Physical and Molecular Properties of Egg-white Lipid Films

A. Handa, A. Gennadios, M.A. Hanna, C.L. Weller, and N. Kuroda

ABSTRACT

Polyethylene glycol-plasticized films were cast from alkaline (pH 11.25), heated (45 °C for 20 min), aqueous egg white (EW) solutions, with or without (10% or 20% w/w of EW) milkfat (two fractions), oleic acid, or egg yolk lysophospholipid (LPL). The lipids did not reduce (P > 0.05) film water vapor permeability. Oleic acid increased (P < 0.05) tensile strength and elongation, and surface sulfhydryl group (SH) concentrations in EW solutions. Oleic acid probably increased negative charges on EW proteins, unfolding protein chains, and exposing SH groups. LPL also slightly increased (P < 0.05) surface SH concentrations in non-heated mixtures. Electrophoretic patterns suggested oleic acid interactions with ovalbumin, ovotransferrin, and lysozyme. No lysinoalanine was in film-forming mixtures based on lysine interactions with lipids (Ball, 1987; Uno-Ohta et al., 1994, 1995), corn zein (Weller et al., 1998), and soy protein (Gennadios et al., 1993; Shellhammer and Krochta, 1993), whey protein (McHugh and Krochta, 1994; Shellhammer and Krochta, 1997a), wheat gluten (Gontard et al., 1999). Functional properties of EW films have been modified through cross-linking by dialdehyde starch (Gennadios et al., 1999). Composite protein-lipid films can combine the effective structural and oxygen barrier properties of protein films with the high moisture barrier characteristics of lipids. Lipid addition was reported to reduce water vapor permeability of cast protein films from caseinates (Avena-Bustillos and Krochta, 1993), whey protein (McHugh and Krochta, 1994; Shellhammer and Krochta, 1997a), wheat gluten (Gontard et al., 1994, 1995), corn zein (Weller et al., 1998), and soy protein (Gennadios et al., 1998b). Furthermore, EW functionality has been altered through interactions with lipids (Ball, 1987; Yuno-Ohta et al., 1996). Our objective was to study the effects of various lipids on selected physical and molecular properties of cast EW films.

INTRODUCTION

There is high interest in edible films and coatings from renewable biopolymers (Krochta and DeMulder-Johnston, 1997), and protein-based films have been reviewed (Gennadios et al., 1994). Egg white (EW) or albumen consists of ovomucin fibers in an aqueous solution of numerous globular proteins (Powrie and Nakai, 1986). Protective EW coatings have been applied on shell eggs (Wong et al., 1996), and mechanical and water vapor barrier properties of cast EW films, plasticized with glycerin, sorbitol, or polyethylene glycol, have been reported (Gennadios et al., 1996). Conversion of surface sulfhydryl groups to disulfide bonds facilitated EW film formation (Handa et al., 1999). Functional properties of EW films have been modified through cross-linking by dialdehyde starch (Gennadios et al., 1999a) and microbial transglutaminase (Lim et al., 1998).

Composite protein-lipid films can combine the effective structural and oxygen barrier properties of protein films with the high moisture barrier characteristics of lipids. Lipid addition was reported to reduce water vapor permeability of cast protein films from caseinates (Avena-Bustillos and Krochta, 1993), whey protein (McHugh and Krochta, 1994; Shellhammer and Krochta, 1997a), wheat gluten (Gontard et al., 1994, 1995), corn zein (Weller et al., 1998), and soy protein (Gennadios et al., 1998b). Furthermore, EW functionality has been altered through interactions with lipids (Ball, 1987; Yuno-Ohta et al., 1996). Our objective was to study the effects of various lipids on selected physical and molecular properties of cast EW films.

MATERIALS & METHODS

Materials

Desugared, spray-dried EW solids (minimum 92% solids, minimum 80% protein, Type P-39) were obtained from Henningsen Foods Inc. (Omaha, Neb., U.S.A.). Milkfat fractions with melting points of 27 °C (middle-melting, M-59, 7, 27) and 37 °C (high-melting, H-56, 21, 37) were from the Center for Dairy Research, Univ. of Wisconsin (Madison, Wis., U.S.A.). Both milkfat fractions had peroxide values of <1.5 meq/kg and free fatty acid contents of <0.3% as oleic acid. Oleic acid was from Mallinckrodt Baker Chemicals (Phillipsburg, N.J., U.S.A.). Egg yolk lysophospholipid (LPL-100), a mixture of lysophosphatidylcholine and lysophosphatidylethanolamine, was from Q.P. Corp. (Tokyo, Japan). Silicone oil (TSA737F) was from Toshiba Silicone Co. Ltd. (Tokyo, Japan).

