Mechanical and Structural Properties of Milk Protein Edible Films Cross-Linked by Heating and γ-Irradiation

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The mechanical properties of cross-linked edible films based on calcium caseinate and two type of whey proteins (commercial and isolate) were investigated. Cross-linking of the proteins was carried out using thermal and radiative treatments. Size-exclusion chromatography performed on the cross-linked proteins showed that γ-irradiation increased the molecular weight of calcium caseinate, while it changed little for the whey proteins. However, heating of the whey protein solution induced cross-linking. For both cross-linked proteins, the molecular weight distribution was $\geq 2 \times 10^3$ kDa. Combined thermal and radiative treatments were applied to protein formulations with various ratios of calcium caseinate and whey proteins. Whey protein isolate could replace up to 50% of calcium caseinate without decreasing the puncture strength of the films. Films based on commercial whey protein and calcium caseinate were weaker than those containing whey protein isolate. Electron microscopy showed that the mechanical characteristics of these films are closely related to their microstructures.

Keywords: *γ-Irradiation; cross-linking; milk protein; edible films*

INTRODUCTION

Increased consumer demand for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging amounts have led to increased interest for edible film research (Chen, 1995). Edible films offer potential solutions to these concerns, by serving as a barrier to water, oxygen, carbon dioxide, and lipid transfer in food systems. Edible films can also improve food system mechanical properties and control the loss of volatile flavors and aromas (McHugh and Krochta, 1994a; Chen, 1995). Furthermore, biodegradable packagings produced from food protein offer the greatest opportunities since their biodegradability and environmental compatibility are assured (Krochta and De Mulder-Johnston, 1997).

Many food proteins such as corn zein, wheat gluten, soy protein isolate, whey protein isolate, and caseins have been formulated into edible films or coatings (Herald et al., 1996; Wu and Bates, 1972; McHugh and Krochta, 1994b). The highly hydrophilic nature of these proteins limits their ability to provide desired edible film functions. To improve the moisture barrier properties of these films, waxes and fatty acids were added to film formulations (Gontard et al., 1994). Another approach for improving the mechanical strength and water resistance of these materials is protein cross-linking. Enzymes, like transglutaminase, have been used to cross-link many food proteins (Chobert et al., 1996) including caseins (Ikura et al., 1980; Motoki et al., 1987). However, the use of enzymes is generally costly, which limits their application on a larger scale.

The use of physical treatments such as ultrasound (Banerjee et al., 1996) and γ-irradiation (Brault et al., 1997; Mezgheni et al., 1998; Ressouany et al., 1998) improved the mechanical strength of these films at much lower costs. γ-Irradiation is slowly becoming accepted in the food industry as a means of improving the shelf life of various fruits and vegetables and eliminating bacterial contamination in meats (Pszczola, 1997). Furthermore, γ-irradiation generates sterile biomaterials which could be used in pharmaceutical or biomedical applications (Kaetsu, 1995).

The increased mechanical strength in γ-irradiated calcium caseinate solutions results from the formation of cross-links which confer elastomeric properties to the material. These cross-links also improve the water resistance of these films (Brault et al., 1997). The present study focuses on the effect of combined physical treatments (heat and irradiation) on the mechanical and structural properties of milk protein-based edible films. The effects of γ-irradiation and thermal treatment of calcium caseinate and whey protein solutions was studied using size-exclusion chromatography. The puncture strength and the viscoelastic properties of film formulations containing different protein ratios was correlated with transmission electron microscopy observations.

MATERIALS AND METHODS

Reagents. Calcium caseinate (Alanate 380, 91.8% w/w protein) was provided by New Zealand Milk Product Inc. (Santa Rosa, CA). Commercial whey protein concentrate

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Table 1. Protein, Ash, Fat, and Lactose Content of Calcium Caseinate (Alanate 380), Commercial Whey Protein Concentrate (WPC, Sapro-75), and Whey Protein Isolate (WPI)

	protein, %	ash, %	H_2O, %	fat, %	lactose, %
calcium caseinate (Alanate 380)[a]	91.8	3.8	3.6	0.7	0.1
commercial whey protein concentrate (WPC)[b]	76.27	3.72	0	5.79	14.22
whey protein isolate (WPI)[c]	90.57	2.32	3.61	none	3.5

[a] Technical sheet, New Zealand Milk Protein Inc., Santa Rosa, CA, 1999. [b] Technical sheet, Saputo Cheeses Ltd., Montreal, PQ, Canada, 1999. [c] Food Research and development Centre, Agriculture and Agri-Food Canada, 1999.

