

Thermal and mechanical characterization of films based on poly(vinyl alcohol) and β -lactoglobulin blends

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ABSTRACT: In this study, we investigated the possibility of creating easy to handle films based on poly(vinyl alcohol) (PVA) and β -lactoglobulin (β lg) blends using the casting method. Four different variables were studied to obtain these films: different proportions of PVA and β lg, different pHs (10, 7, 5.5, and 2.5), several molecular masses of PVA (130,000, 13,000–23,000, and 2000 g/mol), and the denaturation of β lg. The first objective was to obtain films, and the second was to characterize them by differential scanning calorimetry, thermogravimetric analysis, and mechanical testing. Significant variations in behavior were found, and the variables studied modified the blends in different ways. In particular, better results were achieved when the β lg proportion was less than 30 wt %, and when a pH of 2.5 was used in conjunction with a high molecular mass (130,000 g/mol) of PVA. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 41745.

KEYWORDS: biodegradable; blends; differential scanning calorimetry (DSC); films; mechanical properties

Received 29 July 2014; accepted 8 November 2014

DOI: 10.1002/app.41745

INTRODUCTION

The increasing demand for high-quality, long-life foods, together with the environmental need to reduce packaging waste, has created a growing interest in biodegradable polymer films. The food industry generates large amounts of high-protein waste content that could be recovered. The manufacture of dairy products such as cheese, for instance, creates large amounts of serum. Serum is formed by whey proteins, whose main component is β -lactoglobulin (β lg).

β lg is made up of a single chain of 162 amino acids and is a globular protein with a molecular mass of 18,400 g/mol; it is sensitive to thermal denaturation and, at same time, has two internal disulfide bonds and a free thiol group. The distribution of charged polar and nonpolar amino acids along the protein chain creates chemical potential. In whey proteins, including β lg, the negative charge is uniformly distributed along the chain, and consequently, the proteins fold in such a way that most of the hydrophobic groups are buried within the molecule.

The isoelectric point of β lg is between 3.4 and 5.2. At extreme pH, the protein appears as a monomer; at pHs between 3.4 and

5.2, it is found as an octamer; and at neutral pH, it is a dimer.^{1,2} There are several genetic variants of β lg; the most common are A and B, whose difference is two amino acids. Variety A has a valine at position 118 and an aspartic acid in position 64, whereas variety B has alanine and glycine, respectively, in the same positions.

β lg alone is not capable of forming films, so plasticizers are used toward this objective. Polymer blending is a useful strategy for designing new materials with the intermediate properties of those shown by the constituents, so the needs of the market can be satisfied at low cost. Sothornvit and Krochta^{3,4} managed to produce β lg films with propylene glycol, glycerol, sorbitol, sucrose, and poly(ethylene glycol); all of them had low-molecular-weight molecules. On the other hand, several studies have been carried out with poly(vinyl alcohol) (PVA) as a plasticizing polymer of different proteins, such as albumin,⁵ gellan,⁶ gelatin,⁷ soy,⁸ and wheat.⁹ This polymer has interesting features that would justify its potential for blending with β lg: it is a nontoxic, biocompatible, and biodegradable synthetic polymer. Semicrystalline PVA is a water-soluble polymer with good mechanical performance that can replace low-molecular-weight

plasticizers for polymer/protein hybrid systems without undergoing the surface migration that could be harmful for packaging plastics.

The aim of this study was to determine the viability of β lg-based films with PVA as a plasticizer (it is frequently used with proteins but has not been studied very much with this particular protein) and then to characterize the blends. Blends of PVA and β lg were prepared with different variables. The film-formation capacity of the polymer–protein blends was evaluated first. Then, the materials obtained were thermally and mechanically characterized. The compatibility of the system was analyzed according to the glass-transition and crystallization behavior of PVA in the absence and presence of β lg by differential scanning calorimetry (DSC). We also studied the degradation temperatures ($T_{\text{Degradation}}$'s) of the blends via thermogravimetric analysis (TGA), and finally, the mechanical properties of the films were studied via strain–stress tests.

EXPERIMENTAL

Materials

This study used films prepared from blends of PVA and β lg under different conditions. β lg [from bovine milk, $\geq 85\%$ electrophoresis on polyacrylamide gels (PAGE; which is commonly used method for separation of whey proteins)] was supplied by Sigma-Aldrich as a lyophilized powder and had a mixture of A and B variants and a molecular weight of 18,400 g/mol. Three different PVA varieties in the powdered state were used. The first was a PVA supplied by Sigma-Aldrich with a degree of hydrolysis of over 99% and an average molecular weight of 130,000 g/mol, which was much larger than that of the protein. The second was also provided by Sigma-Aldrich and was a PVA with a 98% degree of hydrolysis and a molecular weight settled between 13,000 and 23,000 g/mol, which was similar to that of the protein. The third was a PVA of low molecular weight (2000 g/mol), which was much smaller than that of the protein, and with a 75% degree of hydrolysis; it was supplied by Acros Organics.

Film Production

The films were produced with the casting method with four different variables: different proportions of PVA– β lg, various pHs, the application or lack of application of denaturation on the protein, and as mentioned previously, various molecular weights of the polymer.

