Formation of Free-Standing Sterilized Edible Films from Irradiated Caseinates

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γ-Irradiation was used to produce free-standing sterilized edible films based on milk protein, namely, sodium caseinate and calcium caseinate. The nature of the counterion and also the protein and glycerol concentrations were examined. Irradiation of a solution based on calcium caseinate produced more cross-links than a solution based on sodium caseinate. As a consequence, films based on calcium caseinate showed a better mechanical strength. Glycerol was found to play a double role in enhancing the formation of cross-links within caseinate chains, accounting for the increase of the puncture strength, and acting as a plasticizer, being responsible for the improved film extensibility and viscoelasticity. Moreover, the effect of the irradiation on the mechanical properties was strongly dependent on the glycerol/protein ratio, i.e., the formulation of the films. Films of high quality and a satisfactory mechanical behavior were generated at glycerol/protein ratios of 0.5 and 0.67.

Keywords: *Caseinates; irradiation; sterilized edible films; glycerol; mechanical properties*

INTRODUCTION

Increased consumer demands for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging amounts have led to increased interests for edible films 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 (Chen, 1995).

Lipids, polysaccharides, and proteins were investigated as film-forming agents (Guilbert, 1986; Kester and Fennema, 1986). Milk proteins, such as whey proteins and caseinates, were also extensively studied, owing to their excellent nutritional value and their numerous functional properties, which are important for the formation of edible films (McHugh and Krochta, 1994; Chen, 1995). For instance, caseinates easily form films from aqueous solutions due to their random-coil nature and ability to form extensive intermolecular hydrogen, electrostatic, and hydrophobic bonds, resulting in an increase of the interchain cohesion (McHugh and Krochta, 1994). Moreover, edible films based on milk proteins were reported to be flavorless, tasteless, and flexible, and depending on the formulation, they varied from transparent to translucent (Chen, 1995). Glycerol, a polyol, is well-known for its plasticizing effects and its use in food technology. Glycerol was mixed at different concentrations to calcium caseinate solutions, since solutions of this protein generated films having better properties than sodium caseinate solutions. All these characteristics make them suitable for applications in food science.

Among the films investigated, edible films based on proteins showed the best mechanical properties (Kester and Fennema, 1986; Peyron, 1991). However, their gas and water vapor barrier properties are variable (Kester and Fennema, 1986; Peyron, 1991). The increase of cohesion between protein polypeptide chains was thought to be effective toward the improvement of the barrier properties of the films. For instance, the presence of calcium was reported to decrease the water permeability of caseinate-based films (Avena-Bustillos and Krochta, 1993). Likewise, caseinates were also reported to be enzymatically cross-linked, using transglutaminase (Ikura et al., 1980; Motoki et al., 1987). However, the high production cost and the limited availability of transglutaminase have limited its potential use in food systems. The use of physical treatments, such as irradiation, can also increase the cohesive strength of the protein by the formation of cross-links. Indeed, the irradiation of aqueous protein solutions generates hydroxyl radicals (·OH) that produce stable compounds (von Sonntag, 1987). These radicals are produced upon water radiolysis (Scheme 1) (Fricke and Hart, 1966). Sulfur and aromatic amino acids react more readily with free radicals, than aliphatic amino acids; particularly ·OH reacts readily with aromatic residues (Thakur and Singh, 1994). As an example, when phenylalanine reacts with ·OH, tyrosine isomers are generated (von Sonntag, 1987). Tyrosine is also sensitive to ·OH attack. Indeed, tyrosyl (phenoxyl) radicals (**II**) are produced as a result of hydrogen abstraction by ·OH (Scheme 2). Tyrosyl radicals may then react with other tyrosyl radicals or with tyrosine molecule to form several stable biphenolic compounds, where the phenolic moieties are linked through a covalent bond (Prütz et al., 1983). The 2′,2-biphenol bityrosine (**VII**), which exhibits a characteristic fluorescence, appears to be the major product due to the strong directing effect of the hydroxyl group (Prütz et al., 1983; von Sonntag, 1987). Bityrosine may be more likely to form between two protein chains (intermolecular bonding) than within a single protein (intramolecular bonding). The intermolecular formation

