

Influence of Gallic Acid and Tannic Acid on the Mechanical and Barrier Properties of Wheat Gluten Films

Anna-Sophie Hager, Katleen J. R. Vallons, and Elke K. Arendt*

School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

ABSTRACT: Vital wheat gluten, a byproduct of wheat starch production, is a highly functional ingredient having a unique viscoelasticity that makes it ideal for the production of edible biodegradable films. However, its functional properties must be modified to ensure sufficient strength and elasticity, in addition to water vapor barrier properties. In this study, vital gluten was modified using tannic and gallic acid. It was found that the addition of tannic acid resulted in stiffer, more resistant, and less resilient and flexible films, having as well decreased water vapor permeability. Tannic acid containing films became reddish brown, whereas gallic acid addition did not have an influence on the film appearance. Films containing gallic acid became more elastic. Gallic acid was found to potentially act like a plasticizer. Scanning electron microscopy was used to investigate the ultrastructure of the produced films.

KEYWORDS: *edible biodegradable films, cross-linking, tannins, water vapor permeability, plasticizer*

■ INTRODUCTION

Due to environmental concerns, research on renewable and biodegradable polymers and materials from agricultural resources has considerably increased over the past decade. Unlike petrochemical-based systems, biodegradable polymers can be broken down in the environment. A polymer is referred to as biodegradable when a degradation process carried out by microorganisms and/or enzymes fully reduces the material to natural products such as carbon dioxide, water, methane, or biomass.^{1,2}

Films are defined as stand-alone thin layers of materials that can be used as covers, wraps, or separation layers in food packaging or as direct edible coatings for foods. In addition, films are frequently used as testing structures for the determination of barrier, mechanical, solubility, and other properties provided by certain raw materials.³ The suitability of biopolymers to form acceptable films is determined by solvent characteristics, as well as the film-forming technologies employed.⁴ Besides starch and cellulose, which have been extensively studied, proteins also exhibit great potential for films and coatings.⁵ They can be applied as moisture, gas, or solute barriers in heterogeneous foods or between food and its environment to extend shelf life and improve overall food quality.⁶ The formation and properties of films based on proteins and polysaccharides have been comprehensively studied and reviewed by Gontard et al., Krochta et al., and Cuq et al.^{6–8}

Wheat gluten proteins show a high potential as raw material for technical applications due to their unique intrinsic properties, with numerous publications referring to their outstanding film-forming potential.⁹ Gluten is made up of two main fractions, gliadins (monomeric proteins) and glutenins (larger polypeptide chains linked with disulfide bonds forming a macropolymer). It is a byproduct of the bioethanol fuel and wheat starch industries and is commercially available with constant quality and at relatively low cost.¹⁰ This contributes to gluten's suitability as a raw material for food

application processes, for which the producers and consumers demand a standardized high quality. Due to the unique viscoelasticity of hydrated gluten, the compound is well suited for film formation. This was demonstrated in several studies resulting in transparent, flexible, colorless, and odorless films.^{11–20}

Although research and development of edible and biodegradable coatings and films has intensified in recent years, only a few are used commercially in packaging systems because of limitations in their performance compared to synthetic plastic packaging. With regard to wheat gluten films, the very high water sensitivity and permeability represent significant hurdles to commercial exploitation.⁹ Chemical, physical, enzymatic, and plasticization modifications are currently being investigated to improve the mechanical performance and barrier properties of protein biopolymers.²¹ Cross-linking is an accepted and very powerful method to improve water resistance, cohesion, rigidity, mechanical strength, and barrier properties of films to water.⁵ Cross-linking is the formation of intermolecular bonds among the chains of a polymer. The present study evaluates the potential of gallic and tannic acids to cross-link gluten and improve mechanical and barrier properties of the resultant films. These are food-grade ingredients and suitable candidates for utilization in biodegradable materials due to their natural presence in many plants and wide availability at relatively low prices. In addition, these phenolic compounds are recognized for their antioxidant capacity. Tannic acid, a gallic ester of D-glucose, is a hydrolyzable tannin with high molecular weight due to its multiple phenolic groups. The molecular weight of gallic acid is significantly lower as this compound consists of a single phenolic ring. The interactions between tannins and proteins have been well studied, especially in

Received: March 8, 2012

Revised: May 28, 2012

Accepted: May 30, 2012

Published: May 30, 2012

relation to haze formation in beverages such as beer, wine, or fruit juices. It has been shown that phenolic compounds such as tannic acid form complexes with proline-rich proteins such as gliadin from gluten.^{22,23} The binding of protein to tannin is believed to be primarily due to the formation of multiple hydrogen bonds between the hydroxyl group of the tannin and the carboxyl group of the proteins.²⁴ Tannin–protein complexes may be also stabilized by hydrophobic interactions between the aromatic ring of tannins and hydrophobic regions of proteins. Orliac et al.²⁵ found that plant tannins, which are phenolic compounds produced as secondary plant metabolites, can be used as agents to improve the mechanical properties of sunflower protein films. Zhang et al.²⁶ used tannic acid as a natural phenolic cross-linker, modifying gelatin and improving its mechanical performance. Rivero et al.²⁷ evaluated the cross-linking capacity of tannic acid in plasticized chitosan films, and Emmambux et al.²¹ investigated the property modification of kafirin films with hydrolyzable and condensed tannins.

