Effects of Glycerol Content and Film Thickness on the Properties of Vital Wheat Gluten Films Cast at pH 4 and 11

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Received 23 April 2009; accepted 5 February 2010
DOI 10.1002/app.32235
Published online 12 May 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This study deals with the optical properties and plasticizer migration properties of vital wheat gluten (WG) films cast at pH 4 and 11. The films contained initially 8, 16, and 25 wt % glycerol and were aged at 23°C and 50% relative humidity for at least 17 weeks on a paper support to simulate a situation where a paper packaging is laminated with an oxygen barrier film of WG. The films, having target thicknesses of 50 and 250 μm, were characterized visually and with ultraviolet/visible and infrared spectroscopy; the mass loss was measured by gravimetry or by a glycerol-specific gas chromatography method. The thin films produced at pH 4 were, in general, more heterogeneous than those produced at pH 11. The thin pH 4 films consisted of transparent regions surrounding beige glycerol-rich regions, the former probably rich in gliadin and the latter rich in glutenin. This, together with less Maillard browning, meant that the thin pH 4 films, in contrast to the more homogeneous (beige) thin pH 11 films, showed good contact clarity. The variations in glycerol content did not significantly change the optical properties of the films. All the films showed a significant loss of glycerol to the paper support but, after almost 9 months, the thick pH 11 film containing initially 25 wt % glycerol was still very flexible and, despite a better contact to the paper, had a higher residual glycerol content than the pH 4 film, which was also more brittle. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 3506–3514, 2010

Key words: biopolymers; diffusion; films; proteins; transparency

INTRODUCTION

Wheat gluten (WG) is promising as a potential packaging material in that it has attractive mechanical (a combination of strength and flexibility), good gas barrier (dry conditions), and film-forming properties. Like many protein materials, it needs a plasticizer in order not to be brittle. Glycerol is probably the most commonly tested plasticizer for these materials because of its high plasticization efficiency.[1,2] Because of its small size, glycerol is easier to disperse evenly in the protein matrix than larger plasticizers, such as sorbitol.[3] However, for the same reason, glycerol is prone to migrate to the film surface,[4] and if the film is in contact with a solid or liquid capable of absorbing it, glycerol migrates from the sample. In fact, even without contact, we have observed an extensive bleeding of glycerol from the surface of the films at high relative humidity (RH), probably because glycerol absorbs moisture, which reduces its viscosity. As a result of this migration, the film becomes brittle.[5]

There are several ways to limit migration, but most of these routes have some critical drawback. A larger plasticizer reduces migration but is difficult to disperse evenly.[6,7] Migration may be reduced by placing the protein film between two hydrophobic polymer films. This is not however feasible if the protein film is to be laminated directly onto paper. Regular paper fibers are highly polar and absorb glycerol. To develop a paperboard packaging with a WG-based oxygen barrier, it is of interest to know how the packaging-related properties change with time. In this study, one of the objectives has been to understand the interaction between the paper support and the WG film during storage and aging. It has been observed that glycerol migration can be reduced by using a system with a high degree of protein aggregation.[5] Migration may even be desirable, such as when the film is used as a carrier of, for example, antioxidants and antimicrobial agents that are intended to migrate out to the foodstuff and prolong its shelf life.[8]

Optical properties are of major concern for many potential biopolymer applications. WG is naturally

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Contract grant sponsor: Vinnova, the Swedish Governmental Agency for Innovation Systems; contract grant number: 2004-01656.
Contract grant sponsor: "Glupack" Consortium.

beige and becomes browner with heat treatment because of Maillard reactions. It is probably difficult, if not impossible to get rid of the natural pigments, including carotenoids, because such compounds remain in virtually every cell.[9] Gontard et al.[10] investigated the optical properties of 50-μm-thick WG films containing glycerol (20 g/100 g WG) at pH 2–6. Above pH 4, they found that the opacity increased with increasing ethanol content. At high ethanol content, the films were heterogeneous, as shown by the phase separation of transparent ethanol-soluble gliadin-rich material and opaque insoluble glutenin-rich material. This was confirmed by Hernández-Muñoz et al.[11–13] who showed that films based on extracted gliadin-rich material were significantly more transparent than those based on glutenin-rich material. Irissin-Mangata et al.[2] showed that the change in opacity was independent of glycerol content (10–20 g/g dry matter) for WG films of similar thickness to those of Gontard et al.[10] and Hernández-Muñoz et al.,[11–13] cast at pH 4 and cross-linked with formaldehyde.

This investigation was undertaken to improve the understanding of two packaging-related issues, the optical WG film properties and the glycerol migration. These properties can be adjusted through the choice of plasticizer type and content, solution pH, and film thickness. Films were produced with different glycerol contents at pH of 4 and 11 (casting solution) and film thicknesses of 50 and 250 μm (target thicknesses). The plasticizer was glycerol, which, as mentioned earlier, is a highly efficient plasticizer. Even though the optical properties of WG films have been evaluated before, no comparison between low and high pH films has, to our knowledge, been made where, at the same time, the plasticizer content and film thickness are varied.

The migration from the WG film was measured gravimetrically and with a glycerol-specific previously developed gas chromatography (GC) method.[5] The decision to place the films on a paper support was based on the idea that several future packaging solutions may involve wheat gluten laminated on paper or paperboard.