Film preparation

Aqueous film-forming solutions of dried EW (9 g/100 mL water), polyethylene glycol (average M.W. 400; 60% w/w of dried EW; plasticizer), and silicone oil (0.1% w/w of dried EW; antifoamer) were prepared as in Handa et al. (1999). After pH adjustment (±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 kept at ambient conditions for 4 to 5 h to solidify, placed in an environmental chamber (25 °C and 50% RH) to complete drying (15 h), peeled from plates, and specimens cut for property testing (Handa et al., 1999). EW-milkfat film-forming mixtures were prepared by adding, after pH adjustment (11.25 ± 0.10), middle- or high-melting milkfat fractions (10 or 20% w/w of dried EW). Heated (45 °C for 20 min) mixtures were homogenized (Virtishear, Virtis Co., Gardiner, N.Y., U.S.A.) at 7500 rpm for 30 s, cast, and dried. Films also were prepared by adding oleic acid (10% w/w of dried EW) or lysophospholipid (LPL) (10% or 20% w/w of dried EW) to EW film-forming solutions. After readjusting (±0.10) pH to 11.25 (oleic acid and LPL slightly reduced mixture pH) and heating (45 °C for 20 min), the mixtures were cast and dried. Film-forming mixtures containing oleic acid and LPL were not homogenized since they were easily dispersed. Film-forming mixtures with 20% w/w of dried EW oleic acid were too viscous to cast uniformly, and films were not characterized.

Film physical properties

Prior to testing, film specimens were conditioned (laid flat on Teflon-coated glass plates) for 3 d in an environmental chamber (25 °C and 50% RH). Film water vapor permeability (WVP) was determined gravimetrically at 25 °C and 100/50% RH gradient according to Gennadios et al. (1999b). Total soluble matter (TSM), as percentage of film dry matter solubilized after 4 h immersion in distilled water, was determined as described by Handa et al. (1999). We assumed that the amount of PEG dissolved in water was about the same for all film samples. Therefore, differences in TSM among films were primarily attributed to differences in protein solubility. Film tensile strength (TS) and percentage elongation at break (E) were determined with an Instron Universal Testing Machine (Model 5566, Instron Corp., Canton, Mass., U.S.A.), as described by Handa et al. (1999).
Film color values (L = lightness, a = red to green, and b = yellow to blue) were measured with a portable colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan) according to Gennadios et al. (1998a). TSM, TS, and E for each type of film were determined in triplicate with individually prepared films as replicated experimental units. WVP and color values were replicated 4 and 6 times, respectively. WVP, TSM, and color values for a film were means of measurements taken on 2 sampling units (specimens) from that film. TS and E values were means of measurements on 5 or 6 specimens from the same film.

**Sulphydryl groups**

Concentrations of surface (free) and total (free and buried) sulphydryl (SH) groups in selected film-forming solutions, before and after heating (45 °C for 20 min), were determined using Ellman’s reagent. Solutions were diluted (1:10 v/v) with 0.1 M Tris-glycine buffer (pH 8) containing 0.01M EDTA and centrifuged at 10,000 × g for 30 min. Duplicate samples of 1 mL protein solution were pipetted into centrifuge tubes. One mL of the buffer (pH 8), with (for total SH) or without (for surface SH) 0.5% sodium dodecyl sulfate (SDS), was added, followed by 0.05 mL of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reagent (Sigma Chemical Co., St. Louis, Mo., U.S.A.). After 10 min at 25 °C, absorbance at 412 nm was measured vs reagent blanks (Hitachi U-2000 spectrophotometer, Tokyo, Japan). The molar absorptivity of 13,600 was used to determine concentrations (µM/g of protein) of SH groups. Protein concentrations of film-forming solutions were determined by the well known Folin phenol method using ovalbumin (grade V, Sigma Chemical) as protein standard. SH concentrations were determined in triplicate with individually prepared film-forming solutions as replicated experimental units.