Scheme 1

$$H_2O \xrightarrow{\gamma} \cdot OH + \cdot H + e^-_{aq}$$

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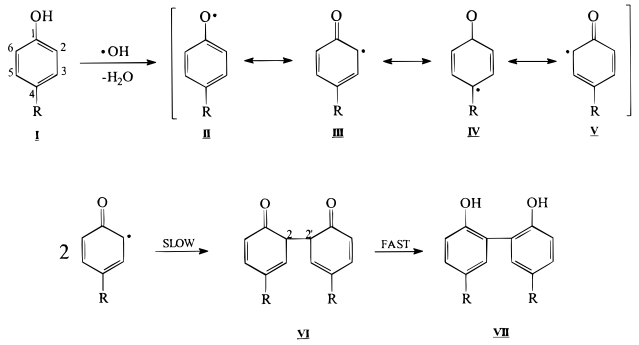
Table 1. Formation of Bityrosine as a Function of Irradiation Dose and Protein Concentration[a]

dose (kGy)	sodium caseinate		calcium caseinate	
	5%	7.5%	5%	7.5%
4	$20730 \pm 762^{4,a}$	$17067 \pm 547^{4,b}$	$28552 \pm 1621^{7,b}$	$17192 \pm 707^{7,b}$
8	$39651 \pm 2095^{5,c}$	$35287 \pm 1893^{5,c}$	$66803 \pm 2391^{8,d}$	$39344 \pm 687^{8,c}$
12	$66271 \pm 1287^{6,f}$	$64735 \pm 769^{6,e}$	$82504 \pm 1650^{9,g}$	$61076 \pm 607^{9,f}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

Scheme 2



of bityrosine is certainly one mechanism for protein aggregation, although other cross-links can also be formed (Davies et al., 1987).

Advantages of the irradiation process are 2-fold: the method is less expensive than using enzymes, and it allows the formation of sterilized films (Rice, 1986).

In this preliminary work, we want to report on the use of γ-irradiation to produce edible and sterilized films based on caseinates, namely, sodium caseinate and calcium caseinate. Moreover, the effect of a plasticizer agent, such as glycerol, on the production of bityrosine and on the mechanical behavior of sterilized edible films was also investigated.

MATERIALS AND METHODS

Materials. Sodium caseinate (alanate-180) and calcium caseinate (alanate-380) were provided by New Zealand Milk Products Inc. (CA, USA). Glycerol plasticizer (99.5%) was obtained from A & C (Montreal, Canada).

Film Formation Method. 5% w/w or 7.5% w/w caseinates were solubilized in distilled water, under stirring. Desired weights of glycerol were added to the solution, and a vacuum was applied to solutions to remove dissolved air. Solutions were then poured in a test tube under a flow of inert atmosphere. Test tubes were exposed to γ-rays with a Co^{60} source (GAMMACELL 220; Nordion International Inc., Kanata, Canada) at a mean dose rate of 2.18 kGy/h for irradiation doses of 4, 8, 12, 15, and 20 kGy. Films were then cast by pipeting 5 mL of the solution onto smooth-rimmed 8.5 cm internal diameter polymethacrylate (plexiglass) plates, sitting on a level surface. Solutions were spread evenly and allowed to dry overnight under a laminary flow hood at room temperature (20 ± 2 °C). Dried films could be peeled intact from the casting surface.

Film Thickness Measurements. Film thickness was measured using a Digimatic Indicator (Mitutoyo, Japan) at five random positions around the film. Depending on the formulation and irradiation dose, the average film thickness was in the range of $(27-64) \pm 2$ μm.