EXPERIMENTAL

Materials

The vital WG powder was kindly supplied by Reppe AB, Lidköping, Sweden. According to the supplier, the gluten protein content (according to Mod NMKL nr6, Kjeltec, Nx6,25) was 85.2%, the starch content (according to Mod. NMKL Nr 6, Kjelldahl. Ewers.) was 5.84% (according to Ewers, pol.), the concentration of fats (according to Soxtec, Lidfett.OA.19, tecator AN 301) was 1.2%, and the ash content (according to NMKL 173 second ed) was 0.86%, all on a dry weight basis. The water content (according to NMKL 231991) was 6.9% on a total weight basis. Glycerol with a concentration of $\geq$99.5 wt % and a water content of $\leq$0.5 wt % was supplied by Karlshamns Tefac AB, Karlshamn, Sweden. Ethanol (95%) was purchased from VWR International AB, Sweden. Acetic acid (99%) and methanol (pro analysis, >99.8%) were supplied by Merck Eurolab GmbH, Germany. Sodium hydroxide and *meso*-erythritol with a purity >99% were obtained from Sigma-Aldrich Chemie GmbH, Germany. (*R*)-(+)-Limonene (D-limonene, purity > 97%) was a Lancaster, England, product. Pyridine (99.5%) and acetic anhydride (>99%) were purchased from Fisher Scientific, Sweden. Blotting paper (grade: 1600) supplied by Munktell Filter AB, Sweden, was used as substrate for the films.

Methods

Film preparation

The films were prepared from a film-forming solution according to the method described by Olabarrieta et al.[5] "Film" will henceforth denote films made of WG and glycerol. A total of 30 or 7.5 g of WG powder, with 8, 16, or 25 wt % glycerol, based on the total weight of the final film, was dispersed in 135 g of 95% ethanol and stirred with a magnetic stirrer during 15 min at room temperature. A total of 90 g of deionized water was then slowly added, and stirring was continued for a further 15 min. The pH of the solutions was then adjusted to 4 or 11, with acetic acid or sodium hydroxide. Afterward, the solution, while being stirred, was heated to 75°C ± 2°C for 20 min (~ 2.5°C/min) in a bath of silicone oil and kept at this temperature for 10 min. The solution was then immediately decanted evenly into circular petri dishes (diameter: 13.5 cm) placed on a level surface. The petri dishes were coated with a release agent layer of poly(tetrafluoroethylene) supported by an aluminum foil (Bytac Type AF-21, Norton Performance Plastics Corp., USA) to enable the dried films to be peeled off. The cast solution was dried into films in a climate room (23°C and 50% RH) for at least 3 days before being tested. The amount of the film-forming solution poured into the petri dish was constantly either 25 g (WG concentration: 13.3 g/100 g solution) or 15 g (3.3 g/100 g solution) to yield a constant weight and minimize thickness variations after drying. This yielded film thicknesses close to the target values of 250 and 50 μm (*vide infra*).

Thickness measurements

The film thickness was measured at a pressure of 100 kPa at 23°C and 50% RH, in accordance with

SCAN-P 7:96,[14] and determined as the average of five measurements taken over the individual specimens, four along the perimeter and one at the center using a Lorentzen & Wettre Type 21 (Lorentzen & Wettre AB, Sweden) micrometer.

Density measurements

The densities of the WG films were obtained at 24°C using the Archimedes principle on a Mettler AE163 balance equipped with a density determination kit (Mettler Toledo, Switzerland). The films were weighed in air and in D-limonene ($\rho = 0.841$ g/cm^3 (20°C) according to the supplier) under ambient conditions. It is believed that the polar WG films would swell less and give more accurate values, in a nonpolar liquid such as D-limonene, than in, for example, water or ethanol. Three measurements per sample were made. The films were equilibrated at 23°C and 50% RH before being taken out for the density measurements.

Mass loss by gravimetry

The aging procedure is largely similar to that presented in the previous study.[5] The film specimens were placed on the blotting paper with the side that had faced the petri dish down and aged in a climate room at 23°C and 50% RH. The glycerol loss was determined on two replicates per sample using the GC method, described later, after aging for 4, 16, and 64 h and 1, 2, 3, 4, 8, 12, and 17 weeks. In addition, the mass loss of the films at the same aging times was gravimetrically determined under the same conditions. At least three replicates from each sample were weighed, and the mean value was calculated.

Optical properties

Film opacity was evaluated according to the test procedure reported by Gontard et al.,[10] but using the ISO 2469:1994(E)[15] method. The films, aged for more than 9 months, were cut and placed in a spectrophotometer cell perpendicular to the beam direction. The absorbance spectra were assessed with a Varian Cary 1E UV–visible Spectrophotometer (Varian Scientific Instruments, UK) in the range of 400–800 nm. The opacity was defined as the area under the absorbance curve and expressed as the product of the absorbance value (A) and the wavelength (nm). Three measurements were made on each sample.

Infrared spectroscopy

Infrared (IR) spectra were obtained using a Perkin-Elmer Spectrum 2000 FTIR spectrometer (Perkin–Elmer, USA) equipped with a MKII Golden Gate™, single reflection ATR system with a diamond crystal, using the Spectrum Version 2.00 program from Graseby Specac, UK. Spectra were recorded for WG films; the surfaces of which were pressed against the ATR-crystal (16 scans at a resolution of 4 cm^{-1}).