This work assesses the effects of tannic and gallic acids on mechanical and barrier properties of wheat gluten films by comparing film thickness, tensile strength, and visual appearance of the films, as well as swelling, solubility, and water vapor permeability. The microstructure of the films produced was investigated using scanning electron microscopy.

MATERIALS AND METHODS

Materials. Tannic and gallic acids and wheat gluten were sourced from Sigma-Aldrich, Munich, Germany (403040, G7384, and G5004, respectively). Glycerol was obtained from Merck (Darmstadt, Germany), acetic acid glacial from BDH Laboratory Supplies (Poole, U.K.), and 100% ethanol from Carbon Group (Ringaskiddy, Ireland). Distilled water was prepared in a Calypso ultrapure water distillery from Fistream International Ltd. (Loughborough, U.K.).

Gluten Characterization. The protein content of the gluten powder was determined using a Leco FP-528 (Leco, St. Joseph, MI, USA). Moisture and fat contents were determined using a CEM SMART System 5 (CEM, Matthews, NC, USA). AACC Method 08-01 was used for the determination of total ash content.

Film Formation. Films were cast according to the method of Emmambux et al.²¹ by dissolving wheat gluten powder ($2.2 \text{ g} \pm 0.1 \text{ mg}$) in 13.5 mL of 100% ethanol and subsequently adding $900 \pm 0.1 \text{ mg}$ glycerol. Tannic and gallic acids (5, 10, 20, and 30% and 1, 2, 5, and 10%, respectively), were dissolved in ethanol prior to the addition of gluten. The pH was adjusted to 4 with glacial acetic acid, and the volume was made up to 30 mL with distilled water. Prior to casting, air was removed from the dispersion by heating at 70°C for 10 min and continuous stirring. Aliquots of 7 g were poured into plastic Petri dishes of 9 cm diameter and gently swirled to evenly cover the bottom. They were placed on a leveled surface in a ventilated oven at 40°C overnight to allow solvent evaporation. Films were stored in the Petri dishes in a proofer at 22°C and 60% relative humidity for at least 24 h before analyses.

Mechanical Properties. Film thickness was measured using a hand-held micrometer Z169048 (Sigma-Aldrich, Arklow, Ireland) and was expressed in micrometers per gram of film-forming solution as an average of 15 measuring points taken randomly for each of three independently produced films. The stress–strain properties of the films were determined at room temperature according to the method of the American Society for Testing and Materials; ASTM D882-97. Films were conditioned at 60% relative humidity and $25 \pm 2^\circ\text{C}$ for at least 24 h prior to measurement. Film strips of 60 mm length and 6 mm width were cut with a scalpel. These samples were then mounted between tensile grips, with the clamped length of the specimen being 30 mm, and subjected to tension with a crosshead speed of 5 mm/s. Both force and elongation were recorded. Tensile properties were determined from two individually cast films with three subsamples tested from each film replicate (i.e., six measurements).

Tannin Binding, Film Swelling, and Solubility. To determine how much of the added tannic or gallic acid was bound to the film, $50 \pm 0.1 \text{ mg}$ of each film was weighed into a microtube and extracted with 1 mL of distilled water. The sample was shaken for 24 h at room temperature. After centrifugation for 10 min at 5000 rpm, the tannin estimation test was performed on the supernatant with a colorimetric assay based on Folin–Ciocalteu phenol reagent as described by Ikawa et al.²⁸ The analyzed films were weighed beforehand, to calculate the percentage of added tannins to bound tannins per film. Furthermore, the film solubility in water was analyzed by performing a Bradford assay on the supernatant.²⁹ To evaluate swelling behavior, samples ($2 \text{ cm} \times 2 \text{ cm}$) were cut from the films, freeze-dried, and weighed (W_i). The films were immersed in distilled water for 2 min. Subsequently, the swollen films were blotted with a filter paper to remove excess liquid and weighed again (W_f).^{5,30} Swelling values were calculated according to the formula

$$\% \text{ swelling} = (W_f - W_i) / W_i \times 100$$

Values represent the average from three measurements on three independently produced films (i.e., nine measurements).

Barrier Properties. To determine water vapor permeability (WVP), polypropylene cups of 55 mm depth and an exposed film area of 44 mm were filled with dry silica gel particles to maintain an internal level of 0% relative humidity (RH). At the top of the cups, a very small air gap was left. Edges were greased with Vaseline, and the films were placed on top of the cups, fixed with rubber bands. The cups were kept in a proofer under controlled conditions of $25 \pm 2^\circ\text{C}$ and 60% RH for 48 h. The water vapor transferred through the film and absorbed by the desiccant was determined from the weight gain of the silica gel recorded at various times, according to the method of Kayserlioglu et al.³¹ Changes in weight were recorded to the nearest 0.1 mg. Steady state conditions were assumed to be reached when the rate of change in weight of the cup became constant over time, that is, approximately 6 h.⁶ The constant rate of weight increase over time was obtained by linear regression, and the water vapor transmission rate (WVTR) was determined as the slope of this curve. The WVP of the film, where x is the film thickness, A is the film surface, P_0 is the vapor pressure of water, and (RH1–RH2) is the relative humidity gradient used in this study,³⁰ was expressed as

$$\text{WVP} = (\text{WVTR} \times x) / AP_0(\text{RH}_1 - \text{RH}_2)$$

Determination was performed in triplicate using three independently produced films.

