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POLYMERIZATION OF TILAPIA (*OREOCHROMIS NILOTICUS*) SKIN BY ELECTRON BEAM IRRADIATION

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ABSTRACT

The culture of tropical tilapia (*Oreochomis niloticus*), have had importance in the last decade due its fast growing, fast reproduction characteristics, high tolerance of climate variations in our country and high disease resistance. The tilapia skin represents from 4.5 % to14 % of fish weight, but is a byproduct that is generally discarded or sold at low cost to feed mills. The general composition of these skins comprises protein, water, minerals and fatty matter where the relative portions of these materials depends of upon animal specie, age, breed, feeding and other animal habits. The putrescible raw animal skins can be chemically and physically treated to make it in non-putrescible stabilized material; it results in a soft and flexible polymeric material. In this work the tilapia skins were exposed to ionizing irradiation from electron beams under comprised doses of 20 kGy and 40 kGy and dose rates of 2.2 kGy/s and 7.4 kGy/s. The raw skins and the chemically degreased skins were the studied materials. The tensile strength and elongation at break were the mechanical parameters evaluated in dry and wet materials. The optical microscopy was used to evaluate some histological and morphological characteristics in irradiated and non-irradiated samples. In this case, in natura samples shows collagen fibers randomically; at irradiated samples, collagen fibers are disposed as a sheaf of straight filaments and it is the same morphology of chemical treated skins. Also, the polymeric product obtained when skins are treated with oxidizing ions were used to compare some results. Irradiated samples shows high integrity and high tensile strength in comparison to the polymeric product obtained by oxidizing ions reaction. These results are discussed.

1. INTRODUCTION

Collagen is the most abundant protein representing nearly 30% of total proteins in the animal body. It is the major component of extracellular matrix and is vital for mechanical protection of tissues, organs, and physiological regulation of cellular environment [1]. The use of collagen is rapidly expanding in cosmetics and pharmaceutical industry. Among various types, type I collagen has been extensively used as biomaterial for the development of tissue engineering constructs and wound dressing systems due to its low antigenic and high direct cell adhesion properties [2].

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Many methods are utilized for polymerization and crosslink collagen and oxidizing ions are the most traditional; chromium and aluminum ions are used commonly [3]. UV irradiation has been used in medical and pharmaceutical research to crosslink collagen and gelatin films [4].
The goal of this work is to study the tilapia skins exposed to ionizing irradiation from electron beams. The raw skins and the chemically degreased skins are the studied materials. Also, the polymeric product obtained when skins are treated with oxidizing ions are used to compare the results.

2. EXPERIMENTAL METHODOLOGY

The tilapia raw skins were kindly available by APTA, a governmental agribusiness technological agency. These skins were scales free, slighted and frosted. The irradiation was performed in atmosphere air on a Job 188 Dynamitron® Electron Beam Accelerator with 1.5 MeV energy under comprised doses of 20 kGy and 40 kGy and dose rates of 2.2 kGy/s and 7.4 kGy/s. The magnitude of absorbed doses are close to the sterilization dose; the dose range used was to assert low degradation level to collagenous material.

The fish skins were irradiated by two modes: *in natura* and previous processed (or "processed" as will be denominated from now in this work). The previous process consists in degrease *in natura* skins with a non-ionic tensoactive (0.5 % v/v) and organic solvent (10 % v/v of hexane) dispersed in water for 1 hour. After, the degreased skins were let in a bath with a proteolitc enzyme aqueous solution (0.1 % w/v).

After irradiation process, the skins were cut from ASTM D882-10 dumbbell shaped polymer samples for uniaxial tensile tests with width 4 mm and length 27 mm by standard molded razor. The samples were obtained from two skin meaning: a) longitudinal (at the scales sense) and transversal (orthogonal of scales sense).

Mechanical tests at samples cut at both meanings were evaluated at room temperature on a universal tensile testing machine Lloyd LXR with control of longitudinal strains in the active zones of samples. The tensile force was measured by a standard load cell (max. 0.1 kN). The engineering stress was determined as the ratio of the axial force to the cross-sectional area of specimens in the stress-free state. The elongation rate was 10.00 mm/min in all assays.

The results are compared to skins polymerized by oxidizing ions as a traditional method, where chromium was the oxidizing ion utilized. The process consists in degrease and remove the remaining meat by proteolitc enzyme process, mentioned above, after, the samples are immersed in green chromium oxide (chromium (III) oxide) aqueous solution (10 % w/v). After 2 hours, the skins are removed from bath and the stretched samples were air dried by two days. These samples are denominated "chemical processed" from now.
The histological analysis was performed with *in natura* samples (defrosted and conserved in formol solution 10 % v/v) and irradiated samples, that are embedded in paraffin mold. The sections (3 μm in thickness) were stretched on water, placed on glass slides, stained with 0.5% w/v toluidine blue, dehydrated and mounted for optical microscopy.

