Mechanical and Barrier Properties of Cross-Linked Soy and Whey Protein Based Films

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Sterilized biofilms based on soy protein isolate (SPI, S system) and a 1:1 mixture of SPI and whey protein isolate (WPI, SW system) were achieved through the formation of cross-links by means of γ-irradiation combined with thermal treatments. The effect of the incorporation of carboxymethylcellulose (CMC) and poly(vinyl alcohol) was also examined. γ-Irradiation combined with thermal treatment improved significantly the mechanical properties, namely, puncture strength and puncture deformation, for all types of films. Irradiated formulations that contain CMC behave more similarly as elastomers. CMC showed also significant improvements of the barrier properties, namely, water vapor permeability, for irradiated films of the S system and for non-irradiated films of the SW system.

Keywords: *γ-Irradiation; cross-linking; soy protein; whey protein; carboxymethylcellulose; biofilms; poly(vinyl alcohol)*

INTRODUCTION

The past decade has seen considerable interest in the development of protein-based biodegradable edible films and coatings due to their application in the food industry as substitute for traditional plastic films (*1*). Many publications have revealed their effectiveness to prevent quality changes in processed food and thus to enhance shelf life by serving as selective barriers to moisture transfer, oxygen uptake, and loss of volatile flavors and aromas, as well as lipid oxidation (*1*, *2*).

Polysaccharides, proteins, and lipids or a combination of any of these macromolecules were investigated as film-forming agents (*2*, *3*). Soy protein isolate (SPI) is a mixture of proteins with different molecular properties. Most soy proteins, ~90%, are storage proteins, viz., globulins. Recent reports pointed out the use of SPI to develop edible and biodegradable films (*4*, *5*). Films produced from SPI were found to be excellent oxygen barriers (*6*, *7*). Whey proteins and caseinates are milk proteins, which have also been extensively studied owing to their excellent nutritional value and their numerous functional properties, which are important for the formation of edible films (*1*, *8*). WPC are concentrated whey (~70% proteins), whereas WPI are whey isolate (~90% proteins). Edible films based on whey proteins were reported to be flavorless, tasteless, and flexible, and, depending on the formulation, they varied from transparent to translucent (*1*). All of these characteristics make them suitable for applications in food sciences and technology.

Protein films exhibit poor water vapor barrier properties due to the hydrophilic nature of their amino acid groups (*2*, *9*). Recent studies have concentrated on improving protein film mechanical and barrier properties (*10*−*12*). The increase of cohesion between protein polypeptide chains was thought to be effective for the improvement of the barrier properties of the films. For instance, the cross-linking of proteins by means of chemical, enzymatic, or physical treatments was reported to improve the permeability as well as the mechanical properties. Attempts have been made to stabilize native proteins by inducing the formation of hydrogen, electrostatic, and covalent bonds (*13*). For example, improvements in protein functionality by cross-linking the soybean 11S protein fraction and whey protein isolate using guinea pig liver transglutaminase has been reported by Yildirim et al. (*14*). However, high production cost and limited availability of transglutaminase have limited its potential use in food systems. Electrostatic complexes between proteins and acidic polysaccharides, such as alginate, pectate, and carboxymethylcellulose (CMC), are also interesting mechanisms. However, these complexes are very unstable due to their sensitivity to pH changes (*15*, *16*).

Heat treatment is well-known to generate cross-links in some proteins, such as soy (*10*) and whey (*17*). Indeed, heating favors soy protein cross-linking by disrupting the protein structure and exposing sulfhydryl and hydrophobic groups (*18*, *19*). Sulfhydryl groups contained in 11S protein were reported to be responsible for the formation of disulfide linkages, resulting thus in the formation of a three-dimensional network (*18*, *19*).

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γ-Irradiation was recently reported to increase the cohesive strength of the protein by the formation of cross-links (*20*). Indeed, the irradiation of aqueous protein solutions generates hydroxyl radicals (•OH) that produce stable compounds (*21*). Sulfur and aromatic amino acids react more readily with free radicals than aliphatic amino acids; particularly, •OH reacts readily with aromatic residues (*22*). As an example, when phenylalanine reacts with •OH, tyrosine isomers are generated (*21*). Tyrosine is also sensitive to •OH attack. Indeed, tyrosyl (phenoxyl) radicals are produced as a result of hydrogen abstraction by •OH. Tyrosyl radicals may then react with other tyrosyl radicals or with a tyrosine molecule to form several stable biphenolic compounds, in which the phenolic moieties are linked through a covalent bond (*20*, *23*). The formation of biphenolic compounds is certainly one mechanism for protein aggregation, although other cross-links can also be formed (*24*). The γ-irradiation method presents some conveniences: it is a well-known process for the sterilization of foods (*25*).

