Effect of Pulsed Light Treatment on the Functional Properties of Casein Films

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Abstract. In this study, the effect of Pulsed Light (PL) treatment as a cross-linking method on the properties of casein films was evaluated. Casein films were prepared from micellar casein concentrate, with 5 g/100g glycerol added as a plasticizer. The UV-curable resins PEG (200) diacrylate and PEG (400) diacrylate were added as photo-initiators. Films were prepared by casting and equilibrated under a range of relative humidity (%RH), then treated with Pulsed Light (PL), with up to 15 pulses of light on each side, at a fluence of 1.57 J/cm² per pulse. The microstructure, mechanical and water barrier properties, water sorption and hydrophobicity of the films were evaluated. PL treatment of casein films with up to 12 pulses increased the films' smoothness and homogeneity, while higher levels of treatment caused structural defects. Mechanical strength and elongation of some of the films, particularly those with added PEG (400) diacrylate, were significantly increased by PL, but no improvement of their water vapor permeability was observed. The incorporation of PEG (200) diacrylate resulted in highly porous film microstructures, and interesting photo-patterning effects observed at high PL doses. These results indicate the potential of PL for creating protein films with unique characteristics and functionality.

Keywords: casein films; Pulsed Light; PEG; cross-linking; photo-polymerization

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1. Introduction

Biopolymer films are often considered as an alternative to plastic films. Protein films in particular have attracted significant attention of researchers and industry in recent years. Biopolymers in general and protein films in particular have great potential for use as food packaging, as well as edible films and coatings (Peterson and others, 1999; Wihodo and Moraru, 2013). Edible films can for instance be placed within a dual texture food, such as pizza, to prevent moisture migration and maintain the texture of each of the layers (Kester and Fennema, 1989). A significant drawback of protein films is that they have a higher water vapor permeability compared to most synthetic polymers (Krochta, 1992; McHugh and Krochta, 1994). Furthermore, protein films are generally weaker and have lower elongation than synthetic films (Hernandez-Izquierdo and Krochta, 2008). Various methods to enhance the physical properties of protein films have been suggested (Wihodo and Moraru, 2013).

Milk proteins, including casein and whey proteins are considered exceptional candidates for biodegradable films (McHugh and Krochta, 1994). Many studies reported on methods to improve the functional properties of films made from casein and casein derivatives, including: pH alteration (Avena-Bustillos and Krochta, 1993), lipid incorporation (Chick and Hernandez, 2002; Sohail et al., 2006; Fabra et al., 2009) and combination with polysaccharides (Fabra et al., 2008, Pereda et al., 2008). A newer method used to improve the functional properties of protein films is photo-induced polymerization, which uses light to initiate and propagate a polymerization reaction to form a cross-linked polymer structure (Baroli, 2006). For photo-polymerization to occur, the backbone of the monomer used needs a photo-polymerizable residue at one or both ends of the molecule (Baroli, 2006). The addition of photoinitiators is generally necessary to initiate the polymerization reaction. The first step in a photo-initiated

polymerization is initiation, which begins when an initiator molecule decomposes into free radicals in the presence of monomers. The instability of carbon-carbon double bonds in the monomer makes them susceptible to reaction with the unpaired electrons in the radical, which "grabs" one of the electrons from the double bond in the monomer. This leaves an unpaired electron at the end of the monomer chain, which thus becomes a free radical (Ifkovits and Burdick, 2007). The next stage is propagation, when the electron transfer and motion of the free radical down the chain take place. The process ends with the termination reaction, which occurs when the growing polymer chain joins with another free radical (Ifkovits and Burdick, 2007).

Photo-polymerization has typically been achieved by continuous UV (Genadios et al., 1998; Rhim et al., 1999; Vaz et al., 2003). Rhim et al. (1999) reported that an UV dose of 51.8 J/m² increased the tensile strength of sodium caseinate films by 2.5%, of egg albumin films by 70.6%, of corn zein film by 20%, and of wheat gluten films by 66.7 %. In another report, the same UV dose (51.8 J/m²) increased the tensile strength of SPI films by 41.9% (Gennadios et al., 1998).