Scanning Electron Microscopy (SEM). Scanning electron micrographs were obtained using a JEOL 5510 SEM system (JEOL, Tokyo, Japan). Freeze-dried films were mounted on aluminum stubs covered with double-sided carbon tape and sputter-coated with gold in a vacuum evaporator. Prepared samples were viewed in a scanning electron microscope at 3–5 kV using a 10 mm working distance.

Statistical Analysis. Multiple samples were compared with PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA and Tukey's honesty significant differences post hoc test were used to describe results at a significance level of $p < 0.05$. Values are presented as the average from at least three measurements \pm standard deviation.

RESULTS AND DISCUSSION

Gluten Characterization and Film Formation. The composition of the gluten powder used for film production was determined as follows: 78.6% protein, 11.7% moisture, 3.1% fat, and 0.97% ash. The gallic acid and tannic acid concentrations utilized for film formation were chosen on the basis of preliminary rheological trials. Frequency sweeps showed that the incorporation of these phenolic compounds caused an increase in complex modulus, thus suggesting a strengthening of the gluten structure (results not shown). This effect was most pronounced at concentrations of 0–30% tannic acid and 0–20% gallic acid. The model of Siebert et al.²² explains this observation, as these authors showed that fewer cross-links

occur between gliadin and tannic acid if an excess of the polyphenol is present. During analysis of the available literature on the formation of edible films, it became clear that the formation of a homogeneous film-forming solution with gluten requires a shift away from the isoelectric point of the protein, the inclusion of a plasticizer, and the presence of ethanol.^{5,12,13,17,31} In this study, acidic conditions (pH 4) were chosen as Vanburen et al.³² had previously reported that tannic acid binding is optimal at this pH. The gluten films cast during this study were homogeneous, flexible, opaque, and slightly yellow in color. Due to the use of a volatile acid to set the pH, there was no acetous taste or objectionable smell of the films. The films were ductile, smooth, and easy to peel off the surface of the Petri dishes. In most cases, the films were strong and flexible enough to be tested. The visual appearance of a film determines the consumer acceptance of a product; as such the slightly yellowish colored films produced in this study are acceptable for many food applications. Gluten films are usually colorless and slightly opaque due to starch residues in the mixture.³³ Tannic acid addition had a significant effect on film color, as they became reddish brown (Figure 1). They also

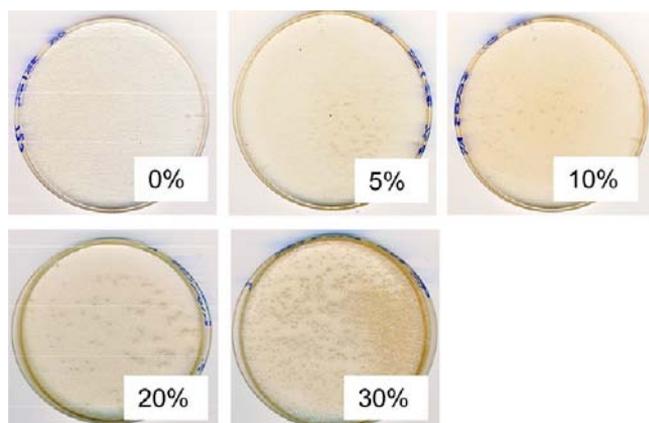


Figure 1. Scans of gluten films containing 0, 5, 10, 20, and 30% tannic acid.

showed several spots on the surface and became more brittle with increasing tannic acid concentration. Contrary to tannic acid modified films, it was observed that film elasticity was positively correlated to increasing gallic acid concentrations. Films with high gallic acid concentrations were very stretchy and easily deformed. Gallic acid addition did not have an effect on the films' visual appearance (Figure 2).

Film Thickness. Tables 1 and 2 show the resulting film properties. Film thickness increased significantly with increasing tannic acid concentration. Scanning electron micrographs (Figure 3) show that films with higher tannic acid concentrations had significant amounts of air bubbles entrapped in the structure. A possible explanation is that during tannic acid mediated gluten cross-linking, air is incorporated into the matrix through mixing and the strong network formed prevents subsequent release, similar to the gluten role in a bread dough. In support of this, it was observed during preliminary fundamental studies (results not shown) that the complex shear modulus for tannic acid modified gluten samples increased, indicating a higher resistance to deformation. This increased gluten strength is likely to contribute to a trapping effect for air bubbles. As a secondary result, the films appear thicker. The addition of gallic acid to the film-forming