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3. RESULTS AND DISCUSSSION

The histological analysis shows a morphology compatible with collagen exclusively. The morphology of fresh raw skin is filamentous but these fibers are randomically distributed on all sample, with some gaps among these morphological formation and they can be disposed as a clusters of fibers where they can be folded (Fig.1). These clusters can be correlated to quasihexagonal packing of collagen fibrils observed by Orgel and co-workers [5].

The chemical process with oxidizing ions allows fibers organization, where they are disposed as a sheaf of straight filaments (Fig.2); also, some gaps interfibers are observed. The addition of chromium allow this morphological observation and it is in great details at Wu *et alli* work [6].

Fig 3. shows irradiated *in natura* skins; the same morphology observed at Fig. 2 is presented in this case. The gaps interfibers are more frequently observed in samples irradiated at 20 kGy and at lower dose rate, that give a fragile aspect to these samples. The irradiated processed skins (Fig. 4) show, also, organized fibers in all sample field but gaps interfibers are less frequent, as shows Fig.4a, obtained in high magnification.

The oxidizing ions crosslink the collagen fibers but they do not disrupt the triple helical structure. By analogy, the absorbed doses, in the level and conditions that were applied, seems only trigger the crosslinkage interfibers.

a) b)

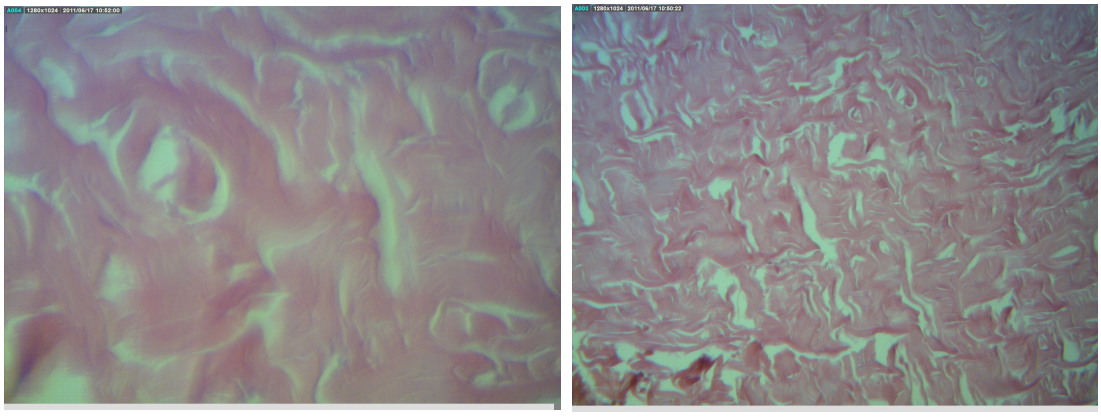

Figure 1. Histological micrographies of fresh raw skin. a) magnification of 400 x and b) magnification of 100 x.

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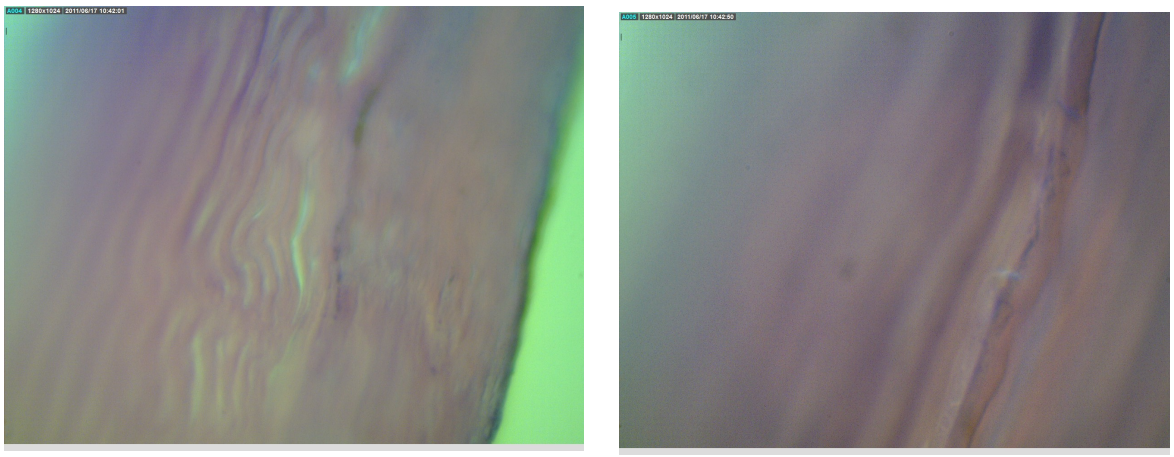

