Effect of Ionizing Radiation on Tilapia (*Oreochromis niloticus*) Protein Biofilms

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Abstract. New alternatives to traditional packaging have considered the use of renewable sources as raw material to form films including proteins. In this paper we used myofibrillar protein from Nile Tilapia (*Oreochromis niloticus*), that in our country, is commercialized at competitive prices comparative to these practiced in developing country. The films were prepared according a casting technique with two levels of plasticizer: 25% and 45% glycerol. The films were irradiated in electron accelerator type Radiation Dynamics, 1.5MeV at dose range from 0kGy to 200kGy. The samples were kept at 25ºC and 58% relative moisture until the analysis. The main properties studied were color, opacity, solubility, and mechanical properties.

1. Introduction

Although traditional packaging made from petroleum fractions has an excellent protection for a great number of products, it becomes a serious concern to the environment, as the majority is not biodegradable. New alternatives consider the use of renewable sources as raw material to form films, among them proteins, cellulose derivatives, alginates, pectin, starches, waxes, acylglycerols, fatty acids [1]. Several studies evolving thermal treatment, use of cross-linking agents, adjustment of pH and exposure to radiation, were carried out in order to improve the film [2].

Radiation of plastics is a way to induce their cross-linking and to improve considerably their performance. As the cross-linking process changes a linear network into a three dimensional one, consequently it evolves a relevant modification of the characteristics of the material [3]. In fact, a certain degree of anchorage avoids intermolecular movements and makes possible the elastic deformation under stress [4].

Ionizing radiation was demonstrated to be effective to improve the caseinates cohesion [5]. When gamma radiation is applied to protein film forming solution it resulted in an improvement in mechanical properties of whey protein films. The radicals formed during radiolysis, specially OH• promote a binding between two adjacent tyrosine molecules forming a bityrosine, that is one of other possible mechanisms occurring and responsible for an improvement in a better resistance to tensile strength [6]. Others papers showed the improvement in soy protein films based on physical cross-linking using gamma radiation [7,8,9].

By the other side, there is little information using ionizing radiation from electron beam machine in improvements of natural polymers. A radiation modification of starch-based plastic sheets was studied and indicated the tensile strength improved due to the formation of intact network structure [10].

In our country, proteins from animal origin were commercialized at competitive prices comparative to these practiced in developing country, in special myofibrillar proteins from fish cultured in captivity. Besides Nile Tilapia (*Oreochromis niloticus*) presents a fast grow and good handling that represents interesting productivity [11].

The mechanical and barrier properties of protein-based films are generally better than those of polysaccharide-based films [11,12]. This is due to the fact that, contrary to polysaccharides which are homopolymers, proteins have a specific structure (based on 20 different monomers) which confers a wider range of potential functional properties, specially high intermolecular binding potential [12]. Besides myofibrillar protein from Nile Tilapia has 3.43% (aminoacid: protein) of tyrosine, a potential cross-linking site under radiation process.

The aim of this paper was to study the effect of ionizing radiation on biofilms made from Nile Tilapia myofibrillar proteins.

2. Material and Methods

2.1. Extraction of proteins

Nile Tilapia (*Oreochromis niloticus*) was submitted to a thermal cooling in a mixture of ice and water cut in wisp pieces to get proteins in *pre-rigor mortis*. The muscle without bones was ground, washed four times with distilled water at 5ºC, in ratio 1:2 (muscle : water) and the washed mince was passed through a sieve (180mm, ABNT 80) to remove upper water. The muscle was reduced in a processor during 10 min. and the mass was passed in another sieve (0.59mm, ABNT-30) to remove final connective tissues [13]. The final mass was lyophilized in a Helotrap CT-60e.

2.2. Preparation of films

The films were obtained according a casting technique, consisting in a dehydration of a film-forming solution (FFS) in a Plexiglas support. FFS were prepared in 1% protein (m/v) with two level of plasticizers: 25% and 45% glycerol. Net protein content determined was 80.98%. pH was kept in 2.7 with glacial acetic acid, using a Tecnal, TEC-2 pHmeter. The SSF were treated thermally at 40ºC during 30min (Tecnal, TE184) [11,14]. The films were dried in controlled room (35ºC, with circulation of air) during 24-48h. The thickness of each film was measured in a digital micrometer (probe of 6.4mm diameter, Mitutoyo) in nine different random points. The films were conditioned at 58% relative moisture and 25ºC in a drier containing saturated aqueous NaBr solution during 4 to 7 days before the characterization.