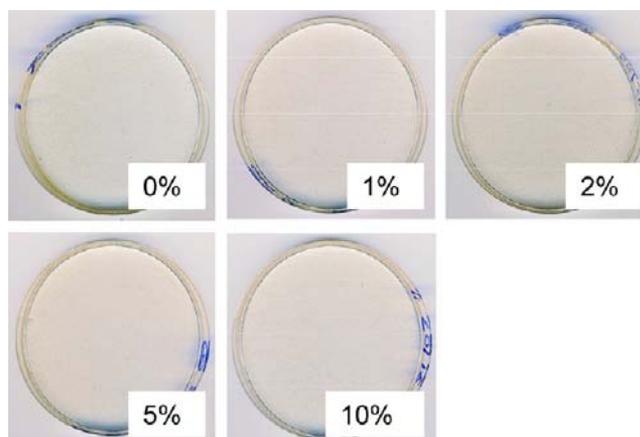


Figure 2. Scans of gluten films containing 0, 1, 2, 5, and 10% gallic acid.

solution did not have an influence on film thickness. The gallic acid molecule is rather small and probably fits into the structure better than the polyphenolic tannic acid compound. On the basis of scanning electron micrographs shown in Figure 3, there are no difference observed in structural coherence between the control and gallic acid containing films.

Mechanical Properties. For an edible film to be of use, it must withstand the normal stresses encountered during its application and the subsequent shipping, storage, and handling of the food. To maintain its integrity and barrier properties, high puncture strengths are generally required; however, film strength must be adjusted in accordance with the intended application of the film. For example, higher film strength can be obtained by incorporating a higher number of and/or better localization of bonds between protein chains. This is due to the mechanical resistance of wheat gluten films' dependence on the type and density of various intermolecular interactions such as disulfide, hydrophobic, and hydrogen bonding in the protein network. It was previously suggested that the addition of tannic acid induces cross-linkage within the gluten structure, which results in an increased mechanical strength.³⁴ The tensile strength of the films is given as the force per unit area (MPa) required to break the film when elongated. The films' extensibility is indicated by measuring its elongation (%). The results showed that tannic acid containing films had higher tensile strength and poorer extensibility than the control (Table 1). Thus, wheat gluten films became stiffer and more resistant but less flexible when modified with tannic acid. This is similar to the observations by Rivero et al.²⁷ for tannic acid cross-linked chitosan films. In addition, wheat gluten films become more rigid with increasing tannic acid concentration. The values shown in Table 2 confirmed the empirically observed influence of gallic acid on the mechanical properties of the wheat gluten films: modification of wheat gluten films resulted in a negative correlation between tensile strength and gallic acid addition levels from 2 to 10% w/w. The film elongation decreased when modified with 1–2% w/w gallic acid. Upon addition of higher concentrations, that is, 5 and 10% w/w, the elongation increased. These results lead to the conclusion that gallic acid does not cross-link wheat gluten but has a plasticizing effect. The data show the same trend as observed by Krochta et al.¹⁵ and Jagadeesh et al.³⁵ for films containing solely plasticizers. In their studies they showed that as the plasticizer concentration increased, tensile strength significantly decreased

Table 1. Properties for Films Containing Increasing Levels of Tannic Acid^a

tannic acid (% of gluten)	film thickness ($\mu\text{m/g}$ film-forming solution)	tensile strength (MPa)	elongation (%)	water vapor permeability $\times 10^{-12}$ (g/m \cdot s \cdot Pa)	swelling (% w/w)	tannic acid bound (% w/w of gluten)	film solubility (mg protein/g film)
0	16.51 \pm 0.73 a	1.08 \pm 0.04 a	204.90 \pm 9.78 a	2.66 \pm 0.11 a	161.8 \pm 18.7 a		10.85 \pm 1.16 ab
5	18.71 \pm 1.72ab	1.36 \pm 0.33 a	87.88 \pm 15.51 b	2.29 \pm 0.18 b	105.4 \pm 8.1 b	4.35 \pm 0.06 a	11.19 \pm 1.01 a
10	20.33 \pm 2.14 b	2.14 \pm 0.37 b	84.05 \pm 8.57 b	1.85 \pm 0.14 c	83.0 \pm 2.7 c	8.88 \pm 0.07 b	9.53 \pm 0.74 b
20	24.41 \pm 2.24 c	2.66 \pm 0.41bc	9.45 \pm 1.80 c	2.49 \pm 0.25 ab	52.2 \pm 2.7 d	18.38 \pm 0.13 c	2.53 \pm 0.44 c
30	26.40 \pm 0.89 c	3.22 \pm 0.57 c	3.59 \pm 0.43 d	2.32 \pm 0.11 b	38.7 \pm 4.6 e	27.59 \pm 0.76 d	0.82 \pm 0.11 d

^aMean values \pm standard deviation of at least triplicates; values followed by the same letter in the same row are not significantly different ($p < 0.05$).