Pulsed Light (PL) technology is another type of light treatment, consisting of intense and short duration pulses of broad spectrum light, in the wavelength range 200 - 1,100 nm, produced using gas discharge lamps (Dunn et al., 1991). The use of PL for microbial inactivation has been reported quite extensively (Uesugi et al., 2007; Woodling and Moraru, 2007; Uesugi and Moraru, 2009; Krishnamurthy et al., 2010; Cheigh et al., 2012), but the potential of this technology for photo-polymerization applications has not yet been explored.

Research suggests that an efficient photo-polymerization reaction occurs when the absorption spectrum of the treated material matches the emission spectrum of the light source (Scherzer et al., 2005) and that the rate of the initiation reaction is proportional to the intensity of

the light source (Ifkovits and Burdick, 2007). The broad spectrum of light and the high light intensity suggest that PL has a great potential to initiate efficient photo-polymerization. PL also offers the advantage of much shorter treatment duration as compared to continuous UV. The objective of this work was to explore the effectiveness of PL as a photo-polymerization technique for improving the structural and mechanical properties of casein films.

2. Materials and Methods

2.1. Film Preparation

Micellar casein (MCC) powder was obtained from the American Casein Company (Burlington, NJ). Protein dispersions of 10 g/100mL concentration were prepared by dispersing MCC powder in ultrafiltered water at 22 ± 2 °C. The protein dispersion was first stirred under moderate agitation on a magnetic stirring plate (Thermix® Stirring Hot Plate, Fisher Scientific, Pittsburgh, PA) for 20 min, followed by intense agitation (21,500 RPM) for 5 min using an UltraTurrax mixer (IKA Works Inc., Wilmington, NC). To ensure complete hydration, the mixture was then heated in glass bottles at 40 °C for 15 min in a water bath, then cooled to 20 °C. After heating, 5 g/100g glycerol (Mallinckrodt Baker, Inc., Phillipsburgh, NJ) was added to the protein mixture as a plasticizer. To further enhance the functional properties of the protein films, 5 g/100g UV-curable resins were added to some of the samples. The resins used in this study were polyethylene glycol (PEG) (200) diacrylate (DA) and PEG (400) DA (Sartomer Company, Inc., Exton, PA), both of which have GRAS status (21 CFR 175). An evaluation of light absorption by casein and the resins was carried out, to ensure that all components of the films absorb light in the UV range. Casein absorbs light from 200 nm to 1100 nm, which coincides

with the spectral range of the PL source, while PEG-200 and PEG-400 show strong absorption peaks between 200-300 nm (Fig. 1).

The protein dispersion with added glycerol and resins was poured onto polystyrene square Petri plates (Fisher Scientific, Pittsburgh, PA). A volume of 10 ml per plate was used, to maintain a constant thickness of the final films. The plates were placed on a leveled bench top and allowed to air dry under a laminar hood for 48 h at 22 ± 2 °C and 35% relative humidity (%RH). The dried films were then peeled off from the plates, then cut in rectangular strips of 1 cm x 3 cm. Film thickness was measured using a hand held caliper (Traceable Digital Caliper, Fisher Scientific, Pittsburgh, PA.), at 3 random positions of the film.

2.2. Pulsed Light Treatment

PL treatments were performed using a RS-3000C SteriPulse System (Xenon Corporation, Woburn, MA). Each film strip was centered individually on an adjustable stainless steel shelf in the PL unit, at 3.2 cm beneath the Xenon lamp, and treated with 0 (control), 9, 12, and 15 pulses of light on each side (the films were treated on one side, then flipped and treated on the other side). The PL dose (fluence) was measured using a pyroelectric head with a Nova II display (Ophir Optronics Inc., Wilminton MA) and expressed in J/cm^2 . Fluence measurements were performed in triplicate. The measured fluence at the sample treatment location was $1.57 \text{ J}/\text{cm}^2$ per pulse.

