Formation of Sterilized Edible Films Based on Caseinates: Effects of Calcium and Plasticizers

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γ-Irradiation was used to produce free-standing sterilized edible films based on caseinate. The effects of calcium ions (Ca^{2+}) and two plasticizers, namely propylene glycol (PG) and triethylene glycol (TEG), were investigated, as was the effect of the irradiation on both the gel formation and mechanical properties of the resulting films. γ-Irradiation provoked formation of bityrosine, i.e., cross-links, accounting for the increase of the puncture strength of films. The presence of PG or TEG enhanced the formation of cross-links, leading to an improved mechanical strength of films. TEG was found to interact more favorably with the caseinate than PG, being responsible for the improved film extensibility. Addition of Ca^{2+} caused the formation of gels. The breaking strength of gels was directly related to the concentration of Ca^{2+}, while the puncture strength of films was found to be almost independent of the calcium concentration. Moreover, a high irradiation dose seemed to affect the protein structure, accounting for the decrease of the breaking strength of gels and for the depreciation of the mechanical behavior of films.

Keywords: *Caseinates; irradiation; calcium ions; propylene glycol; triethylene glycol*

INTRODUCTION

Increased consumer demands for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging volume have led to increased interests in the preparation of biopolymers films and coatings (Chen, 1995). Milk proteins, such as whey proteins and caseinates, have been extensively studied as film-forming agents, 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).

Edible films based on proteins were found to possess satisfactory mechanical properties (Kester and Fennema, 1986; Peyron, 1991). However, they also exhibit poor water vapor barrier properties (Guilbert, 1986). The increase of cohesion between protein polypeptide chains was thought to be effective toward the improvement of the barrier properties of the films. The cross-linking of caseinates with calcium ions (Avena-Bustillos and Krochta, 1993) or with transglutaminase (Ikura et al., 1980; Motoki et al., 1987) has been reported. More recently, γ-irradiation was also reported to be an effective method in enhancing the cohesion within caseinate (Brault et al., 1997). Upon radiolysis of an aqueous protein solution, hydroxyl radicals ($^{\bullet}OH$) are generated (von Sonntag, 1987). Aromatic amino acids react readily with these $^{\bullet}OH$ (Thakur and Singh, 1994). For instance, tyrosine amino acids react with $^{\bullet}OH$ to produce tyrosyl radicals (**II**) (Scheme 1). Tyrosyl radicals may then react with other tyrosyl radicals or with tyrosine molecules to form several stable biphenolic compounds, in which 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). Formation of bityrosine is certainly one mechanism for protein aggregation, although other cross-links can also be formed (Davies et al., 1987). The γ-irradiation method presents some conveniences: it is a well-known process for the sterilization of goods (Thakur and Singh, 1994), and it is less expensive than using enzymes.

Our assumption was that the combination of both calcium ions and γ-irradiation would generate films with an improved cohesion, making them suitable for packaging and/or coating purposes. The addition of plasticizers and calcium ions was also assumed to enhance the mechanical strength of films and gels based on caseinate.

MATERIALS AND METHODS

Materials. Caseinate (alanate 380) was provided by New Zealand Milk Products Inc. (Santa Rosa,CA). Propylene glycol (PG) and triethylene glycol (TEG) plasticizers (99.5%) were obtained from A&C (Montreal, PQ, Canada).

Film Formation Method. Caseinate (5% w/w) was solubilized in distilled water, under stirring. Desired weights of additives were added to the solution, and a vacuum was applied to solutions to remove dissolved air. Solutions were then poured into a test tube under a flow of inert atmosphere. Test tubes were exposed to γ-rays with a ^{60}Co source at the Canadian Irradiation Center (Gammacell 220; MDS-Nordion International Inc., Kanata, ON, Canada) at a mean dose rate of 2.18 kGy/h for irradiation doses of 8, 16, 32, 64, 96, and 128 kGy at 20 ± 2 °C. Films were then cast by pipetting 5 mL of the solution onto smooth-rimmed 8.5 cm i.d. polymethacrylate (plexiglass) plates, sitting on a leveled surface. Solutions were spread evenly and allowed to dry overnight at

