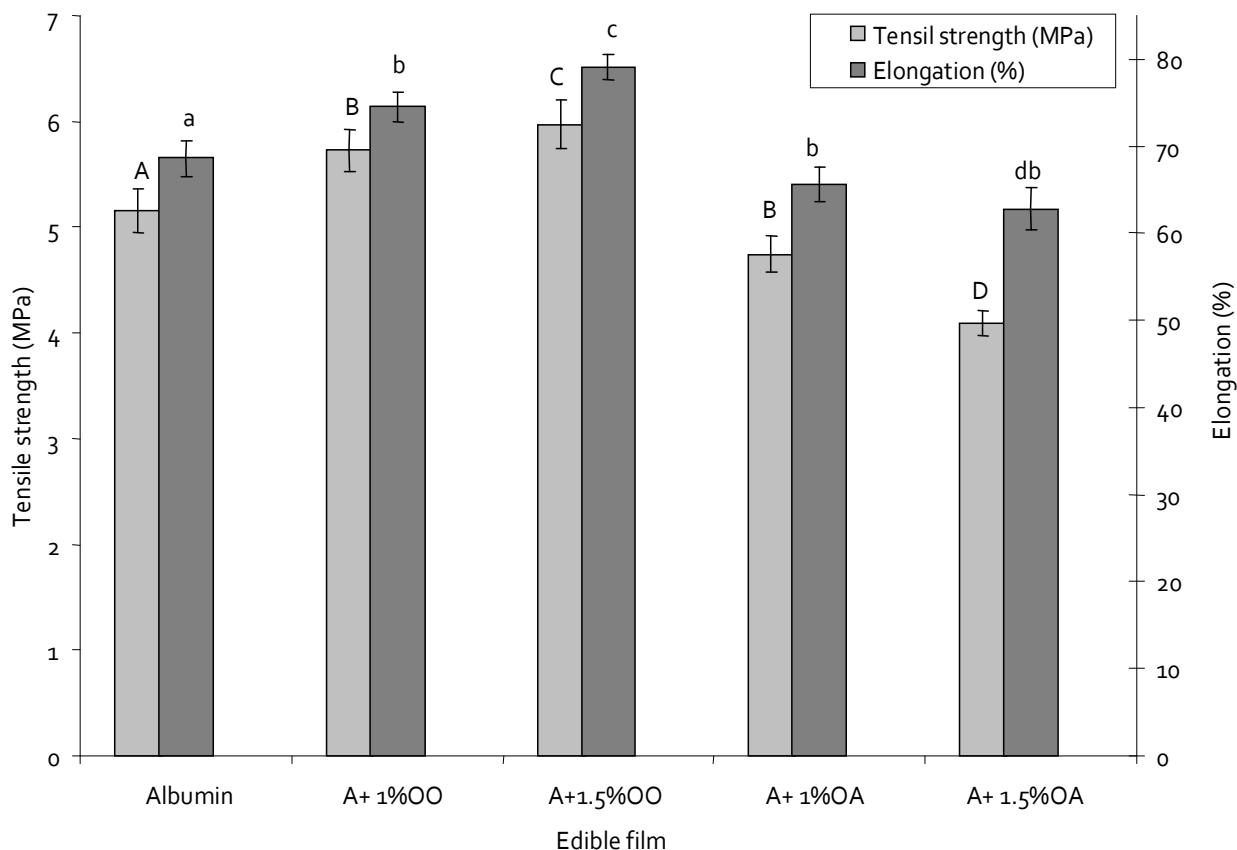
OP of albumin films with (1 and 1.5%) olive oil and oleic acid, respectively are summarized in Figure 4. These results indicate that there was a significant effect of albumin films on oxygen ( $P < 0.05$ ) with different concentrations of lipids. The oxygen permeability obtained for albumin film (control) was 2.2 (cm<sup>3</sup>µm /m<sup>2</sup> .d. bar); this result is similar with the work done by Qiu-Ping and Wen-Shui (2008) and it is lower than the results of Wong et al. (1992) who reported that the OP was 3.3 cc µm/m<sup>2</sup> .day. kPa.