2.3. Irradiation

The films were irradiated at room temperature by EB, with beam current of 2.01mA and acceleration energy of 0.550MeV, generated by the Radiation Dynamics Inc. (USA). Irradiation doses varied from 25kGy to 200kGy. Dosimeter was carried out using cellulose triacetate – CTA (Fuji Photo Film Co., Tokyo, Japan).

2.4. Color and opacity measures

Color was measured in a miniscan colorimeter (HunterLab, XE) and the conditions consisted of D65 luminance ("day light"), 10º incident angle, 30mm diameter entrance cell, using a CIELab color scale. The parameters L, a and b were determined and color difference was calculated by equation 1 [15]:

$$•E = [(L - L_s) + (a - a_s) + (b - b_s)]^{0.5} \qquad \text{Eq. 1}$$

where L_s*, a_s* and b_s* were CIELab parameters for blank standard.

Opacity was measured by HunterLab method [15] using the same equipment for color and determined by equation 2:

$$Y = (Yp / Yb) \times 100 \qquad \text{Eq. 2}$$

where Yp and Yb were CIELab parameters for black and blank standards, respectively.

Color and opacity measurements were made in triplicata.

2.5. Solubility

Films with known moisture were cut (2cm diameter) and immersed in distilled water with soft agitation and at 25°C (room with controlled moisture and temperature). After 24h, films were dried until constant weight. Solubility is expressed in terms of dry mass loose. Measurements were in triplicate [11].

2.6. Mechanical properties

Mechanical properties of films were determined by tensile tests using rectangular samples of 100 mm X 16 mm, initial grips separation of 80 mm and cross-head speed of 0.9 mm/s [16]. The tensile strength (force/initial cross-sectional area) and deformation at break ($\Delta l / l_0$) were determined with the software Texture Expert V.1.15 (SMS) directly from the stress X strain curves, and the elastic modulus was calculated as the inclination of the linear initial portion of this curve. A total of twelve specimens were tested for each film and dose type.

2.7. Statistical analysis

All data was submitted to a statistical treatment, consisting of anova and Duncan multi range test (Statistica 5.1, StatSoft, 1998).

3. Results and discussion

Color, Opacity and solubility measurements for films containing 25% glycerol are presented in Table I and for films with 45% glycerol in Table II. Films with 45% glycerol presented fewer values than those with 25% glycerol, agreeing with results of Paschoalick [14] that attributed this fact to dilution effect by the glycerol, once it is colorless. Radiation dose caused a slightly increase of color reaching the major value in 150kGy dose for both cases.

Radiation dose had no marked trend on opacity results although values oscillated. Glycerol plasticizer affects the opacity results in similar way of color measurements.

The increase of plasticizer level affected the solubility, as expected. The least value of solubility was obtained for 25% glycerol film at dose 150kGy and 200kGy (statistically not significant). Solubility values of films based on Nile Tilapia myofibrillar protein are inferior to the greatest of protein films in literature. We can mention as example: films based on sardine myofibrillar protein (from 33% to 47%), films based on rice protein concentrate (from 35.3% to 76.4%), films based on isolated milk whey protein (above 50%), films based on isolated soy protein (from 26.81% to 39.41%) [13].

TABLE I: OPACITY, COLOR AND SOLUBILITY MEASUREMENTS IN FUNCTION OF ABSORBED DOSE FOR FILMS CONTAINING 25% GLYCEROL.

Dose(kGy)	Opacity	Color	Solubility
0	4.6 ± 0.8 ab	17.9 ± 0.6 ab	16.3 ± 1.5 a
25	5.4 ± 0.4 b	20.0 ± 2.3 bc	17.5 ± 0.6 a
50	4.5 ± 0.4 ab	15.6 ± 1.4 a	16.1 ± 1.8 a
100	3.8 ± 0.9 a	18.2 ± 1.0 ab	17.1 ± 1.7 a
150	5.4 ± 0.8 b	21.4 ± 1.8 c	15.5 ± 1.5 a
200	3.6 ± 0.5 a	19.0 ± 1.3 bc	15.4 ± 0.6 a

Means values followed by different letters in the same column are significantly different ($p<0.05$).