2.3. Film Conditioning

After PL treatment, the film strips were stored in glass desiccators over supersaturated solutions of LiCl, MgCl_2 , $\text{Mg}(\text{NO}_3)_2$, NaCl and KCl, which resulted in different %RH values in

the headspace. Water was used to create a headspace of %RH close to 100%. Films were allowed to equilibrate at 22 ± 2 °C for 3-4 days. After conditioning, the water activity (a_w) of the films was measured at 25 °C using an AquaLab Series 3 hygrometer (Decagon Devices, Inc., Pullman, WA).

2.4. Evaluation of Film Microstructure

The microstructural features of the films were observed using scanning electron microscopy (SEM). Film specimens were first dehydrated and then sputter-coated with Au using a Denton Vacuum Desk II Cold Sputter Etch Unit (Denton Vacuum, Moorestown, NJ). The sputter-coated samples were viewed with a Leica Stereoscan 440 SEM (Leica Cambridge Ltd., Cambridge, England), at a voltage of 10 kV and a maximum magnification of 1500X, at the Cornell Center for Materials Research (Ithaca, NY).

2.5. Mechanical Properties of the Films

Mechanical properties were measured using a TA-XT2 texture analyzer (Stable Micro Systems, Texture Technologies Corp., Scarsdale, NY). The experiments were performed at approximately 40% RH and 22 ± 2 °C. Each specimen was tested immediately after being taken out of the desiccator, to minimize dehydration.

Film tensile strength and % elongation at break were evaluated according to ASTM Standard D 882-02 (ASTM 2003). The initial grip separation was 30 mm, and the test crosshead speed was 0.1 mm/s. A minimum of 8 samples were measured for each film type and two replications were conducted. Tensile strength (MPa) was calculated as the peak force (N) divided

by initial cross-sectional area (m^2). Film elongation was reported in percent of length increase divided by the original film length.

The study was replicated twice. Due to slight differences in % RH between the two replicates, the results of each replicate were reported separately.

2.6. Water Vapor Permeability (WVP)

Glass jars with metal lids and metal rings lined with neoprene-band seal to ensure air tightness (Ball Corporation, Muncie, IN) were used to measure water vapor transmission rate (WVTR) of the films. The dimension of a jar without the lid was 7.37 cm x 7.37 cm x 5.84 cm (2.9 in x 2.9 in x 2.3 in). To mount the film strip, a rectangular window of 1 cm x 3 cm was cut out from the center of the metal lid and the film specimen was taped to the lid. The shape and size of the film strips were chosen to match the profile of the PL lamp and ensure uniformity of the PL treatment.

Each glass jar was filled with deionized water to expose the underside of the film to 100% RH. The whole assembly was placed in an acrylonitrile-styrene desiccator cabinet (Fisher Scientific, Pittsburgh, PA). The temperature in the desiccator cabinets was maintained at 22 ± 2 °C by placing them in a temperature controlled chamber. Values of % RH of 11%, 32%, 53%, 75%, and 84% were achieved using supersaturated solutions of LiCl, MgCl_2 , $\text{Mg}(\text{NO}_3)_2$, NaCl, and KCl, respectively. Equilibrium was reached within 3 h, at which time the assembly was weighed again. Four more data points were collected at 3 h intervals, over a 12 h period.

To avoid the existence of a stagnant air layer above the film (Gennadios et al., 1994), small hand held fans were placed inside the desiccator cabinets to provide forced convection conditions and constant air flow. The % RH and temperature in the desiccators were monitored

with a digital thermometer-hygrometer (Fisher Scientific, Pittsburgh, PA). The height of the stagnant air gaps inside the jars were measured before and after the experiments using a handheld caliper (Fisher Scientific, Pittsburgh, PA). The mean of initial and final stagnant air gap height was used for WVP calculation. The stagnant air gap height of all samples was always less than 14 mm to ensure accurate WVP determination (McHugh et al., 1993).