(Sapro-75, 76.27% w/w protein) was purchased from Saputo Cheeses Ltd. (Montreal, PQ, Canada). Whey protein isolate (WPI, 90.57% w/w protein) was prepared at the Food Research Center of Agriculture and Agri-food Canada. Whey protein isolate was produced from permeate obtained by tangential membrane microfiltration. Fresh skim milk was microfiltered 3-fold at 50 °C using an MF pilot cross-flow unit as described previously by St-Gelais et al. (1995). The proteins contained in the permeate were concentrated 25-fold at 50 °C by ultrafiltration using a UF pilot unit equipped with a Romicon membrane (PM 10, total surface area 1.3 m^2). The concentrate was diafiltered 5-fold by constant addition of water and freeze-dried before use in order to obtain WPI. Carboxymethyl cellulose sodium salt (CMC, low viscosity) was obtained from Sigma Chemicals (St. Louis, MO). Glycerol (99.5%, reagent grade) was purchased from American Chemicals Ltd. (Montreal, PQ, Canada). Acetronitrile (99.95%) was obtained from Anachemia Chemicals (Montreal, PQ, Canada). All products were used as received without further purification.

Method for Film Preparation. All formulations were based on 5% w/w total protein, 2.5% glycerol, and 0.25% CMC. Different protein sources were used for the film formulations. The content in protein, fat, lactose, and ashes is summarized in Table 1. The components were solubilized in distilled water, under stirring, and the solutions were heated at 90 °C for 30 min. They were then degassed under vacuum to remove dissolved air and flushed under nitrogen according to Brault et al. (1997). Solutions were irradiated at a total dose of 32 kGy in a ^{60}Co underwater calibrator unit (UC-15; 17.33 kGy/h) (MDS Nordion, Kanata, ON, Canada) at the Canadian Irradiation Center. Films were then cast by pipetting 5 mL of the solution onto smooth-rimmed 8.5-cm internal diameter Petri dishes 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 were peeled intact from the casting surface.

Film Thickness Measurements. Film thickness was measured with a Mitutoyo Digimatic Indicator (Tokyo, Japan) at six random positions around the film. Depending on the formulation and irradiation dose, the average film thickness was in the range 45−60 (±2 μm).

Size-Exclusion Chromatograhy. Soluble protein were obtained by filtering 0.5% (w/v) of the protein solution through 0.45-μm nylon membrane filters (VWR, Nalge, Mississauga, ON, Canada). Size-exclusion chromatography was performed on the soluble protein fraction using a Varian Vista 5500 HPLC coupled with a Varian Auto Sampler model 9090. Proteins were determined on a standard UV detector set at 280 nm. Two Supelco (Bellefonte, PA) Progel TSK PWH and GMPW columns followed by two Waters Hydrogel columns (Waters, Mississauga, ON, Canada) (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 kD 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^{-1}. The aqueous portion of the eluant was 0.02 M tris buffer (pH = 8.0) and 0.1 M NaCl. The molecular weight calibration curve was established by using a set of protein molecular weight markers MW-GF-1000 (Sigma) ranging from 2×10^3 to 29 kD.

Determination of Insoluble Matter. The average dry weight of the films was determined on seven films by drying them in an oven at 45 °C until constant weight was achieved (6 or 7 days). Seven more films were dropped in 100 mL of boiling water for 30 min. The flasks were removed from the heat and the films remained in the water for another 24 h. After 24 h, the solid films were removed and dried in the oven as previously described. Results are calculated by the following equation:

$$\text{insoluble matter} = [\text{dry weight (solid residues)}/\text{dry weight (untreated film)}] \times 100 \quad (1)$$

Mechanical Properties of Films. Puncture tests were carried out on a Stevens LFRA texture analyzer model TA/1000 (New York, NY), as described previously by Gontard et al. (1992). Films were equilibrated for 48 h in a desiccator containing a saturated NaBr solution ensuring 56% relative humidity. A cylindrical probe (0.2-cm diameter) was moved perpendicularly to 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. To avoid any thickness variation, the puncture strength values were divided by the thickness of the film. The force-deformation curves were recorded. Viscoelastic properties were evaluated using relaxation curves. The same procedure was used, but the probe was stopped and maintained at 3-mm deformation. The parameter Y was calculated using the equation:

$$Y(1\ \text{min}) = (F^0 - F^1)/F^0 \quad (2)$$

where F^0 and F^1 were forces recorded initially and after 1 min of relaxation, respectively (Peleg, 1979). A low relaxation coefficient ($Y \rightarrow 0$) indicates high film elasticity whereas a high coefficient ($Y \rightarrow 1$) indicates high film viscosity.

Transmission Electron Microscopy (TEM). Dry films were first immersed in a solution of 2.5% glutaraldehyde in cacodylate buffer, washed and postfixed in 1.3% osmium tetroxide in collidine buffer. Samples were then dehydrated in acetone (25, 50, 75, 95, and 100%) before embedding in a SPURR resin. Polymerization of the resin proceeded at 60 °C for 24 h. Sections were made with an ultramicrotome (LKB 2128 Ultratome) using a diamond knife and transferred on Formvar-carbon coated grids. Sections were stained 20 min with uranyl acetate (5% in 50% ethanol) and 5 min with lead citrate. Grids were observed with an Hitachi 7100 transmission electron microscope operated at an accelerating voltage of 75 keV.