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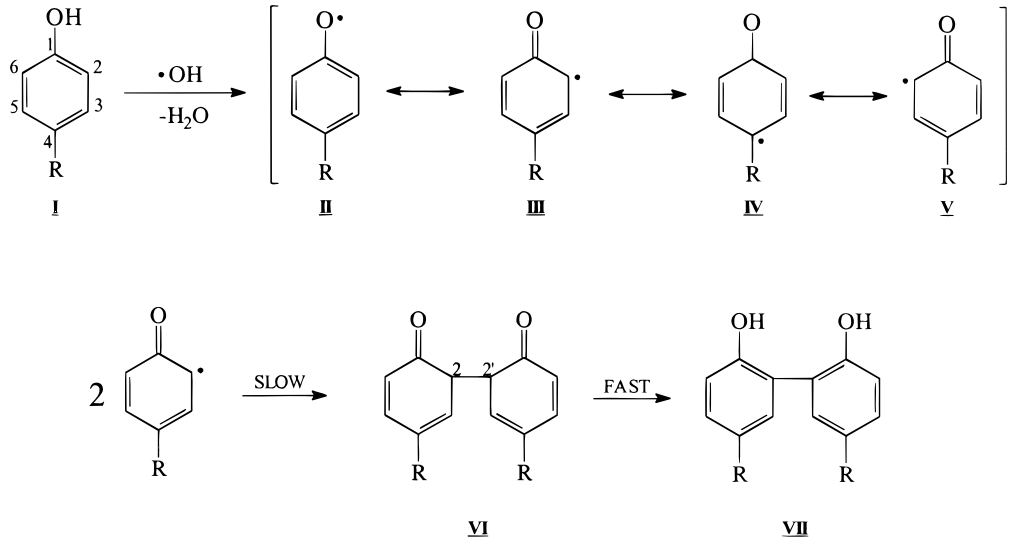
Scheme 1



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 30−50 ± 2 μm.

Fluorescence Measurements. The formation of bityrosine was measured in protein solution (0.05%) solubilized in HEPES buffer (20 mM, pH 7), using a Varian 2070 spectrofluorometer (Varian, Palo Alto, CA), according to a procedure reported previously (Davies et al., 1987). Fluorescence measurements were done at 325 nm excitation and 410 nm emission.

Mechanical Properties. Puncture tests were carried out using a Stevens LFRA texture analyzer Model TA/1000 (Stevens, New York, NY), as described previously (Gontard et al., 1992). Films were cut 4 cm in diameter and equilibrated with a sodium bromide saturated solution in a desiccator, to ensure 56% relative humidity. 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. To avoid any thickness variations, the puncture strength measured value was divided by the thickness of the film. The force−deformation curves were recorded.

Gel Formation Method. Gels were formed using a modified procedure (Sakamoto et al., 1994). Solutions were prepared and irradiated as described previously. The desired concentration of calcium ions was added to the irradiated solution. A 1.25 mL aliquot of the latter solution was poured into wells with care to avoid entrapment of air bubbles. Each well was 7 mm in diameter and 45 mm in height. Wells were sealed and placed into a water bath at 90 °C for 4 h. After that time, wells were cooled to room temperature and stored at 4 °C overnight.

Gel Breaking Strength. The strength of gels was evaluated by measuring the breaking strength using a Stevens LFRA texture analyzer Model TA/1000, according to a procedure described previously (Sakamoto et al., 1994). A cylindrical probe (3 mm in diameter) was moved over the center of each well, and then the sample was compressed at a constant speed (1 mm/s). The breaking strength was measured and read on the force vs deformation curve by the value of the first force peak.

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 samples (films and gels type) were tested.

RESULTS

Formation of Bityrosine. Bityrosine (i.e. crosslinks) was produced upon γ-irradiation, as suggested by fluorescence analysis. The amount was directly related to the irradiation dose (Figures 1−3). In the absence of calcium ions (Ca^{2+}) and plasticizers, the bityrosine increased significantly with the irradiation dose until 64 kGy (Figure 1). At doses >64 kGy, the irradiation process did not influence significantly the dimerization process of tyrosine (Figure 1). Plasticizers, such as PG and TEG significantly improved the amount of bityrosine produced by γ-irradiation (Figure 1). The amount was related to the irradiation dose. According to the statistical analysis, a dose of 96 kGy was optimal for caseinate solutions containing 2.5% PG and 5% TEG, while for caseinate solutions containing 5% PG or 2.5% TEG, the production of bityrosine was not optimal even at 128 kGy.

In the presence of Ca^{2+}, the same behavior occurred, i.e., a significant increase of the production of bityrosine with the exposure to γ-irradiation (Figures 2 and 3). According to the statistical analysis, when the concentration of Ca^{2+} was 0.125%, a plateau occurred at 64 kGy with 2.5% PG, 2.5% TEG, and formulations without plasticizers. The maximum of bityrosine produced for formulations with 5% TEG was monitored at 128 kGy; however, no significant differences were found between 96 and 128 kGy ($P \leq 0.05$). In the presence of 2.5% PG, 2.5% TEG, or 5% PG, the production of bityrosine was not optimal even at 128 kGy (Figure 2). When the concentration of Ca^{2+} was increased to 0.25%, the production of bityrosine was not optimal even at 128 kGy, in the presence of 2.5%PG, 2.5%TEG, 5% TEG, or 5% PG (Figure 3). An optimal dose was obtained at 96 kGy for formulation without plasticizers.