**Available lysine**

Available lysine concentrations in selected film-forming solutions, before and after heating (45 °C for 20 min), were determined using o-phthalaldehyde (OPA) and N-acetyl-L-cysteine (NAC) (Medina Hernández and García Alvarez-Coque, 1992). Following dilution (1:100 v/v) of film-forming solutions with deionized water, duplicate samples of 0.5 mL were pipetted into centrifuge tubes, and 2.5 mL of OPA-NAC reagent were added. After 10 min at 25 °C, absorbance at 335 nm was measured vs reagent blanks (Hitachi U-2000 spectrophotometer). The molar absorptivity of 6830 was used to determine concentrations (µM of NH₂/g of protein) of available lysine in samples. Reported values were means of 3 replicates with individually prepared film-forming solutions as replicated experimental units.

**Electrophoresis**

Molecular characteristics of EW protein in heated (45 °C for 20 min) and non-heated film-forming solutions were compared through electrophoretic patterns. Film-forming solution samples for electrophoretic analyses were prepared as described by Handa et al. (1999). Native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE were performed according to Laemmli (1970), using a 4% stacking gel (STC-808 electrophoresis unit, TEFCO, Tokyo, Japan). Separating gels of 8% and 4% to 20% gradient were used for native PAGE and SDS-PAGE, respectively. Gels were stained with Coomassie brilliant blue R-250 (0.1% w/v) in methanol/acetic acid/water (40%/10%/50% v/v/v) and were destained in methanol/acetic acid/water (10%/7.5%/82.5% v/v/v). The standard protein mixture (Mark 12, TEFCO, Tokyo, Japan) ranged in molecular weight from 200 kDa (myosin) to 2.5 kDa (insulin A chain).

**Statistical analysis**

Means and standard deviations for measured film properties were compared and differences (P < 0.05) among means by Duncan’s multiple range test were detected using the General Linear Models procedure in SAS Release 6.08 (SAS Institute Inc., Cary, N.C., U.S.A.).

---

**RESULTS & DISCUSSION**

**Water vapor permeability**

Interest in expanding use of milkfat in food products has been noted (Kaylegian et al., 1993) and milkfat addition to edible films and coatings has been suggested (Shellhammer and Krochta, 1997a,b). However, the two milkfat fractions we used did not affect (P > 0.05) WVP of EW films (Table 1). This was attributed to the high content of short- and medium-chain (C₃–C₁₀) fatty acids in milkfat (Kaylegian et al., 1993) since moisture barrier ability of lipids decreases with decreasing hydrocarbon chain length (Fennema et al., 1993; Shellhammer and Krochta, 1997b). Similarly, milkfat (10% to 30% w/w of protein) did not notably change WVP of cast soy protein films (Gennadios et al., 1998b). In contrast, emulsion films containing 60% whey protein and 40% milkfat had lower WVP than control whey protein films (Shellhammer and Krochta, 1997a). However, the milkfat fraction used in that study had a higher melting point (~45 °C), and, therefore, greater hydrophobicity than the fractions we used.

Oleic acid has reduced WVP of wheat gluten (Gontard et al., 1994) and soy protein (Gennadios et al., 1998b) films. However, oleic acid did not affect (P > 0.05) WVP of EW films in our study (Table 1). King et al. (1984) observed an increase of negative charge in EW proteins treated with oleic acid. Oleic acid likely increased negative charges (and hydrophilicity) along EW 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). Saturated fatty acids (e.g., myristic, palmitic, or stearic acids) reduced WVP of whey protein (McHugh and Krochta, 1994), wheat gluten (Derksen et al., 1995), and soy protein (Gennadios et al., 1998b) films. However, we could not incorporate these saturated fatty acids into EW film-forming solutions since EW coagulated at a lower temperature than the melting points of those acids. LPL at 10% (w/w of EW) slightly increased (P < 0.05) film WVP (Table 1). This likely resulted from the hydrophilic groups (hydroxyl and amino) carried by LPL.

**Total soluble matter**

Total soluble matter is related to film hydrophilicity and structural integrity. Small reductions in TS of EW films (P < 0.05) resulted from addition of 10% oleic acid, 20% LPL, and 20% high-melting milkfat (Table 1). Similarly, oleic acid and milkfat reduced TSM of soy protein films (Gennadios et al., 1998b). However, from a practical standpoint, added lipids did not substantially affect TS of EW films. Overall, TSM values of our EW films were notably greater than those reported for fish myofibrillar (Cuq et al., 1996), soy (Gennadios et al., 1998b), and rice bran (Gnanasambandam et al., 1997) protein films. Note, however, that protein films in the reported studies contained lower amounts of hydrophilic plasticizers than our EW films. Due to

