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Composition Factors Affecting the Water Vapor Permeability and Tensile Properties of Hydrophilic Zein Films

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Transparent unplasticized zein films were prepared in aqueous ethanol or acetone. Tensile properties indicated that films prepared in acetone were stronger but less flexible than those prepared in ethanol. Both types of films, however, were too brittle for most applications. Films containing a glycerol:poly(propylene glycol) ratio of 1:3 exhibited elongation values almost fifty times greater than glycerol-plasticized films. Incorporation of cross-linking agents into zein films resulted in approximately a 2–3-fold increase in tensile strength values. Water vapor barrier properties were best for unplasticized zein films cross-linked with 20% polymeric dialdehyde starch. Incorporation of plasticizer into zein films resulted in an almost doubling in water vapor permeability values.

Keywords: *Hydrophilic; film; zein; tensile properties; plasticizer; cross-linking agents; water vapor permeability*

INTRODUCTION

Agriculturally derived alternatives to the polyolefin packaging materials currently used by the food industry provide an opportunity to strengthen the agricultural economy and reduce importation of petroleum and petroleum products. Recently, considerable research has been reported on the preparation and evaluation of biopolymer films derived from renewable resources to replace petroleum-based packaging material. Many of these studies have reported on mechanical and barrier properties of corn zein (Park and Chinnan, 1990; Aydt et al., 1991; Gennadios et al., 1993, 1994). Zein, a prolamine, is soluble in a variety of organic solvents and forms characteristically hard and glossy films and coatings. Yamada et al. (1995) have shown that the solvent in which the film is prepared, can affect its water permeability. Zein films are generally brittle and require the addition of plasticizer. Park et al. (1994) found that glycerol-plasticized films were very brittle and their elongation improved significantly when poly(ethylene glycol) was used as the plasticizer. Cross-linking agents have been used to improve the tensile strength and water-resistance properties of zein films (Yamada et al., 1995) and zein–starch molded plastics (Lim and Jane, 1993; Spence et al., 1995). Cross-linked formaldehyde-free zein fiber, with good breaking tenacity and elongation, has been prepared with polycarboxylic acids (Yang et al., 1996).

In this study, film blends of various plasticizer and cross-linking agents with zein, prepared in aqueous ethanol or acetone, were screened and evaluated for tensile and water vapor barrier properties. The objective of this research was to increase film elongation and strength without significantly compromising water vapor barrier properties.

MATERIALS AND METHODS

Materials. Corn zein (F-4000) protein was obtained from Freeman Industries (Tuckahoe, NY). Glycerol, 95+% (GLY), poly(ethylene glycol), average MW ca. 400 (PEG), and 1,2,3,4-butanetetracarboxylic acid, 99% (BTCA) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI), and poly(propylene glycol), MW 400 (PPG), was from polysciences, Inc. (Warrington, PA). Epichlorohydrin, formaldehyde (37% solution), gluteraldehyde (Grade I, 50% solution), and polymeric dialdehyde starch (PDS) were obtained from Sigma Chemical Co. (St Louis, MO), and citric acid (CA), monohydrate, was from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Film Formation. A total of 1 g of corn zein and plasticizer, in various proportions, was dissolved in 10 mL of 80% ethanol or 70% acetone, to yield a 10% (w/w) mixture. The mixture was heated with stirring at 60 °C for 10 min. Cross-linked films were prepared by adding the cross-linking agent to the mixture which was then heated at 70 °C for 30 min in 20 mL autoclave bottles. Zein proteins were cross-linked with either 3% formaldehyde, glutaraldehyde, and epichlorohydrin or 6% of either CA or BTCA based on zein content. Corn zein protein films were also cross-linked with 5, 10, 15, and 20% PDS. Ethanolic solutions were cast in polystyrene Petri dishes, Thomas Scientific (Swedesboro, NJ), and acetone solutions in Teflon Petri dishes, Norton Performance Plastics Corp. (Akron, OH), 100 mm in diameter. The solutions were dried in a vacuum oven, adjusted to 10 inches mercury, at 40 or 60 °C for acetone or ethanol respectively. The dried films were stored in a desiccator at 52% relative humidity (RH) until testing.

Film Thickness Measurements. A micrometer (Tumico, St. James, MN) was used to measure film thickness. Measured thicknesses are the mean values of 10 random measurements.