The values of olive oil films (1 and 1, 5%) were slightly lower ( $P < 0.05$ ) than that of the control film. Khwaldia et al. (2005) reported that the addition of anhydrous milk fat to a sodium caseinate film reduced its OP. Similarly, OP of wheat gluten composite films decreased when oil was

added to organic solutions because the hydrophobic characteristics of these components reduce the water content of the films and thus their oxygen solubility (Gontard et al., 1999; Miller and Krochta, 1997). The values of oleic acid films are slightly lower ( $P < 0.05$ ) than that of the control film. In contrast, the addition of a fatty acid through an emulsion with proteins can increase the OP of the resulting film (Wu et al., 2002) and also addition of lipids to gelatin/ triacetin film increased their OP (Bertana et al., 2005). Those authors suggested that this behavior was caused by the creation of microscopic holes in the film body. Gas permeability of edible films and coatings depend on several factors, such as the integrity of the film, the ratio between crystalline and amorphous zones, the hydrophilic-hydrophobic ratio and the polymeric chain mobility. The interaction between the film-forming polymer and the presence of a plasticizer or other additives are also important factors in film permeability as reported by Garcia et al. (2000).

### Tensile strength and elongation at break

Different kinds of lipid films lack the structural integrity of films prepared from protein or polysaccharide (Gontard et al., 1995). Therefore, incorporated lipids in different con-



**Figure 5.** Effect of different concentrations of olive oil and oleic acid on elongation at break of albumin films ( $P < 0.05$ ). <sup>A, B, C, D</sup> Significant difference ( $P < 0.05$ ) between tensile strength (MPa) in edible films while <sup>a, b, c, d</sup> significant difference ( $P < 0.05$ ) between elongation in different edible films.

centrations may reduce protein film strength. Generally, high tensile strengths are necessary for edible protein films in order to withstand the normal stress encountered during their application, subsequent shipping, and food handling. However, flexibility of edible protein films, and elongation at break should be adjusted according to the intended application of edible films. Tensile strength of control albumin film was 5.35 MPa. This value is comparable to a previously reported value (Iwata et al., 2000).

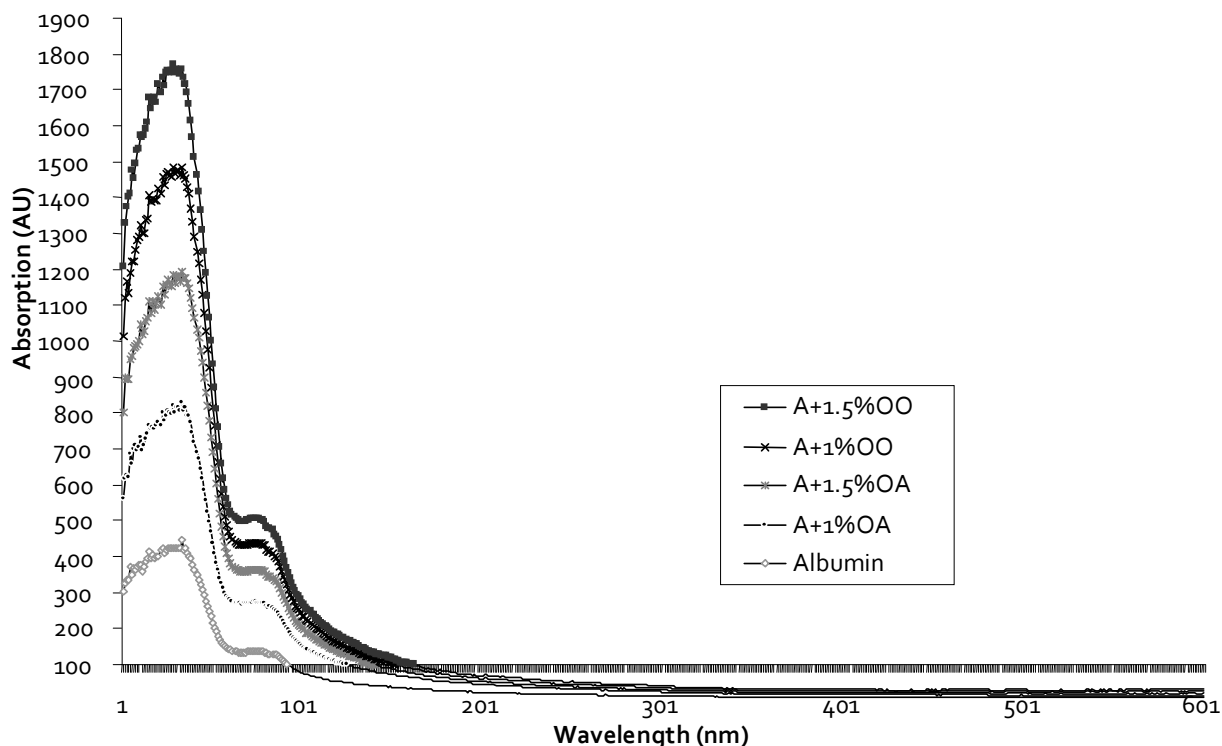
Tensile strength of films was changed by the incorporation of lipid materials together with 1% and 1.5% (v/v) olive oil and oleic acid as shown in Figure 5. The effect of different concentrations of unsaturated fatty acid (oleic acid) on tensile strength of albumin films led to a slight reduction ( $P < 0.05$ ); other monounsaturated fatty acids (olive oil) in different concentrations slightly increased tensile strength. These findings support those of Hagenmaier and Shaw (1990) who studied the formation of hydroxypropylmethyl cellulose-fatty acid film.