Fluorescence Measurements. The formation of bityrosine was measured using a Spectrofluorometer 2070 (Varian, CA), according to a procedure reported previously (Davies et al., 1987).

Mechanical Properties. Puncture tests were carried out using a Stevens LFRA Texture Analyzer Model TA/1000 (NY, USA), as described previously (Gontard et al., 1992). Films were cut into a 4 cm diameter section and equilibrated with a sodium bromide saturated solution in a desiccator, to ensure 56aw. 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 the hardness and deformation capacity of the film. In order to avoid any thickness variations, the puncture strength measured value was 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 film was then allowed to relax. The force−time relaxation curves were recorded for 1 min following deformation. The parameter $Y(1\ \text{min})$, a dimensionless ratio, was used to represent the decay of the force and was calculated according to $Y(1\ \text{min}) = (F^0 - F^1)/F^0$ where F^0 and F^1 were forces recorded initially and after 1 min of relaxation, respectively (Peleg, 1979). A low relaxation coefficient ($Y \rightarrow 0$) indicates high film elasticity.

Statistical Analysis. Analysis of variance and Duncan multiple-range tests with $P \leq 0.05$ were employed to analyze statistically all results. The Student t test was utilized at the time of the analysis of variance and paired-comparison with $P \leq 0.05$ (Snedecor and Cochran, 1978). For each measurement, three replicates of three film types were tested.

RESULTS AND DISCUSSION

Film Formation. As expected, films obtained from nonirradiated and irradiated caseinate solutions were free-standing, colorless, transparent, tasteless, and flavorless. Irradiation of both sodium and calcium caseinate solutions resulted in a significant increase of bityrosine, as suggested by fluorescence analysis (Table 1). This aggregation might account for the insolubility of films obtained from irradiated solutions, while films obtained from nonirradiated solutions are water-soluble. Depending on the concentration of the protein, the production of bityrosine was found to be different for both counterions, i.e., calcium vs sodium. In fact, at 5% (w/w) caseinate contents, the calcium caseinate produced significantly more bityrosine than sodium caseinate, for doses ranging between 4 and 12 kGy (Table 1). However, at higher concentration of protein (7.5% w/w), the sodium caseinate produced significantly more bityrosine than calcium caseinate, only at 12 kGy, whereas at 4 and 8 kGy the amount of bityrosine produced was independent of the nature of the counterions (Table 1). However, the differences observed at 7.5% concentration are probably unimportant. Furthermore, both caseinates produced significantly more bityrosine at a concentration of 5% (w/w), for doses ranging between 4 and 12 kGy. However, the differences were slight for sodium caseinate. This latter observation suggests that more cross-links are produced at a concentration of 5% (w/w).

The irradiation process did not show any significant effects on the puncture strength, at either concentration of the protein (Table 2). In other words, films produced with a high concentration of protein or during a long exposure to γ-irradiation did not necessarily generate a more resistant film. The main difference observed

Table 2. Variation of the Puncture Strength of Caseinate Edible Films with the Irradiation Dose[a]

dose (kGy)	sodium caseinate		calcium caseinate	
	5%	7.5%	5%	7.5%
0	$16.2 \pm 0.4^{3,a}$	$14.5 \pm 1.8^{3,a}$	$16.8 \pm 0.9^{5,a}$	$17.4 \pm 0.4^{4,b}$
4	$15.0 \pm 0.3^{4,b}$	$14.5 \pm 0.2^{3,c}$	$16.3 \pm 1.5^{5,b}$	$16.4 \pm 0.0^{5,d}$
8	$15.3 \pm 0.7^{4,c}$	$14.2 \pm 0.2^{3,e}$	$17.4 \pm 0.5^{5,c}$	$16.3 \pm 0.2^{5,f}$
12	$14.7 \pm 0.2^{4,d}$	$14.3 \pm 0.5^{3,g}$	$17.2 \pm 1.0^{5,e}$	$16.7 \pm 0.2^{5,h}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