Water Vapor Permeability (WVP) Determination. The apparatus and methodology are described in ASTM E96-80 (ASTM, 1980) "Water Method", as modified by McHugh et al. (1993). Four replicates of each film type were tested at 30 ± 2 °C. Air velocities were approximately 150 m/min across the films. Cast films were sealed on Plexiglas cups containing 9 mL of distilled water. There was an air gap of 0.6 cm between the water and the underside of the film. Test cups were placed in a desiccator cabinet maintained at 0% RH with calcium sulfate. Cups were weighed a minimum of five times at intervals no less than 3 h apart. The water vapor transmission rate was calculated from a linear regression of the slope of weight loss vs time. Water vapor transmission rate (WVTR) was calculated by dividing the slope by the test cell mouth area. Permeance [WVTR/($p_2 - p_3$)] was calculated at 30 °C as describe by McHugh et al. (1993), where p_2 and p_3 are the corrected partial pressure at the inner surface of the film and the film outer surface, respectively. Water vapor permeability

was calculated as the product of the permeance and average thickness of the film.

Tensile Property Measurements. Tensile properties were determined using an Instron model 1122 tensile tester with a 2000 g load cell. Specimens were 5.0 mm wide, and were in the range 0.08–0.2 mm thick. Exact thicknesses were measured using a micrometer. A gauge length of 25 mm and an extension rate of 5 mm/min were used. Five replicates were run for each material. Samples were conditioned overnight in a desiccator held at 52% RH by means of a saturated solution of sodium hydrogen sulfate in a dish in the bottom of the desiccator. Individual specimens were stored in the desiccator until just prior to being put in the Instron jaws. The jaw clamps were operated using 45 psi air pressure. Data were collected and analyzed for average standard deviation using the DOS-based Series IX, Version 6, Instron software.

RESULTS AND DISCUSSION

Three quantitative parameters were obtained from stress–strain measurements: tensile strength (TS), elongation to break (ETB), and initial modulus. The film's strength and flexibility are described by TS and ETB values. Initial modulus is a direct measurement of the stiffness of the film and is of critical importance in applications where the degree of resistance to stretching is an important factor. Transparent unplasticized zein films were prepared in aqueous ethanol and acetone. Films prepared in acetone were stronger but less flexible than those prepared in ethanol as indicated by their mechanical properties shown in Table 1. In addition films from acetone tended to be less uniform in appearance than those from ethanol. Regardless of the solvent used to prepare these films they were too brittle for most applications and required addition of plasticizer to improve their flexibility.

Table 1. Mechanical Properties of Unplasticized Zein Films[a]

solvent (aqueous)	TS (MPa)	CV	ETB (%)	CV	modulus (MPa)	CV
80% ethanol	10.9b	9.0	3.4a	24.0	551b	12.0
70% acetone	14.4a	10.4	1.5b	17.4	1273a	4.5

[a] Means in the same column with no letter in common are significant at $p < 0.05$ using the Bonferroni LSD multiple-comparison method.

Table 2. Effect of Plasticizer on Mechanical Properties of Zein Films[a]

plasticizer (30%)	TS (MPa)	CV	ETB (%)	CV	modulus (MPa)	CV
control[a]	10.9a	9.0	3.4c	24.0	551a	12.0
GLY[b]	7.0bc	9.3	2.6c	13.9	498a	7.3
PEG[b]	5.7c	31.3	44.4b	57.2	199b	41.0
PPG[b]	9.4ab	39.4	2.8c	42.9	608a	18.4
GLY:PPG[b,c]	5.1c	17.2	117.8a	34.0	135b	21.4
GLY:PEG[b,c]	4.9c	10.4	26.6c	39.4	180b	14.5

[a] Means in the same column with no letter in common are significant at $p < 0.05$ using the Bonferroni LSD multiple-comparison method. [b] Films were prepared in 80% aqueous ethanol. [c] The ratio of plasticizers GLY:PPG or GLY:PEG was 1:3.

Effect of Plasticizer. The effect of different plasticizers or combinations of plasticizers on the mechanical properties of zein films is shown in Table 2. Up to 30% glycerol could be incorporated into zein films with no significant decrease in TS, however flexibility was not improved over unplasticized films as indicated by their ETB values. Films prepared with PEG were more