Gas chromatography

The method has been fully described elsewhere,[5] and only a general overview description is therefore given here. WG films were mixed with methanol to elute glycerol, and the extract was mixed with *m*-erythritol, dissolved in methanol, as internal standard. After drying in nitrogen, the mixture was acetylated with acetic anhydride (with pyridine as catalyst), and the content of the acetylated glycerol was obtained using gas chromatography (Hewlett-Packard 5890 GC system with a DB5-MS column: J&W, Folsom, CA, USA; 60 m × 0.25 mm i.d.; 0.25-μm film thickness). The internal standard calibration between *m*-erythritol and glycerol is described by Olabarrieta et al.[5]

RESULTS AND DISCUSSION

The effects of varying thickness and pH on the optical properties

When the thin pH 4 films were drying, regions with a beige color appeared in the solution. These remained in the solid film, and they tended to be more intense with higher glycerol content [Figs. 1(a) and 2]. These beige regions were surrounded by transparent regions, which made the films appear almost colorless with good contact clarity. The transparent regions were thinner than the beige regions, and IR spectroscopy revealed that the beige regions were richer in glycerol, as indicated by the ratio of the glycerol 850 cm^{-1} peak to the WG amide II peak (centered at 1537 cm^{-1}). This method was appropriate because the presence of the plasticizer does not influence the secondary protein structure.[16] It was assumed that the amide II peak could always be used in the calculations regardless of the actual protein structure (glutenin/gliadin), and that any residual ethanol/acetic acid did not affect the results. Ethanol dissolves gliadin, and it is thus probable that the transparent regions were rich in gliadin, while the beige regions were rich in undissolved glutenin, as suggested by Gontard et al.[10] The glycerol content in the glutenin-rich regions was, on average, about 2.5 times higher than in the gliadin-rich regions, as exemplified in Figure 3.

If the film-forming solution was slightly agitated after casting, the beige regions were more evenly distributed over the final film resulting in fewer transparent regions. In the pH 11 films, the beige color covered the whole sample (at least for the 16 and 25 wt % films), and the optical appearance was

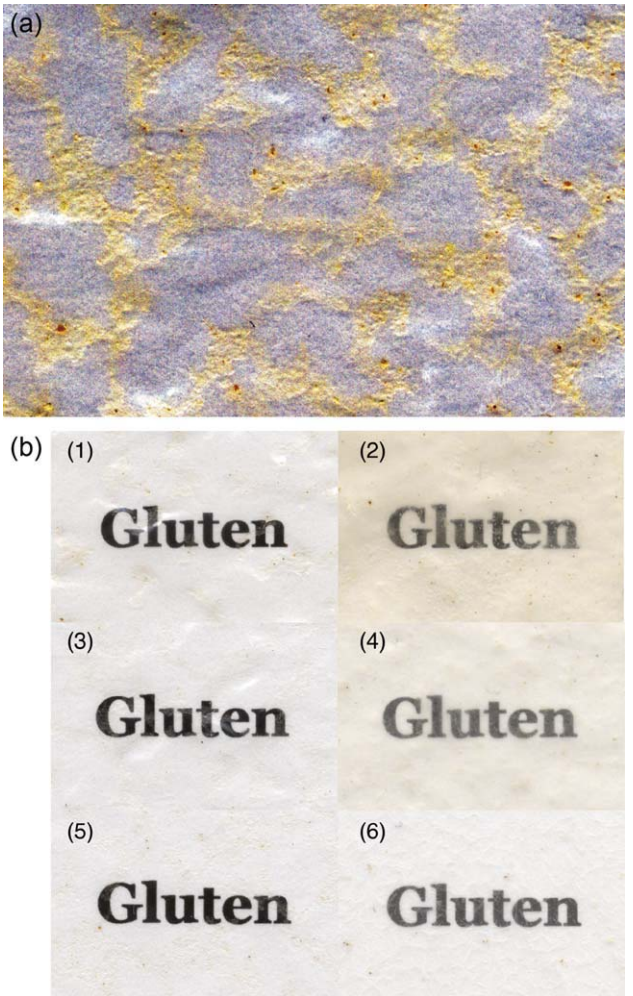

Figure 1 (a) Scanned images of the thin WG films placed on top of a white paper with the text "gluten": (1) A-25-t, (2) B-25-t, (3) A-16-t, (4) B-16-t, (5) A-08-t, and (6) B-08-t. The width of each picture is 21.6 mm. The films were of similar thicknesses except for B-25-t that was thicker (83 μm), and (b) shows a magnified computer-enhanced picture of the A-25-t film. Samples in Figures 1 and 2 were aged for >9 months. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

more uniform, indicating a more uniform distribution of glycerol, glutenin, and gliadin [Fig. 1(a)]. Interestingly, the pH 4 film two-phase structure, where the pigments were mostly in one phase, favors overall higher transparency [cf. pH 4 and 11 thin films in Fig. 1(a) and Table I]. The absorption values in Table I are higher or in the same range as those reported previously.[2,10–13] It is known that the degree of aggregation/polymerization is higher at pH 11 than at pH 4.[5] Apparently, the mechanisms occurring at pH 11 favor a more uniform distribution of glycerol, gliadin, and glutenin but also an enhanced general browning as discussed later.