Water vapor permeability (WVP) was calculated according to the method of Gennadios et al. (1994). First, a linear regression analysis of weight loss as a function of time was performed to ensure that steady state slopes were achieved. The regression coefficients (R^2) were greater than 0.970 for all samples. WVTR was calculated as the slope divided by the area of the film exposed in the test jar (3 cm²):

$$WVTR = \frac{Slope}{Area} \quad (1)$$

To account for the diffusion of water vapors through the stagnant air layer inside the test jar, the corrected partial pressure of water vapor at the underside of the film (p_2) was calculated as:

$$WVTR = \frac{P * D * L_n \frac{P - p_2}{P - p_1}}{R * T * \Delta z} \quad (\text{kg/m}^2\text{s}) \quad (2)$$

The corrected water vapor partial pressure at the inner surface of the film was used to calculate water vapor permeance:

$$Permeance = \frac{WVTR}{p_2 - p_3} \quad (\text{kg/m}^2\text{sPa}) \quad (3)$$

The water vapor permeability (WVP) was finally determined by multiplying the corrected permeance and film thickness:

$$WVP = Permeance * Film Thickness \quad (g \text{ m/m}^2\text{sPa}) \quad (4)$$

where,

P = Total pressure (1.013×10^5 Pa at 25 °C)

D = Diffusivity of water through air (2.5×10^{-5} m²/s at 25 °C)

R = Gas law constant (8.306×10^6 Pa-cm³/g-mol-K)

T = Absolute temperature (298.15 K)

Δz = Mean stagnant air gap height, $(z \text{ original} - z \text{ final})/2$ (mm)

p_1 = Water vapor partial pressure at solution surface inside the test jar, at 25 °C
(3.168×10^3 Pa)

p_2 = Corrected water vapor partial pressure at the underside of the film (Pa)

p_3 = Water vapor partial pressure at film outer surface inside the cabinet (Pa)

The location of the water vapor pressure values used in Equation 2 and Equation 3 is illustrated schematically in Fig. 2. Three samples of each film type were measured in each replicate and the study was replicated twice.

2.7. Moisture Sorption Isotherms

Water activity (a_w) of the films was measured as described above. To determine the moisture content, the equilibrated films were quickly transferred to a 55-mm diameter aluminum dish (Fisher Scientific, Pittsburgh, PA) and weighed using an analytical balance (Denver Instrument, Denver, CO). The samples were then dried in an Isotemp Standard Lab Oven (Fisher Scientific, Pittsburgh, PA) at 103 ± 2 °C for 24 h, re-weighed and their moisture content calculated based on the moisture loss during drying. Three replicate measurements were

conducted. Moisture sorption isotherms were built by plotting the measured moisture content of the films vs. a_w .

The GAB (Guggenheim–Anderson–de Boer) model was used to fit the sorption isotherm data. Monolayer values for moisture were calculated according to Iglesias and Chirife (1982):

$$M = \frac{m_0 C_G k a_w}{(1 - k a_w)(1 - k a_w + C_G k a_w)} \quad (5)$$

where M is the equilibrium moisture content at a water activity a_w , m_0 is the monolayer value, C_G is the Guggenheim constant, and k is the constant correcting properties of the multilayer molecules relative to the bulk liquid.

The GAB constants were determined by re-writing Equation 5 as Equation 6, followed by plotting a_w/M as a function of a_w .

$$\frac{a_w}{M} = \frac{k}{m_0} \left(\frac{1}{C_G} - 1 \right) \cdot a_w^2 + \frac{1}{m_0} \left(1 - \frac{2}{C_G} \right) \cdot a_w + \frac{1}{k \cdot m_0 \cdot C_G} \quad (6)$$

The constants of the second degree polynomial obtained by fitting the data were then used to calculate C_G and k .