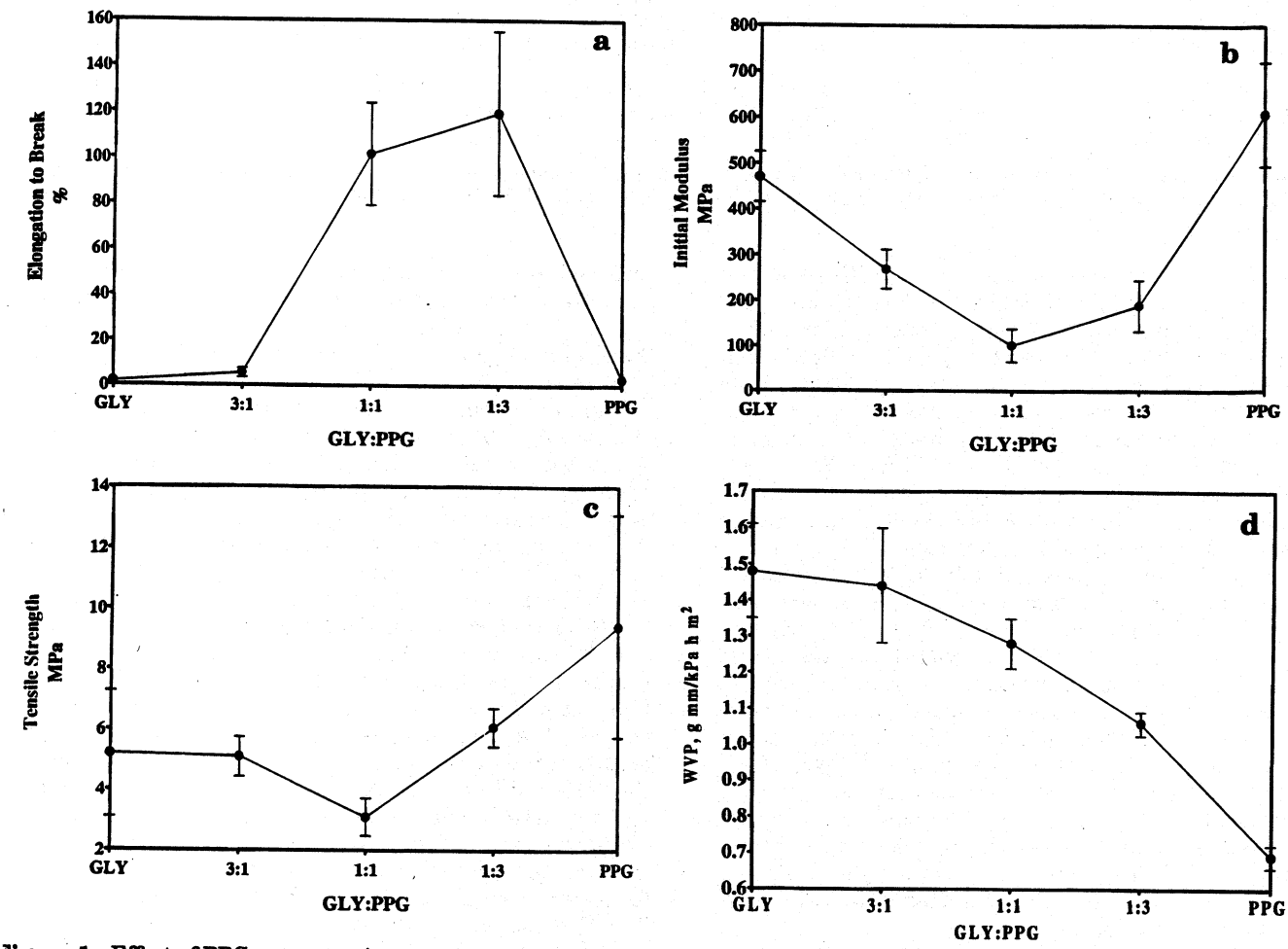

Figure 1. Effect of PPG concentration on zein film tensile and water vapor barrier properties: (a) elongation to break (ETB); (b) initial modulus; (c) tensile strength (TS); (d) water vapor permeability (WVP).

Table 3. Effect of Cross-Linking Agent on Zein Film Tensile Properties[a]

cross-linking agent	TS (MPa)	CV	ETB (%)	CV	modulus (MPa)	CV
control[b,c]	10.9c	9.0	3.4ab	24.0	551b	12.0
formaldehyde[c]	28.9a	9.0	2.4b	8.3	1230a	8.9
glutaraldehyde[c]	21.4ab	3.3	4.1a	34.1	1085a	3.9
epichlorohydrin[c]	19.5b	32.8	2.1b	14.3	1230a	8.9
CA[c]	16.1bc	24.2	2.8ab	14.3	633bc	19.3
BTCA[c]	19.1b	22.0	3.4ab	21.4	780b	18.1
control[b,d]	14.4a	10.4	1.5b	17.4	1273a	4.5
formaldehyde[d]	4.6c	21.7	2.2ab	22.7	428d	30.4
glutaraldehyde[d]	7.6b	18.4	2.6a	23.1	668bc	21.0
epicholorhydrin[d]	4.0c	5.0	1.7ab	17.7	453cd	14.8
CA[d]	too brittle to test					
BTCA[d]	10.2b	21.6	2.2ab	27.3	770b	21.2

[a] For each type of preparation, mean values with no letter in common are significant different at $p < 0.05$ by Bonferroni LSD multiple-comparison method. [b] Films not cross-linked. [c] Films were prepared in 80% aqueous ethanol. [d] Films were prepared in 70% aqueous acetone.