Table 3. Variation of the Puncture Deformation of Caseinate Edible Films with the Irradiation Dose[a]

dose (kGy)	sodium caseinate		calcium caseinate	
	5%	7.5%	5%	7.5%
0	$2.3 \pm 0.2^{2,a}$	$2.6 \pm 0.2^{3,a}$	$2.3 \pm 0.1^{3,4,a}$	$2.7 \pm 0.1^{4,a}$
4	$2.3 \pm 0.1^{2,b}$	$2.5 \pm 0.2^{3,b}$	$2.2 \pm 0.1^{3,b}$	$2.5 \pm 0.1^{5,b}$
8	$2.3 \pm 0.1^{2,c}$	$2.7 \pm 0.1^{3,c}$	$2.3 \pm 0.2^{3,4,c}$	$2.6 \pm 0.2^{4,5,c}$
12	$2.3 \pm 0.2^{2,d}$	$2.6 \pm 0.2^{3,d}$	$2.4 \pm 0.1^{4,d}$	$2.3 \pm 0.2^{5,d}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

came from the nature of the counterion, i.e., calcium vs sodium. For the same irradiation dose, calcium caseinate films had higher puncture strength values than sodium caseinate films, independent of the concentration of the protein.

The puncture deformation of the films was found to be independent of the irradiation treatment, the nature of the counterion, and the concentration of the protein (Table 3).

These preliminary results have clearly demonstrated that calcium caseinate generates more bityrosine, i.e., cross-links, upon exposure to γ-irradiation, than sodium caseinate. As a result, films from calcium caseinate exhibit a greater mechanical strength. However, these films were found to crumble easily, to be brittle, and to exhibit poor deformation properties. The use of plasticizers was thus necessary to obtain films with an acceptable mechanical strength and flexibility. Plasticizers increase the melt viscosity to facilitate molding or extruding, but also flexibility (Champetier and Monnerie, 1969; Stevens, 1990). It is believed that the thermal motion of the low molecular weight plasticizers increases the polymer's free volume and the segmental motion of the polymer chains (Champetier and Monnerie, 1969; Stevens, 1990).

Glycerol Effects. At first glance, the presence of glycerol gave films showing some opaqueness, which seemed to be proportional to the glycerol concentration. The amount of bityrosine formed upon irradiation was found to be significantly more important in the presence of glycerol, than in the absence (Table 4). The production of bityrosine was significantly dependent on the irradiation dose and on the formulation, i.e., the concentration of both protein and glycerol. The more pronounced effect of the irradiation process on the formation of bityrosine, and hence on the formation of cross-links, was observed at formulations corresponding to 2.5% w/w glycerol/7.5% w/w caseinate, at doses ranging between 4 and 20 kGy, and to 2.5% w/w glycerol/5% w/w caseinate, at doses >12 kGy (Table 4). Lower amounts of bityrosine, i.e., cross-links, were produced at the other two formulations, i.e., 5% w/w glycerol/5% w/w caseinate for doses >12 kGy and 5% w/w glycerol/7.5% w/w caseinate (Table 4) for doses ranging between 4 and 20 kGy. The production of bityrosine was significantly activated at doses ≥4 kGy, independently of both protein and glycerol concentrations (Table 4).