Statistical Analysis. Analysis of variance and Duncan multiple-range tests with $p \leq 0.05$ were used to analyze all results statistically. For puncture strength and deformation to puncture measurements, three replicates of seven films were tested. For viscoelasticity measurements, three replicate of three films were tested. The Student's t test was used and paired-comparison with $p \leq 0.05$ (Snedecor and Cochran, 1978).

RESULTS AND DISCUSSION

Size-Exclusion Chromatography. Figure 1 shows the elution curves obtained for native, heated, or irradiated calcium caseinate. Heating calcium caseinate at 90 °C for 30 min increased the molecular weight 3−4-fold (Figure 1b). However, when the protein was submitted to γ-irradiation at a dose of 32 kGy, cross-linking occurred and the molecular weight distribution peak shifted to higher molecular weights. In Figure 1a,b, a very small residual peak was present at 30-mL elution volume. This small protein peak could be attributed to

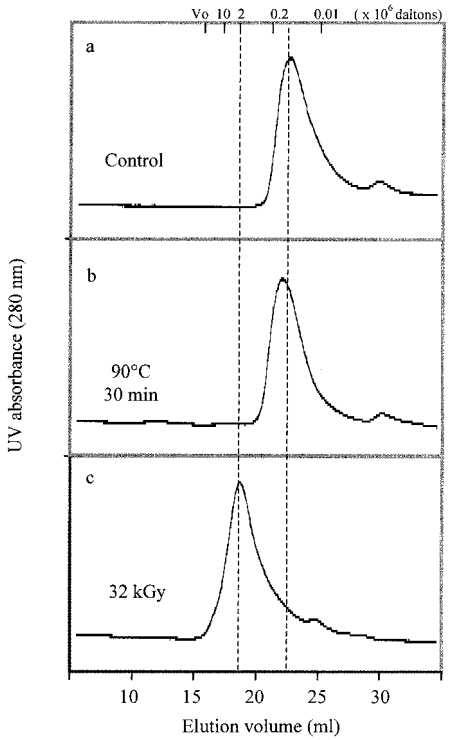

Figure 1. Elution curves for calcium caseinate (Alanate 380): (a) native; (b) heated at 90 °C for 30 min; (c) irradiated at 32 kGy.

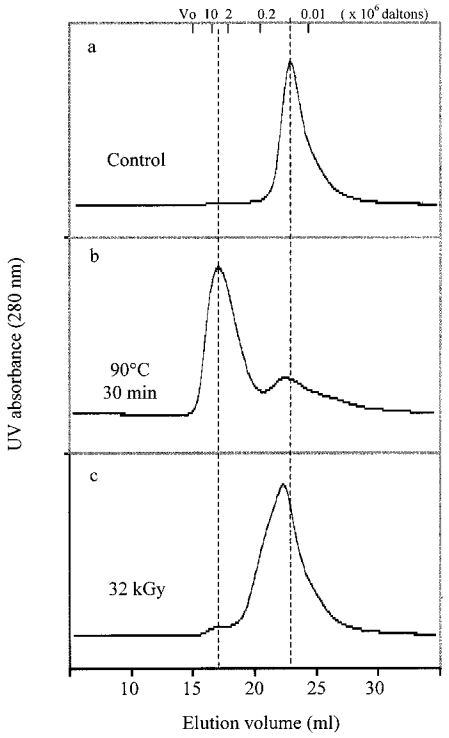

Figure 2. Elution curves for commercial whey proteins (WPC): (a) native; (b) heated at 90 °C for 30 min; (c) irradiated at 32 kGy.

low mass un-cross-linked or intramolecularly cross-linked proteins. In Figure 1c, when the solution was irradiated at 32 kGy, this small residual peak shifted to 25-mL elution volume and decreased in intensity. No synergistic effect of combining heating and γ-irradiation on the size-exclusion chromatography patterns of protein solutions was observed (not shown). This observation indicates that the cross-linking of caseinate by irradiation is more efficient than by heating. Based on the protein calibration curve, the molecular weight distribution of the cross-linked soluble calcium caseinate fraction was $\geq 2 \times 10^3$ kDa, an increase greater than 60-fold (Figure 1c). Previous studies demonstrated that γ-irradiation induced the formation of bityrosine (Davies, 1987; Brault, 1997; Mezgheni et al., 1998; Ressouany et al., 1998). The conditions leading to the formation of cross-links in peptides have been widely investigated (Prütz et al., 1983). Although bityrosine is expected to be the major component formed during γ-irradiation due to the strong characteristic fluorescence, other mechanisms for protein cross-linking should also be considered (Davies et al., 1987). Bityrosine is more likely to form between two protein chains (intermolecular bonding) than within a single protein, accounting for the increase in molecular weight (Figure 1c). However, intramolecular bonding should not be totally excluded. In Figure 1a,b, a very small residual peak is present at 25-mL elution volume. This small protein peak could be attributed to low mass un-cross-linked or intramolecularly cross-linked proteins. In Figure 1c, when the irradiation was carried out at 32 kGy, this small residual peak disappeared, an indication that the cross-linking of caseinate by irradiation was more efficient than by heating. Ressouany et al. (1998) demonstrated that the maximum cross-linking density was obtained at an irradiation dose of 64 kGy for similar calcium caseinate solutions. A new small residual peak at 20-mL elution volume was probably incompletely cross-linked caseinate.