Edible films and coatings discussed previously contain plasticizers. These additives are important as they improve the mechanical properties, viz., the flexibility, but also reduce the melt viscosity to facilitate the processability of the biofilms through reduction in intermolecular forces between adjacent polymer chains (*26*, *27*). Moreover, plasticizers were reported to play a second important role. Indeed, glycerol, a polyol, was found to enhance significantly the formation of cross-links within milk proteins chains, namely, caseinates (*20*). This effect was explained by the preferential binding concept elaborated by Gekko and Timasheff (*28*). Similar behaviors were reported with other plasticizer additives, such as propylene glycol and triethylene glycol (*29*).

Several more or less successful approaches were attempted to improve properties of soy protein films (*4*, *6*, *7*, *16*). Despite these numerous studies on soy proteins, most of them were conducted using simple model systems that contained only soy proteins dispersed in water. Few studies dealt with complex systems (*30*, *31*). It is important to study complex systems, for instance, soy proteins in combination with lipids, carbohydrates, etc., to utilize most effectively the functional behavior of soy proteins (*30*). Moreover, film color and flavor are important factors in terms of consumer acceptance of edible films. It is known that the common disadvantage of soy protein is its beany flavor (*30*). The addition of whey has been shown to be effective in diminishing the undesirable flavor, but fundamental research on the effect of mixing whey with SPI on its functional properties is lacking (*14*, *30*). Therefore, this investigation was conducted with the aim to produce biofilms based on soy protein isolate (SPI) alone (S system) and in combination with whey protein isolate (WPI) (SW system) by means of γ-irradiation and thermal treatment. The combination of γ-irradiation with heat is thought to generate films with improved mechanical properties and water vapor permeability. Moreover, the effects of the incorporation of CMC and poly(vinyl alcohol) (PVA) on both mechanical and barrier film properties were also assessed.

MATERIALS AND METHODS

Materials. SUPRO 500E SPI was provided by Dupont Campbell Protein Technologies (St. Louis, MO). WPI was lyophilized and dried for 3 h in a vacuum oven at 80 °C (model 19 laboratory oven, Precision Scientific Inc., Chicago, IL) from the solution purified at the Food Research and Development Centre (St-Hyacinthe, PQ, Canada). Low-viscosity CMC sodium salt was purchased from Sigma Chemical Co. (St. Louis, MO), and lyophilized PVA, 98% hydrolysis, was purchased from Aldrich Chemical Co. (St. Louis, MO). Sodium carbonate monohydrate reagent and glycerol (99.5%) were obtained from American Chemicals Ltd. (Montréal, PQ, Canada). Phosphorus pentaoxide was obtained from NDH Inc. (Toronto, ON, Canada).

Film Formation. SPI and WPI were solubilized in distilled water, under stirring, at 90 °C, to obtain an SPI/WPI ratio of 1:1, with a total protein concentration of 5% (w/v) in the film-forming solution. The pH was adjusted at 8.5 with 1 M Na_2CO_3, and when necessary, 0.25% (w/v) of CMC or 0.5% (w/v) of PVA was added. After complete solubilization, 2.5% (w/v) of glycerol was added, and the solution was then degassed under vacuum to remove dissolved air. The different protein solutions were poured into separate 240 mL amber bottles (Anachemia Sciences, Montreal, PQ, Canada) and irradiated together at the Canadian Irradiation Centre (CIC) at a dose of 32 kGy and a mean dose rate of 31.24 kGy/h, using a ^{60}Co source UC-15A (MDS-Nordion International Inc., Kanata, ON, Canada). Films were then cast by pipetting 5 mL of the solution onto smooth-rimmed 8.5 cm (i.d.) polymethacrylate (Plexiglas) plates, sitting on a leveled surface. Solutions were spread evenly and allowed to dry overnight at room temperature (20 ± 2 °C) in a climatic chamber (45−50% RH). Dried films could be peeled intact from the casting surface. The overall experiment was performed in two separate replications.

Film Thickness Measurements. Film thickness was measured using a Digimatic Indicator (Mitutoyo, Tokyo, Japan) at five random positions around the film, by slowly reducing the micrometer gap until the first indication of contact. Depending on the formulation, the average film thickness was in the range of 45−65 ± 2 μm.