Table 2. Properties for Films Containing Increasing Levels of Gallic Acid^a

gallic acid (% of gluten)	film thickness ($\mu\text{m/g}$ film-forming solution)	tensile strength (MPa)	elongation (%)	water vapor permeability $\times 10^{-12}$ (g/m \cdot s \cdot Pa)	swelling (% w/w)	gallic acid bound (% w/w of gluten)	film solubility (mg protein/g film)
0	16.51 \pm 0.73 a	1.08 \pm 0.04 a	204.90 \pm 9.78 a	2.66 \pm 0.11 ab	161.8 \pm 18.2 a		10.85 \pm 1.16 a
1	16.11 \pm 1.51 a	1.04 \pm 0.11 ab	196.44 \pm 26.10 a	2.74 \pm 0.14 a	256.25 \pm 15.06 b	0.11 \pm 0.03 a	30.46 \pm 2.29 b
2	16.33 \pm 2.60 a	0.94 \pm 0.10 bc	187.70 \pm 19.62 a	3.55 \pm 0.35 c	196.33 \pm 19.14 c	0.32 \pm 0.02 b	44.34 \pm 4.87 c
5	16.36 \pm 1.29 a	0.85 \pm 0.10 c	238.23 \pm 11.19 b	2.08 \pm 0.14 d	187.70 \pm 18.92 ac	0.71 \pm 0.10 c	91.43 \pm 12.70 d
10	16.66 \pm 0.71 a	0.53 \pm 0.07 d	296.8 \pm 13.98 c	2.45 \pm 0.06 bd	189.40 \pm 6.88 c	1.55 \pm 0.19 d	97.06 \pm 5.11 d

^aMean values \pm standard deviation of at least triplicates; values followed by the same letter in the same row are not significantly different ($p < 0.05$).

while the extensibility significantly increased. Soares and Soldi³⁶ proposed that plasticizers reduce the intermolecular interactions and increase the amount of hydrogen bonding. Polar groups (–OH) of the plasticizer are believed to form polymer–plasticizer hydrogen bonds, replacing polymer–polymer interactions and hence leading to lower values for tensile strength.

Tannin Binding, Film Swelling, and Solubility. Water uptake and solubility are important considerations if films are to be used in a high-moisture environment. Wheat gluten films are generally water sensitive due to the hydrophilic nature of many of the amino acids constituting the protein chains and the substantial amount of hydrophilic plasticizer (glycerol).¹⁴ Influences of tannic acid concentration on the swelling behavior of wheat gluten films are shown in Table 1. The water uptake in percent of initial film weight decreased significantly with increasing amount of tannic acid. Unmodified gluten films immersed in distilled water showed significant swelling, and water regain accounted for about 160% w/w after 2 min. Increased tannic acid concentrations reduced the degree of swelling to approximately 50% w/w and lower. It was proposed that the increased degree of cross-linking decreased the availability of gluten for water interaction. Similar results were obtained for gelatin films cross-linked with tannic acid.^{26,30} Influences of various concentrations of gallic acid on the swelling capacities of films are shown in Table 2. Swelling ratios increase with increasing concentrations of gallic acid, compared to the unmodified wheat gluten film, with the addition of 10% gallic acid causing a 17% increase.

As shown in Table 1, the solubility of the films decreased significantly when the ratio of added tannic acid was increased to 20–30%. However, up to an addition level of 10% there is no significant difference from control. Reduced solubility indicates that there are a higher number of cross-links, which results in lower protein chain mobility and, thus, reduced three-dimensional rearrangements of the network during moisture sorption.¹⁴ Incorporation of gallic acid had an opposite effect. As shown in Table 2, the solubility of gluten proteins increases significantly upon addition of the phenolic compound, with up to 97% of gluten protein being solubilized (for 10% w/w gallic

acid containing films). This was expected due to the hydrophilic nature of this phenolic compound.

As shown in Table 1, nearly all of the added tannic acid was bound under the previously described film-forming conditions. This result confirms that setting the film-forming solution to pH 4 is optimal for tannin binding.³² Table 2 shows that the percentage of gallic acid bound to the protein network was very low. For example, when 10% w/w gallic acid was added, only 1.55% was bound, thus illustrating the low affinity of gluten for the phenolic compound.

Barrier Properties. Because the main function of an edible film or coating is often to impede moisture transfer between the coated food and the surrounding atmosphere or between two components of a heterogeneous food product, it is essential that the WVP is as low as possible.³⁷ In this study, film WVP was shown to be dependent on the number of polar groups that the polymer contained. This is caused by the sorption of migrating water molecules to polar groups, thereby facilitating water transport.⁶ The low pH used in this study was responsible for proteins' unfolding, thus exposing their hydrophilic residues. Consequently, the affinity of gluten proteins for water molecules was increased, allowing vapor to penetrate more readily.⁶ Table 1 shows that tannic acid addition has the potential to decrease WVP. However, upon an addition level of 10% w/w, the WVP increased again. The initial improvement of barrier properties is probably related to the tight gluten–tannic acid network, which likely occupies previously hydrophilic hydroxyl groups and traps small air bubbles inside the matrix, resulting in a reduced water molecule sorption in the cross-linked film. It can be assumed that as the cross-link density inside the film increases, so too does the water vapor barrier effect.³⁸ The incorporation of >10% tannic acid resulted in brittle films. The scanning electron micrographs revealed multiple small cracks in the film structure, thus potentially allowing the water vapor to pass through (Figure 4). Another explanation for the increase in WVP of the gluten–tannic acid film, above a certain concentration, might be that the extremely low volatility of tannic acid at the processing temperature prevented elimination of the nonbound tannin fraction. If this is the case, the free tannic acid would itself bind water. As such, an