---

**Table 1—Water vapor permeability (WVP) and total soluble matter (TSM) of cast egg white (EW) films containing middle-melting milkfat (MMM), high-melting milkfat (HMM), oleic acid, or egg yolk lysophospholipid (LPL)**

<table>
<thead>
<tr>
<th>Lipid in film (%)</th>
<th>Thickness (µm)</th>
<th>RH inside cup (%)</th>
<th>WVP (g mm⁻¹ m⁻¹ kPa)</th>
<th>TSM (%)</th>
<th>Lipid in film (%)</th>
<th>Thickness (µm)</th>
<th>RH inside cup (%)</th>
<th>WVP (g mm⁻¹ m⁻¹ kPa)</th>
<th>TSM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% lipid</td>
<td>98±5</td>
<td>72±1±2</td>
<td>8.3±0.5</td>
<td>48.4±0.6</td>
<td>10% lipid</td>
<td>99±6</td>
<td>72±0±1±5</td>
<td>8.5±0±2</td>
<td>46.7±0.8a</td>
</tr>
<tr>
<td>10% MMM</td>
<td>106±3</td>
<td>73.3±0±6</td>
<td>8.3±0±2</td>
<td>45.7±0.6</td>
<td>10% oleic acid</td>
<td>106±3</td>
<td>71±7±1±1</td>
<td>9.2±0±5</td>
<td>47.7±0.2d</td>
</tr>
<tr>
<td>20% LPL</td>
<td>108±2</td>
<td>72±4±0±7</td>
<td>8.8±0±5±d</td>
<td>46.3±0.1-</td>
<td>20% LPL</td>
<td>108±2</td>
<td>72±4±0±7</td>
<td>8.8±0±5±d</td>
<td>46.3±0.1-</td>
</tr>
</tbody>
</table>

aMeans of 4 replicates ± standard deviations.
bMeans of 3 replicates ± standard deviations.
cMeans in same column followed by same letter not different (P>0.05).
their high solubility in water, EW films may be more appropriate for water-soluble packaging applications.

**Tensile strength and elongation**

Lipid films lack the structural integrity of protein or polysaccharide films (Gontard et al., 1995). Therefore, incorporated lipids may reduce protein film strength. For example, added lipids reduced TS or puncture strength of wheat gluten (Gontard et al., 1994), milk protein (Banerjee and Chen, 1995; Shellhammer and Krochta, 1997a), and soy protein films (Gennadios et al., 1998b). In our study, milk-fat fractions and LPL either did not affect (P > 0.05) or slightly reduced (P < 0.05) TS of EW films (Table 2). However, oleic acid increased (P < 0.05) EW film E (by 43%) and so did 20% HMM (Table 2). Contrary to other lipids, oleic acid substantially increased TS by 15% (Table 2). As mentioned, oleic acid introduced negative charges (carboxyl groups) to EW proteins contributing to EW film formation (Handa et al., 1999). Also, we reported that heating increased concentration of surface SH groups in EW film-forming solutions (Table 3), although not as much as egg yolk lysophospholipid (LPL) (Gennadios et al., 1998b). In confirmation, heating (45 °C for 20 min) increased (P < 0.05) surface SH concentration in EW, EW–oleic acid, and EW-LPL solutions (Table 3). This was attributed to heat-induced protein unfolding since, for the same type of solution, heating did not affect (P > 0.05) total SH concentration (Table 3).

**Color**

Confirming previous studies (Gennadios et al., 1996, 1998a; Handa et al., 1999), our EW films cast from highly alkaline (pH 11.25) film-forming solutions were transparent. This was expected since highly alkaline conditions have been reported to result in transparent EW gels (Kitabatake et al., 1988; Handa et al., 1998) and lipid films did not lessen film transparency. The EW films had greater L values than soy protein (Gennadios et al., 1998b) or wheat gluten (Roy et al., 1999) films. The effect of lipid addition on L values was inconsequential (Table 2). Lipids slightly reduced film yellowness and increased greenness as evidenced by greater (P < 0.05) b values, respectively (Table 2). These small color differences were attributed to variations in film-forming ingredients.