Mechanical Properties. Puncture tests were carried out using a Stevens LFRA texture analyzer model TA/1000 (Stevens, NY), as described previously (*32*). Film samples (8.5 cm in diameter) were equilibrated in a desiccator at 56% RH with sodium bromide saturated solution. Then film samples were placed in the middle of two acrylic plates with a hole 3.2 cm in diameter, and the holder was held tightly by two screws. A cylindrical probe (2 mm in diameter) was moved perpendicularly at the film surface at a constant speed (1 mm/s) until it passed through the film. Strength and deformation values at the puncture point were used to determine hardness and deformation capacity of the film. Puncture strength was calculated by multiplying the reading value (grams) by the gravitational force; to avoid any thickness variations, the puncture strength calculated value was divided by the thickness of the film and was expressed in newtons per millimeter. The force−deformation curves were recorded, and the results were expressed in millimeters. Viscoelastic properties were evaluated using relaxation curves. The same procedure was used, but the probe was stopped and maintained at 3 mm deformation. The film was then allowed to relax. The force−time relaxation curves were recorded for 1 min following deformation. The relaxation coefficient Y(1 min), a dimensionless ratio, was used to represent the decay of the force and was calculated as $Y(1\text{ min}) = (F^0 - F^1)/F^0$, where F^0 and F^1 were forces recorded initially and after 1 min of relaxation, respectively (*33*). A low relaxation coefficient ($Y \rightarrow 0$) indicates high film elasticity, whereas a high relaxation coefficient ($Y \rightarrow 1$) indicates high film viscosity.

Water Vapor Permeability (WVP). WVP of films was determined gravimetrically using a modified ASTM (*34*) procedure. The films were sealed with silicone sealant high-vacuum grease from Dow Corning (Midland, MI) in a glass permeation cell containing phosphorus pentaoxide (0% RH, 0 mmHg water vapor pressure). All cast films were shiny on the side facing the casting plate surface and dull on the side facing the frying air during the measurements. The glass permeation cells were 3.8 cm (i.d.), 8.3 cm (o.d.), and 13.0 cm tall, with an exposed area of 12.56 cm^2. The cup was placed in a desiccator maintained at 100% RH (17.54 mmHg water vapor pressure,

at 20 °C) with distilled water. The water vapor transferred through the film and absorbed by the desiccant was determined from the weight gain of the cell. The assemblies were weighed initially and at 1, 2, 3, 4, 5, 6, 24, and 48 h intervals. Linear regression analysis gave very significant correlation ($r = 0.995$). Steady state conditions were assumed to be reached when the change in weight became constant over time (≈6 h), and the weights were recorded over 24 h for all samples. The WVP was determined as follows (*35*−*37*): WVP = $(wx)/AT(p_2 - p_1)$ (g·mm/m^2·day·mmHg), where w is the weight gain of the cup over time ($T = 24$ h), x is the thickness (mm), A is the area of exposed film (m^2), and $p_2 - p_1$ is the vapor pressure across the film (mmHg).

Size Exclusion Chromatography (SEC). Size exclusion chromatography was performed using a Varian Vista 5500 HPLC coupled with a Varian autosampler model 9090. Detection of the protein solution was done using a standard UV detector set at 280 nm. Two Supelco Progel TSK PWH and GMPW columns followed by two Waters Hydrogel columns (2000 and 500) were used for the molecular weight determination of the cross-linked proteins. The total molecular weight exclusion limit was 25×10^3 kDa based on linear poly(ethylene glycol) (PEG). The eluant (80% v/v aqueous and 20% v/v acetonitrile) was flushed through the columns at a flow rate of 0.8 mL/min. The aqueous portion of the eluant was 0.02 M tris buffer (pH 8) and 0.1 M NaCl. The molecular weight calibration curve was established using a series of protein molecular weight markers (Sigma, MW-GF-1000) ranging from 2×10^3 to 29 kDa. All soluble protein solutions (0.5% w/v) were filtered on 0.45 μm prior to injection.

Statistical Analysis. Analysis of variance was employed to analyze statistically all results, using the program SPSS (SPSS Inc., version 6.1) Specific differences between types of films were determined by least significance difference (LSD). The Student t test was utilized to test the difference between irradiated and non-irradiated samples. All comparisons were made at a 5% level of significance. For each measurement, three replicates of seven film types were tested.

RESULTS AND DISCUSSION

SEC. Denatured SPI showed two main molecular weight peaks, in the range of ∼60 and ∼2000 kDa (Figure 1a). When γ-irradiation was combined with heating, further cross-links were generated in both systems investigated, the S system and the SW system, as suggested by the size exclusion chromatography elution patterns (Figures 1 and 2).