TABLE II: OPACITY, COLOR AND SOLUBILITY MEASUREMENTS IN FUNCTION OF ABSORBED DOSE FOR FILMS CONTAINING 45% GLYCEROL.

Dose(kGy)	Opacity	Color	Solubility
0	2.5 ± 0.2 a	13.7 ± 0.3 a	23.1 ± 4.2 a
25	2.9 ± 0.6 ab	13.3 ± 2.5 a	24.7 ± 1.1 a
50	4.6 ± 1.6 b	14.4 ± 0.6 a	24.0 ± 2.6 a
100	3.1 ± 0.6 ab	15.4 ± 1.2 a	24.1 ± 3.0 a
150	3.5 ± 0.3 ab	21.3 ± 0.5 b	24.4 ± 1.8 a
200	2.9 ± 0.9 ab	18.6 ± 1.3 c	25.0 ± 1.6 a

Means values followed by different letters in the same column are significantly different ($p<0.05$).

Mechanical properties are presented in Tables III, IV and V and in Figures 1, 2 and 3. Tensile strength showed a slightly trend to increase with increment of radiation dose (Table III and Figure 1). Tensile strength value reached the major value for film with 45% glycerol at 100kGy ($p<0.05$), followed by values at 50kGy and 150kGy (statistically different from values at 0kGy). This tendency occurred also for films with 25% but the maximum value at 100kGy was not statistically different from values at 150kGy and 200kGy. Considering plasticizer effect, the major values for tensile strength were obtained for films containing 25% glycerol. Moreover, the resistance of these radiated films was slightly higher than similar films produced without radiation [16].

TABLE III: TENSILE STRENGTH FOR FILMS WITH 25% AND 45% GLYCEROL IN FUNCTION OF RADIATION DOSE

	Tensile Strength (Mpa)	
Dose (kGy)	25% Glycerol	45% Glycerol
0	6.74 ± 1.83 a	2,13 ± 0.64 a
25	7.26 ± 0.97 a	2,49 ± 0.57 ab
50	6.91 ± 1.70 a	3,40 ± 0.34 b
100	9.24 ± 0.70 b	4,32 ± 0.69 c
150	7.46 ± 1.47 ab	3,19 ± 0.55 b
200	7.80 ± 1.19 ab	2,94 ± 0.93 ab

Means values followed by different letters in the same column are significantly different (p<0.05).

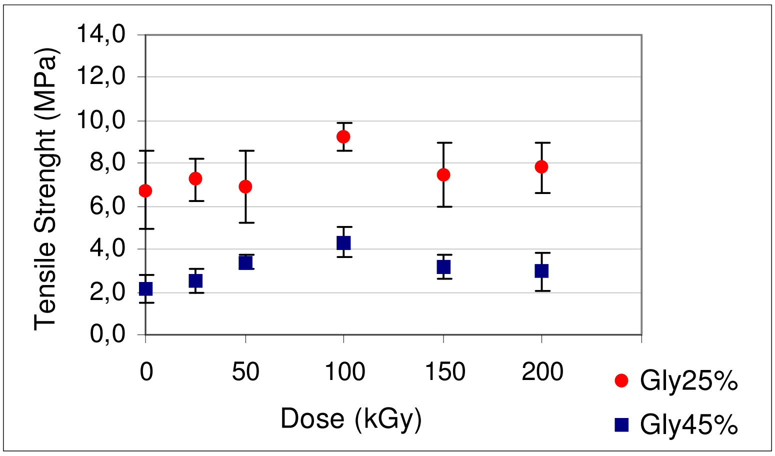

FIG.1. Effect of radiation doses on tensile strength of films with different level of plasticizer.

From Table IV and Figure 2 it can be observed that elongation values had no influence from radiation dose (p<0.05). Effect of plasticizer resulted in major values for films containing 45% glycerol. Major tensile strength values and elongation values that not suffer reduction indicates that irradiation contributed to a better resistant films at 100kGy dose. Contrarily to the resistance, the elasticity of these radiated films was generally lower than those of similar films produced without radiation [16].