2.8. Water Contact Angle Measurements

The water contact angles of the films were measured using a Tantec CAM-Plus goniometer (Schaumburg, IL), by delivering a droplet of deionized water to the surface of the film and viewing it through the eyepiece of the goniometer (Goddard and Hotchkiss, 2007). A minimum of 10 measurements were performed for each film type. The average angle values were used as a measure of surface hydrophobicity.

2.9. Statistical Analysis

Two-way ANOVA tests with an interaction effect were performed on the values of tensile strength, % elongation, WVP and water contact angles to determine any significant differences in the properties of casein films due to PL treatment and relative humidity conditions. Any differences between means were compared based on the Tukey-Kramer HSD test, with significance defined at $p \leq 0.05$. All statistical analyses were performed using JMP version 7.0 (SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1. Microstructural Features of the Pulsed Light Treated Casein Films

Electron micrographs of the casein films revealed a visible increase in surface smoothness and homogeneity after PL treatment with up to 12 pulses (18.8 J/cm^2) per side, as shown in Fig. 3 (top row). At 15 pulses (23.5 J/cm^2), PL treatment caused some cracking on the film surface. Irradiating proteins in solid state could lead to cross-linking or molecular degradation, depending on the nature of the protein and irradiation dosage (Rhim et al., 1999). The presence of the fine cracks in the films treated with high doses of PL in this study suggests that the 15 pulses treatment may be in the range where light-induced degradation of the casein matrix starts to occur. This is very important to note, since such structural defects will affect the films' mechanical and barrier properties.

The incorporation of PEG (400) DA in casein resulted in films that closely resembled the appearance of casein films, with occasional micrometer sized circular pores in their structure. The addition of PEG (200) DA to casein resulted in a “sponge-like” structure of the films, with fairly large pores, in the range of tens of μm (Fig. 3, bottom row). PL did not cause noticeable

changes to the microstructure of casein films with PEG (400) DA. For the casein films with PEG (200) DA, PL with 15 pulses drastically reduced the size of the surface pores. The observed ‘closing’ of the pores bears a resemblance with the UV patterning reported by Yu et al. (2008) and Choi et al. (2008), and is an indication of cross-linking within the film’s matrix due to PL.

3.2. Effect of Resin Addition and PL on Surface Hydrophobicity of Casein Films

Wetting by water is always an issue of concern for any packaging material, and particularly for food packaging. The water contact angles (WCA) for the films produced in this work are shown in Fig. 4. The casein films and films with PEG (200) DA had WCA values of about 50°, which indicates a moderately hydrophilic character. For the films with PEG (400) DA, an average WCA of 11.5° was obtained, which indicates a fully wetting behavior. PL treatment induced some changes in WCA of the films. WCA of casein films with PEG (400) DA increased significantly after PL with 15 pulses as compared to the untreated films ($p \leq 0.05$). WCA of films treated with 15 pulses were significantly lower compared to the other treatment levels. This increase in the wetting characteristics of the films after PL treatment is consistent with previous reports about the effect of UV on the wetting properties of materials (Zubkov et al., 2005). Overall, the high wettability of the casein films with added resins suggests that they may not be suitable for applications where contact with water is a concern.

3.3. Effect of PL on Water Sorption Properties of Casein Films

For all types of films generated in this study, J-shaped isotherms were obtained (Fig. 5), which is typical for most protein-based matrices (Jangchud and Chinnan, 1999; Cho and Rhee, 2002; Foster et al., 2005). PL treatment did not have any significant effect on the moisture

sorption properties of the casein films. For the casein films with added resins, a slight decrease in moisture sorption capacity was observed for the PL treated films at high water activity levels.