The total mass of the films at the end of the process was set at 250 mg; to achieve this objective, we used an initial total mass of 250 mg of PVA plus β lg powder, in the right proportions, dissolved in 15 g of distilled water in each case. The process began with the solution of PVA powder with high, medium, or low molecular mass in 15 g of water. The protein powder was then added to bring the total mass of solid materials up to 250 mg. This meant that blends of compositions up to 50% β lg were obtained progressively by the addition of more protein to PVA.

Then, the pH of each solution was adjusted. In general, proteins are very sensitive to changes in the pH of the medium because they possess different properties as their structure changes, so a decision was made to analyze the effect of that variable on the blend proper-

ties. In this way, various samples of the films were prepared at four different pHs: two extremes of pH 10 and 2.5, neutral pH 7, and pH 5.5, which was very close to the isoelectric point of β lg where the tendency toward aggregation between molecules was at its highest. The process continued with the homogenization of mixtures by magnetic stirring for at least for 1 h.

The effect of the denaturation process on the protein was also taken into consideration. With the application of a heat treatment for denaturation, the β lg's most reactive zones were exposed, and interactions with other molecules were increased. So the aim was to take advantage of this characteristic to improve the linkages with the polymer chains. Hence, the effect of heat treatment was determined by a comparison of films with and without denaturation. Upon the adjustment of the pHs, films without protein denaturation were poured into Petri dishes, and solvent evaporation was followed for 3 days at room temperature. The finished films were stored at 24°C and at a relative humidity of 45%. In the case of films with protein denaturation, after the pH adjustment and the final agitation of the solutions, they were heated to 90°C for 30 min. After this, the procedure followed was the same as that used the ones without denaturation.

DSC

Thermal analysis of the blends was carried out with a TA Instruments DSC Q200 under nitrogen flow. The samples were placed in hermetically sealed aluminum pans; an empty pan was used as a reference. The DSC was calibrated with the melting temperature (T_m) and enthalpy of indium.

The glass-transition temperature (T_g), which corresponded to the temperature where the baseline changes, and T_m , which corresponded to an endothermic peak in the thermogram, of the polymer and its blends with β lg were studied. The aim was to study the effects of the composition, pH variation, variation in molecular mass of PVA and denaturation process on the protein at both temperatures of transition.

The average sample weight was 7 mg, and the heat treatment was as follows: heating from 15 to 250°C at a rate of 10°C/min, cooling to 15°C at a rate of 5°C/min, and subsequent heating to 250°C at a rate of 10°C/min to eliminate the moisture effect on the films under the protection of a nitrogen flow.

TGA

The thermal analysis consisted of a thermogravimetric test with a TA Instruments TGA Q50 instrument. Film samples were placed in a testing plate and heated from room temperature to 600°C at a rate of 10°C/min. Using this test, we could analyze the start of $T_{\text{Degradation}}$ of the materials according to their proportions of PVA/ β lg, different pHs, and different types of PVA along with the samples with and without denaturation.

Mechanical Testing

Tensile tests were used to analyze the compatibility of the blends with pure components in an effort to find an intermediate behavior between them. It is known that protein-based films are fragile and not easy to handle, so the objective of blending with the PVA polymer was to improve the ductile performance.

Table I. Film-Formation Possibilities Under Different Conditions

PVA M_w (g/mol)	pH	Denaturation	Composition (wt % β lg)
130,000	10	No	0–50%
130,000	10	Yes	0–50%
130,000	7	No	0–50%
130,000	7	Yes	0–50%
130,000	5.5	No	0–30%
130,000	5.5	Yes	0–30%
130,000	2.5	No	0–50%
130,000	2.5	Yes	0–50%
13,000–23,000	10	No	0–40%
13,000–23,000	10	Yes	0–40%
13,000–23,000	7	No	0–30%
13,000–23,000	7	Yes	0–40%
13,000–23,000	5.5	No	0–30%
13,000–23,000	5.5	Yes	0–30%
13,000–23,000	2.5	No	0–30%
13,000–23,000	2.5	Yes	0–40%
2000	10	No	0–50%
2000	10	Yes	0–50%
2000	7	No	0–40%
2000	7	Yes	0–40%
2000	5.5	No	0–40%
2000	5.5	Yes	0–40%
2000	2.5	No	0–50%
2000	2.5	Yes	0–40%

M_w , weight-average molecular weight.

A double-columned Instron machine (model 5565) was used to conduct the mechanical properties characterization. The distance between clamps was set at 50 mm, and the crosshead displacement rate was 5 mm/min. All of the variables were recorded with the software provided with the equipment (Bluehill v. 2.0). The tensile measurements were made in accordance with EN ISO 527-3, part 3. In particular, the Young's modulus, tensile strength, and strain were determined because they were directly connected to the PVA content; PVA acted as plasticizer or matrix of blends, as has been shown in previous studies.^{4,15–19} At least 10 samples of each film were analyzed, strips $10 \times 100 \text{ nm}^2$ were cut (with the average thickness being $30 \mu\text{m}$), the test temperature was set at $23 \pm 2^\circ\text{C}$, and the relative humidity was set at $50\% \pm 5\%$.

RESULTS AND DISCUSSION

Fabrication

Table I shows the film-formation possibilities under different conditions of pH, composition, PVA molecular mass, and denaturation process.