TABLE IV: ELONGATION FOR FILMS WITH 25% AND 45% GLYCEROL IN FUNCTION OF RADIATION DOSE

	Elongation (%)	
Dose (kGy)	25% Glycerol	45% Glycerol
0	22.0 ± 8.1 a	41.6 ± 27.3 a
25	28.0 ± 21.0 a	51.2 ± 22.9 a
50	29.1 ± 10.9 a	55.3 ± 24.3 a
100	25.3 ± 17.8 a	66.7 ± 18.3 a
150	22.1 ± 15.6 a	34.5 ± 18.7 a
200	25.4 ± 10.7 a	42.8 ± 22.1 a

Means values followed by different letters in the same column are significantly different ($p<0.05$).

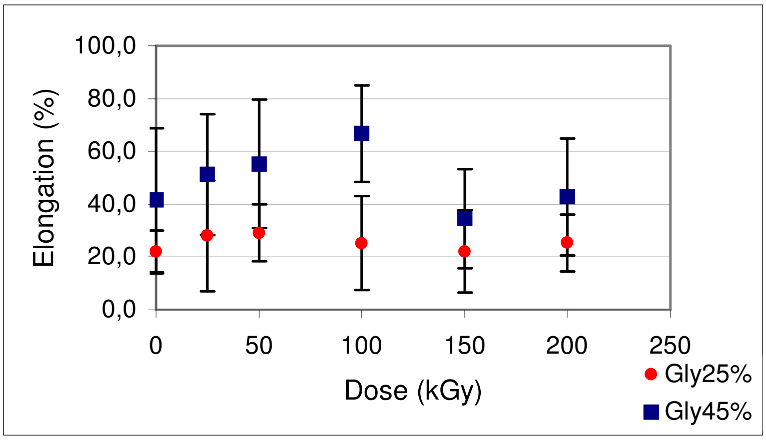

FIG.2. Effect of radiation doses on percentage elongation of films with different level of plasticizer.

Young modulus remained practically constant with increase of radiation dose, as can be observed in Table V and Figure 3. In this case, these results agree with those obtained by Garcia and Sobral [16].

TABLE V: YOUNG MODULUS FOR FILMS WITH 25% AND 45% GLYCEROL IN FUNCTION OF RADIATION DOSE

	Young Modulus	
Dose (kGy)	25% Glycerol	45% Glycerol
0	5.21 ± 0.46 a	1.81 ± 0.29 ab
25	5.40 ± 0.77 a	1.96 ± 0.25 ab
50	5.00 ± 0.60 a	1.83 ± 0.27 ab
100	5.17 ± 0.61 a	1.82 ± 0.26 ab
150	5.17 ± 1.11 a	2.08 ± 0.73 b
200	5.45 ± 0.65 a	1.62 ± 0.35 a

Means values followed by different letters in the same column are significantly different ($p<0.05$).

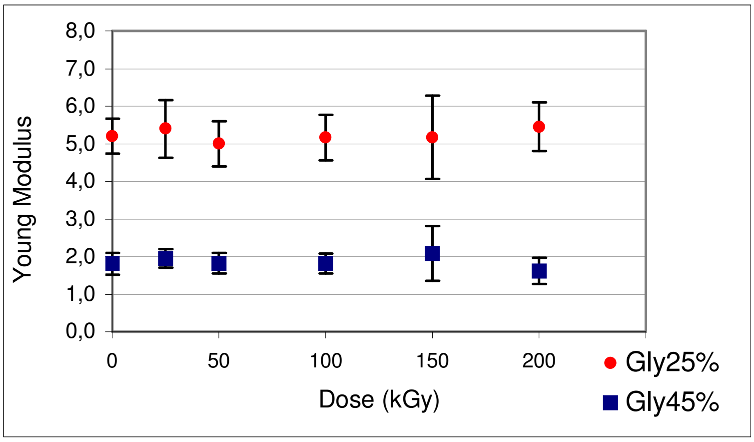

FIG.3. Effect of radiation doses on young modulus of films with different level of plasticizer.

4. Conclusion

Radiation from electron beam on films based on myofibrillar protein caused an slightly increase on tensile strength value in an absorbed dose of 100kGy, while elongation value at this dose had no reduction. Other properties studied like color, opacity and solubility seemed not be significantly affected by radiation.

5. References

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