Table 4. Effect of Plasticizer on Film Tensile Properties of Cross-Linked Zein Films[a]

plasticizer	TS (MPa)	CV	ETB (%)	CV	modulus (MPa)	CV
control[b]						
15% GLY	5.2a	12.6	4.4b	11.4	499a	15.2
15% PEG	4.3a	32.6	3.4b	44.1	323b	25.4
30% GLY: PPG[c]	5.1a	17.2	117.8a	34.0	135c	21.4
formaldehyde						
15% GLY	11.8a	8.5	5.7b	11.0	482a	33.0
15% PEG	11.2a	13.4	3.0b	6.7	500a	17.4
30% GLY:PPG[c]	5.2b	9.6	118.0a	10.1	112b	11.6
glutaraldehyde						
15% GLY	19.8a	4.5	3.0b	17.0	412a	15.5
15% PEG	10.4b	19.2	2.8b	7.1	397a	19.1
30% GLY:PPG[c]	1.4c	7.1	224.0a	13.8	25b	44.0
epichlorohydrin						
15% GLY	16.6a	13.6	2.9b	10.4	498b	11.7
15% PEG	18.5a	11.9	2.2b	9.1	884a	11.7
30% GLY:PPG[c]	1.6b	12.5	297.0a	6.1	26c	23.1
CA						
15% GLY	2.5b	44.0	1.8b	72.2	246a	57.3
15% PEG	8.0a	31.3	6.3b	57.1	305a	15.1
30% GLY:PPG[c]	0.4b	25.0	128.0a	16.4	10b	30.0
BTCA						
15% GLY	8.2a	30.5	2.9b	34.5	528a	8.1
15% PEG	5.6a	21.4	5.6b	26.8	327b	31.8
30% GLY:PPG[c]	0.8b	22.0	67.1a	34.6	21c	57.1

[a] Films prepared in 80% aqueous ethanol. For each cross-linking agent, mean values in the same column with no letter in common are significantly ($p < 0.05$) different by the Bonferroni LSD multiple-comparison method. [b] Films not cross-linked. [c] The ratio of plasticizers GLY:PPG was 1:3.

flexible than glycerol plasticized films, with little significant difference in TS (Table 2). Park et al. (1994) reported that zein films containing only glycerol were very brittle (ETB of 4%) which improved to 94% when the ratio of mL of PEG/g of protein was 0.39. They observed that glycerol tends to migrate to the surface of new corn zein films within a few hours after preparation, resulting in a loss of film flexibility (Park et al., 1992). This could be attributed to the hydrophobic nature of the zein film matrix; however, films prepared with the more hydrophobic plasticizer, PPG, were also brittle with poor elongation (Table 2). Significant improvement in zein film flexibility, however, was achieved with blends of GLY and PPG. Films containing a GLY: PPG ratio of 1:3 exhibited ETB values almost fifty times greater than GLY-plasticized films (Table 2). A similar increase in elongation was not observed when PEG replaced PPG in the plasticizer blend. It appears that there is a synergy between GLY and PPG in the film, through certain secondary forces, which can be attributed to the PPG methyl side chain. This effect greatly reduces the intermolecular forces between protein polymers thus facilitating extensibility of the film. There was no detectable separation of plasticizers in the film.

As can be seen in Figure 1 the most significant change in tensile properties for films containing 30% plasticizer occurred with changes in plasticizer composition. For example, decreasing the ratio of GLY:PPG in zein films from 3:1 to 1:1 and 1:3 increased ETB values from less than 10% to approximately 100% and 120%, respectively (Figure 1a). This change in plasticizer composition resulted in a decrease in initial modulus values from approximately 300 MPa to 100 and 200 MPa with correspondingly small changes in TS values (Figure 1b,c). In addition, WVP values, and the corresponding variation between zein films with the same plasticizer composition, decreased with increasing PPG concentration (Figure 1d). This variation could be attributed to preferential separation of GLY over PPG from the film.