The moisture isotherms were fitted using the GAB model, and the constants C_G , m_0 and k were calculated (Table 1). The high R^2 values confirmed that this model was a good fit for the experimental data. The k values obtained for all the casein films are in the range observed for other food materials (Quirijns et al., 2005). Negative values were obtained for C_G , which is a physical impossibility. This has been attributed previously to insufficient data in the low a_w region ($a_w < 0.11$) (Quirijns et al., 2005), which was also the case in this study. Nonetheless, Quirijns et al., (2005) demonstrated that sorption isotherms are not sensitive to C_G when based on experimental data obtained at $a_w > 0.11$. The m_0 values obtained for all film types were much higher than the values generally obtained for food systems. This has been reported before for foods containing plasticizers such as glycerol and sorbitol (Sherwin and Labuza, 2005). Therefore, the GAB parameters shown in Table 1 can be used to accurately describe the sorption isotherms in the a_w range from 0.2 to 0.95, but no further inferences can be made about the effect of treatment conditions on the GAB parameters.

3.4. Effect of PL on the Mechanical Properties of Casein Films

The mechanical properties of the films obtained in this work were comparable to those of other UV treated protein matrices, at similar a_w values (Rhim et al., 1999; Gennadios et al., 1998). Overall, the tensile strength (TS) of the casein films decreased significantly ($p < 0.05$) as a_w increased. For all film types, TS decreased exponentially with a_w (Fig. 6), which is a common behavior for many food biopolymers. The numerical parameters of the exponential dependency are shown in Table 2. These changes in mechanical strength with a_w were expected, since the

presence of water molecules reduces the hydrogen bonding between the protein chains and increases molecular spacing, which decreases mechanical strength (Cho and Rhee, 2002; Hernandez-Izquierdo and Krochta, 2008). The resins had a plasticizing effect on the casein matrix, as observed from the significantly lower TS values of the films with incorporated resins compared to the casein film. The addition of the plasticizers changed the mechanical resistance behavior of the films from brittle (high TS, low elongation) to ductile (lower TS, higher elongation). This effect was particularly noticeable at $a_w < 0.5$.

The film % elongation (or ductility) was also significantly affected by a_w ($p < 0.05$), although the effect of a_w did depend on the type of film. The % elongation – a_w correlation followed an exponential relationship, although this was not statistically significant in most cases (low R^2 values – not shown). As seen in Fig. 7, the % elongation of the casein films increased with a_w , while most of the films with incorporated resins became less elastic with increasing a_w . This behavior is probably due to the fact that plasticized films were very weak, which caused them to break easily as their a_w increased. For the films not treated with PL, the elasticity of the plasticized films, particularly those with PEG (200) DA, of $a_w < 0.8$ was much higher (about 120% elongation at $a_w \sim 0.15$) than for the casein films (about 20% elongation at $a_w \sim 0.15$). At $a_w > 0.8$, all films had comparable levels of elasticity (50-60% elongation) (Fig. 7).

For the casein films without resins, PL did not cause a statistically significant change in tensile strength. The exception were the films treated with 15 pulses, for which the mechanical strength decreased, due to the presence of cracks in their structure (Fig. 3C). PL-treated casein films had significantly higher % elongation as compared to the non-treated films ($p < 0.05$). This suggests some degree of cross-linking and the formation of a slightly more ductile network after

PL treatment, since the formation of covalent bonds will allow the network to deform more before reaching the breaking point.

The effect of PL was more noticeable for the films with incorporated resins. For the films with PEG (400) DA, a significant increase in tensile strength after PL was observed in both replicates ($p < 0.05$) (Fig. 6, Table 2). However, the effect of PL on the % elongation of these films was only statistically significant in Replicate 1 ($p < 0.05$). For the films with PEG (200) DA, the increase in tensile strength after PL was statistically significant only in Replicate 2 ($p < 0.05$), while the % elongation decreased significantly after PL in both replicates ($p < 0.05$). These effects may be explained by the highly porous microstructure for the casein films with PEG (200) DA (Fig. 3). Overall, despite the increase in mechanical strength of some of the PL treated films, the tensile strength and elongation of all casein films produced were still lower compared to those of synthetic films at equivalent humidity level (Gennadios et al., 1994).