Formation of Gels. Gels were not formed in unirradiated samples and in the absence of Ca^{2+}, independent of the irradiation dose. However, gels were obtained once Ca^{2+} was added (Figures 4 and 5). Depending on the formulation, gels were formed at doses corresponding to 16 or 32 kGy.

At 0.125% Ca^{2+}, gels appeared at 16 kGy for caseinate solutions without plasticizers and those containing PG

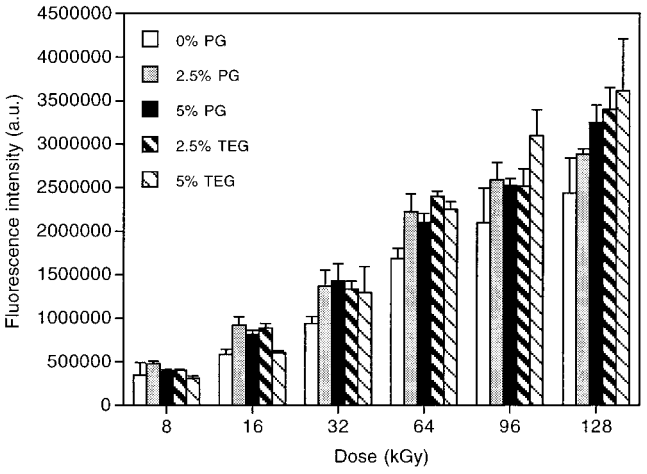


Figure 1. Formation of bityrosine as a function of irradiation dose in the absence of calcium.

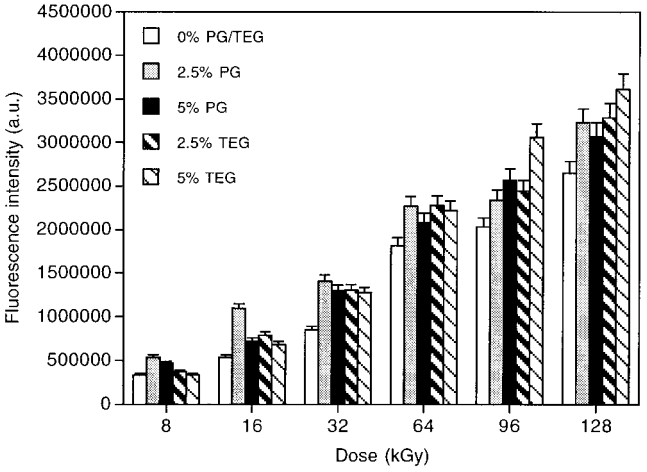


Figure 2. Formation of bityrosine as a function of irradiation dose in the presence of 0.125% calcium.

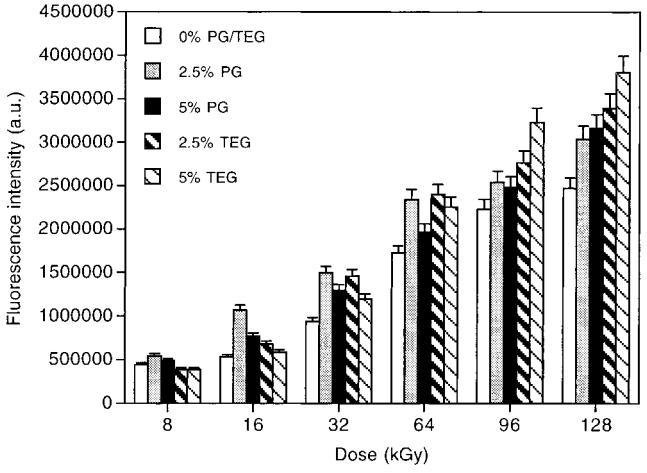


Figure 3. Formation of bityrosine as a function of irradiation dose in the presence of 0.25% calcium.

(Figure 4). Gels appeared at 32 kGy when TEG was added instead of PG (Figure 4). The breaking strength of gels with or without plasticizer increased significantly with the irradiation dose, followed by a significant decrease at still higher doses. For instance, gels containing 2.5% PG or 5% PG or without plasticizers showed a maximum of the breaking strength at 32 kGy (Figure 4). Moreover, an increase of the concentration of PG decreased significantly the breaking strength of

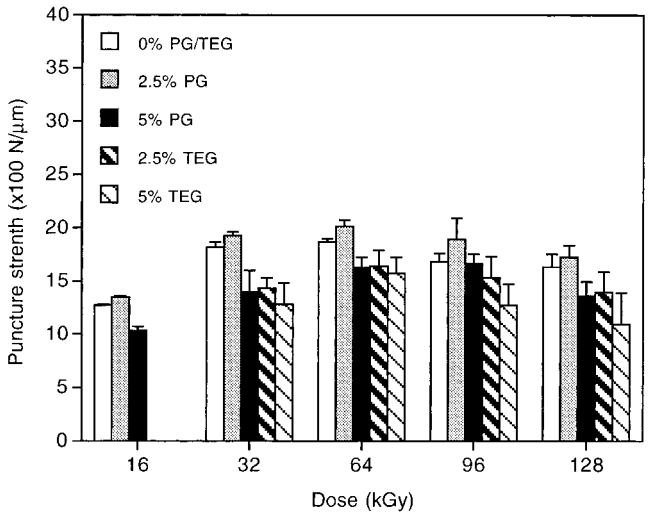


Figure 4. Effect of irradiation dose on the puncture strength of gels in the presence of 0.125% calcium.