Effect of Cross-Linking Agents. Film flexibility and TS was also improved with the addition of cross-linking agents. Yang et al. (1996) suggested that zein fibers are brittle and exhibit poor breaking tenacity because they are prepared in alcohol solutions which do not permit the zein proteins to unfold. As a result the bulky side groups prevent the polypeptides from getting close to each other and to align in an ordered structure or in an effective orientation within the film matrix. Incorporation of cross-linking agents into zein films prepared in ethanol resulted in approximately a 2–3-fold increase in TS values (Table 3). These films tended to be stiff with significantly higher modulus values than the control and those cross-linked with

Table 5. Zein–Dialdehyde Starch Cross-Linked Films[a]

dialdehyde starch (%)	TS (MPa)	CV	ETB (%)	CV	modulus (MPa)	CV
0	10.9a	9.0	3.4a	24.0	551c	12.0
5	15.8a	31.6	1.9b	52.6	1074ab	12.4
10	17.5a	17.7	1.7b	11.8	1145ab	8.7
15	13.6a	48.5	1.8b	11.1	919b	9.7
20	17.1a	18.7	1.8b	18.8	1213a	12.2
20[b]	13.5a	29.6	2.6ab	11.5	569c	18.5

[a] Films prepared in 80% aqueous ethanol. Means in the same column with no letter in common are significant at $p < 0.05$ using the Bonferroni LSD multiple-comparison method. [b] Films contained 15% GLY.

Table 6. Water Vapor Permeability of Zein Films[a]

film type	solvent	plasticizer	WVP (g mm/kPa h m^2)
zein	ethanol	none	0.620c ± 0.036
zein	acetone	none	0.577c ± 0.009
zein	ethanol	GLY:PPG (30%)[b]	1.060ab ± 0.032
zein	acetone	GLY (15%)	1.010b ± 0.108
zein/PDS (20%)	ethanol	none	0.533c ± 0.031
zein/PDS (20%)	ethanol	GLY:PPG (30%)[b]	1.15a ± 0.100

[a] Values with no letters in common are significantly different $p < 0.05$ using the Bonferroni LSD multiple-comparison method. [b] The ratio of mixed plasticizers was 1:3.

either CA or BTCA. Formaldehyde cross-linked films had the highest TS values, however since formaldehyde is considered toxic, cross-linking agents, such as CA and BTCA, were attractive alternatives. Films prepared using these cross-linking agents, exhibited TS values that were greater than the control and modulus values that were significantly lower than the other cross-linked films (Table 3). Cross-linked films prepared in 70% acetone had TS values lower than the control and were too brittle to test when cross-linked with citric acid. Modulus values for zein films cross-linked in acetone were generally lower than those prepared in ethanol.

Since cross-linked films prepared in acetone had TS values less than the control, the effect of plasticizer on film tensile properties was investigated only for films prepared in ethanol. Plasticizer concentrations in zein films were limited to 15% for GLY or PEG and 30% for GLY/PPG since higher concentrations imparted a greasy appearance and feel to the films. ETB values for the control and cross-linked zein films were significantly greater for films containing 30% GLY/PPG in a 1:3 ratio compared to 15% of either GLY or PEG (Table 4). Except for the control, the TS values for films prepared with a blend of plasticizer were always lower than those containing the other plasticizers tested.

Zein proteins were also cross-linked with polymeric dialdehyde starch (PDS). Clear zein films cross-linked with up to 20% PDS were prepared and had higher TS (though not statistically significant) and modulus values compared to films containing no oxidized starch (Table 5). Zein–PDS films containing 15% GLY had slightly lower TS values, and modulus values were approximately half that of the cross-linked unplasticized zein films. Addition of more plasticizer resulted in opaque films which were not uniform.

Water Vapor Barrier Properties. Water vapor permeability was compared for cross-linked and non-cross-linked zein films in the presence and absence of plasticizer (Table 6). Zein films containing no plasticizers had the lowest WVP values. Of these, films cross-linked with 20% PDS exhibited the best water barrier properties. Although not statistically significant, films prepared in acetone had lower WVP values than those prepared in ethanol. Incorporation of plasticizer into zein films significantly reduced water vapor barrier properties resulting in an almost doubling in WVP values. It is worth mentioning that zein films prepared in acetone containing 15% GLY and zein–PDS films containing 30% GLY/PPG were rough and not homogeneous. This lack of homogeneity is probably demonstrated by their high coefficients of variation values which were 10.7% and 8.7%, respectively.

Future studies will be directed toward improving tensile and water vapor barrier properties of zein films crosslinked with oxidized starch. The effect of concentration and mixtures of conventional and novel plasticizers on these properties will be investigated.

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