The concentrations of protein higher than 50% β lg did not form good films, and that specific proportion of β lg was only possible in some cases, as shown in Table I. The films obtained

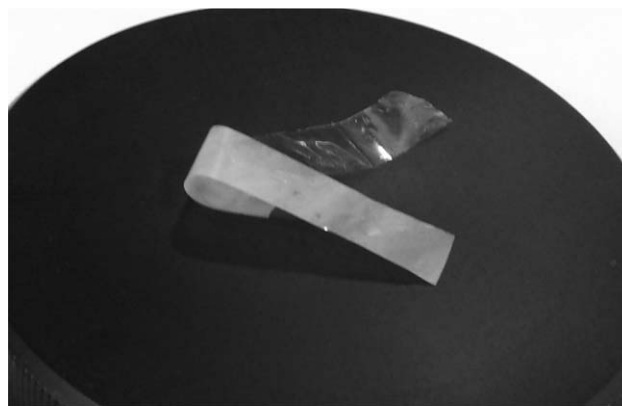


Figure 1. Two examples of 80% PVA–20% β lg films made at pH 2.5 and 5.5 with a denaturation process and with a high molecular mass of PVA. The white sample correspond to pH 5.5, and the transparent one corresponds to pH 2.5.

with medium PVA molecular mass admitted lower quantities of protein than the films created with high and low molecular masses of polymer. This suggested that there was a lower compatibility of components when a PVA of medium molecular mass was used. At the same time, the denaturation process in those films seemed to be more effective than on the films obtained with high and low molecular masses because that treatment allowed the formation of films with higher quantities of protein when β lg was denaturated. On the other hand, extreme pHs usually provided more flexibility to the films, and the detachment process was easier. Figure 1 shows two examples of 80% PVA–20% β lg films made at pH 2.5 and 5.5.

DSC Analysis

The DSC technique is a very useful method for studying the miscibility state of polymer blends because the T_g of polymer blends is strongly affected by the degree of phase separation. Polymer–polymer miscibility is usually determined by a comparison of the T_g 's of single polymers with the T_g 's of blends.

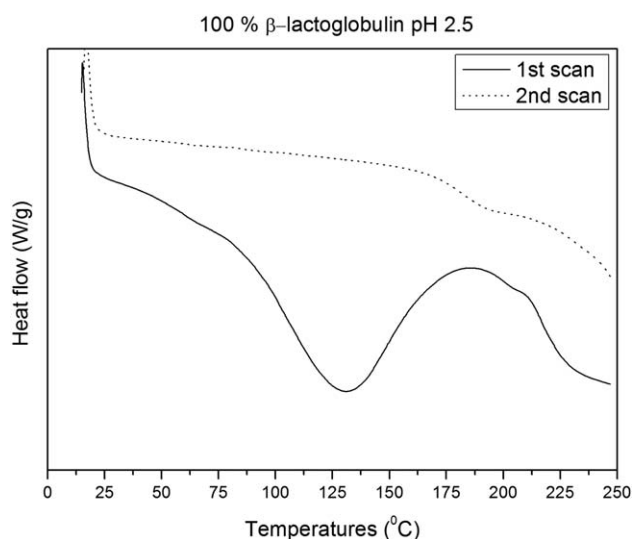


Figure 2. First and second scan of a sample of 100% β lg at pH 2.5 with exothermic transitions upward.

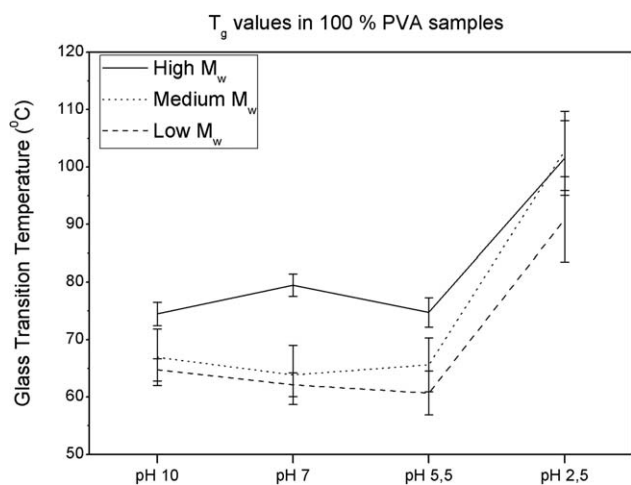


Figure 3. T_g 's of 100% PVA samples at different pHs.

Polymer alloys are achieved when components present intermolecular interactions; in this case, a material may possess a single phase with a single T_g . Mixtures with a lower degree of miscibility present several phases with various T_g 's.

We expected that PVA and β lg would show some degree of interaction because this polymer has been shown to have interactions with other natural molecules, such as starch, cellulose, gelatin, chitosan, wheat protein, and soy proteins.^{10,11} In our study, we started analyzing the behavior of pure β lg and pure PVA, and then, we analyzed the thermal characteristics of the mixtures.