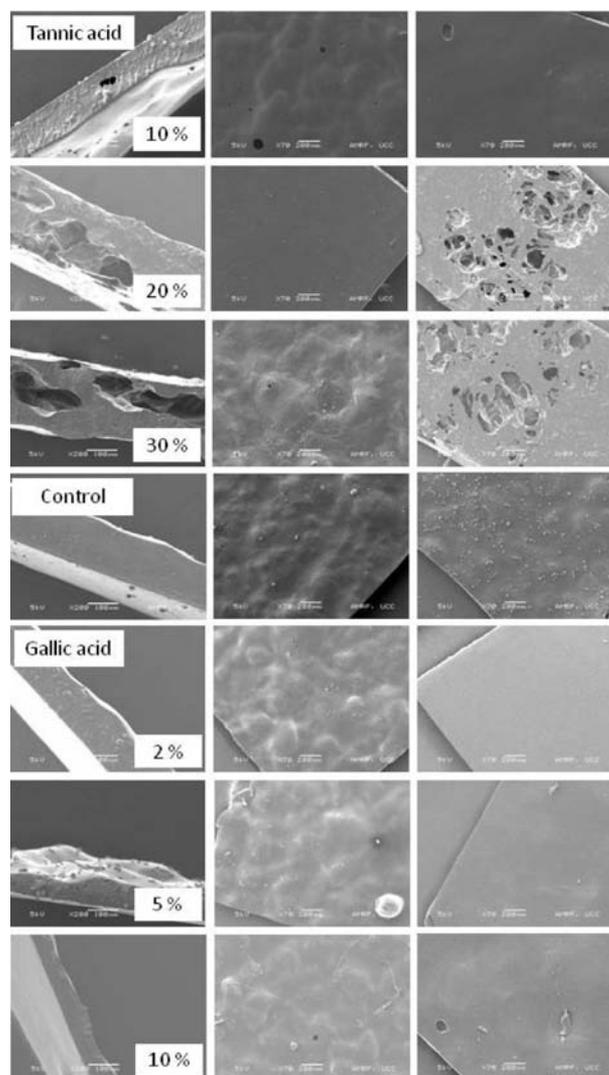


Figure 3. Scanning electron micrographs of gluten films containing different ratios of tannic (10, 20, and 30%) or gallic acid (2, 5, and 10%) as compared to control.

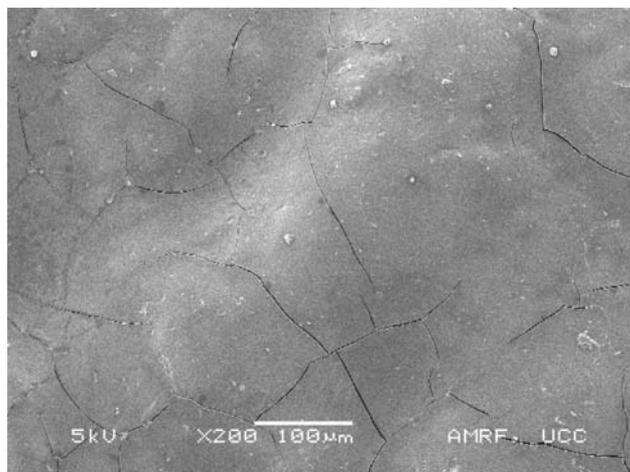


Figure 4. Representative scanning electron micrograph of the surface of tannic acid film showing multiple cracks.

excess of tannins would occupy free space in the network and interfere with film homogeneity,²⁵ allowing easier passage of

water. Cao et al.³⁰ found that cross-linking gelatin films with tannic acid had only a small influence on the WVP. The presumed reason was that tannic acid has many hydroxyl groups, which could combine with water, and so the apparent WVP did not change. However, in this study tannic acid cross-linking was found to have a significant positive influence on wheat gluten films (at certain addition levels), decreasing the WVP by up to 30% (for films containing 10% w/w tannic acid).

Table 2 shows the influences of gallic acid on WVP. Much like a plasticizer, gallic acid addition initially increases WVP. Different authors observed the same trend when adding increasing amounts of glycerol to their film formulations.^{39,40} Kowalczyk and Baraniak⁴¹ also observed an increase in WVP when glycerol was added to films made of pea protein isolate. Gallic acid, like glycerol, is a very low molecular substance, which can easily penetrate into the protein network; hence, the cumulative addition increasingly disrupts intermolecular interactions among polypeptide chains. Cuq et al.⁴² proposed that this plasticizer-mediated reorganization of the protein network increases free space within the network and consequently promotes water diffusion into the film matrix. However, at concentrations >2% gallic acid (% w/w of gluten), the WVP decreased compared to the control. This is probably due to the excess small phenolic gallic acid filling otherwise free space in the film network.