The significant improvement of the production of bityrosine in the presence of glycerol can be explained by the preferential binding concept elaborated by Gekko and Timasheff (1981). These authors have suggested that the presence of glycerol in an aqueous solution of protein increases the chemical potential of the protein. Such an increase of the chemical potential corresponds to a decrease of the solubility of the protein in the glycerol−water system. Since glycerol is essentially hydrophilic, it interacts strongly with water, thus enhancing the hydrophobicity of the protein. In globular proteins, most hydrophobic groups are buried in the interior of the protein, while the polar residues are located preferentially on the surface. Aromatic amino acids are hydrophobic components, and they are not buried inside the protein, owing to their bulky structure. They are thus located on the surface of the protein, together with the hydrophilic amino acids (Nakai and Li-Chan, 1987). However, these nonpolar aromatic amino acids will be repelled by the highly polar glycerol−water solvent. In other words, the nonpolar groups would prefer to migrate into the interior of the protein out of contact with solvent, in order to relieve the situation. However, such migration is hindered by the tight packing of the three-dimensional structure of proteins and by the fact that hydrophobic groups are covalently linked to the polypeptide chain of the protein. As a result, the converse takes place; i.e., the water and glycerol molecules redistribute themselves in the vicinity of the polar groups of the protein (Gekko and Timasheff, 1981). This phenomenon, called preferential binding, leads to a greater availability of the hydrophobic domains containing aromatic amino acids, such as tyrosine molecules. In the presence of glycerol, tyrosine residues are then able to react more easily upon exposure to γ-irradiation, accounting for the increase of bityrosine production.

A greater amount of bityrosine means an increase of cross-links within polypeptide macromolecules. Steric

Table 4. Effect of Glycerol Concentration and Irradiation Dose on the Formation of Bityrosine[a]

dose (kGy)	calcium caseinate 5%			calcium caseinate 7.5%		
	0% glycerol	2.5% glycerol	5% glycerol	0% glycerol	2.5% glycerol	5% glycerol
4	$28552 \pm 1621^{1,a}$	$20503 \pm 941^{11,b}$	$23126 \pm 1482^{18,c}$	$17192 \pm 1621^{1,a}$	$43513 \pm 1328^{6,b}$	$20803 \pm 928^{11,c}$
8	$66803 \pm 2391^{2,d}$	$66439 \pm 1805^{12,d}$	$81304 \pm 2395^{19,f}$	$39344 \pm 687^{2,d}$	$97240 \pm 4388^{7,L}$	$57961 \pm 897^{12,f}$
12	$82504 \pm 1650^{3,g}$	$75782 \pm 1206^{13,j}$	$85465 \pm 1392^{20,j}$	$61076 \pm 607^{3,g}$	$103811 \pm 1653^{8,h}$	$92734 \pm 1901^{13,j}$
15	$89584 \pm 1817^{4,k}$	$134112 \pm 1328^{14,m}$	$125394 \pm 1551^{21,n}$	$95587 \pm 1252^{4,j}$	$128415 \pm 1232^{9,k}$	$117110 \pm 1398^{14,L}$
20	$129044 \pm 931^{5,o}$	$163519 \pm 1126^{15,q}$	$158853 \pm 1289^{22,r}$	$139249 \pm 1697^{5,m}$	$184986 \pm 1581^{10,n}$	$151133 \pm 1653^{15,o}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

Table 5. Effect of Glycerol Concentration and Irradiation Dose on the Puncture Strength of Caseinate Edible Films[a]

dose (kGy)	calcium caseinate 5%			calcium caseinate 7.5%		
	0% glycerol	2.5% glycerol	5% glycerol	0% glycerol	2.5% glycerol	5% glycerol
0	$16.8 \pm 0.9^{1,a}$	$5.6 \pm 0.2^{5,c}$		$17.4 \pm 0.4^{1,a}$	$11.0 \pm 1.0^{3,b}$	$4.3 \pm 0.2^{4,c}$
4	$16.3 \pm 1.5^{1,d}$	$5.9 \pm 0.1^{5,f}$	$2.7 \pm 0.2^{10,g}$	$16.4 \pm 0.0^{2,d}$	$11.2 \pm 0.5^{3,L}$	$4.4 \pm 0.2^{4,f}$
8	$17.4 \pm 0.5^{1,f}$	$6.9 \pm 0.1^{6,j}$	$3.3 \pm 0.1^{11,12,k}$	$16.3 \pm 0.2^{2,g}$	$10.4 \pm 1.5^{3,h}$	$4.6 \pm 0.1^{4,j}$
12	$17.2 \pm 1.0^{1,L}$	$7.0 \pm 0.2^{6,n}$	$3.8 \pm 0.4^{11,13,o}$	$16.7 \pm 0.2^{2,j}$	$10.5 \pm 0.9^{3,k}$	$5.8 \pm 0.2^{5,L}$
15		$10.5 \pm 0.4^{7,q}$	$3.6 \pm 0.3^{11,12,13,r}$		$12.5 \pm 1.4^{3,m}$	$5.2 \pm 0.2^{6,n}$
20		$10.1 \pm 0.4^{8,t}$	$4.0 \pm 0.3^{13,u}$		$12.1 \pm 0.1^{3,o}$	$5.7 \pm 0.1^{5,p}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