Irradiation of the S system at 32 kGy induced a decrease of the 60 kDa peak and an increase of a peak at ∼200−2000 kDa (Figure 1b). The shift of the low molecular weight moiety to higher values is interpreted by an enhancement of protein aggregation of the S system due to the formation of bityrosine (i.e., cross-links). It is clear from Figure 1 that the combination of γ-irradiation with thermal treatment generates a much more important protein aggregation because further cross-links are formed: bityrosine in addition to disulfide bonds. On the basis of the protein calibration curve, the effect of protein aggregation enhanced the molecular weight by >15-fold (Figure 1). The integration of the peak area indicated that this increase of the molecular weight affected only 15% of the total soy proteins. Previous studies have demonstrated that γ-irradiation of caseinate solutions induced the formation of bityrosine, resulting in an enhancement of the molecular weight of caseinate by more than 60-fold (*11*, *12*, *20*, *29*, *38*, *39*). Because the content of tyrosine in soy proteins is less than in caseinate, 3.3 versus 4.0%, respectively (*4*), less bityrosine (i.e., cross-links) is generated upon γ-irradiation, accounting thus for an aggregation behavior of least importance in soy proteins with respect to caseinate.

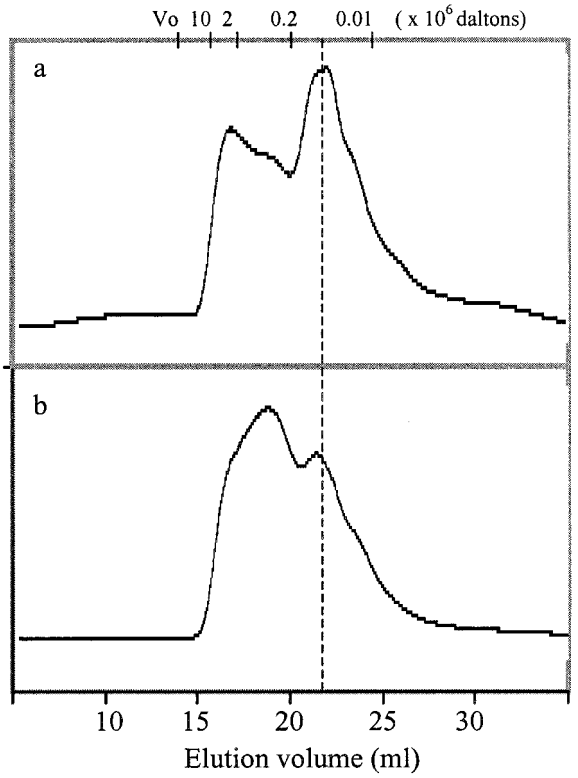

Figure 1. Elution profile of SPI (a) heated and (b) heated in combination with γ-irradiation at 32 kGy.

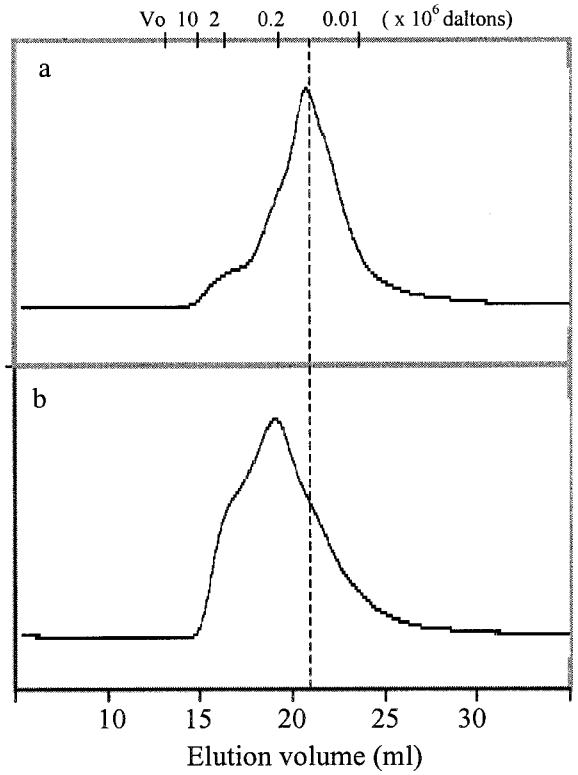

Figure 2. Elution profile of a 1:1 mixture of SPI and WPI (a) heated and (b) heated in combination with γ-irradiation at 32 kGy.