Scanning Electron Micrographs. Figure 3 presents scanning electron micrographs of the morphology of surfaces and cross sections of tannic acid containing, gallic acid containing, and control wheat gluten films. The images illustrate that both the untreated and tannic acid cross-linked films had smooth dense surfaces typical for protein films. However, it is also obvious that the addition of tannic acid affected the microstructure of wheat gluten films, due to specific tannin–gluten interactions. With increasing tannic acid concentration, more and more free space was present in the structure. These voids are between strands of the molecule polymers and may be related to the formation of channels that provide less resistance to flow during the moisture transfer process.²⁶ With regard to gallic acid containing films, the top and bottom surfaces were similar in appearance to the gluten control sample. The cross-sectional views showed that there was no observable difference in coherence between the different films. There were no visible cracks, and the hole seen in the 10% w/w gallic acid sample represents an exception rather than a repeatedly occurring structural feature. In some micrographs, starch granules can be seen. Originating from the wheat gluten powder, they were likely expelled during the film formation.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: e.arendt@ucc.ie. Phone: +353 21 490 2064. Fax: +353 21 427 0213.

Funding

Funding for A.-S.H. was received through an EMBARK scholarship granted by the Irish Research Council for Science, Engineering and Technology (IRCSET). IRCSET's initiatives are funded by the National Development Plan of Ireland under the auspices of the Department of Education and Science. This research was partly funded also by FIRM Ireland.

Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Albertson, A. C.; Karlsson, S. Chemistry and biochemistry of polymer biodegradation. In *Chemistry and Technology of Biodegradable Polymers*; Griffin, G., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1994; pp 7–17.
- (2) Olabarrieta, I. *Strategies to Improve the Aging, Barrier and Mechanical Properties of Chitosan, Whey and Wheat Gluten Protein Films*; KTH, Kungliga Tekniska Hogskolan: Stockholm, Sweden, 2005.
- (3) Valencia-Chamorro, S. A.; Palou, L.; Del Rio, M. A.; Perez-Gago, M. B. Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. *Crit. Rev. Food Sci. Nutr.* **2011**, *51* (9), 872–900.
- (4) Wang, L.; Liu, L.; Hohnes, J.; Kerry, J. F.; Kerry, J. P. Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films. *Int. J. Food Sci. Technol.* **2007**, *42* (9), 1128–1138.
- (5) Tropini, V. Wheat gluten films cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide. *Ind. Crops Prod.* **2004**, *20*, 281–289.
- (6) Gontard, N.; Guilbert, S.; Cuq, J. L. Edible wheat gluten films – influence of the main process variables on film properties using response-surface methodology. *J. Food Sci.* **1992**, *57* (1), 190–195.
- (7) Krochta, J. M. Edible protein films and coatings. In *Food Proteins and Their Applications*; Damodaran, S., Ed.; Dekker: New York, 1997; pp 529–550.
- (8) Cuq, B.; Gontard, N.; Guilbert, S. Proteins as agricultural polymers for packaging production. *Cereal Chem.* **1998**, *75* (1), 1–9.
- (9) Hongkang, Z.; Gauri, M. Biodegradable protein-based films from plant resources: a review. *Environ. Prog. Sustainable Energy* **2010**, *29* (2), 203–220.
- (10) Mauricio-Iglesias, M.; Peyron, S.; Guillard, V.; Gontard, N. Wheat gluten nanocomposite films as food-contact materials: migration tests and impact of a novel food stabilization technology (high pressure). *J. Appl. Polym. Sci.* **2010**, *116* (5), 2526–2535.
- (11) Gontard, N.; Duchez, C.; Cuq, J. L.; Guilbert, S. Edible composite films of wheat gluten and lipids – water vapor permeability and other physical properties. *Int. J. Food Sci. Technol.* **1994**, *29* (1), 39–50.
- (12) Gontard, N. Edible and biodegradable films: Study of wheat gluten film-forming properties. *C. R. Acad. Agric. Fr.* **1994**, *80* (4), 109–118.
- (13) Gennadios, A. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **1993**, *41* (11), 1835–1839.
- (14) Angellier-Coussy, H.; Gastaldi, E.; Gontard, N.; Guillard, V. Influence of processing temperature on the water vapour transport properties of wheat gluten based agromaterials. *Ind. Crops Prod.* **2011**, *33* (2), 457–461.
- (15) Krochta, J. M.; Baldwin, E. A.; Nisperos-Carriedo, M. *Edible Coating and Films to Improve Food Quality*; Technomic Publishing: Lancaster, PA, 1994; pp 379.
- (16) Mangavel, C.; Barbot, J.; Gueguen, J.; Popineau, Y. Molecular determinants of the influence of hydrophilic plasticizers on the mechanical properties of cast wheat gluten films. *J. Agric. Food Chem.* **2003**, *51* (5), 1447–1452.
- (17) Gallstedt, M.; Mattozzi, A.; Johansson, E.; Hedenqvist, M. S. Transport and tensile properties of compression-molded wheat gluten films. *Biomacromolecules* **2004**, *5* (5), 2020–2028.
- (18) Kuktaite, R.; Plivelic, T. S.; Cerenius, Y.; Hedenqvist, M. S.; Gallstedt, M.; Marttila, S.; Ignell, R.; Popineau, Y.; Tranquet, O.; Shewry, P. R.; Johansson, E. Structure and morphology of wheat gluten films: from polymeric protein aggregates toward superstructure arrangements. *Biomacromolecules* **2011**, *12* (5), 1438–1448.
- (19) Hernandez-Munoz, P.; Villalobos, R.; Chiralt, A. Effect of thermal treatments on functional properties of edible films made from wheat gluten fractions. *Food Hydrocolloids* **2004**, *18* (4), 647–654.
- (20) Sanchez, A. C.; Popineau, Y.; Mangavel, C.; Larre, C.; Gueguen, J. Effect of different plasticizers on the mechanical and surface properties of wheat gliadin films. *J. Agric. Food Chem.* **1998**, *46* (11), 4539–4544.
- (21) Emmambux, M. N.; Stading, M.; Taylor, J. R. N. Sorghum kafirin film property modification with hydrolysable and condensed tannins. *J. Cereal Sci.* **2004**, *40* (2), 127–135.
- (22) Siebert, K. J.; Troukhanova, N. V.; Lynn, P. Y. Nature of polyphenol–protein interactions. *J. Agric. Food Chem.* **1996**, *44*, 80–85.
- (23) Asano, K.; Shinagawa, K.; Hashimoto, N. Characterization of haze-forming proteins of beer and their roles in chill haze formation. *J. Am. Soc. Brew. Chem.* **1982**, *40*, 147–154.
- (24) Haslam, E. Polyphenol–protein interactions. *Biochem. J.* **1974**, *139* (1), 285–288.
- (25) Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. Effects of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer* **2002**, *43* (20), 5417–5425.
- (26) Zhang, X.; Do, M. D.; Casey, P.; Sulistio, A.; Qiao, G. G.; Lundin, L.; Lillford, P.; Kosaraju, S. Chemical modification of gelatin by a natural phenolic cross-linker, tannic acid. *J. Agric. Food Chem.* **2010**, *58* (11), 6809–6815.
- (27) Rivero, S.; Garcia, M. A.; Pinotti, A. Crosslinking capacity of tannic acid in plasticized chitosan films. *Carbohydr. Polym.* **2010**, *82* (2), 270–276.
- (28) Kayserlioglu, B. S.; Stevels, W. M.; Mulder, W. J.; Akkas, N. Mechanical and biochemical characterisation of wheat gluten films as a function of pH and co-solvent. *Starch/Staerke* **2001**, *53* (8), 381–386.
- (29) Cao, N.; Fu, Y. H.; He, J. H. Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids* **2007**, *21* (4), 575–584.
- (30) Ikawa, M.; Schaper, T. D.; Dollard, C. A.; Sasner, J. J. Utilization of Folin–Ciocalteu phenol reagent for the detection of certain nitrogen compounds. *J. Agric. Food Chem.* **2003**, *51*, 1811–1815.
- (31) Bradford, M. M. Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye binding. *Anal. Biochem.* **1976**, *72* (1–2), 248–254.
- (32) Vanburen, J. P.; Robinson, W. B. Formation of complexes between protein and tannic acid. *J. Agric. Food Chem.* **1969**, *17* (4), 772–777.
- (33) Cho, S. W.; Gallstedt, M.; Hedenqvist, M. S. Effects of glycerol content and film thickness on the properties of vital wheat gluten films cast at pH 4 and 11. *J. Appl. Polym. Sci.* **2010**, *117* (6), 3506–3514.
- (34) Kayserlioglu, B. S.; Bakir, U.; Yilmaz, L.; Akkas, N. Drying temperature and relative humidity effects on wheat gluten film properties. *J. Agric. Food Chem.* **2003**, *51* (4), 964–968.
- (35) Jagadeesh, D.; Reddy, D. J. P.; Rajulu, A. V. Preparation and properties of biodegradable films from wheat protein isolate. *J. Polym. Environ.* **2011**, *19* (1), 248–253.
- (36) Soares, R. M. D.; Soldi, V. The influence of different cross-linking reactions and glycerol addition on thermal and mechanical properties of biodegradable gliadin-based film. *Mater. Sci. Eng. C: Mater. Biol. Appl.* **2010**, *30* (5), 691–698.
- (37) Bourtoom, T.; Chinnan, M. S. Preparation and properties of rice starch-chitosan blend biodegradable film. *LWT–Food Sci. Technol.* **2008**, *41* (9), 1633–1641.
- (38) Irissin-Mangata, J.; Bauduin, G.; Boutevin, B. Bilayer films composed of wheat gluten film and UV-cured coating: water vapor permeability and other functional properties. *Polym. Bull.* **2000**, *44* (4), 409–416.
- (39) Park, H. J. Water vapor permeability and mechanical properties of grain protein-based films as effected by mixtures of polyethylene glycol and glycerin plasticizers. *Trans. Am. Soc. Agric. Eng.* **1994**, *37* (4), 1281–1285.
- (40) Gontard, N.; Guilbert, S.; Cuq, J. L. Water and glycerol as plasticizers affect mechanical and water-vapor barrier properties of an edible wheat gluten film. *J. Food Sci.* **1993**, *58* (1), 206–211.
- (41) Kowalczyk, D.; Baraniak, B. Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. *J. Food Eng.* **2011**, *105* (2), 295–305.

(42) Cuq, B.; Gontard, N.; Cuq, J. L.; Guilbert, S. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *J. Agric. Food Chem.* **1997**, *45* (3), 622–626.