Figure 2. Histological micrographies of chemical processed skin. a) magnification of 40 x and b) magnification of 1000 x.

a) b)

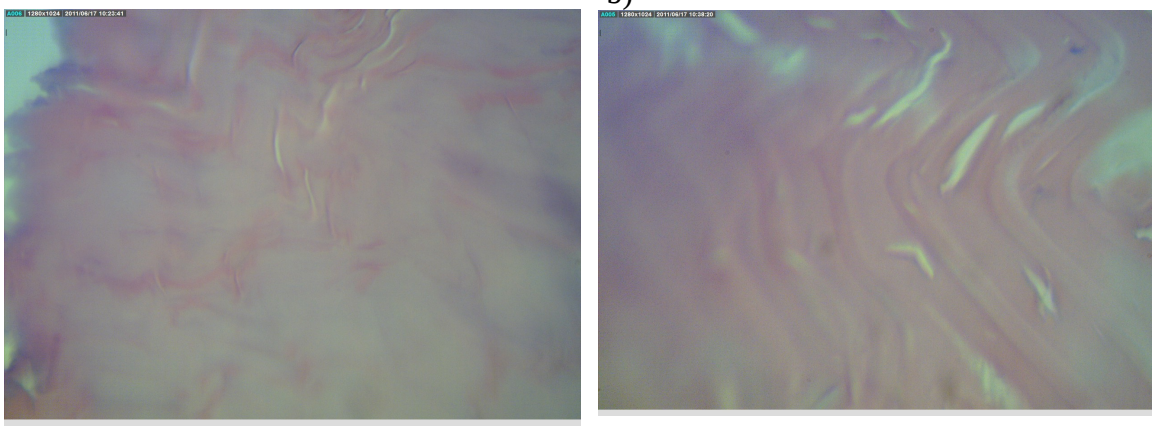

Figure 3. Histological micrographies of irradiated skin. a) *in natura* skin at 20 kGy and 7.4 kGy/s - magnification of 40 x and b) *in natura* skin at 20 kGy and 2.2 kGy/s - magnification of 100 x.

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a) b)

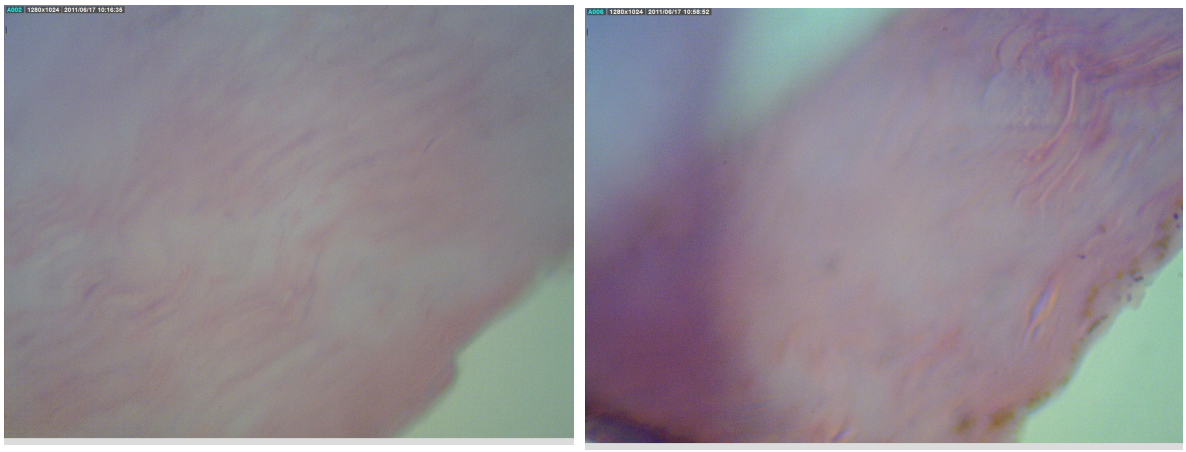

Figure 4. Histological micrographies of irradiated skin. a) processed skin at 40 kGy and 7.4 kGy/s - magnification of 100 x and b) processed skin at 40 kGy and 2.2 kGy/s - magnification of 40 x.

The mechanical analysis shows interesting results. The tensile strength of longitudinal cut at dry *in natura* skin (Fig.5) show the same behavior in all dose rates tested: the average value at 40 kGy is higher than 20 kGy, but the standard deviations are great in all samples and the values are close each other. In all cases, the average values and their standard deviation are lower than polymerized skins by chemical process.