Unfortunately, pure β lg did not present a T_g (see Figure 2), so the miscibility study could not be developed as in traditional studies. Two transitions observed in the first scan were attributed¹² to the denaturation process (marked with an arrow at 65°C) and the rupture of the linkages generated in that process (the endothermic peak near 130°C), respectively. In the second scan, only one transition could be seen near 180°C; this was

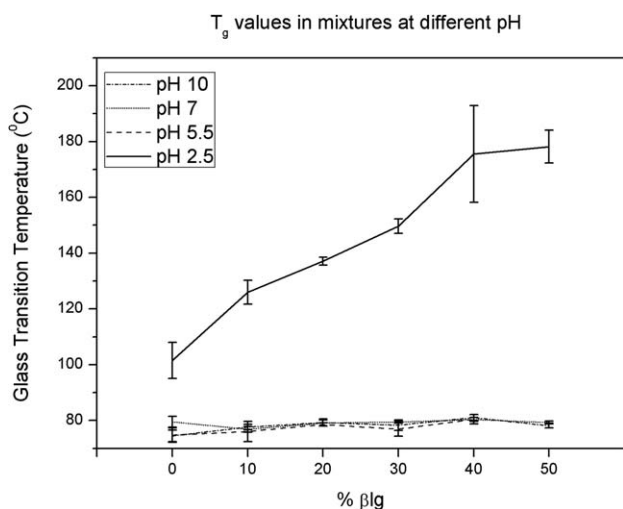


Figure 4. T_g 's in blends formed with a high molecular mass of PVA and the denaturation process of protein.

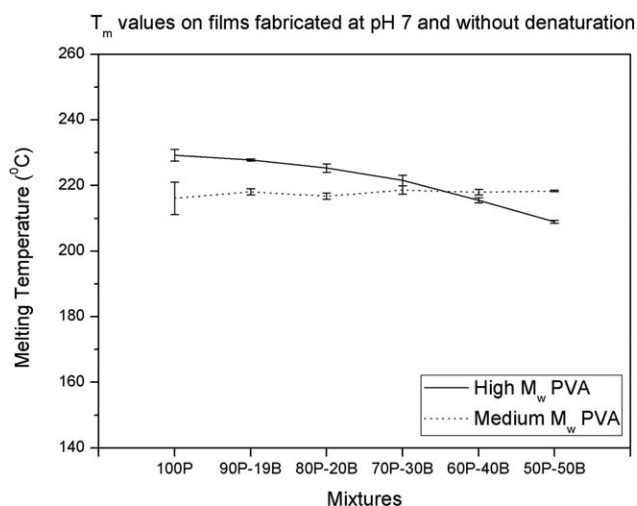


Figure 5. T_m values of films produced at pH 7 and without a denaturation process. PVA with a low molecular mass had a very low crystallinity, so no comparison was made with it.

probably due to the rupture of linkages remaining from the first scan.

An analysis of the behavior of the pure PVA polymers showed that their thermograms had a similar appearance when samples were made at pH 10, 7, and 5.5. All of them presented a T_g and T_m at higher values when the molecular mass was increased. The low-molecular-mass polymer was less crystalline than the others, so the melting peak was quite attenuated.

In the first scan, the transition values were very low according to the bibliography; this was attributed to the moisture in the films, which acted as a plasticizer. So a decision was taken to make a second scan to remove the water's plasticizing effect, and so, the thermal study of PVA and the mixtures was made with these data.

When the behavior of the pure PVA samples was analyzed at pH 2.5, different results were obtained: no melting transition was observed, so the samples had lost their crystallinity, and the

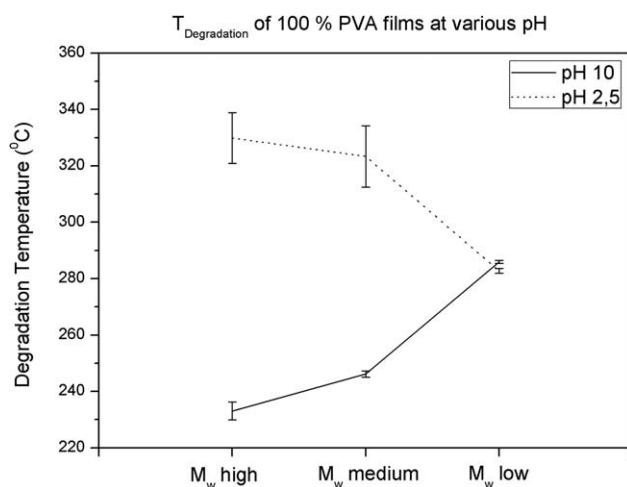


Figure 6. $T_{\text{Degradation}}$ values measured in films of 100% PVA content produced at pH 10 and 2.5.

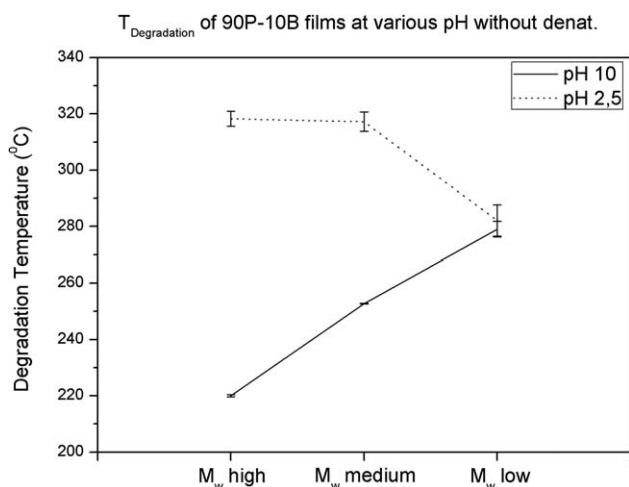


Figure 7. $T_{\text{Degradation}}$ values measured in films with a 90% PVA–10% β lg content produced at pH 10 and 2.5 and without a denaturation process.

T_g values reached nearly 30°C. This was a higher temperature compared to the values obtained with the films formed at pH 10, 7, and 5.5 (see Figure 3).