When SPI was mixed with WPI in a 1:1 ratio (SW system), the elution pattern exhibited a main peak at ∼60 kDa, with a shoulder at ∼2000 kDa (Figure 2a). The irradiation of the SW system at 32 kGy increased

Table 1. Puncture Strength of Protein-Based Edible Films[a]

film	composition (ratio)	puncture strength (N/mm) non-irradiated	puncture strength (N/mm) irradiated (32 kGy)
S	SPI:Gly (2:1)	31.53 ± 2.34[b]	43.30 ± 2.75[b]*
S1	SPI:Gly:CMC (20:10:1)	48.63 ± 2.67[d]	52.20 ± 2.52[d]*
S2	SPI:Gly:CMC:PVA (20:10:1:2)	52.49 ± 2.25[e]	59.00 ± 3.10[d]*
SW	SPI:WPI:Gly (1:1:1)	28.60 ± 2.40[a]	40.32 ± 2.87[a]*
SW1	SPI:WPI:Gly:CMC (10:10:10:1)	32.79 ± 2.83[b]	41.27 ± 3.52[ab]*
SW2	SPI:WPI:Gly:CMC:PVA (10:10:10:1:2)	37.48 ± 1.64[c]	46.07 ± 3.56[c]*

[a] S = films based on SPI; SW = films based on mixture of SPI and WPI; SPI = soy protein isolate; WPI = whey protein isolate; Gly = glycerol; CMC = carboxymethylcellulose; PVA = poly(vinyl alcohol). Means followed by different letters in each column are significantly different ($p \leq 0.05$). Means followed by an asterisk in each row are significantly different ($p \leq 0.05$).

Table 2. Puncture Deformation of Protein-Based Edible Films[a]

film	composition (ratio)	puncture deformation (N/mm) non-irradiated	puncture deformation (N/mm) irradiated (32 kGy)
S	SPI:Gly (2:1)	6.20 ± 0.30[e]	6.31 ± 0.25[c]
S1	SPI:Gly:CMC (20:10:1)	4.80 ± 0.34[c]	6.21 ± 0.35[c]*
S2	SPI:Gly:CMC:PVA (20:10:1:2)	5.07 ± 0.35[d]	5.85 ± 0.26[b]*
SW	SPI:WPI:Gly (1:1:1)	3.71 ± 0.30[a]	4.78 ± 0.45[a]*
SW1	SPI:WPI:Gly:CMC (10:10:10:1)	4.12 ± 0.49[b]	4.76 ± 0.44[a]*
SW2	SPI:WPI:Gly:CMC:PVA (10:10:10:1:2)	4.38 ± 0.21[b]	4.83 ± 0.26[a]*

[a] S = films based on SPI; SW = films based on mixture of SPI and WPI; SPI = soy protein isolate; WPI = whey protein isolate; Gly = glycerol; CMC = carboxymethylcellulose; PVA = poly(vinyl alcohol). Means followed by different letters in each column are significantly different ($p \leq 0.05$). Means followed by an asterisk in each row are significantly different ($p \leq 0.05$).

protein aggregation, as confirmed by the shift of molecular weight from ~60 to ~200 kDa and the increase of the intensity of the high molecular weight moiety at 2000 kDa (Figure 2b). The formation of bityrosine induced by γ-irradiation is responsible for this shift of molecular weight toward higher values (Figure 2b). However, the protein aggregation in the SW system is not as important as in the S system, the shift being <5-fold versus >15-fold, respectively. This behavior is due to the fact that WPI contains even fewer tyrosine residues than SPI, 2.5 versus 3.3%, respectively (*4*). With respect to other complex systems investigated so far, the molecular weight shift was reported to be more important in the caseinate/WPI system (*12*). The higher amount of tyrosine in caseinate (4.0%) accounts for this behavior.

Nevertheless, the use of γ-irradiation has proven to be efficient because it resulted in an enhancement of cross-links in both systems investigated.

Mechanical Properties. Cross-links confer to any material elastomeric properties, if the cross-link density does not exceed a critical value (*27*, *40*). Indeed, the higher this value is, viz., the higher the branched chains are, the more rigid is the material. The effect of the combination of γ-irradiation with heat treatment in the S and SW systems on the mechanical properties of films was also investigated, namely, the puncture strength and puncture deformation.

Tables 1 and 2 illustrate a significant increase of the puncture strength as well as a significant increase of the puncture deformation following the irradiation of the different formulations at 32 kGy. These results clearly emphasize that the combination of γ-irradiation with thermal treatment enhances the formation of cross-links enabling, thus, the formation of a film with improved mechanical properties. According to Brault et al. (*20*), the use of γ-irradiation can increase the mechanical properties of films by the formation of bityrosine between two protein chains. Heat treatment (30 min, 90 °C) could also promote cross-linking via the formation of disulfide and hydrophobic bonds. According to Lindsay (*41*), the cysteine groups present in proteins can undergo polymerization via sulfhydryl−disulfide interchange reactions during heating to form a continuous covalent network upon cooling.