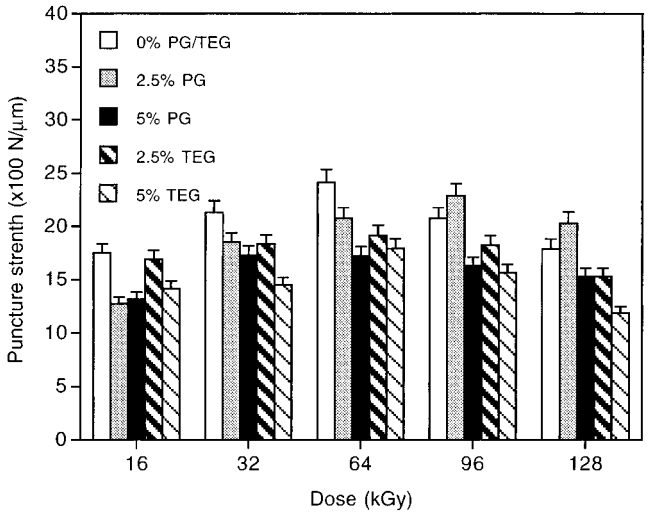


Figure 5. Effect of irradiation dose on the puncture strength of gels in the presence of 0.25% calcium.

gels (Figure 4). The breaking strength of gels having TEG was not significantly affected ($P > 0.05$) by the irradiation process.

At 0.25% Ca^{2+}, gels appeared at 16 kGy for each formulation investigated. The breaking strength of these gels was enhanced with respect to 0.125% Ca^{2+} (Figures 4 and 5). Moreover, at 0.25% Ca^{2+} the breaking strength of gels increased significantly upon exposure to γ-rays up to 64 kGy, followed by a significant decrease at higher doses (Figure 5). Depending on the nature of the plasticizer (i.e. PG vs TEG), the breaking strength of gels increased significantly upon irradiation, followed by a plateau and then by a significant decrease ($P \leq 0.05$). Gels having 2.5% PG showed a maximum of the breaking strength at 96 kGy, while those containing 5% PG showed a plateau going from 32 to 128 kGy (Figure 5). The breaking strength of gels having 2.5% TEG varied slightly with the dose. A maximum was reached at 64 kGy (Figure 5). The effect of the dose was more evident for gels containing 5% TEG: it increased significantly up to 64 kGy, followed by a significant decrease at 128 kGy (Figure 5).

Mechanical Properties of Films. The trend of the puncture strength of films with the irradiation dose was relative to the formulation. The puncture strength of

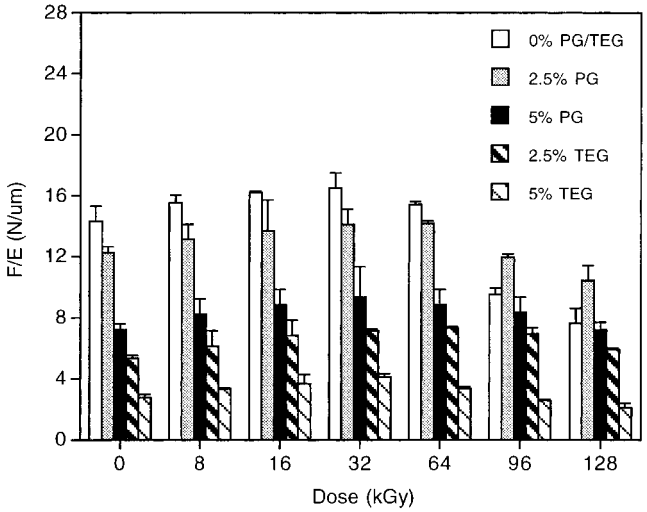


Figure 6. Effect of irradiation dose on the puncture strength of caseinate edible films in the absence of calcium.