Table 6. Effect of Glycerol Concentration and Irradiation Dose on the Puncture Deformation of Caseinate Edible Films[a]

dose (kGy)	calcium caseinate 5%			calcium caseinate 7.5%		
	0% glycerol	2.5% glycerol	5% glycerol	0% glycerol	2.5% glycerol	5% glycerol
0	$2.3 \pm 0.1^{12,a}$	$5.7 \pm 0.4^{6,b}$		$2.7 \pm 0.1^{1,a}$	$4.1 \pm 0.4^{3,b}$	$7.7 \pm 0.2^{4,c}$
4	$2.2 \pm 0.1^{1,c}$	$6.1 \pm 0.2^{6,7,L}$	$8.5 \pm 0.4^{10,f}$	$2.5 \pm 0.1^{1,2,d}$	$3.8 \pm 0.3^{3,L}$	$9.4 \pm 0.5^{5,6,f}$
8	$2.3 \pm 0.2^{1,2,g}$	$6.6 \pm 0.1^{7,8,j}$	$9.5 \pm 0.1^{11,j}$	$2.6 \pm 0.2^{1,2,g}$	$4.0 \pm 0.3^{3,h}$	$11.0 \pm 0.1^{7,j}$
12	$2.4 \pm 0.1^{2,k}$	$7.0 \pm 0.3^{8,9,L}$	$10.3 \pm 0.3^{11,13,m}$	$2.3 \pm 0.2^{2,j}$	$4.0 \pm 0.3^{3,k}$	$9.1 \pm 0.3^{5,L}$
15		$6.2 \pm 0.2^{6,7,o}$	$9.6 \pm 0.6^{11,12,p}$		$3.9 \pm 0.4^{3,m}$	$11.6 \pm 0.6^{7,n}$
20		$7.3 \pm 0.3^{9,r}$	$10.8 \pm 0.7^{13,s}$		$4.2 \pm 0.2^{3,o}$	$11.2 \pm 0.6^{7,p}$

[a] Means followed by the same number in each column are not significantly different at the 5% level. Means followed by the same letter in each row are not significantly different at the 5% level. For each measurement, three replicates of three film types were tested.

exclusion chromatography experiments should be carried out to find out how much the aggregation behavior affects the molecular weight of the protein. Moreover, these experiments might enable the determination of the cross-link density, which is expressed by the ratio of the average number molecular weights of the uncross-linked and cross-linked polymers (Champetier and Monnerie, 1969; Stevens, 1990).

Cross-links confer to any material elastomeric properties, if the cross-link density does not exceed a critical value (Champetier and Monnerie, 1969; Stevens, 1990). Indeed, the higher this value is, i.e., the greater the number of branched chains, the more rigid is the material. The effect of an increase of cross-links, induced by glycerol, on the mechanical properties of films was also investigated, namely puncture strength and puncture deformation.

Tables 5 and 6 illustrate a decrease of the puncture strength and an increase of the puncture deformation with an increase of the glycerol contents, whatever the concentration of the protein. These results clearly emphasize the plasticizing effect of glycerol, which can be interpreted by the ability of highly hydrophilic glycerol to reduce internal hydrogen bindings within the protein (i.e., protein−protein or water−protein bindings), thereby decreasing the internal forces and resulting in an increase of intermolecular spacing. Similar results were already reported concerning the plasticizing effect of glycerol on other films, such as starch and hydroxypropylated starch (Mark et al., 1966).