As expected, the opacity was higher and more pH independent for the thicker samples, although the

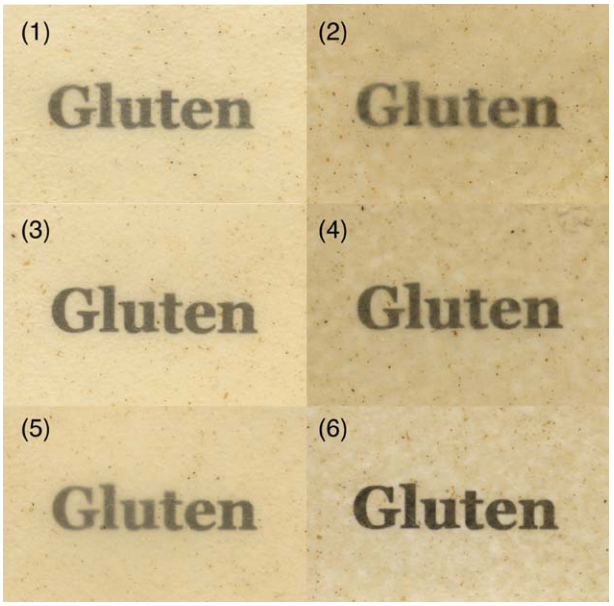

Figure 2 Scanned pictures of the thick WG films placed on top of a white paper with the text "gluten": (1) A-25-T, (2) B-25-T, (3) A-16-T, (4) B-16-T, (5) A-08-T, and (6) B-08-T. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

color was more beige/brown in the case of the pH 11 films [Fig. 1(b) and Table I]. Small brown/red particles were observed in all the films and in the thin pH 4 films especially, if not exclusively, in the beige regions [Fig. 1(b)]. These were probably due to a mixture of undissolved glutenins, starch, and/or shell products.

pH effect on the film protein structure

The shape of the intense amide I IR absorption region, presented in Figure 4, yields information about the secondary protein structure. Only qualitative analysis,

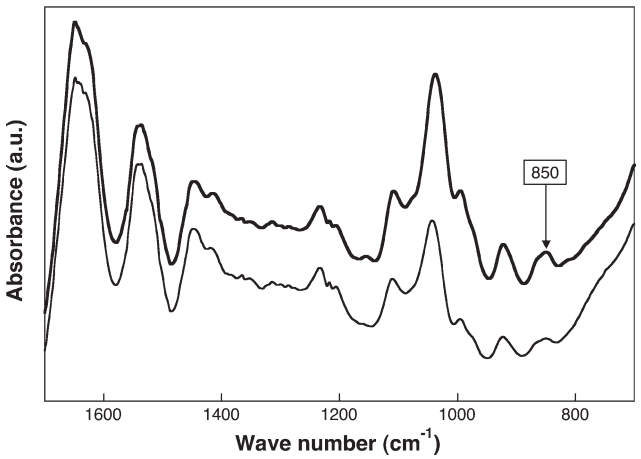

Figure 3 IR spectra of the pH 4 thin film (aged >9 months) in a beige region (bold line) and a transparent region (thin line).

TABLE I
Effect of pH, Glycerol Content, and Thickness on Film Opacity

Sample[a]	pH	Gluten[b] (g/100 g)	Glycerol[c] (wt %)	Ethanol[d] (g/100 g)	Thickness (μm)	Opacity (*A*·nm)	Ref.
A-25-T	4	13.3	25	57	268 (17)	488 (4)	
A-25-t	4	3.3	25	57	51 (4)	102 (7)	
A-16-T	4	13.3	16	57	239 (4)	495 (3)	
A-16-t	4	3.3	16	57	55 (9)	123 (2)	
A-08-T	4	13.3	8	57	233 (12)	479 (5)	
A-08-t	4	3.3	8	57	58 (5)	99 (6)	
B-25-T	11	13.3	25	57	252 (8)	491 (11)	
B-25-t	11	3.3	25	57	49 (6)	294 (21)	
B-16-T	11	13.3	16	57	219 (16)	515 (6)	
B-16-t	11	3.3	16	57	46 (6)	333 (4)	
B-08-T	11	13.3	8	57	232 (18)	453 (7)	
B-08-t	11	3.3	8	57	43 (6)	287 (21)	
	4	13	16.7	45	53	84 (7)	2
	4	2.5	16.7	45	50 (3)	88	10
	4	7.5	16.7	45	50 (3)	71	10
	4	12.5	16.7	45	50 (3)	94	10
	4	7.5	16.7	70	50 (3)	250	10
	5	10	16.7	57.5	50 (3)	139	10
	5	7.5[e]	10–25	65	52 (7)	35 (2)	12
	5	7.5[f]	10–40	65	52 (7)	79 (3)	12
	5	7.5[g]	10–40	44	52 (7)	102 (3)	12
	6.75	7.5[e]	10–25	65	55 (5)	32	13
	6.75	7.5[h]	10–25	65	55 (5)	37	13
	5	7.5[e]	10–25	65	40 (8)	34 (3)	11
	5	7.5[g]	10–25	44	40 (8)	101 (10)	11