Another observation that needs to be made is that crosslinking may in fact hinder the biodegradability of the casein films. For example, Rimdusit et al. (2008) reported that in six weeks, the carbon dioxide emission from cross-linked methyl cellulose (MC) films was reduced around 80% compared to pure MC. For casein films, both cross-linked and not cross-linked, there is no information on the extent of biodegradability and this would have to be determined in future work.

3.5. Effect of PL on Water Vapor Barrier Property of Casein Films

The water vapor permeability (WVP) values for all films are shown in Fig. 8. These WVP values do not account for the direction of the water vapor diffusion process.

PL treatment significantly changed the WVP of casein films ($p < 0.05$). PL-treated casein films had larger WVP values than untreated films, particularly at high % RH gradients. PL treatment did not significantly change WVP of casein films with PEG (200) DA or PEG (400) DA. These results are rather contradictory when compared to the changes in mechanical properties reported above, and may be due to the increase affinity for water molecules of the PL treated films.

The WVP values for the films with incorporated resins were generally higher than for the casein films. For the highest % RH gradient (88.41%), the WVP of the casein films with added resins were one to two orders of magnitude larger than for the casein films (Fig. 8). The increase in WVP as a result of adding plasticizers is known, since plasticizers reduce intermolecular forces along protein chains and increase the polymer free volume (Sothornvit and Krochta, 2000). The high WVP values observed at high % RH gradient values for the casein films with PEG (200) DA can also be explained by their porous structure. While for applications where moisture transport needs to be prevented this is a disadvantage, such films may be useful in situations where some degree of moisture transport is necessary, i.e. packaging of fresh fruits and vegetables, where partial permeability to water vapors of the packaging material is required to allow for respiration.

4. Conclusions

PL treatment seemed to induce some cross-linking in casein films, but excessive PL doses also caused light-induced degradation and structural defects. The surface topography of casein films also became smoother after PL treatment. Mechanical strength and elongation of casein films with added PEG (400) DA, a UV-curable resin, were increased by PL treatment,

although no improvement of their water vapor permeability was observed. The incorporation of PEG (200) DA resulted in highly porous film microstructures, which may be suitable for specific applications that require water vapor transport, such as packaging of fresh produce. Overall, PL has some potential for inducing changes in the functional and structural properties of casein films, although the properties in the films obtained in this study were still inferior to synthetic polymeric films.

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475

TABLES

Table 1. GAB model constants and goodness of the fit (R^2) for all film types at different PL treatment levels

Film type	PL Treatment	C_G^a	k^a	m_0^b	R^2
Casein	0 pulses (Control)	-79.45	0.91	0.18	0.86
	9 pulses (14.1 J/cm ²)	-41.44	0.93	0.17	0.91
	12 pulses (18.8 J/cm ²)	-23.28	0.93	0.16	0.91
	15 pulses (23.5 J/cm ²)	-17.17	0.95	0.14	0.97
Casein + PEG (200) DA	0 pulses (Control)	-17.16	0.88	0.26	0.90
	9 pulses (14.1 J/cm ²)	-28.22	0.88	0.24	0.91
	12 pulses (18.8 J/cm ²)	-27.52	0.88	0.25	0.94
	15 pulses (23.5 J/cm ²)	-25.92	0.86	0.24	0.91
Casein + PEG (400) DA	0 pulses (Control)	-18.61	0.92	0.19	0.91
	9 pulses (14.1 J/cm ²)	-17.71	0.90	0.18	0.94
	12 pulses (18.8 J/cm ²)	-14.57	0.92	0.17	0.86
	15 pulses (23.5 J/cm ²)	-12.58	0.91	0.16	0.86

^a GAB constants

^b Monolayer moisture content (kg/kg d.b.)