Figure 2 shows the elution curves obtained for the commercial whey proteins (WPC), before (Figure 2a) or after heating (Figure 2b), or irradiated (Figure 2c). γ-Irradiation induced very little molecular weight changes in WPC, and no more increase of molecular weight was obtained by combining heating and γ-irradiation (not shown). Only a broadening of the elution peak can be observed in Figure 2c. This feature is not surprising, considering that whey proteins contain less tyrosine residues than caseins (Wong et al., 1996). Our results support the report by Davies (1987), who determined bityrosine content by fluorescence, that in the case of α-casein, the bityrosine concentration quadrupled following a low dose of irradiation (0.25 kGy), while it increased 10-fold in the case of BSA. Although whey proteins contain BSA in small amount, we expected a much more potent effect of γ-irradiation at high dose of 32 kGy on the molecular weight of whey proteins. It should be emphasized that tests were run on irradiated WPI, yielding similar results (not shown in Figure 2). The globular whey proteins are more prone to intramolecular cross-linking, leading to little change in molecular weight. As expected, when the whey protein solution was heated for 30 min at 90 °C, it readily underwent cross-linking via the formation of disulfide bonds. The solution contained two distinct molecular weight fractions. The molecular weight of the predominant fraction was $\geq 2 \times 10^3$ kDa, while the smallest fraction can be attributed to un-cross-linked protein or intramolecularly cross-linked protein. Similar results were obtained with heated or irradiated WPI (not shown in Figure 2). These results are consistent with those reported by Hoffmann et al. (1997) on the molecular mass distributions of heat-induced beta-lactoglobulin;

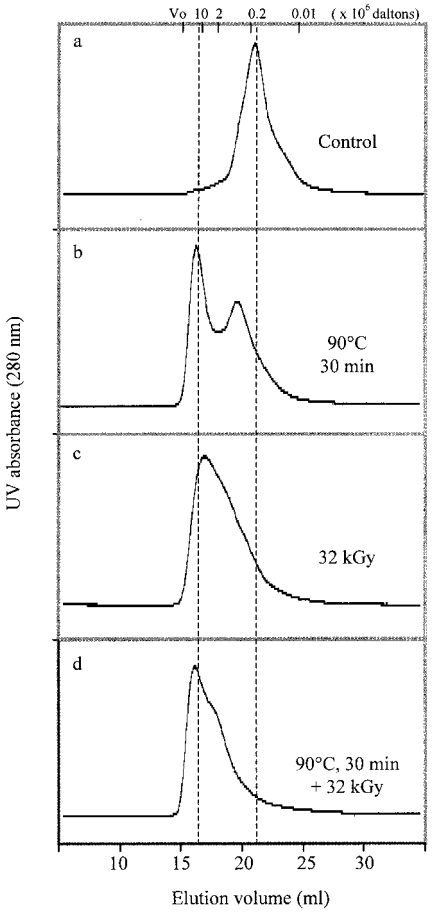

Figure 3. Elution curves for whey protein isolate (WPI) and calcium caseinate with ratio of 50−50: (a) control; (b) heated at 90 °C for 30 min; (c) irradiated at 32 kGy; (d) combined heat and irradiation treatment.

these authors were able to separate aggregates having a molecular mass of up to 4×10^3 kDa.

Figure 3 shows the molecular mass changes in the case of a 50−50% mixture of WPI and caseinate before (Figure 3a) or after heating (Figure 3b), irradiated (Figure 3c), or heated at first then treated with irradiation (Figure 3d). Following the weight calibration curve, the combination of heating and irradiation increased the molecular weight of the proteins to more than 10×10^3 kDa (Figure 3d), compared to $0.2\ 10 \times 10^3$ kDa for the native protein solution (Figure 3a).

The size-exclusion chromatography experiments clearly show the conditions leading to an increase in molecular weight in calcium caseinate and whey proteins. Mezgheni et al. (1998) reported that the cross-links generated by γ-irradiation significantly improved the mechanical strength of calcium caseinate-based edible films. Similarly, Rayas et al. (1997) improved the tensile strength of wheat protein films using cystein as a cross-linking agent. Cross-links confer elastomeric properties due to the formation of branched chains that increase the rigidity of a material. When the cross-linking density is sufficiently high, it increases the water resistance of the film (Gontard et al., 1994). Li et al. (1999) demonstrated that UV radiation reduced the water solubility and increased the tensile strength of whey protein-based films. Such a feature is beneficial for the development of biodegradable films and coatings. To evaluate the water solubility of the cross-linked materials, swelling experiments were performed; the results are shown below.