The effect of incorporated edible lipids on tensile strength of composite films gave the smallest tensile strength. As mentioned, oleic acid introduced negative

charges (carboxyl groups) to albumin proteins (King et al., 1984; Ball, 1987). The improve texture and increasing film tensile strength is due to poor structural properties integrity; different lipids also have reduced extensibility of protein films. Probably, the increased surface charge intensified repulsive forces unfolding protein chains and exposing reactive (SH) groups that contributed to film formation upon drying (Gontard et al., 1994; Banerjee and Chen, 1995; Gennadios et al., 1998). In confirmation, albumin films containing olive oil in different concentration had higher (79.12 %) ( $P < 0.05$ ) elongation than the control albumin films (68.61%).

Contrary to other lipids, oleic acid substantially increased elongation of soy protein films (Gennadios et al., 1998). Also, corn zein films had been plasticized with oleic acid (Lai and Padua, 1998). Similarly, oleic acid with different concentrations decreased ( $P < 0.05$ ) albumin film elongation (by a value of 65.63 %). This suggests occurrence of structural modification (unfolding) of albumin protein chains through association with oleic acid and olive oil. Unfolding enabled increased interaction through disulfide, hydrogen, and hydrophobic bonds, resulting in a more homogeneous and more extensible





**Figure 6.** UV-visible spectra of albumin film, albumin - olive oil film (1% and 1.5%), albumin - oleic acid film (1% and 1.5%).

film structure.

### Opacity

Film opacity is a critical property function if the film is used as a surface food coating. Visual characteristics of albumin film such as gloss, color, and transparency can affect consumer acceptability and even food quality. Opacity films are characterized by low values of the area below the absorption curve. It was observed that the addition of olive oil and oleic acid improves the barrier properties to UV radiation in the range of 200 to 280 nm. The impact of lipids (1 and 1.5% of olive oil and oleic acid, respectively) on opacity is shown in Figure 6. The color of the film with lipids was yellow and the color intensity depended on film thickness. The addition of lipids increased the film opacity, and the opacity increased significantly ( $P < 0.05$ ) as lipids concentration increased. The same trend was observed by Bertan et al. (2005), Perez-Mateos et al. (2009) and Quezada-Gallo et al. (2000). This may be attributed to the physical state of the lipids at room temperature.

Perez-Mateos et al. (2009) had stated that sunflower oil caused whiteness in cod gelatin film due to the light scattering effect of the emulsion. The addition of C16 and C18 to a filmogenic solution of gelatin resulted in opaque films, whereas the presence of Brazilian elemi had little effect on opacity (Bertan et al., 2005). Overall, the results

indicate that opacity results are strongly related with lipid migration during film preparation, which could be visualized as a cloudy appearance of the films.

### Conclusions

In conclusion, we succeeded in having composite films (Albumin / lipid) with improved mechanical properties relative to albumin films without lipids. Albumin films without lipids can be made through emulsion technology. However, the film became more opaque depended on Hunter system ( $L^*$ ,  $a^*$  and  $b^*$ ),  $\Delta E$ , YI, WI and opacity. Olive oil was more effective than oleic acid in reducing the WVP and solubility in the water, Tensile strength and percentage elongation at break increased with higher concentration of lipids and films with olive oil showed better mechanical properties overall than those with oleic acid and control films. Finally, the AFM results show that the surface of albumin films were much more uniform. When lipids were applied a trend towards higher roughness, values was obtained in films with olive oil, as compared to those with oleic acid, which may be due to the different behaviour of both oils as drying takes place.

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