In the presence of glycerol, the irradiation dose affected significantly the puncture strength, depending on the formulation of the caseinate solution. For instance, when the formulation was 2.5% w/w glycerol/5% w/w caseinate, a significant increase of the puncture strength with the irradiation dose was observed from 8 kGy, whereas at a formulation of 5% w/w glycerol/7.5% w/w caseinate a significant increase of the puncture strength occurred from 12 kGy (Table 5). Moreover, the effect of irradiation on puncture strength was less pronounced at a caseinate concentration corresponding to 7.5% w/w (Table 5). Likewise, the puncture strength of the films was found to be strongly related to the formulation of caseinate solutions. Indeed, the measured puncture strength was significantly more important at formulations corresponding to 2.5% w/w glycerol/7.5% w/w caseinate and 2.5% w/w glycerol/5% w/w caseinate, than those measured at formulations corresponding to 5% w/w glycerol/7.5% w/w caseinate and 5% w/w glycerol/5% w/w caseinate (Table 5). The puncture strength values can be related to the amounts of bityrosine produced, i.e., cross-links, during the irradiation process, which were greater at the following formulations: 2.5% w/w glycerol/7.5% w/w caseinate and 2.5% w/w glycerol/5% w/w caseinate (Table 4).

Except for 2.5% w/w glycerol/7.5% w/w caseinate, the puncture deformation increased significantly with the irradiation dose in the presence of a plasticizer, indicating that the irradiation treatment generated elastic, i.e., flexible, films. These results were further confirmed by a significant decrease ranging between 4 and 5% of the relaxation coefficient upon irradiation, for formulations investigated. At 5% w/w glycerol/7.5% w/w caseinate, the increase of the puncture deformation with the irradiation dose was generally significant from 4 kGy upward and was the most important (Table 6). At 2.5% w/w glycerol/5% w/w caseinate and 5% w/w glycerol/5% w/w caseinate, the puncture deformation increased significantly with irradiation dose from 8 kGy, while at 2.5% w/w glycerol/7.5% w/w caseinate the puncture deformation was not significantly affected by the irradiation treatment (Table 6). The effect of the irradiation dose on the puncture deformation was more important at a caseinate concentration of 7.5% than it was at 5%. As noticed for the puncture strength, puncture deformation values were strongly dependent on the formulation of films. The highest puncture deformation values measured were at formulations of 5% w/w glycerol/5% w/w caseinate and 5% w/w glycerol/7.5% w/w caseinate, while the lowest puncture deformation values were found for the formulation corresponding to 2.5% w/w glycerol/7.5% w/w caseinate (Table 6). These results can be interpreted in terms of bityrosine contents, i.e., cross-links or branched chains. It seems that too many cross-links are produced at 2.5% w/w glycerol/7.5% w/w caseinate, leading to a stiff film, whereas the amount of branched chains produced at 5% w/w glycerol/5% w/w caseinate and 5% w/w glycerol/7.5% w/w casein-

Table 7. Effects of the Glycerol/Caseinate Ratio on the Mechanical Behavior of Films Irradiated at 20 kGy[a]

	glycerol/caseinate ratio mechanical property			
	0.33	0.5	0.67	1
puncture strength (%)	+10 ± 0.1	+80 ± 0.3	+30 ± 0.6	+15 ± 1.2
puncture deformation (%)	+2 ± 0.1	+30 ± 0.1	+45 ± 2.0	+25 ± 2.0

[a] (+) indicates an increase of the mechanical property measured after exposure to γ-ionization corresponding to 20 kGy. (−) indicates a decrease of the mechanical property measured after exposure to γ-ionization corresponding to 20 kGy. For each measurement, three replicates of three film types were tested.

ate seemed to be just enough to confer viscoelastic properties to the films.