On the second scans obtained for each mixture, at different pHs, comparisons were made to determine the effect of protein on the T_g of the pure PVA and then on its T_m ; we expected that changes in the transition temperatures would be indicative of some interactions between components.

When the T_g 's of the mixtures were analyzed, four main conclusions were obtained. First, the blends showed a large increase in the T_g values when pH 2.5 was used in the production process; the same as was observed in samples of pure PVA. Second, higher T_g 's were seen with a higher content of β lg, as shown in the examples given in Figure 4. Third, the T_g 's increased when higher molecular masses of PVA were used. Fourth, the denaturation process applied to protein did not generate clear differences when the films were made with PVAs with high and low molecular masses; in both cases, the behavior was irregular.

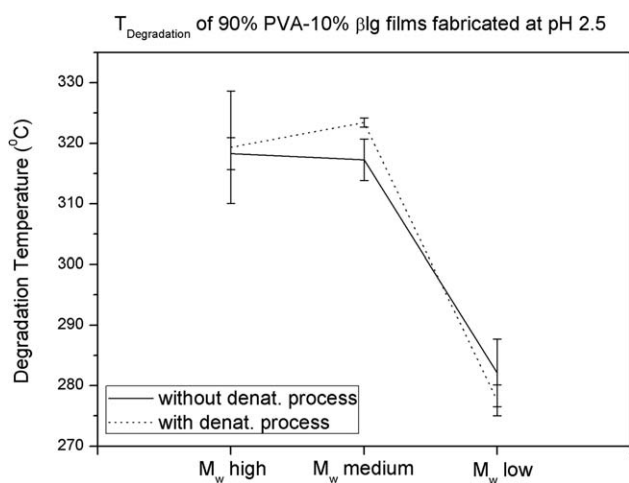


Figure 8. $T_{\text{Degradation}}$ values obtained in 90% PVA–10% β lg films produced at pH 2.5 with the denaturation process and without it.

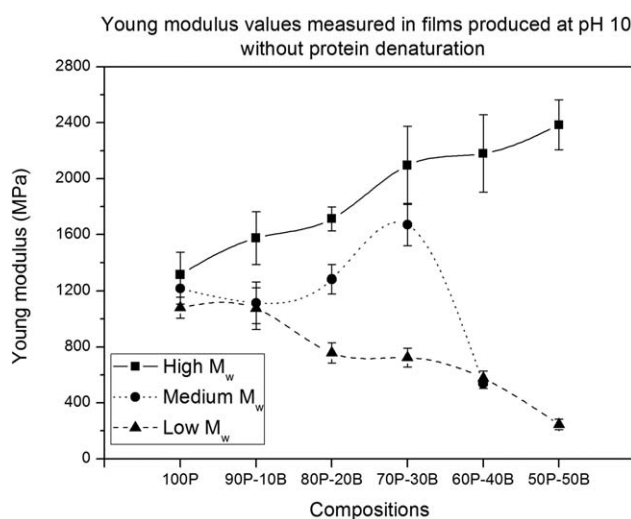


Figure 9. Young's modulus values at different compositions and with various PVAs.

When PVA of medium molecular mass was used, the denaturalized films had higher T_g values, with an average of about 6°C.

As shown in Figure 4, we concluded that pH values of 10, 7, or 5.5 hardly changed the T_g 's in the pure PVA and blends. However, at pH 2.5, the T_g of this polymer showed an increase of about 30°C in its value at all of the levels studied. This behavior was clearly maximized when the β lg quantities increased. However, when films formed at pH values of 10, 7, or 5.5 were used, the pH's contribution to T_g was modest, about 6–7°C only. These observations may be explained by the electrostatic interactions, which increased the T_g values of the materials when pH 2.5 was used in the formation of the films.

When we analyzed the T_m of blends, the following could be said. First, the films created at pH 2.5 had no melting point; in other words, the polymer became completely amorphous. Second, the T_m values were higher when the molecular mass of PVA was increased because of the larger size of the molecules.

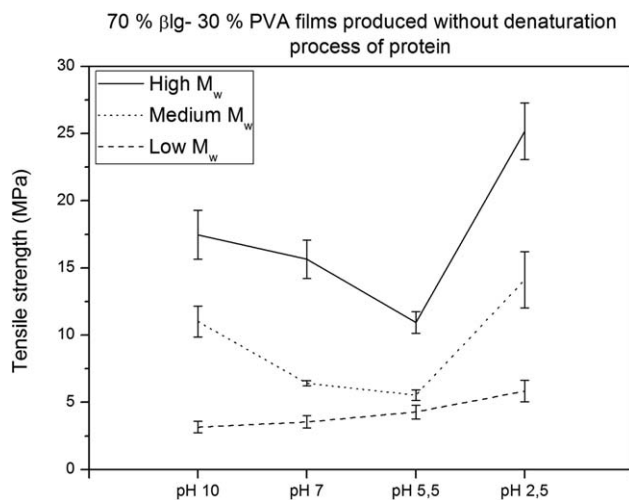


Figure 10. Tensile strength values at different pHs with various molecular weights of PVA.