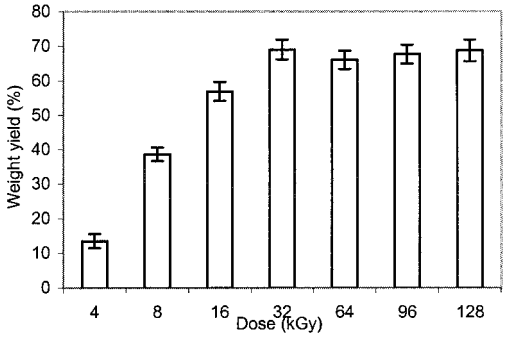

Figure 4. Fraction of insoluble matter as a function of the irradiation dose. Results are expressed as the percentage in solid yield after soaking the films 24 h in water.

Table 2. Fraction of Insoluble Matter As Affected by Heating and Irradiation[a]

type of film	insoluble matter, %	
	heating	heating + irradiation
caseinate	18	84
whey protein concentrate	45	45
caseinate−whey protein isolate	29	84

[a] Results are expressed as the percentage in solid yield after soaking the films 24 h in water.

Insolubility of Irradiated Films. Figure 4 shows the insolubility results obtained for calcium caseinate films irradiated at different doses. The proportion of the insoluble fraction increases with the irradiation dose up to 32 kGy when 70% of the film remained insoluble after 24 h. These results are supported by the size exclusion chromatography results (Figures 1−3) which suggest that a maximum cross-linking density was obtained at about 32 kGy. Table 2 shows the insolubility obtained for calcium caseinate, WPC, and calcium caseinate−WPI (50−50%) films, after heating at 90 °C, 30 min, or heating in combination with irradiation at 32 kGy, i.e., the optimal irradiation dose for maximum cross-linking. Results showed that percentage of insoluble matter recovered for heated (90 °C, 30 min) calcium caseinate films was 18% and increased to 84% when the films were heated and irradiated at 32 kGy. Similar results were obtained for films made from calcium caseinate−WPI (50−50%). The percentages of insoluble matter were 29% for heated films and 84% for heated and irradiated films. In contrast, no combined effect of heating and irradiation was observed for WPC films. The size-exclusion chromatography results combined with the solubility measurements indicate that the irradiation of calcium caseinate led to the formation of an insoluble fraction of high molecular weight, which accounts for 70% of the dry matter and a soluble protein fraction of molecular weight $\geq 2 \times 10^3$ kDa. Heating treatment combined with irradiation led to the formation of an insoluble fraction, accounting for 84% of the dry matter with similar molecular weight ($\geq 2 \times 10^3$ kDa). This observation can be explained by an increase of intramolecular cross-linking in the protein solution. Ressouany et al. (1998) suggested that a maximum cross-linking density was obtained at a dose of 64 kGy. However, these results were obtained with caseinate films irradiated at a mean dose rate of 1.5 kGy/h. In the present study, films were irradiated at a much higher dose rate (38.1 kGy/h), which increased the efficiency of the cross-linking process. Visual observation of the films that were stored in the water for 24 h showed that the aqueous phase of the films irradiated

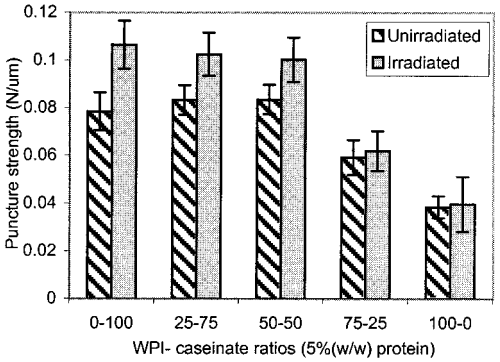

Figure 5. Puncture strength of unirradiated and irradiated (32 kGy) whey protein isolate (WPI)−calcium caseinate films. Ratios express the proportion in WPI or calcium caseinate for a formulation based on 5% w/w total protein solution. For instance, the formulation 25−75 represents 1.25 g of WPI protein and 3.75 g of calcium caseinate protein per 100 g of protein solution.