The values in parentheses are standard deviations.
[a] The first letter indicates whether the films have been produced from an acidic (A, pH = 4) or a basic (B, pH = 11) solution; the number indicates the glycerol concentration (wt %), and the second letter indicates whether the film is thick (T) or thin (t).
[b] Wheat gluten concentration (g/100 g solution).
[c] Glycerol content based on the total weight of the dry film.
[d] Ethanol concentration (g/100 g solution).
[e] Gliadin-rich protein concentration (g/100 g solution).
[f] Gliadin-rich protein concentration (g/100 g solution), sample polymerized with cystein.
[g] Glutenin-rich protein concentration (g/100 g solution).
[h] Gliadin-rich protein concentration (g/100 g solution), sample crosslinked with formaldehyde.

based on a comparison of relative intensities, seemed meaningful here because of the many overlapping peaks.[17] These include the peaks centered at: ~ 1668 (β-turns), ~ 1650 (α-helices and random coils), ~ 1632 (intramolecular β-sheets), and ~ 1618 cm^{-1} (intermolecular β-sheets, implying a high level of hydrogen bonding).[16,18] Here, analysis was made on aged films (>9 months). The band at ~ 1650 cm^{-1} was prominent in the pH 4 film spectrum, whereas the region including the 1632 and 1618 cm^{-1} bands was pronounced in the pH 11 films. The pH 11 films seemed to have reached a greater aggregation, as indicated by a high content of β-sheets; the pH 4 films, on the other hand, showed more features of a less aggregated material (α-helices and random coils), in agreement with the results on thicker films reported by Olabarrieta et al.[5]

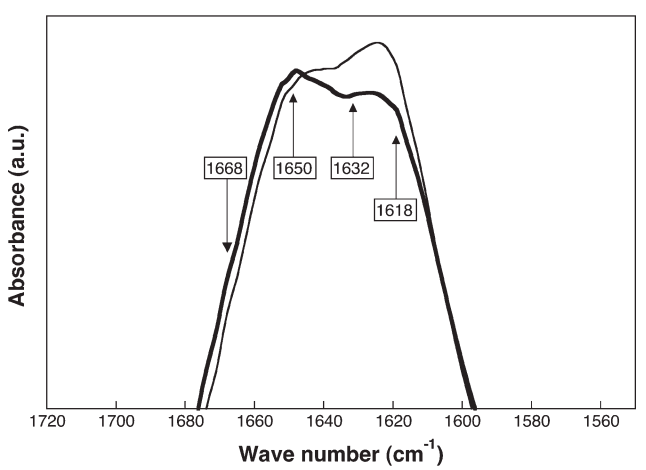

Figure 4 IR spectra of the aged (>9 months) thick pH 4 (bold line) and pH 11 (thin line) films in the amide I region.

pH and film browning

Apart from the naturally occurring pigments, nonenzymatic browning (Maillard) reactions between reducing sugar and amino acids, induced by heat

treatment and/or variations in pH, also determine the final film color. Several amino acids in WG are known to cause browning. Ashoor and Zent[19] determined the degree of browning of L-amino acids, naturally present in proteins, after they had been heat treated at 121°C for 10 min. Proline and glutamine, the most abundant nonpolar and polar amino acids in WG, belonged to the group having an intermediate browning capacity, whereas glutamic acid and the thiol-containing cysteine belonged to the low brown-producing group. Petriella et al.[20] showed that the browning reaction increased with increasing pH and increasing temperature in the reaction medium, and it was also observed that the browning rate was independent of water activity (a_W). More recently, Ajandouz and Puigserver[21] investigated the effect of pH, ranging from 4 to 12, on Maillard reaction kinetics. The reaction was much faster under alkaline conditions. Accordingly, the more beige/brown pH 11 films, observed also by Gennadios et al.,[22] were a result of more extensive Maillard reactions. Figure 5 and Table I show that the opacity was not significantly affected by the glycerol concentration, but that it was dependent on the pH of the cast solution for the thin films. In addition, its value was strongly influenced by the thickness. It should be noted that any effects on the optical properties of film surface roughness have not been explicitly considered. The results, on the thin films, were in agreement with those of Gontard et al.[10] who also reported that film opacity decreased with decreasing pH at an ethanol concentration greater than 35%. Note again that they did not investigate systems above pH 6.