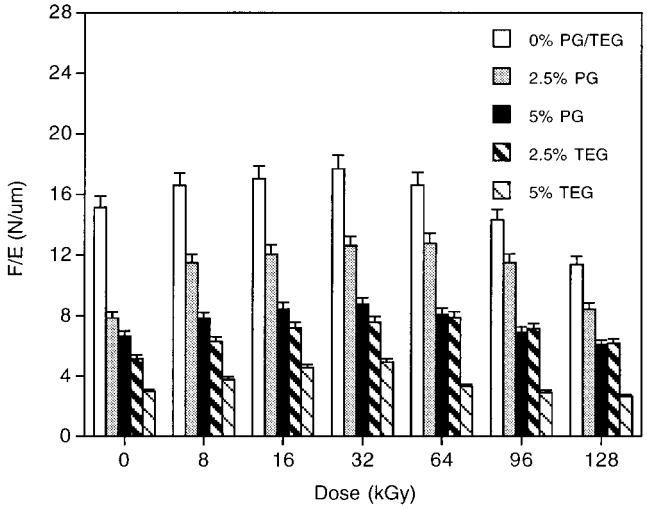


Figure 7. Effect of irradiation dose on the puncture strength of caseinate edible films in the presence of 0.125% calcium.

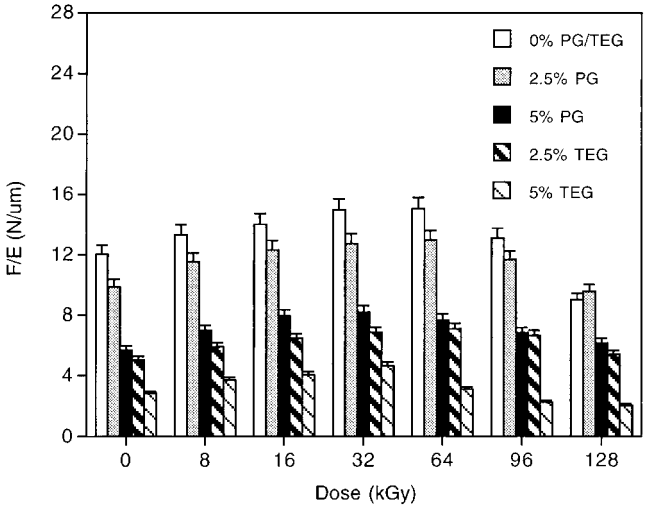


Figure 8. Effect of irradiation dose on the puncture strength of caseinate edible films in the presence of 0.25% calcium.

films formed with caseinate only, i.e., without calcium ions and plasticizers, increased significantly ($P \leq 0.05$) with the dose (Figure 6), followed by a plateau at 16 kGy and by a significant decrease ($P \leq 0.05$) at higher doses (96 kGy). Films containing PG showed a significant decrease ($P \leq 0.05$) of puncture strength values at any doses with respect to 0% PG except at a concentration of 2.5%, at which the puncture strength was higher at 96−128 kGy. The puncture strength of films containing 2.5% PG increased significantly ($P \leq 0.05$) with the dose, followed by a plateau from 32 to 64 kGy and by a significant decrease ($P \leq 0.05$) at 96−128 kGy (Figure 6). The increase of the concentration of PG to 5% caused a further decrease of the puncture strength. The dose was found to modify superficially the puncture strength of films having 5% PG (Figure 6). Films with TEG also showed lower puncture strength values, at any dose with respect to 0% TEG. The irradiation dose was found to poorly affect the puncture strength of films containing 2.5% TEG. A maximum was reached at 32−96 kGy. At 128 kGy, the puncture strength decreased significantly ($P \leq 0.05$). The puncture strength of films having 5% TEG increased significantly with the dose up to 16 kGy, where a plateau was reached and extended to 32 kGy (Figure 6). A significant decrease was then noted (Figure 6). Moreover, the puncture strength was found to be significantly lower ($P \leq 0.05$) at 5% TEG than at 2.5% TEG.

Addition of 0.125% Ca^{2+} ions caused a significant enhancement ($P \leq 0.05$) of puncture strength values of films based on caseinate without plasticizers, a significant decrease ($P \leq 0.05$) for films containing 2.5% PG, and no significant effects ($P > 0.05$) for films having 5% PG and TEG (Figures 6 and 7). Without plasticizers, the irradiation dose did not influence significantly ($P > 0.05$) the puncture strength except at 128 kGy, at which a significant decrease ($P \leq 0.05$) was detected (Figure 7). The puncture strength of films containing plasticizers showed a different behavior upon irradiation. Indeed, the puncture strength of films containing 2.5% PG increased significantly ($P \leq 0.05$) up to 8 kGy, at which a plateau was reached and extended to 96 kGy (Figure 7). At doses corresponding to 96−128 kGy, a significant decrease was observed (Figure 7). The irradiation process had a slight effect on the puncture strength of films having 5% PG. An increase was noted upon γ-irradiation, followed by a plateau (8−64 kGy) and by a decrease at 96−128 kGy. Similar effects were found for films containing 2.5% TEG (Figure 7). The puncture strength for formulations containing 5% TEG was maximum at doses corresponding to 16 kGy. At doses >32 kGy, the puncture strength decreased significantly ($P \leq 0.05$). Addition of 0.25% Ca^{2+} ions caused a significant decrease ($P \leq 0.05$) of puncture strength values for formulations without plasticizers, while no siginificant effects were detected for those containing plasticizers (Figures 6−8). In the absence of plasticizers, the puncture strength of films increased significantly ($P \leq 0.05$) with the dose, followed by a plateau (8−64 kGy) and then by a significant decrease ($P \leq 0.05$) at higher doses (Figure 8). Puncture strength values of films with plasticizers were significantly lower ($P \leq 0.05$) (Figure 8). The irradiation process caused a slight change of the puncture strength values for films having 5% PG or 2.5% or 5% TEG. Nevertheless, the puncture strength increased significantly ($P \leq 0.05$), reached a plateau, and decreased at doses corresponding to 96−128 kGy. The puncture strength of films containing 2.5% PG increased significantly ($P \leq 0.05$) at 32 kGy, where a plateau was reached and extended to 96 kGy. A significant decrease ($P \leq 0.05$) was observed at 128 kGy.