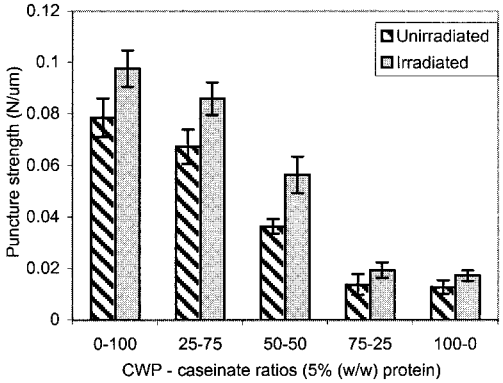

Figure 6. Puncture strength of unirradiated and irradiated (32 kGy) commercial whey protein−calcium caseinate films. Ratios express the proportion in WPC or calcium caseinate for a formulation based on 5% w/w total protein.

at 4 kGy was highly turbid, while no turbidity was noticed in the case of the films irradiated at a dose ≥32 kGy. Therefore, the reduced weight of the films in the water might be mainly due to the un-cross-linked, soluble small molecular mass proteins.

Enzymatic cross-linking by horseradish peroxidase has been used to cross-link soy protein edible films (Stuchell and Krochta, 1994). Cross-linking did not improve further the water vapor permeability of these films as compared to heat-treated films. The films treated with the enzyme had higher soluble matter levels, which suggests an increase in low molecular weight material. These authors concluded that horseradish peroxidase was not specific enough for use in edible films and that more specific enzymes such as transglutaminase should be used. However, transglutaminase is far more expensive than horseraddish peroxidase, which greatly limits its use in the development of edible films. The present research shows that γ-irradiation, which induces the cross-linking of tyrosine residues in a manner similar to peroxidase (Matheis and Whitaker, 1987), is a method specific enough for the development of edible films and is particularly cost-efficient when used on a large-scale basis. Moreover, protein cross-linking by γ-irradiation increased water-resistance, and it has been demonstrated that tyrosine−tyrosine cross-links improved the mechanical resistance of these films (Mezgheni et al., 1998; Ressouany et al., 1998). In light of these results, a dose of 32 kGy was chosen in order to evaluate the effect of γ-irradiation on the mechanical properties of edible films based on calcium caseinate and whey proteins.

Mechanical Properties of Calcium Caseinate−Whey Protein Films. Figure 5 shows the puncture strength variations of films cast from solutions containing different WPI−calcium caseinate ratios (5% w/w total protein solution). For instance, a protein ratio of 50−50 corresponds to 2.5% WPI protein and 2.5% calcium caseinate protein. Addition of WPI in the formulations did not significantly affect ($p \leq 0.05$) the puncture strength of the films up to a WPI−calcium caseinate ratio of 50−50. At higher WPI concentrations, the puncture strength of the films was significantly reduced ($p \leq 0.05$) and reached a minimal value of 0.04 N/μm for the films based on WPI only. γ-Irradiation significantly increased ($p \leq 0.05$) the mechanical properties of the films by inducing cross-links between protein chains. For instance, for films based only on calcium caseinate (0−100), γ-irradiation increased the puncture strength by more than 35%. This result is superior to the one reported by Ressouany et al. (1998). These authors used a dose rate of 2.18 kGy/h, while the present experiments were carried out at a dose rate of 17.33 kGy/h. A higher dose rate apparently increased the efficiency of the cross-linking mechanism. For the films containing an equal WPI−caseinate ratio (50−50), cross-linking increased by 20%. However, at WPI ratios higher than 50%, γ-irradiation did not affect the puncture strength, probably because the intermolecular cross-links were only generated between caseinate proteins. Statistical analysis confirmed that the films cast from solutions containing a WPI−caseinate ratio of 0−100, 25−75, and 50−50 did not significantly differ from one another, whether irradiated or not. The high puncture strength of films containing 50% WPI, comparable to pure calcium caseinate, suggests other favorable interactions than intermolecular bonding between whey protein isolate and calcium caseinate. The puncture strength obtained for films made from mixtures of calcium caseinate and WPI might be indicative of their phase behavior. A greater cohesiveness between WPI and calcium caseinate would be expected at WPI−caseinate ratios of 25−75 and 50−50.

For the films containing commercial whey proteins (WPC, Sapro-75) (Figure 6), the puncture strength of the films significantly decreased ($p \leq 0.05$) with increasing whey protein concentration. These results are not surprising considering that the WPC contains substantial amounts of impurities such as lactose and fats which could act as internal plasticizers in the films. Results depicted in Figures 5 and 6 also show that γ-irradiation had a more potent effect on films richer in calcium caseinate. No statistical differences ($p > 0.05$) were noted between irradiated and control films at WPC−caseinate ratios of 75−25 and 100−0. As established in Figures 1 and 2, the radiative treatment was more effective on calcium caseinate than on whey proteins in terms of molecular weight increase.