**Table 2**—Tensile strength (TS), elongation at break (E), and color values (L, a, and b) of cast egg white (EW) films containing middle-melting milkfat (MHM), high-melting milkfat (HMM), oleic acid, or egg yolk lysophospholipid (LPL)

<table>
<thead>
<tr>
<th>Lipid in film (% w/w of EW)</th>
<th>TS* (MPa)</th>
<th>E* (%)</th>
<th>Lb</th>
<th>ab</th>
<th>bb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% lipid</td>
<td>4.69±0.06</td>
<td>62.6±1.8</td>
<td>95.62±0.18</td>
<td>–0.71±0.07</td>
<td>4.44±0.11</td>
</tr>
<tr>
<td>10% LPL (non-heated)</td>
<td>4.75±0.28</td>
<td>64.2±1.9</td>
<td>95.66±0.19</td>
<td>–0.87±0.09</td>
<td>4.15±0.12</td>
</tr>
<tr>
<td>10% LPL (heated)</td>
<td>4.36±0.25</td>
<td>54.0±3.7</td>
<td>96.13±0.21</td>
<td>–1.02±0.12</td>
<td>5.13±0.41</td>
</tr>
<tr>
<td>10% HMM</td>
<td>4.54±0.35</td>
<td>43.1±5.3</td>
<td>95.90±0.22</td>
<td>–1.00±0.08</td>
<td>5.15±0.12</td>
</tr>
<tr>
<td>20% HMM</td>
<td>4.06±0.04</td>
<td>44.1±7.0</td>
<td>96.40±0.14</td>
<td>–1.16±0.09</td>
<td>5.59±0.37</td>
</tr>
<tr>
<td>10% oleic acid</td>
<td>5.77±0.20</td>
<td>89.2±1.6</td>
<td>95.86±0.14</td>
<td>–1.40±0.12</td>
<td>6.00±0.34</td>
</tr>
<tr>
<td>10% LPL</td>
<td>4.80±0.17</td>
<td>77.7±5.1</td>
<td>95.54±0.10</td>
<td>–1.08±0.10</td>
<td>5.40±0.19</td>
</tr>
<tr>
<td>20% LPL</td>
<td>4.56±0.06</td>
<td>84.5±2.5</td>
<td>95.34±0.11</td>
<td>–1.40±0.19</td>
<td>6.22±0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid in solution (% w/w of EW)</th>
<th>Surface SH (µM/g protein)</th>
<th>Total SH (µM/g protein)</th>
<th>Available lysine (µM NH₂/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% lipid (non-heated)</td>
<td>6.77±0.90</td>
<td>40.27±0.76</td>
<td>37±10</td>
</tr>
<tr>
<td>0% lipid (heated)</td>
<td>13.16±0.24</td>
<td>40.26±0.69</td>
<td>353±12</td>
</tr>
<tr>
<td>10% oleic acid (non-heated)</td>
<td>15.9±0.15</td>
<td>36.9±1.18</td>
<td>347±13</td>
</tr>
<tr>
<td>10% oleic acid (heated)</td>
<td>23.2±0.31</td>
<td>36.4±0.89</td>
<td>349±9</td>
</tr>
<tr>
<td>10% LPL (non-heated)</td>
<td>8.54±1.19</td>
<td>39.1±1.03</td>
<td>387±16</td>
</tr>
<tr>
<td>10% LPL (heated)</td>
<td>13.42±0.42</td>
<td>38.1±0.73</td>
<td>373±13</td>
</tr>
<tr>
<td>20% LPL (non-heated)</td>
<td>8.55±0.12</td>
<td>38.37±1.62</td>
<td>401±11</td>
</tr>
<tr>
<td>20% LPL (heated)</td>
<td>13.57±0.57</td>
<td>36.9±1.38</td>
<td>393±13</td>
</tr>
</tbody>
</table>

Means of 3 replicates ± standard deviations.

Means of 6 replicates ± standard deviations.

Means of 3 replicates ± standard deviations.

Means of 6 replicates ± standard deviations.

Means in same column followed by same letter not different (P>0.05).

Means in same column followed by same letter not different (P>0.05).

Means in same column followed by same letter not different (P>0.05).

*SH values, respectively (Table 2). These small color differences were attributed to variations in film-forming ingredients.

**Table 3**—Concentrations of surface SH groups, total SH groups, and available lysine in non-heated and heated (45 °C for 20 min) egg white (EW) film-forming solutions containing oleic acid or egg yolk lysophospholipid (LPL)

- a Means of 3 replicates ± standard deviations.
- b Means in same column followed by same letter not different (P>0.05).
- c Means in same column followed by same letter not different (P>0.05).
Therefore, LPL-induced protein unfolding was likely limited and was subsequently obscured by the substantial protein structure modification effect of heating.