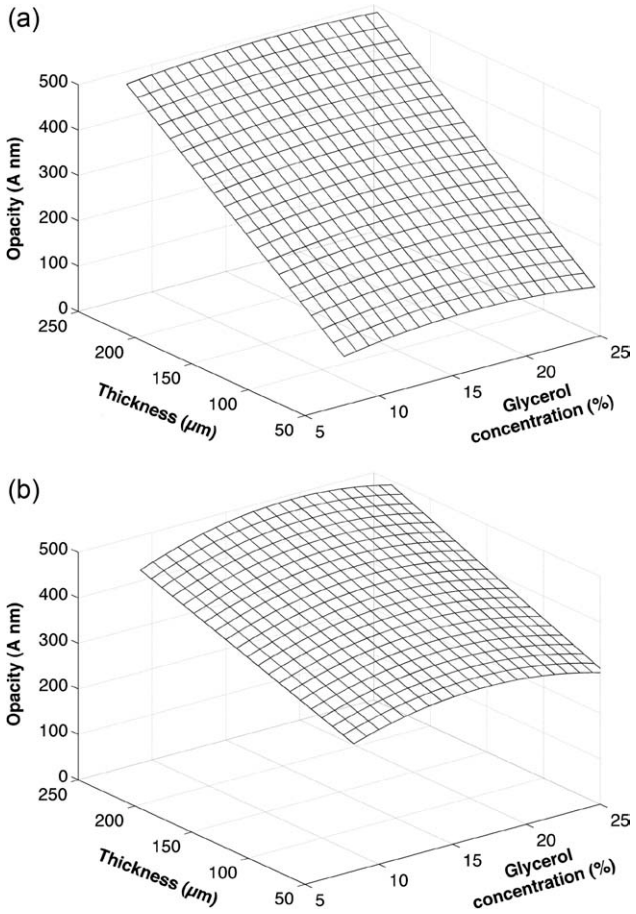

Figure 5 Opacity as a function of film thickness and glycerol concentration at pH 4 (a) and at pH 11 (b). Samples were aged for >9 months.

Film shape during aging (effects of thickness and pH)

The initially flat films lost contact to various extents with the blotting paper during the aging. The thin films became undulated and lost contact relatively rapidly (within a month), but the thick films with 8 wt % glycerol lost contact with the blotting paper already within a few hours although they were still relatively flat. As more glycerol migrated from the 16 and 25 wt % glycerol films than from the 8 wt % glycerol films, these experienced a greater degree of shrinkage and became more undulated. The thicker films, except for those with 8 wt % glycerol, became slightly curved with time, but good contact with the blotting paper was still maintained. For at least the first month, many films were in contact with the blotting paper along most of their surface. At longer times (>1 month) the enhanced curvature of the thick films resulted in a smaller contact area with the blotting paper. However, the thick films made at pH 11 were in good contact with the blotting paper at the last observation (after almost 9 months). At that stage, the corners of the films made at pH 4 were pointing up and away from the blotting paper, and contact was maintained only in the middle of the films. When an attempt was made to bend/flatten these films they broke. However, the films made at pH 11 were still very flexible. As glycerol tends to migrate through direct contact with a solid- or a liquid-absorbing environment, the degree of film–paper contact was obviously important for the amount and kinetics of migration.

Glycerol distribution during aging

Figure 6 shows how the glycerol concentration ratio between the upper and lower film surfaces (neglecting any influence of residual volatiles) changed with time. This ratio was obtained as the ratio of the 850 cm^{-1}/1537 cm^{-1} IR-peak ratio between the upper and lower sides. Almost all the films had a higher glycerol concentration at the lower side, and, in the case of the thin films, the difference increased with time. Olabarrieta et al.[5] also showed, but on thicker films, that the glycerol content was higher on the

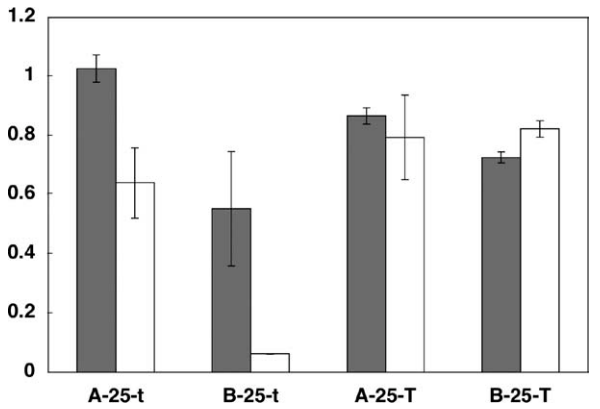

Figure 6 Glycerol concentration ratio between the upper and lower film surfaces. "Gray bar" and "white bar" refer to the pristine and the aged films, respectively (>9 months).

lower side. The glycerol seemed to migrate toward the lower side when the film was being formed, probably as a consequence of its low viscosity and the gravitational force. In the case of the thin films, the accumulation of glycerol on the lower side may have been a consequence of the undulation and poor contact with the blotting paper.

GC analysis

Before considering the mass-loss kinetics (gravimetry), it was important to understand what was actually migrating. Besides glycerol, moisture is present, and it is also possible that there is residual ethanol and/or acetic acid (pH 4).[5] Figure 7 illustrates a comparison between GC and gravimetric loss data. It was observed that the GC and gravimetric loss curves were insignificantly different for all the samples which had initially 16/25 wt % glycerol. Because of the low glycerol content, the 8 wt % data

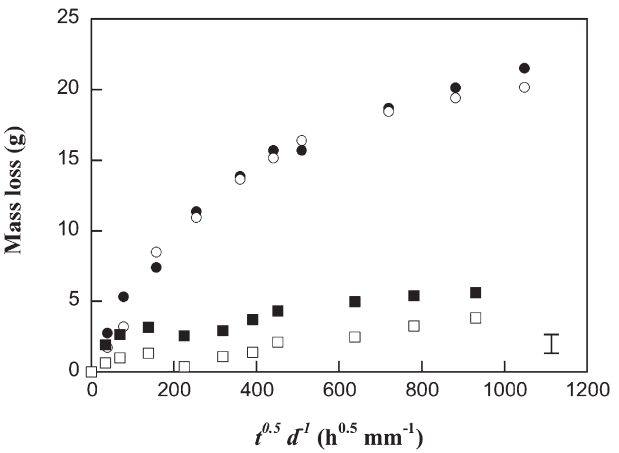

Figure 7 Glycerol mass loss as a function of time for A-25-t: (●) GC, (○) gravimetry and A-08-t: GC (■), gravimetry (□). The *x*-axis data are normalized with respect to the initial sample thickness.