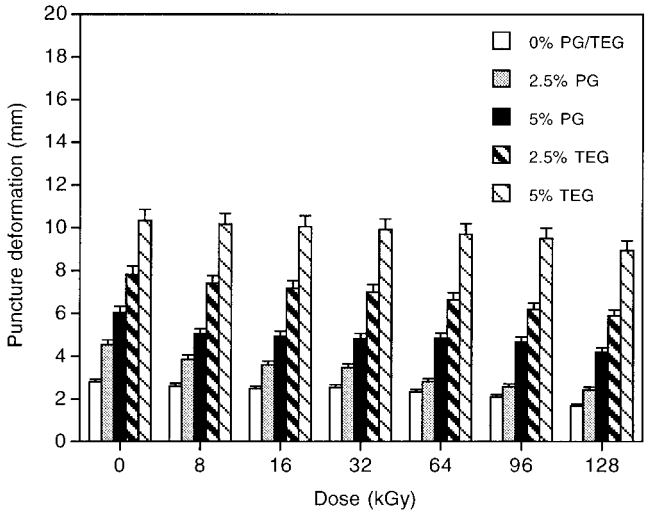


Figure 9. Effect of irradiation dose on the puncture deformation of caseinate edible films in the absence of calcium.

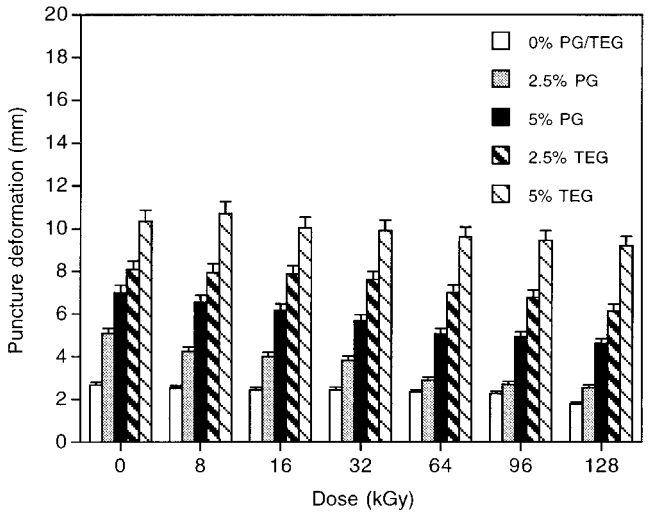


Figure 10. Effect of irradiation dose on the puncture deformation of caseinate edible films in the presence of 0.125% calcium.

The trend of the puncture deformation of films with the irradiation dose was relative to the formulation. The puncture deformation of films formed with caseinate only was not influenced by the irradiation process except at doses >64 kGy, at which it decreased significantly ($P \leq 0.05$) (Figure 9). In all cases, the puncture deformation of films increased significantly when plasticizers were added (Figures 9−11). The puncture deformation of films containing 2.5% PG and without calcium ions showed a significant decrease ($P \leq 0.05$) upon exposure to γ-rays, from 0 to 128 kGy (Figure 9). At 5% PG the puncture deformation decreased significantly ($P \leq 0.05$) from 0 to 8 kGy. However, further irradiation caused a stabilization of the puncture deformation up to 96 kGy. A second decrease of the puncture deformation occurred at 128 kGy (Figure 9). The puncture deformation of films having 2.5% TEG did not vary from 8 to 32 kGy (Figure 9). However, a significant decrease ($P \leq 0.05$) was observed between 0 and 16 kGy and between 64 kGy and 128 kGy (Figure 9). When films contained 5% TEG, the puncture deformation was not affected by γ-irradiation, but at high doses (i.e. 96−128 kGy) a significant decreased was noticed (Figure 9).