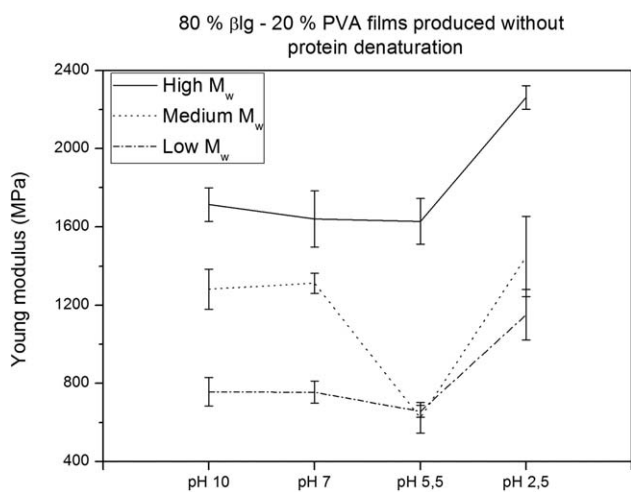


Figure 11. Young's modulus values at different pHs with various molecular weights of PVA.

When we varied the protein quantity and used a PVA of a high molecular mass, this brought about a decrease in the T_m values, but this effect was not detected when we used a PVA of medium molecular mass (see Figure 5). This behavior indicated that in the first case, as is common when a lower molecular mass element is added to a polymer, the crystallization process was hindered in some way. This means that some kind of interaction was happening between components. However, this did not seem to occur in the second case because no T_m difference was measured; this indicated a lower level of interaction. On the other hand, no important variations in the T_m 's were seen when we applied the denaturation process to the protein.

TGA

The TGA technique was used to analyze the thermal stability of the polymers with the application of a temperature scan. In this case, the aim was to corroborate the behavior recorded in the DSC experiments. Different results were expected from films

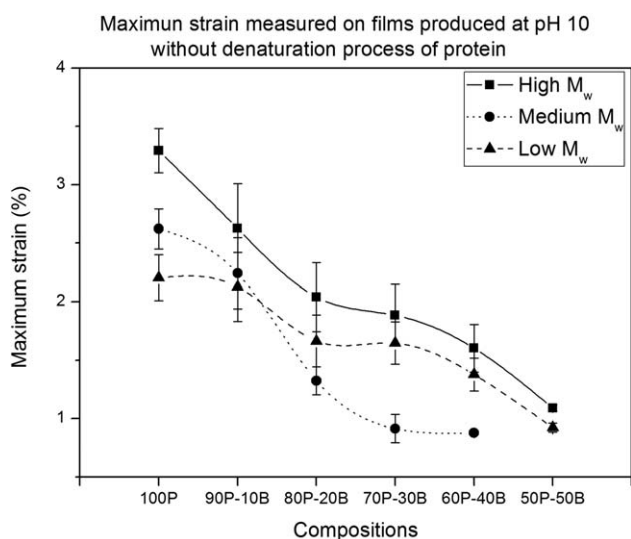


Figure 12. Maximum strain variations measured with various compositions and with different molecular weights of PVA.

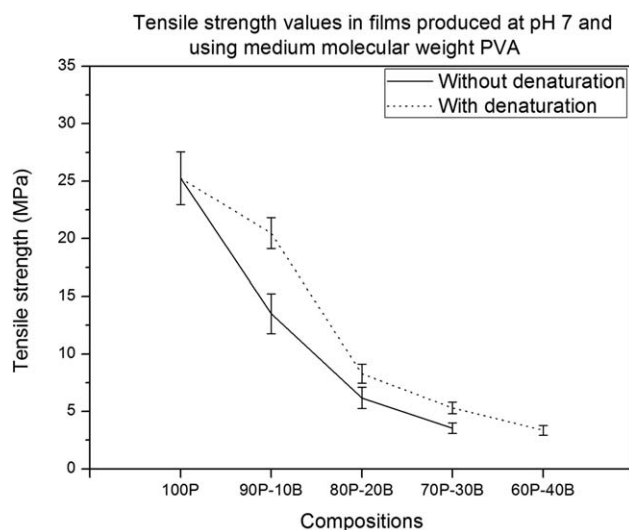


Figure 13. Tensile strength variations measured with various compositions.

created at pH 2.5 compared to those created at pH 10, 7 or 5.5. An additional objective was to confirm the behavior detected when the effect of the denaturation process of protein was studied; it was surprisingly undefined when high and low molecular masses of PVA were used.

When the effects of the pH and molecular mass of the PVA in the mixtures were analyzed in $T_{\text{Degradation}}$ (see Figures 6 and 7), the results indicate that films based on pH 2.5 were clearly different from those obtained with other pHs. In this way, the samples at acidic pH presented higher $T_{\text{Degradation}}$'s than those created at pH 10, 7, or 5.5.

Therefore, we concluded that the films with electrostatic interactions in their structure presented high $T_{\text{Degradation}}$'s and high T_g values, as was demonstrated in the DSC experiments. At pH 10, 7, or 5.5, interactions between the components were not so strong, and that fact was reflected on $T_{\text{Degradation}}$'s and T_g values obtained.

Furthermore, blends obtained with higher molecular masses of PVA presented lower $T_{\text{Degradation}}$ values. This behavior was attributed to the length of the polymeric chain: longer chains were present when the molecular weight of a polymer was increased, and they had the greater possibility of being interrupted than the shorter ones. The $T_{\text{Degradation}}$'s recorded for mixtures with low molecular masses of PVA were closer to the behavior of the pure protein.