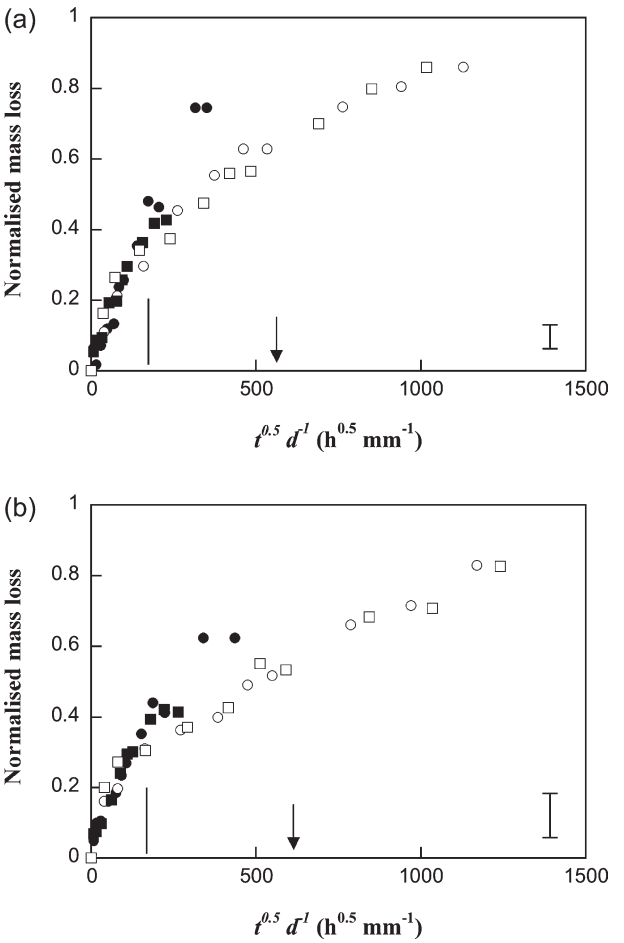

Figure 8 Normalized glycerol loss as a function of time for (a) pH 4: (●) A-25-T, (■) A-16-T, (○) A-25-t, and (□) A-16-t, and (b) pH 11: (●) B-25-T, (■) B-16-T, (○) B-25-t, and (□) B-16-t. The line and the arrow indicate, respectively, about 1 month for the thick and the thin films. The standard deviation was calculated for each data point in the figure and for clarity only the maximum of these is shown; this was also the case in Figures 8 and 9. In the absence of known initial thicknesses for the A-25-T and B-25-T samples measured at the last time (these were prepared in a later batch), they had to be estimated. The two high value A-25-T [Fig. 8(a)] and B-25-T [Fig. 8(b)] thickness data points were therefore calculated from eq. (1) (*vide infra*) using a d_i value from the previous round (left value) or a thickness measured directly at the actual time (not using eq. (1), right value).

were very uncertain (low values and bad contact with the paper) and were not considered in the evaluations. The GC/gravimetric data indicated that glycerol was the essential migrating species, but it is possible that volatiles (e.g., water) migrate with the glycerol. Nevertheless, we consider glycerol to be the migrating component in the loss kinetics calculations.

Mass-loss kinetics

Figures 8 and 9 show the loss of glycerol as a function of the square root of time, normalized with

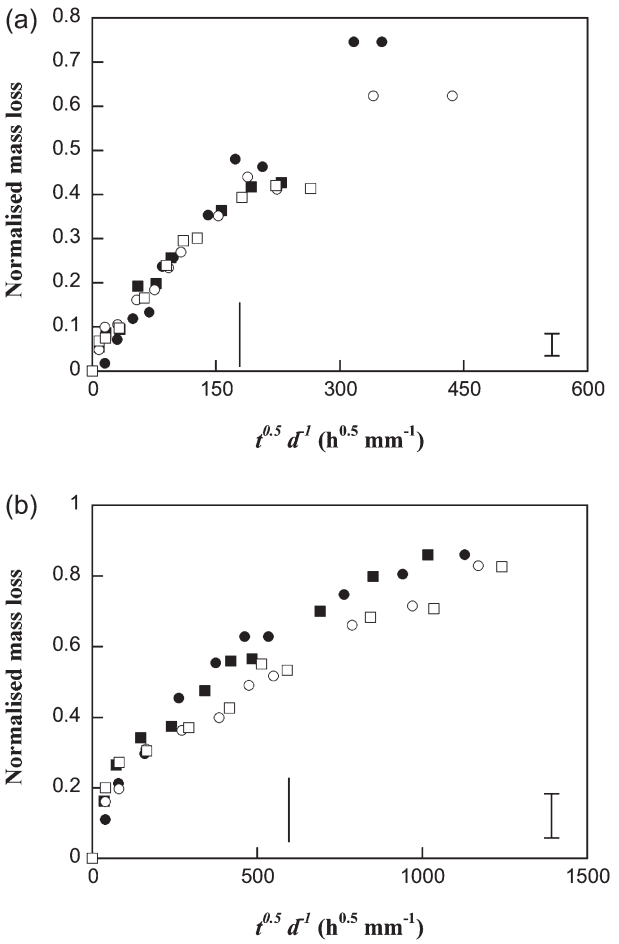

Figure 9 Normalized glycerol loss as a function of time for thick (a) (●) A-25-T, (■) A-16-T, (○) B-25-T, and (□) B-16-T, and thin (b) (●) A-25-t, (■) A-16-t, (○) B-25-t, and (□) B-16-t films. The line indicates about 1 month.