**Available lysine**

Thermal treatments of proteins at alkaline pH can lead to formation of lysinoalanine through condensation of lysine residues with dehydroalanine (Cheftel et al., 1985). Lysinoalanine in edible protein-based films would be undesirable because of reported toxic effects.

**Electrophoresis**

In native PAGE patterns of EW-oleic acid solutions, both ovalbumin and ovotransferrin moved to the anode (lanes 3 and 4, Fig. 1), providing further evidence that oleic acid introduced negative charges. Ovalbumin and ovotransferrin in EW treated with oleic acid were reported to elute at more negative pH by DEAE-Sephacel chromatography (King et al., 1984). Heating (45 °C for 20 min) also resulted in a much fainter ovotransferrin band in native PAGE patterns for EW and EW–LPL solutions (lanes 2, 6, and 8, Fig. 1), suggesting occurrence of notable heat-induced aggregation of ovotransferrin. This confirmed our previous finding that ovotransferrin was heavily involved, likely through S–S bonding, in EW film formation from alkaline, heated solutions (Handa et al., 1999).

No differences were observed among SDS–PAGE patterns of EW protein in EW, EW–oleic acid, or EW–LPL solutions when S–S bond-cleaving 2-mercaptoethanol had been used (Fig. 2). However, without 2-mercaptoethanol, differences in electrophoretic patterns of EW protein were observed (Fig. 3). Oleic acid promoted formation of protein aggregates that did not enter the gel (lanes 4 and 5, Fig. 3). Such protein aggregation was attributed to S–S formation since it was not evident in presence of 2-mercaptoethanol (Fig. 2). This further supported the hypothesis that oleic acid increased net charge of EW proteins causing protein chain unfolding, exposing buried SH groups, and, upon film drying, leading to extensive S–S formation. Increased protein aggregation due to oleic acid resulted in fainter bands for ovotransferrin, lysozyme, and, to a lesser extent, ovalbumin (lanes 4 and 5, Fig. 3). Therefore, all three of these major EW protein fractions

**Fig. 1—Native PAGE patterns for egg white (EW) protein in alkaline film-forming solutions with or without (45 °C for 20 min) heating and with or without (10% or 20% w/w of EW) oleic acid (OA) or yolk lysophospholipid (LPL) (lane 1 = no lipid, no heating; lane 2 = no lipid, heating; lane 3 = 10% OA, no heating; lane 4 = 10% OA, heating; lane 5 = 10% LPL, no heating; lane 6 = 10% LPL, heating; lane 7 = 20% LPL, no heating; lane 8 = 20% LPL, heating).**

**Fig. 2—SDS-PAGE patterns with 2-mercaptoethanol in sample solvent system for egg white (EW) protein in alkaline film-forming solutions with or without (45 °C for 20 min) heating and with or without (10% or 20% w/w of EW) oleic acid (OA) or yolk lysophospholipid (LPL) (lane 1 = molecular weight standard; lane 2 = no lipid, no heating; lane 3 = no lipid, heating; lane 4 = 10% OA, no heating; lane 5 = 10% OA, heating; lane 6 = 10% LPL, no heating; lane 7 = 10% LPL, heating; lane 8 = 20% LPL, no heating; lane 9 = 20% LPL, heating).**

**Fig. 3—SDS-PAGE patterns without 2-mercaptoethanol in sample solvent system for egg white (EW) protein in alkaline film-forming solutions with or without (45 °C for 20 min) heating and with or without (10% or 20% w/w of EW) oleic acid (OA) or yolk lysophospholipid (LPL) (lane 1 = molecular weight standard; lane 2 = no lipid, no heating; lane 3 = no lipid, heating; lane 4 = 10% OA, no heating; lane 5 = 10% OA, heating; lane 6 = 10% LPL, no heating; lane 7 = 10% LPL, heating; lane 8 = 20% LPL, no heating; lane 9 = 20% LPL, heating).**