The results discussed so far demonstrate that the mechanical behavior of the films and effects of the irradiation treatment are strongly sensitive to the glycerol/protein ratio (Table 7). The highest amount of bityrosine, i.e., cross-links, occurred at a ratio of 0.33 (2.5% glycerol/7.5% caseinate) (Table 4), leading to very high puncture strength values (Table 5) and very low puncture deformation values (Table 6). Moreover, at a glycerol/protein ratio of 0.33, the mechanical properties did not show significant variations with the irradiation dose: the puncture strength showed an increase of only 10%, while the puncture deformation showed an increase of only 2%. However, when this ratio was increased, the mechanical properties were found to be different. Indeed, the highest effect of the irradiation dose on the puncture strength was observed at a ratio of 0.5 (2.5% glycerol/5% caseinate) (Table 5). At this glycerol/protein ratio, the amount of bityrosine produced was still important, but lower than 0.33 (Table 4). The puncture strength was found to be more affected by the irradiation process: an increase of 80% was noticed between the nonirradiated and irradiated samples at 20 kGy. On the other hand, the most important effect of the irradiation on the deformation was observed at a ratio of 0.67 (5.0% glycerol/7.5% caseinate): an increase of 45% was measured after irradiation at 20 kGy. These findings can be explained by the fact that the lowest amounts of cross-links were produced at a ratio of 0.67, as confirmed by puncture strength values (Table 5). As a consequence, films behave more similarly as elastomers. The largest increase of puncture deformation (45%) by the irradiation treatment was observed at a ratio of 0.67, suggesting that cross-links produced are near-optimal at a glycerol/caseinate ratio of 0.67, irradiated at 20 kGy.

Among the formulations investigated, films obtained from the irradiation process exhibited the best mechanical strength and flexibility at glycerol/protein ratios of 0.5 and 0.67. Investigations of thermal properties of calcium caseinate films and of permeabilities will be carried out in order to make a correlation between mechanical, thermal, and permeability properties of these films. The biodegradability of calcium caseinate films using *Pseudomonas fragi* is currently under investigation. Results of these studies will be presented in a later report.

CONCLUSION

This investigation has clearly demonstrated the usefulness of γ-irradiation for making free-standing sterilized edible films, based on caseinates. Calcium caseinate was found to yield films having better mechanical strength than sodium caseinate. Addition of glycerol has significantly increased the formation of cross-links within protein chains. This effect was explained by the preferential binding concept. Moreover, glycerol played a second role; i.e., it was found to improve the mechanical strength and to increase the film flexibility. Depending on the glycerol/caseinate ratio, the irradiation treatment was beneficial for the toughness and the flexibility of the films. Among the formulations investigated, the largest effects of irradiation dose on the mechanical properties of edible films were found at glycerol/caseinate ratios of 0.5 and 0.67. These results are a direct consequence of the bityrosine, i.e., cross-links, produced upon irradiation. Indeed, they lead to a branching of polypeptide chains to form a three-dimensional network. If the number of branched chains is not too high, i.e., optimal, the resulting network demonstrates a viscoelastic behavior. The three-dimensional network constructed upon irradiation of calcium caseinate and the interactions between the protein and glycerol molecules contribute to the mechanical behavior of the films. Hence, inadequate irradiation period or inadequate glycerol/caseinate ratio will strongly affect the structure of the film, and thus its mechanical behavior.

It is believed that these promising films might find application as microencapsulating agents of flavors and medicaments, in coating of fruits, vegetables, and cheese, as well as in food packaging. Preliminary coating tests with solutions of calcium caseinate and glycerol on strawberries, sliced potatoes, and apples were performed. Films obtained from irradiated solutions have reduced considerably the water loss for strawberries during the storage and have delayed the oxidation (browning) of potatoes and apples.

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