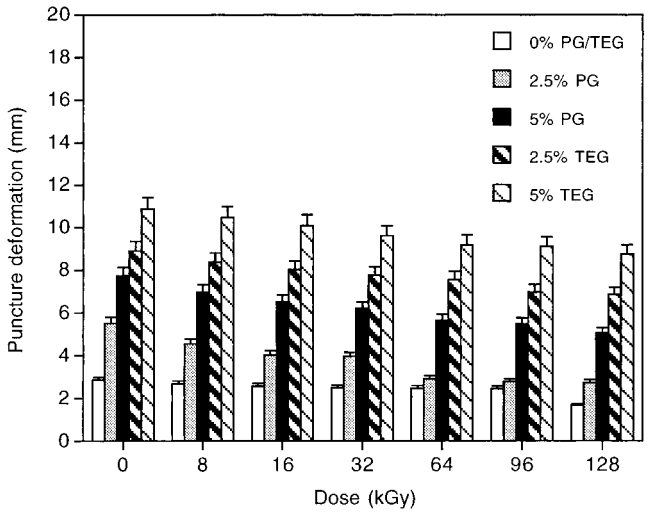


Figure 11. Effect of irradiation dose on the puncture deformation of caseinate edible films in the presence of 0.25% calcium.

Addition of Ca^{2+} did not influence the puncture deformation values of films (Figures 9−11). In the presence of 0.125% Ca^{2+}, the puncture deformation varied slightly with the irradiation dose. The puncture deformation for formulations without plasticizers was not influenced by the irradiation process except at higher doses (96−128 kGy), at which it decreased significantly. Addition of PG or TEG caused a significant increase ($P \leq 0.05$) of puncture deformation values. The puncture deformation of films containing 2.5% PG decreased significantly ($P \leq 0.05$) from the beginning of the irradiation (i.e. from 0 to 8 kGy) (Figure 10). The puncture deformation value then did not vary until 32 kGy (Figure 10). However, at doses >32 kGy a further decrease of the puncture deformation occurred (Figure 10). The puncture deformation for films having 5% PG or 2.5% TEG did not vary upon exposure to γ-rays except at doses >64 kGy, at which a significant decrease ($p \leq 0.05$) was observed as compared to 0 kGy (Figure 10). The puncture deformation for films with 5% TEG began to decrease at doses >16 kGy. In the presence of 0.25% Ca^{2+}, the behavior of the puncture deformation toward the irradiation was somewhat different, except for films without plasticizers (Figures 10 and 11). A significant decrease ($P \leq 0.05$) of the puncture deformation occurred with the irradiation dose (i.e. from 0 to 64 kGy) for films with 2.5% PG or 5% PG (Figure 11). The puncture deformation of films with PG decreased from 0 to 8 kGy; it reached then a plateau up to 32 kGy for films having 2.5% PG and up to 128 kGy for films with 5% PG (Figure 11). A further decrease arose at doses >32 kGy for films having 2.5% PG (Figure 11). The puncture deformation of films with TEG decreased significantly ($P \leq 0.05$) at 16 kGy and reached then a plateau up to 64 kGy at 2.5% TEG and up to 128 kGy for films having 5% TEG (Figure 11). A longer exposure to γ-irradiation (i.e. 96 kGy) induced a further decrease of the puncture deformation in films with 2.5% TEG (Figure 11).

DISCUSSION

In all cases, the amount of bityrosine formed upon irradiation (i.e. cross-links) was improved in the presence of PG and TEG (Figures 1−3). A similar behavior was recently reported with glycerol (Brault et al., 1997)

and was explained by the preferential binding concept (Gekko and Timasheff, 1981).

Likewise and as expected, calcium ions did not affect significantly the formation of bityrosine, since they were added after the ionization process. Calcium ions, the irradiation process, and the heating were essential for the formation of gels. Calcium ions are well-known to act as a firming agent. The irradiation process was reported to affect the conformation of proteins (von Sonntag, 1987). The heating process was reported to be necessary to induce structural changes within the protein, in order that it becomes receptive to Ca^{2+} to form a gel (Barbut and Foegeding, 1993). The increase of the concentration of Ca^{2+} resulted in a significant increase of the puncture strength of gels. This behavior can be explained by interactions of Ca^{2+} with the caseinate. It is known that Ca^{2+} ions favor electrostatic interactions between two adjacent carboxylic groups of different polypeptidic chains (Hongsprabhas and Barbut, 1997). These interactions contribute to a more dense structure of the protein and, thus, to an increase of the puncture strength of gels and films.

The presence of PG and TEG caused a decrease of the breaking strength of gels and films and an increase of the puncture deformation of films (Figures 4−11). Likewise, the breaking strength of gels and films decreased and the puncture deformation of films increased with an increase of the concentration of PG and TEG. These results clearly emphasize the plasticizing effect of both PG and TEG. Similar results were recently reported with glycerol (Brault et al., 1997). In any case, TEG was found to be a more effective plasticizer than PG. In other words, TEG is more able to reduce internal hydrogen bindings within the caseinate (i.e. caseinate−caseinate or water−caseinate bindings), thereby decreasing the internal forces and resulting in an increase of intermolecular spacing. This effectiveness of TEG with respect to PG might be explained by the structure of these plasticizers. Indeed, TEG has four sites (two hydroxy groups and two ether groups) that might interact with caseinate, while PG has only two sites (two hydroxy groups).