On the other hand, the study of the denaturation effect on the $T_{\text{Degradation}}$ of mixtures had nearly the same results as those obtained from the DSC analysis (see Figure 8). Samples made of high and low molecular masses of PVA did not appear to follow a defined pattern, but when a PVA of medium molecular mass was used, the denaturation process raised the $T_{\text{Degradation}}$ values of the films. Therefore, we concluded that the thermal treatment in this case improved the capacity of proteins to interact with surrounding molecules; this seemed to generate a stronger net in the material, and so, higher $T_{\text{Degradation}}$'s were measured. In contrast, other effects seemed to prevail over the

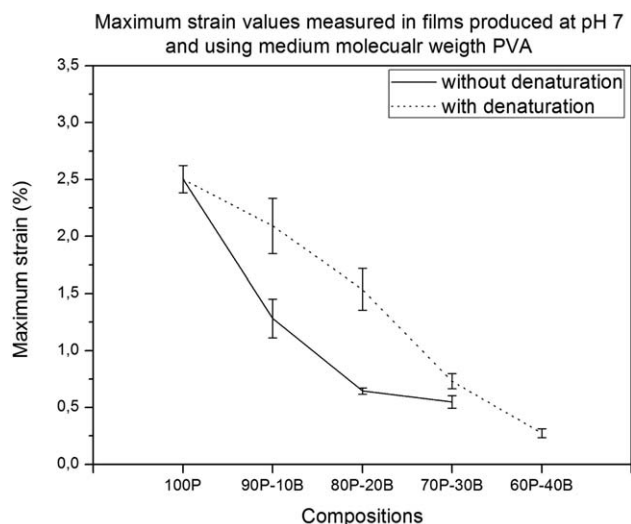


Figure 14. Maximum strain variations measured with various compositions.

effect of denaturation when high and low molecular masses of PVA were used. Thus, the results obtained in the DSC experiments were corroborated with TGA.

Mechanical Characterization

The aim with these tests was to analyze three mechanical properties of the films produced under different conditions: Young's modulus, tensile strength, and tensile strain.

As was expected, we observed that the films produced with a higher molecular weight of PVA had better mechanical properties than those made with lower molecular weights (see Figure 9).

When the pH in the film production process was varied, a higher tensile strength and a higher Young's modulus were achieved at pH 2.5, but the fragility also increased in those

films. In contrast, the films produced at pH 5.5, near the isoelectric point of the protein, showed poor mechanical properties (see Figures 10 and 11).

Higher protein contents produced brittle films with low strength as the plasticizer effect of PVA was less intense and the matrix where the protein was dispersed was less dense (see Figure 12). In all cases, low deformation values were recorded because the experiments were done at very low relative humidity so as to increase the rates of the experiments.

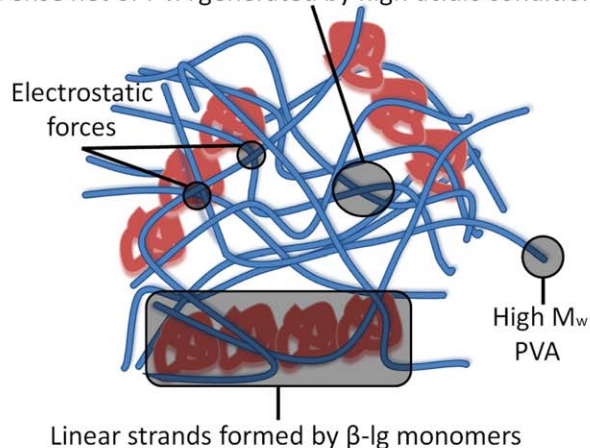
The protein denaturation process did not seem to play a significant role when high and low molecular weights of PVA were used. However, when a PVA of intermediate molecular weight was used to produce films, higher tensile strengths, deformation capabilities, and Young's moduli were achieved (see Figures 13 and 14).

Our aim, when we blended PVA with β lg, was to provide enough plasticity to the protein so that handling without any detrimental effects resulted. When less than 50% in β lg was added, this objective was achieved in nearly all cases. A reduction in the PVA proportions in the films reduced their mechanical properties, but the molecular weight of PVA used, the pH values in the production process, and the application or lack of application of the denaturation treatment to the protein also modified the results.

When a high-molecular-weight polymer was used in the production process, the mechanical properties were improved over those measured in films made with a low molecular weight. It is also worth mentioning that when medium-molecular-weight PVA was used, the weakest films were formed, so they were more difficult to handle and had poorer mechanical properties. This was indicative of a low level of compatibility between the components.

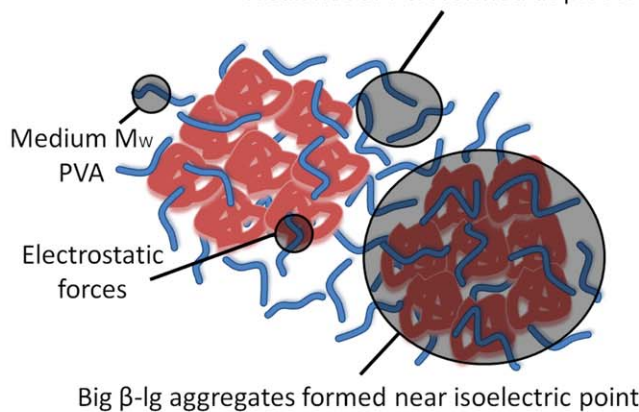
When different pHs were used to produce films, different results were obtained, as was the case in other protein–PVA systems.¹²