**EW** has substantial lysine and alanine contents (5.12 and 4.96 g/100 g dry wt, respectively) (Cook and Briggs, 1986). Heating (45 °C for 20 min) did not affect (P > 0.05) available lysine contents in EW, EW-oleic acid, or EW-LPL film-forming solutions (Table 3). Available lysine in EW solutions also was not affected notably by oleic acid, while it increased (P < 0.05) with LPL (Table 3). The increased levels of measured NH₃ groups in EW–LPL solutions were attributed to NH₃ groups inherent to LPL. We concluded that heating at pH 11.25 during EW film preparation did not form lysinoalanine.
interacted with oleic acid as reported by King et al. (1984). Slightly fainter ovo transferrin, lysosome, and ovalbumin bands (but no aggre-
gate formation) also was observed in SDS–PAGE patterns from non-
heated EW–LPL solutions (lanes 6 and 8, Fig. 3) as compared to non-
heated controls (lane 2, Fig. 3). Therefore, possibly LPL also promot-
ed limited protein unfolding and S–S formation from exposed SH

groups.

From SDS–PAGE, we had concluded that heating of alkaline EW film-forming solutions promoted protein aggregation through S–S
formation (Handa et al., 1999). Similarly, without 2-mercaptoethanol,
SDS-PAGE patterns from heated (45 °C for 20 min) EW, EW-oleic
acid, and EW-LPL solutions had fainder ovo transferrin and lysosome
bands (lanes 3, 5, 7, and 9, Fig. 3) than non-heated solutions (lanes
2, 4, 6, and 8, Fig. 3). S–S formation was likely involved since no
such differences in band intensity between heated and non-heated
solutions were noted for SDS–PAGE patterns of 2-mercaptoethanol-
treated samples (Fig. 2). Oleic acid and, to a lesser extent, egg yolk
LPL interacted with EW protein in film-forming solutions yielding
cast films of improved functional properties (greater TS and E, and
lower TSM). The potential of altering EW film properties through use
of other anionic surfactants (e.g., SDS) or free fatty acids (e.g., lau-
ric acid) merits investigation. However, reducing WVP of hydro-
phlic EW films would probably require cross-linking treatments
(e.g., Maillard reaction) that promote covalent bonds other than S–
S bonds.

REFERENCES

Avena-Bustillos, R.J. and Krochta, J.M. 1993. Water vapor permeability properties of
caseinate-based edible films as affected by pH, calcium cross-linking and lipid con-

JAOCS 64: 1718-1725.


Cook, P. and Briggs, G.M. 1986. The nutritive value of eggs. Ch. 7 in Food Chemistry and
Toxicology, A. Gennadios, C.L. Weller, M.A. Hanna, and G.W. Froning. 1996. Mechanical and

protein-based edible films. Ch. 15 in Emulsions for Successful Utilization of Renewable Re-

Gennadios, A., Froning, G.W., Kuroda, N., and Hanna, M.A. 1999b. Tensile,
solubility, and electrophotographic properties of egg white films as affected by surface

wheat gluten and lipids: water vapor permeability and other physical properties. Int.

Gontard, N., Marchesseau, S., Cuq, J-L., and Guibert, S. 1995. Water vapor perme-
30: 49-56.


solubility and electrophotographic properties of egg white films as affected by surface

two Egg-White Lipid Films . . .


Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head


Li, T-J., Mine, Y., and Tung, M.A. 1997. Transglutaminase cross-linked egg white
protein films: Tensile properties and oxygen permeability J. Agric. Food Chem. 46:
4022-4029.

Mares, B.A. 1990. Correlation of protein sulphydryls with the strength of heat-


protein, assay using o-phthalaldehyde/N-acetyl-L-cysteine spectrophotometric

Mine, Y. 1992 Sulphydryl groups changes in heat-induced soluble egg white aggre-

Mine, Y. 1995 Recent advances in the understanding of egg white protein functional-

Montenegro, M., Hamann, D.C., and Ball, H.R. Jr. 1984. Mechanical failure character-

Poffen, W.R. and Nakai, S. 1986. The chemistry of eggs and egg products. Ch. 6 in
Publishing, Westport, CT.

molecular properties of wheat gluten films cast from heated film-forming solutions.
J. Food Sci. 64: 57-60.

as affected by lipid type and amount. J. Food Sci. 62: 390-394.

Shelhammer, T.H. and Krochta, J.M. 1997b. Water vapor barrier and rheological prop-
erties of simulated and industrial milkfat fractions. Trans. ASAE 40: 1119-1127.


tion properties of ovalbumin as affected by fatty acid salts. J. Food Sci. 61: 908-910, 920.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.

Ms 0829 received 2/16/99; revised 4/19/99; accepted 5/23/99.