The irradiation treatment affected significantly ($P \leq 0.05$) the breaking strength of gels and films (Figures 4−8). For all cases investigated, the trend of the puncture strength with the dose was an increase, followed by a stabilization and a decrease. The puncture strength values can be associated with the amount of bityrosine produced, i.e., cross-links, during the irradiation process. A greater amount of bityrosine means an increase of cross-links within polypeptide macromolecules. Cross-links confer to any material flexibility and/or rigidity, depending on the cross-link density; that is, the higher this value is, i.e., the higher branched chains are, the more rigid the material (Stevens, 1990). Hence, the increase of the breaking strength of gels and films is related to the formation of cross-links between caseinate polypeptide. However, as the dose is increased, the breaking strength of gels and films decreased. This uncommon trend might be explained by a partial fragmentation or conformational changes of the caseinate at very high irradiation doses, mainly 96 and 128 kGy. Proteins irradiated at very high doses were reported to be more susceptible to molecular damage of the primary, secondary, tertiary, and quaternary structures (Garrisson, 1987). The ionizing irradiation interacts with a protein molecule and may cause a permanent damage in the form of covalent bond breaks or conformational changes (Poitier et al., 1994). Such effects at high irradiation doses were recently observed with β-galactosidase from *Escherichia coli* (Poitier et al., 1991). More work (for instance, X-ray crystallography of the protein) is needed to clearly identify structural modifications of the caseinate at high doses.

The puncture deformation exhibited an unusual behavior toward the irradiation treatment. Indeed, the puncture deformation either was not affected by the irradiation or it decreased, depending on the formulation. In some cases, it showed first a decrease, followed by a stabilization and then by a second decrease at higher doses. These results suggested that high cross-link density is quickly reached, at lower doses, giving a rigid film rather than an elastic one. The further decrease that occurred at higher doses (>64 kGy) might be explained once again by a partial fragmentation or conformational changes of the caseinate at these doses.

The presence of calcium ions did not really modify the mechanical behavior of films except the puncture strength for films without plasticizers. Interactions of calcium ions with caseinate, i.e., interactions between calcium ions and two adjacent carboxylic groups of different polypeptidic chains (Hongsprabhas and Barbut, 1997), are rather electrostatic, whereas the formation of bityrosine involves covalent bindings. Electrostatic bindings are weaker than covalent bindings (Atkins, 1990) and require thus a lower puncture strength. An increase of the puncture strength was not necessarily expected for the addition of calcium ions, but rather a stabilization as observed (Figures 6−8). However, it must be pointed out that the decrease of the puncture strength with the addition of calcium ions is not completely understood. Nevertheless, the increase of the cohesion by calcium ions is well-known to increase the barrier properties of edible films (Avena-Bustillos and Krochta, 1993). Water vapor and gas permeability investigations are currently underway to find out whether a synergetic effect of both the effect of the γ-irradiation and the presence of calcium ions exists.

To evaluate the biodegradability of our films, experiments using *Pseudomonas aeruginosa* will be carried out with films irradiated at 4 and 64 kGy, i.e., with films having different cross-link densities. Preliminary results have shown that the film irradiated at 4 kGy (low cross-link density) undergoes a faster biodegradation than the one irradiated at 64 kGy (high cross-link density). Results of this investigation will be presented in a later paper.

CONCLUSION

This investigation has clearly demonstrated that γ-irradiation is responsible for the generation of bityrosine, i.e., cross-links, accounting for the making of free-standing sterilized edible films, based on caseinates. Addition of PG or TEG has significantly increased ($P \leq 0.05$) the formation of cross-links. This effect was explained by the preferential binding concept. Moreover, PG and TEG improved the mechanical strength and the flexibility of films. TEG was, however, found to be a more efficient plasticizer than PG. The chemical structure of TEG might account for this effectiveness. Calcium ions allowed the formation of gels based on caseinates, and their breaking strength was directly related to the concentration of these ions. This effect

was rationalized by interactions of calcium ions with caseinate that lead to a denser structure. High irradiation doses were found to affect severely the mechanical properties of films. It was assumed that caseinate underwent a partial fragmentation or conformational changes at high doses. The formation of bityrosine upon γ-irradiation led to a branching of polypeptide chains to form a three-dimensional network. This three-dimensional network and interactions between the protein and plasticizer molecules contribute to the mechanical behavior of the films. However, an inadequate irradiation period will strongly affect the structure of the film and thus its mechanical behavior.

It is believed that these caseinates might find several practical implementations. For instance, they might be used as films in food packaging; as coating agents for cheese, fruits, and vegetables, and as microencapsulating agents of flavors and medicaments.

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