The enhancement of the mechanical behavior of the films was found to be strongly related to the formulations. In both systems investigated, the effect of the irradiation on the puncture strength was weaker in the presence of glycerol only (S and SW). In the S system a significant improvement ($p \leq 0.05$) of ~37% was observed, whereas in the more complex SW system, γ-irradiation increased significantly the puncture strength of ~41% (Table 1). The addition of CMC increased significantly the puncture strength of both systems (S1 and SW1): from 31.5 to 48.6 N/mm in the S system treated thermally and from 28.6 to 32.8 N/mm in the SW system following a similar treatment (Table 1). γ-Irradiation resulted in significant increases of the puncture strength of ~7 and ~26% in the S and SW systems, respectively (Table 1). The incorporation of PVA in both systems (S2 and SW2) enhances even more the puncture strength, going from 48.6 to 52.5 N/mm in the heated S system and from 37.5 to 46.1 N/mm in the non-irradiated SW system (Table 1). As for the previous formulations, once γ-irradiation was applied to both systems that contained CMC and PVA (S2 and SW2), a significant improvement of the puncture strength values occurred: ~12% in the S system versus ~23% in the SW system (Table 1).

Between the formulations investigated in both systems, the measured puncture strength values were highest in irradiated S2 and SW2 formulations, viz., in the presence of CMC and PVA (Table 1). The puncture strength values can be related to the amount of cross-links produced, which can be bityrosine and disulfide bridges, during the irradiation process. Consequently, cross-links were significantly more important in the formulations that contained the most excipients, especially PVA, viz., S2 and SW2 (Table 1). This result can be explained by the fact that PVA also undergoes cross-linking following γ-irradiation (*42*). As mentioned earlier, upon radiolysis hydroxyl radicals (•OH) are generated from water (*20*, *21*). These radicals are the main precursors of macroradicals and could react with PVA chains by hydrogen abstraction.

If we compare only irradiated formulations in S systems, the presence of CMC (S1) in addition to γ-irradiation had a more pronounced effect on the puncture strength value, with a 21% increase of the puncture strength (Table 1). Addition of PVA to this mixture resulted in an additional 13% increase of puncture strength. In the SW system, a more pronounced effect was obtained in the presence of both CMC and PVA (SW2), in addition to γ-irradiation representing an increase of 12% of puncture strength as compared to 2% in the presence of CMC only. Hence,

more cross-links were generated in S1 and SW2 formulations. These results showed that γ-irradiation is less efficient in increasing the puncture strength in formulations containing WPI than in S formulations. According to SEC results, γ-irradiation induced fewer molecular weight changes in the presence of whey protein. These results could be explained by the fact that WPI contains fewer tyrosine residues than SPI (2.5 and 3.3%, respectively) (*4*), reducing the efficiency of γ-irradiation to produce bityrosine in whey protein as compared to soy protein. Similar results were also obtained by Vachon et al. (*12*). In their study, γ-irradiation was less efficient in producing cross-linking in whey protein than in casein. However, heat treatment combined with γ-irradiation led to the formation of an insoluble fraction. These results suggest that both heating and γ-irradiation are efficient in producing intramolecular cross-linking in the protein solutions. Heating produces more disulfide linkages, whereas γ-irradiation produces more bityrosine linkages.

Finally, the puncture strengths of the biofilms were significantly more important in the non-irradiated and irradiated S system than in the SW system (Table 1). Consequently, the generation of cross-links was more important in the S system.

Except for the S formulation, the puncture deformation increased significantly upon γ-irradiation at 32 kGy (Table 2), indicating that the irradiation treatment generated elastic, that is, flexible films. In formulations that contained only glycerol, S and SW, γ-irradiation had a significant impact on only the SW system. Indeed, following the irradiation treatment, a significant improvement of ~22% of the puncture deformation was observed in SW (Table 2). The improvement of the elasticity is further confirmed by the significant decrease of ~27% of the relaxation coefficient. The relaxation coefficient of the irradiated SW system was 0.64 as compared to 0.87 for the non-irradiated SW system (data not shown). The addition of CMC decreased significantly the puncture deformation in the S system, from 6.20 to 4.80 mm in the S1 formulation treated thermally (Table 2). In the SW system, the opposite behavior occurred, viz., a significant increase of the puncture deformation from 3.71 to 4.12 mm upon the addition of CMC (Table 2). The addition of CMC did not change significantly the relaxation coefficient in the S system (S, 0.58; S1, 0.59). However, incorporation of CMC in the SW system resulted in a significant ($p \leq 0.05$) reduction of the relaxation coefficient, with lower values in the SW1 formulation. Values for SW and SW1 were, respectively, 0.87 and 0.59. This is indicative of more elasticity in SW, as previously reported by Peleg (*33*). Except for the S system, γ-irradiation resulted in significant increases of the puncture deformation. Depending on the formulation, the increase varied from 9 to 22% (Table 2). The incorporation of PVA in both systems (S2 and SW2) enhances the puncture deformation from 4.80 to 5.07 mm in the non-irradiated S2 formulation and from 4.12 to 4.38 mm in the non-irradiated SW2 formulation (Table 2). A significant decrease of ~11% of the relaxation coefficient resulted in S2 (from 0.59 to 0.52), whereas in the SW system the relaxation coefficient did not vary significantly (~0.58, not shown). On the other hand, the puncture deformation in S2 was significantly lower than the puncture deformation in S, 5.07 versus 6.20 mm (Table 2). The puncture deformation value in the SW2 formulation was significantly higher with respect to SW formulation (Table 2).