respect to film thickness. The thickness was not measured as a function of time, but it was expected to decrease as a consequence of the migration of glycerol. The actual thickness (d_a) was therefore used in the normalization, and the change was approximated roughly by considering an isotropic change in volume with time and assuming that the glycerol and glycerol-free material volumes were additive:

$$d_a = \left[1 + \frac{w_\infty(1 - w_n)}{\frac{\rho_2}{\rho_1} - w_\infty(1 + w_n) + w_\infty{}^2 w_n \frac{\rho_1}{\rho_2}}\right]^{-\frac{1}{3}} d_i, \quad (1)$$

where d_i and w_∞ are the thickness and glycerol weight fraction at the start of the desorption experiment, and w_n, ρ_1, and ρ_2 are the normalized glycerol weight fraction, glycerol density, and WG density, respectively. The densities were estimated by extrapolation of the measured glycerol/gluten film densities. The extrapolation of the pH 4 film densities yielded a glycerol value of 1.26 g cm^{-3}, which was the same as that given by the supplier (23°C). The extrapolation of the pH 11 film data yielded a different value (1.24 g cm^{-3}), and the former value was therefore used in the calculations. Although the densities in several cases were higher for the films made at pH 11 than for the films made at pH 4, the extrapolated value of the pH 4 film was used in all the calculations (ρ_2 = 1.30 g cm^{-3}). Although this estimation involves a number of approximations, it is a better description of the system than using a constant time-independent thickness.

Figure 8(a,b) shows that, considering the scatter in data, the loss rate was independent of the initial glycerol concentration in the case of the 16 and 25 wt % films. The films with 8 wt % glycerol showed, at longer times, the slowest loss, probably because of their poorer contact with the blotting paper (illustrated with A-08-t in Fig. 7). Because of the large scatter, probably because of the low absolute glycerol content, these data are not discussed further, and the data discussions below refer, unless otherwise stated, to the samples with the higher concentrations of glycerol. It should be mentioned that the first three "short-term" values (excluding the origin), the last point of the thick 25 wt % films and the remaining data, were obtained from three different batches. In several cases, the initial three glycerol loss values fell outside (above) the general trend. On the other hand, the last thick 25 wt % data point fitted in each case (pH 4 and 11) relatively well into a possible common desorption curve together with the rest of the (shorter term) data [see e.g., Fig. 8(a,b)]. The last data point for the 25 wt % thin films, obtained as in the case of the thick 25 wt % films from a separate batch, was not shown because of the large uncertainty in the actual thicknesses; nevertheless, they were not unrealistic when considering an extension toward longer times of the mass-loss (desorption) curves in Figure 8(a,b). An explanation of the deviation between the short-term and long-term thin-film loss values (long-term defined basically as when the difference between the thin film and the thick film data became noticeable) could be the poor contact between the film and the blotting paper at longer times. It is important to note that, apart from the scatter in mass loss data (error bars in the figures), there is an additional uncertainty in the x-axis data values due to the scatter in thicknesses used (cf. the standard deviation in Table I). A poor film-to-paper transfer rate due to improper contact should have the highest impact on thin films. Figure 8(a) indicates that the choice of pH had little effect on the thick-film glycerol loss rate at short/intermediate times. However, at the longest time [thick films with 25 wt % glycerol, Fig. 9(a)] and considering also the later thin-film desorption data [Fig. 9(b)], the loss rate seemed, despite the scatter in data, to be faster for the pH 4 films. This is in accordance with what was

observed earlier,[5] and it is suggested that it is due to a lower degree of aggregation and consequently a more open protein structure at low pH.

CONCLUSIONS

This study is unique in that it has systematically compared the optical and mass-loss kinetics over a broader-than-ever pH interval, three different glycerol loadings, and the effects of two very different thicknesses. The most interesting findings were as follows:

- the thin films with the phase-separated pH 4 structure yielded better contact clarity than the corresponding more homogeneous pH 11 analogs.
- the greater protein aggregation at pH 11 seemed to favor a lower degree of phase separation, but it also led to inferior optical properties (a homogeneous beige/brown film without transparent regions).
- glycerol was more readily observed in the glutenin-rich phase, suggesting, in addition to the gliadin/glutenin phase separation in the ethanol/water solution, that it played a role in the formation of the pH 4 film structure. However, the overall measured opacity was not significantly altered by the change in glycerol content.
- the GC method appeared to be a valuable tool for determining the glycerol-specific migration kinetics. Comparisons between GC and gravimetric data suggested that the latter essentially measured glycerol migration.
- the degree of contact between the film and the paper was of great importance for the extent and rate of migration. Overall, the loss of glycerol was extensive and indicated that care must be taken when laminating a glycerol-plasticized polymer to an absorbing material such as paper.

Martin Svensson at Svenska Lantmännen AB is acknowledged for valuable discussions. Bo Johansson, Reppe AB, Sweden, is thanked for supplying wheat gluten powder. Jiebing Li is acknowledged for technical support regarding the GC measurements.

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