482 **Table 2.** Parameters of the Tensile strength (TS) – water activity (a_w) exponential relationship
 483 ($TS = ae^{ba_w}$) and goodness of the fit (R^2) for all film types

Film type	Casein			Casein + PEG(200)DA			Casein + PEG(400)DA		
Parameters Treatment	a	b	R^2	a	b	R^2	a	b	R^2
0 (Control)	25.14	-4.55	0.85	11.28	-3.37	0.89	5.49	-2.58	0.90
9 pulses	22.65	-4.36	0.91	8.18	-2.73	0.94	7.70	-2.67	0.94
12 pulses	25.14	-4.39	0.92	9.26	-3.11	0.92	7.81	-2.59	0.93
15 pulses	19.35	-3.84	0.88	9.08	-2.93	0.92	8.19	-2.66	0.86

FIGURES

Figure 1. Light absorption spectra for casein dispersion and the liquid photoinitiators (PEG-200 and PEG-400)

Figure 2. Schematic diagram of the water vapor permeability measurement rig, indicating the locations of water vapor pressure and the air gap (headspace) height

Figure 3. SEM images (1500 X) of casein films (A, B, C) and casein films with PEG (200) diacrylate (D, E, F) before and after PL treatment: [A, D] – 0 pulses; [B, E] – 12 pulses; and [C, F] – 15 pulses. Photos for films treated with 9 pulses of light look similar to those treated with 12 pulses and are not shown.

498

499 **Figure 4.** Water contact angle of casein films, casein films with PEG (200) DA, and casein
500 films with PEG (400) DA, after different PL treatments: untreated (□); treated with 9 pulses (■);
501 treated with 12 pulses (■); and treated with 15 pulses (■). Asterisks indicate significant
502 differences within a composition group, at $\alpha = 0.05$.

Figure 5. Effect of PL treatment on the moisture sorption isotherms of the three types of films (casein, casein with PEG (200) DA and with PEG (400) DA, after PL treatment. The symbols indicate measured values: 0 pulses ($\square$); 9 pulses ($\blacktriangle$); 12 pulses ($\bullet$); and 15 pulses ($\blacklozenge$); and the lines GAB predictions: 0 pulses (—); 9 pulses ($\cdots$); 12 pulses (--); and 15 pulses ($\text{-}\cdot\cdot\cdot$)

Figure 6. Tensile strength of casein films after PL treatment with 0, 9, 12, and 15 pulses and conditioning at different % RH conditions (combined data from Replicate 1 and Replicate 2). Data points represent average values and error bars indicate 1 standard deviation for measurements within a replicate. The symbols indicate measured values: 0 pulses ($\diamond$); 9 pulses ($\bullet$); 12 pulses ($\blacktriangle$); and 15 pulses ($\blacksquare$); and the lines indicate regression lines: 0 pulses (—); 9 pulses (— —); 12 pulses (·····); and 15 pulses (— — —).

Figure 7. % Elongation of casein films after PL treatment with 0, 9, 12, and 15 pulses and conditioning at different % RH conditions (combined data from Replicate 1 and Replicate 2). Data points represent average values and error bars indicate 1 standard deviation for measurements within a replicate. The symbols indicate measured values: 0 pulses ($\diamond$); 9 pulses ($\bullet$); 12 pulses ($\blacktriangle$); and 15 pulses ($\blacksquare$); and the lines indicate exponential trendlines: 0 pulses (—); 9 pulses (---); 12 pulses (.....); and 15 pulses (— — —).

Figure 8. Water vapor permeability of casein films (□), casein with PEG (200) DA films (■) and casein with PEG (400) DA films (■) after PL treatment with 0, 9, 12, and 15 pulses and conditioning at different % RH (combined data from Replicate 1 and Replicate 2).