As noticed for the puncture strength, puncture deformation values were strongly dependent on the formulation of films. The highest values of puncture strength were obtained for S1 and S2. The highest measured puncture deformation value was obtained with the S and S2 formulations (Table 3). Therefore, the presence of excipients in the S system did not influence the elastic behavior of the biofilms, whereas the addition of CMC and PVA to SW improved the elasticity of the films, as confirmed by the decrease of the relaxation coefficient in SW1 and SW2 with respect to the SW system. Values were, respectively, 0.59, 0.57, and 0.87 for the SW1, SW2, and SW systems.

The contribution of γ-irradiation was more important in S1 and SW formulations. In both cases, a significant enhancement of ~23% of the puncture deformation was obtained, whereas lowest contributions of the γ-irradiation on the puncture deformation values were found for the formulations corresponding to S1, S2, SW1, and SW2. These results can be interpreted in terms of bityrosine content, that is, cross-links or branched chains. It seems that too many cross-links are produced in S, S2, SW1, and SW2, leading to a stiff film, whereas the amount of branched chains produced in S1 and SW formulations seemed to be just enough to confer viscoelastic properties to the films. The puncture deformation of the biofilms was significantly more important in the non-irradiated and irradiated S systems than in the SW system (Table 2). Therefore, the addition of WPI in the formulation tended to give stiffer films with respect to SPI, as confirmed by the higher value of the relaxation coefficient found in SW (0.87) versus S (0.58) formulations (data not shown).

The results discussed so far demonstrate that the mechanical behavior of the biofilms and the effect of the irradiation treatment are strongly sensitive to the formulation (Table 1). The highest effect of γ-irradiation on the formation of bityrosine, that is, cross-links, in the S and SW systems, leading to increases of, respectively, 27 and 29% of the puncture strength value (Table 1). However, γ-irradiation did not reduce the puncture deformation (Table 2). It seems that too many cross-links were produced, leading to a stiff three-dimensional network. On the other hand, the most important effect of the irradiation on the deformation was observed when CMC was added to the formulation S1 (Table 2): an increase of 23% was measured after irradiation. These findings can be explained by the fact that lower amounts of cross-links were produced in S1 with respect to S, as confirmed by the low effect of γ-irradiation on the puncture strength values (Table 1). As a consequence, films behave more similarly as elastomers.

The highest improvement of the mechanical properties following γ-irradiation in the SW system was observed in the SW formulation: an increase of 22% was noticed between the non-irradiated and irradiated samples (Table 2). It seems that the cross-links generated with this formulation were not too many but rather sufficient to confer good elastomeric properties to the film (Table 2). Although the addition of CMC to the formulation, SW1, lowered the amount of cross-links, as inferred from the lower increase of the puncture strength value, viz., 21%, an important increase of 13% of the deformation occurred after γ-irradiation was applied (Table 2), which is not insignificant.

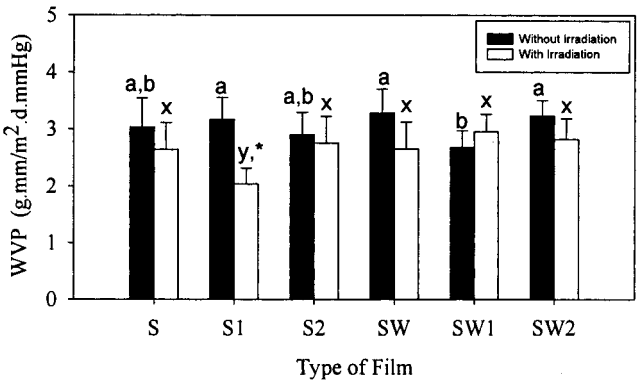

Figure 3. WVP of films based on SPI and a 1:1 mixture of SPI and WPI. Means with different letters are significantly different ($p < 0.05$). Irradiated samples with asterisks are significantly different ($p < 0.05$) from the non-irradiated ones.