Figure 7 shows the viscoelasticity coefficient of films irradiated or unirradiated. A low viscoelasticity coefficient means that the material is highly elastic, while a high coefficient indicates that the material is more viscous and easily distorted. As discussed by Mezgheni (1997), γ-irradiation decreases the viscoelasticity coefficient of caseinate films, resulting in a more elastic material. An addition of whey proteins (WPC) by 25% of total protein did not change the viscoelasticity coef-

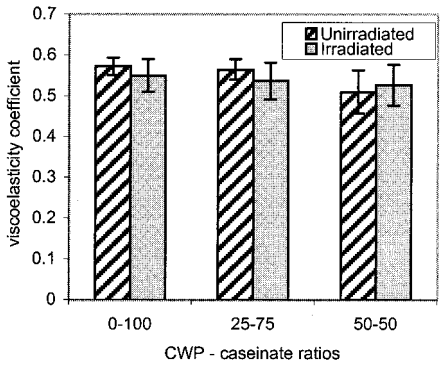

Figure 7. Viscoelasticity coefficient for unirradiated and irradiated (32 kGy) WPC−calcium caseinate films.

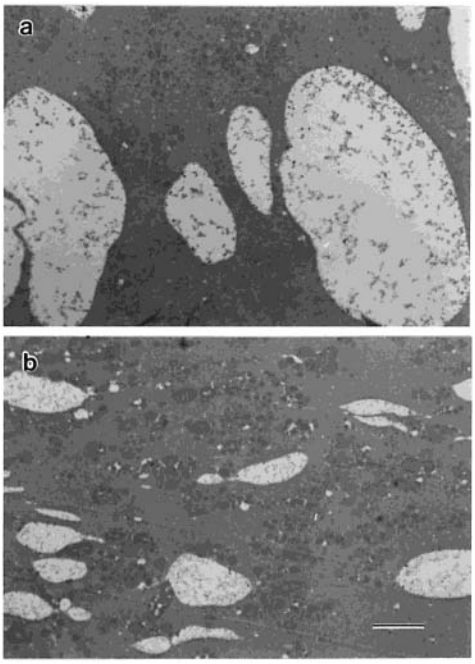

Figure 8. Cross sections of (a) unirradiated or (b) irradiated (32 kGy) calcium caseinate films. (9 mm bar = 3 μm).

ficient ($p \leq 0.05$). No statistical differences ($p > 0.05$) were found between films unirradiated or irradiated. However, the decrease from the 0−100 to the 50−50 formulations was found to be statistically significant ($p \leq 0.05$).

Microstructure Observations. Cross-sections of the films were observed by using transmission electronic microscopy (TEM). Figure 8 shows the micrographs that were obtained for cross-sections of films made from calcium caseinate. The micrographs show that the structure of these films was slicker and highly porous. Similar observations were made by Frinault et al. (1997) on casein films prepared by a modified wet spinning process. However, the microstructure of the films that were cast from irradiated solutions (Figure 8b) is clearly more dense than that of the films cast from unirradiated solutions (Figure 8a). Cross-links, which are present in the irradiated films, increase the molecular proximity of the protein chains. This increased molecular proximity as well as the additional molecular bonds, proved by size exclusion chromatography (Figures 1−3), directly influence the macroscopic characteristic of the films in terms of mechanical strength and water-resistance shown by physical measurements (Figures 4−7). Cross-sections of films containing variable amounts

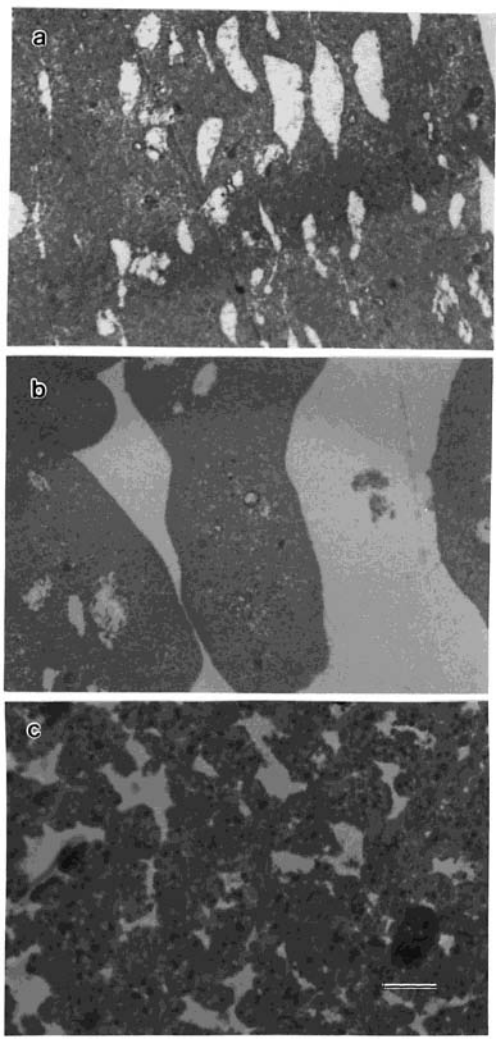

Figure 9. Cross sections of irradiated (32 kGy) WPC−calcium caseinate films with the ratio: (a) 50:50; (b) 75:25; (c) 100:0 (9 mm bar = 3 μm).