Dense net of PVA generated by high acidic conditions



(a)

Weak net of PVA formed at pH 5.5



(b)

Figure 15. Simplified structure of the films (a) obtained with a high- M_w PVA at pH 2.5 and with β lg concentrations of lower than 30 wt % and giving the best results and (b) obtained with a medium M_w PVA at pH 5.5 and with β lg concentrations of higher than 30 wt % and giving the worst results. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Extreme acid pH produced the highest mechanical properties, on the other hand, a pH near the isoelectric point of β lg led to films with much lower mechanical properties. These results were in accordance with those seen in thermal analysis, where higher T_g and $T_{\text{Degradation}}$ values seemed to bring about more intense interactions with extreme pH instead of basic or neutral pH. When the polymer–polymer and protein–polymer intermolecular bonds were promoted, then the material net was stronger, but its fragility was increased. The fragility problem was partially solved in films produced with a medium molecular weight of PVA when the thermal denaturation of β lg was used, although at other molecular weights, it did not seem to promote better characteristics.

Relations Between the Studied Variables and the Blend Structures

The four variables examined in this study were shown have some kind of effect on the blends but with different significances. Protein quantities limited the tendency of the protein to aggregate; in this study, we observed that when the protein–water concentrations exceeded 5 mg/mL (in films with 70% PVA–30% β lg, 60% PVA–40% β lg, and 50% PVA–50% β lg), the aggregation process of β lg was clearly distinguishable, and the films showed clear behavioral differences after production.

On the other hand, the pH value in solution produced two main effects. One effect observed was that pH 2.5 generated a large number of mainly hydrogen bonds in PVA, and as a result, the T_g increased significantly, the fusion temperature peak on DSC thermograms disappeared, and $T_{\text{Degradation}}$ rose. In addition, the protein experienced a different structural behavior when the pH was varied.^{1,2,13,14} At extreme pHs, the protein was found as a monomer, but it had a great tendency to aggregate and form linear strands, more at pH 2.5 than at pH 10. When β lg was mixed with PVA, we observed that the strands spread out in the highly bonded PVA matrix, and the blends showed increases in both T_g and $T_{\text{Degradation}}$ and, at the same time, were easier to handle. When films were produced at pH 7, the protein acquired a dimmer form, so bigger aggregates were distributed in the PVA matrix, which was less bonded than at acidic conditions; this resulted in weaker films. Near the isoelectric point, at pH 5.5, protein tends to form big aggregates or clusters made up of various monomers bonded by electrostatic forces; when they were inserted on weakly bonded PVA films, they had poor handling and thermal properties.

Moreover, the molecular mass choice of PVA was shown to be an important factor in the obtainment of good films. Better results were achieved when a high molecular mass was used, and the worst was achieved when a medium molecular mass polymer was used; this led to the conclusion that in the second case, the protein aggregation effect was bigger than in the other cases. It negatively affected both the film homogeneity and the properties. When the thermal denaturation process was applied to films made with a medium molecular mass, their handling and mechanical properties underwent an improvement. At the same time, $T_{\text{Degradation}}$ increased slightly; this showed that this treatment was useful when a protein nature predominated. However, this thermal treatment did not seem to have a signifi-

cant effect when high- and low-molecular-mass PVAs were used in film production, so we concluded that in those blends, the polymer nature predominated.

Figure 15 shows a hypothetical structure of the best and worst blends based on the experimental results obtained.

CONCLUSIONS

The first objective of developing films based on mixtures of PVA and β lg was achieved. The results obtained with the formation of the films confirm that the pH, molecular mass of the polymer, denaturation of the protein, and quantity of protein changed the way the protein and polymer interacted, and therefore, the properties of the films were different. The four parameters affected the interactions between PVA and β lg but in different ways.

We observed that the main factor that affected the formation of films was its composition, so when a certain quantity of protein was exceeded, 30 wt %, the plasticization effect of the polymer was too weak, and fragile films that were hard to detach were formed. In addition, the influence of the pH and molecular mass of PVA were also decisive factors in the attempt to obtain good films: acidic conditions and high-molecular-mass PVA helped to bring about better results.

When films produced under low pH conditions were analyzed by DSC and TGA, they showed very different T_g 's and $T_{\text{Degradation}}$'s than those films produced under other conditions. These transitions raised their values; this demonstrated that molecules under these conditions had more interactions between them, formed a stronger net, and as a result, the modulus of elasticity and mechanical strength were higher. The effect of the denaturation process applied to the protein was slight and, in most cases, was covered by the effects of the pH and the molecular mass of the polymer.

The observation of interactions between β lg and PVA with IR spectroscopy was tried, but no successful results were obtained; perhaps another method could have been used to achieve better results. With regard to the production process, it would be interesting to test film production from melted components as it is the most common way of producing plastic films industrially. At this moment in time, these studies have not started yet.

ACKNOWLEDGMENTS

The authors are grateful for funds from the Basque Government, Department of Education, Universities and Research (contract grant numbers GIC10/152-IT-334-10 and GIC12/161-IT-632-13) and the Ministry of Economy and Competitiveness (contract grant number BIO2010–21542-C02-01), and Juan Carlos Arboleya and Idoia Olabarrieta from AZTI–Tecnalia are thanked for their technical advice.

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