The largest increase of puncture deformation (23%) by the irradiation treatment was observed with S1 and SW formulations, suggesting that cross-links produced are near optimal in these formulations, irradiated at 32 kGy.

WVP. Figure 3 presents the WVP for the formulations investigated, expressed in g·mm/m^2·24h·mmHg. There is no significant impact of the formulation on the WVP in the S system (Figure 3). Values of WVP range between 2.9 and 3.16 in the non-irradiated S system. The contribution of the irradiation treatment was found to be significant only in the S1 formulation, viz., in the presence of CMC. Indeed, upon irradiation the WVP of S1 went from 3.16 to 2.03, representing a decrease of ~36% (Figure 3). This behavior could be explained by the increase of protein−protein interactions resulting from the formation of bityrosine, viz., cross-links, which results in a decrease of the diffusivity of the permeant (*43*). As for the SW system, the sole significant impact coming from the formulation was observed in the SW1 formulation. The presence of CMC gave films with a lower WVP with respect to SW and SW2 formulations: 2.68 versus 3.23−3.28, respectively. Unlike the S system, the irradiation treatment did not influence the WVP of films obtained in the SW systems. Moreover, the addition of WPI did not bring any value to the barrier properties.

The addition of CMC was effective in both systems investigated. In the S system, its effect was combined with γ-irradiation, whereas in the SW system the effect was observed in the non-irradiated formulation. The impact of CMC could be attributed to its characteristic to form electrostatic complexes with proteins (*15*, *16*). However, the effect of CMC can also be explained by one of the basic functions of CMC, which is to impart viscosity to the aqueous phase, thereby stabilizing the other ingredients or preventing syneresis (*43*). Although more cross-links were generated in the presence of PVA, this excipient did not further bring down the permeability of the corresponding films with respect to CMC.

The WVP values obtained with our systems are comparable to those reported earlier by Stuchell and Krochta (*4*) and Jo et al. (*10*) for soy protein films. Non-irradiated films showed WVP values in the ranges of 2.9−3.2 for the S system and 2.7−3.3 for the SW system, whereas the WVP values for irradiated films were in the ranges of 2.0−2.75 for the S system and 2.65−2.95 for the SW system. Therefore, it can be concluded that the addition of WPI did not have a significant impact on the barrier properties of the biofilms. Nonetheless, it is worth emphasizing that the barrier properties for the SW system, viz., a 1:1 mixture of SPI/WPI, seem to be more efficient than those reported for the SPI/PEO system: 2.65-3.3 versus 34.6−46.1, respectively (*31*).

Conclusion. γ-Irradiation treatment generated cross-links, increasing the molecular weight of soy protein. This investigation has clearly demonstrated the usefulness of γ-irradiation to improve mechanical properties of films based on SPI (S system) and on mixtures of SPI with WPI (SW system). Both mechanical properties, puncture strength and puncture deformation, increased with the irradiation treatment. Films based on SPI (S system) presented higher puncture strength and puncture deformation values than SW system. It must be emphasized that for either films based on SPI (S system) or based on SPI with WPI (SW system), there was an increasing trend of puncture strength values as CMC and PVA were added. CMC increased significantly the puncture strength in both systems (S and SW). Puncture strength had its higher value with PVA in the S system. PVA was the more efficient compound in increasing the puncture strength in S and SW systems, whereas in relation to the viscoelasticity, it reduced significantly this property, contributing to a low relaxation coefficient in both systems (S and SW). The contribution of CMC in viscoelasticity was favorable only in the SW system, when it decreased significantly the relaxation coefficient. The WVP values for irradiated films were smaller than for un-irradiated ones, except for one case of the SW1 system. The addition of PVA showed no contribution on permeability, whereas CMC presented some improvements but not a trend. In the S system, CMC reduced significantly the WVP value when combined with γ-irradiation, and in the SW system, CMC caused a significant decrease for non-irradiated films.

ABBREVIATIONS USED

CMC, carboxymethylcellulose; PVA, poly(vinyl alcohol); PDD, puncture deformation; PS, puncture strength; RH, relative humidity; S, films based on soy protein isolate; SPI, soy protein isolate; SW, films based on mixture of soy protein isolate and whey protein isolate; WPC, whey protein concentrate; WPI, whey protein isolate; WVP, water vapor permeability; PEO, poly(ethylene oxide).

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