of WPC and calcium caseinate were also evaluated. The films were cross-linked both by heat and irradiation (32 kGy). Figure 9 shows the micrographs of films containing WPC−calcium caseinate ratios of 50−50, 75−25, and 100−0. The films made of WPC only (100−0) have a granular structure and contain numerous small masses that may be attributed to impurities such as fat, lactose, and mineral salts. Addition of calcium caseinate to the formulations rendered their microstructure smoother and slicker. However, major differences are seen between the micrographs of films 50−50 (Figure 9a) and 75−25 (Figure 9b) in terms of pore size. The pores are obviously much larger in the case of the films cast from a solution containing a protein ratio of 75−25. The variations in pore size distribution of these films might be correlated in part with the variations in puncture strength. As previously hypothesized, the internal structure might be indicative of the protein phase behavior. A great difference between the microstructure of films 75−25 and 100−0 (Figure 9c) can also be observed. The topography of the films varies from a porous structure to a more granular one (Figure 9c). Similar correlation between microstructure and mechanical strength were seen in films based on WPI (Figure 10). However, the structure of films containing WPI were generally more dense and homogeneous and comparable to calcium caseinate films. The cross section

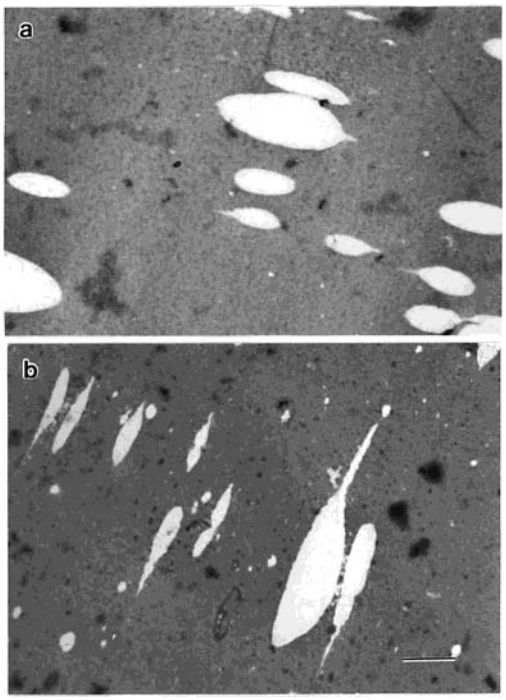

Figure 10. Cross sections of WPI−calcium caceinate films: (a) heated at 90 °C for 30 min; (b) heated at 90 °C for 30 min and irradiated at 32 kGy (9 mm bar = 3 μm).

of WPI−caseinate films (50−50) heated at 90 °C for 30 min shows larger average pore sizes (Figure 10a) than the same films both heated and irradiated at 32 kGy (Figure 10b). After combined heat and irradiation treatment, the microstructure of the films was more dense, which may be caused by the higher average molecular mass, shown in Figure 3c. A close relationship is observed between the microstructure of the films and their puncture strengths. Films made from calcium caseinate showed large pore sizes, indicating a highly porous structure. As a consequence, significantly ($p \leq 0.05$) higher puncture strength values were obtained for those films. The addition of WPI in the film forming solution increased significantly ($p \leq 0.05$) the molecular interaction by covalent disulfide cross-linking and hydrophobic bonds. Similar results have also been observed by Banerjee et al. (1996), who found a more closely knit matrix of proteins and other constituents in milk-protein based edible films submitted to ultrasound treatment. As observed previously, more than 50% of WPI could be added to calcium caseinate films without detrimental effect on puncture strength. This observation could be correlated with the porous and dense structure observed in calcium caseinate and calcium caseinate−WPI films. In contrast, incorporation of WPC in the film forming solution reduced significantly ($p \leq 0.05$) the puncture strength due to the granular structure of WPC films induced by the presence of impurities (fat, lactose, and mineral salts).

Conclusion. This report shows that γ-irradiation was efficient for inducing cross-links in calcium caseinate edible films. Unlike enzyme treatments, γ-irradiation would be particularly cost-efficient when used on a large-scale basis. The solubility measurements demonstrate that the treatment are selective enough to produce films containing a high ratio of insoluble matter. Combination of radiative and thermal treatments of the films based on calcium caseinate and whey proteins resulted in an increase in the puncture strength of the films. The mechanical properties of the films were influenced by the type of whey protein used. WPI could be added in equal amount to calcium caseinate without decreasing the puncture strength of the films. In contrast, the addition of WPC rapidly decreased the puncture strength of these films, probably due to the presence of impurities contained in the commercial product, which may disrupt protein−protein interactions. The observation of the microstructure of films by transmission electron microscopy revealed that all films were characterized by a highly porous structure. However, pore sizes varied depending on the protein ratio and influenced the mechanical behavior of the films.

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