Tensile strength of longitudinal cut at humid *in natura* skins have an opposite behavior: the average value at 20 kGy is lower than 40 kGy and all results are higher than humid skin sample polymerized by chemical process. These results suggest moisture at irradiated *in natura* skins allow high mechanical resistance face dryness at irradiated *in natura* skins.

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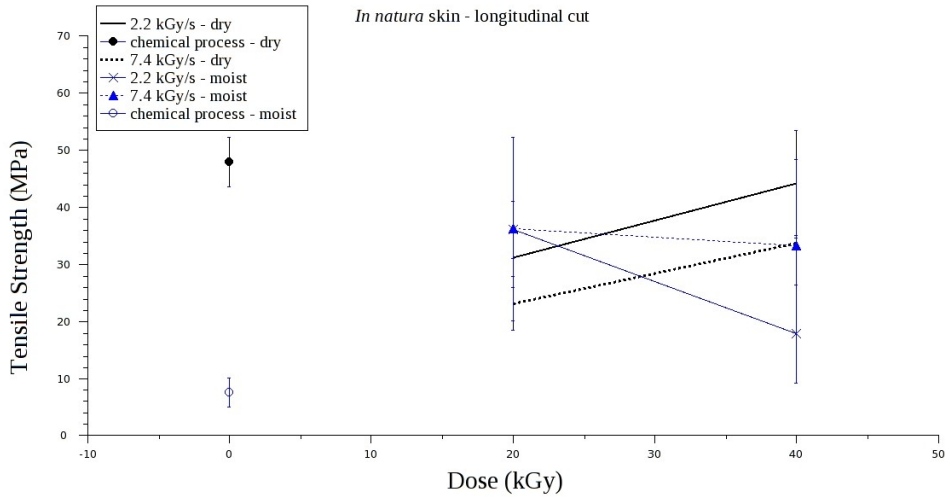

Figure 5. Tensile strength of *In natura* skin (longitudinal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

The tensile strength at transversal cut of dry *in natura* skins (Fig. 6) increase with absorbed dose (in a distinct dose rate); analyzing both dose rates performed in this study, tensile strength increase at the lower dose rate.

For humid samples this behavior is contrary. Also, all values are greater than dry or moist skins polymerized by chemical process, that suggests irradiation process improve the polymerization process in this kind of sample.

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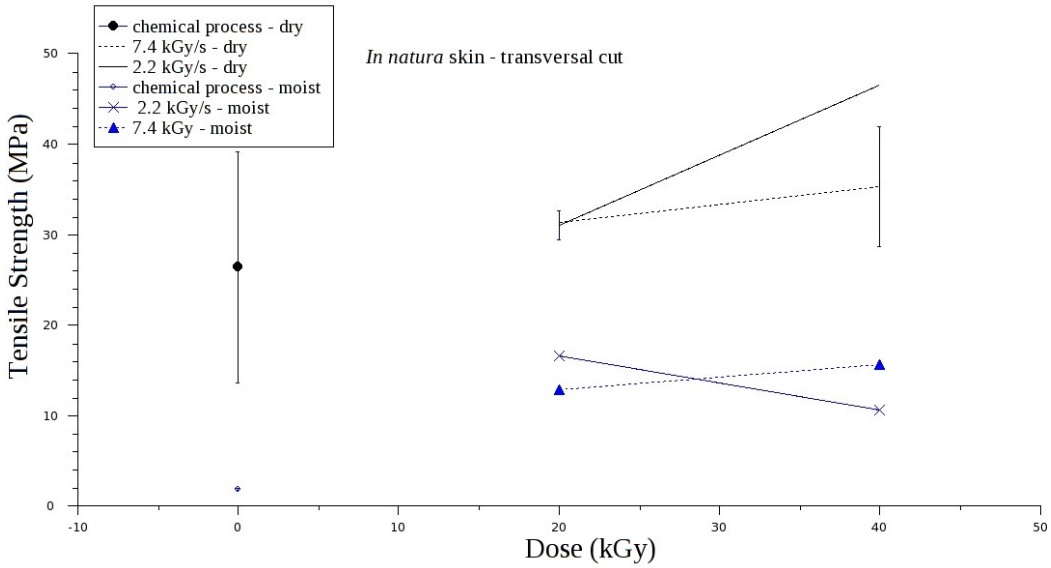

Figure 6. Tensile strength of in natura skin (transversal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

If processed skins are used (Fig. 7), the tensile strength at dry samples is higher than at dry skins polymerized by chemical process and independent of absorbed dose and dose rate (if considering average and standard deviation values). Moist irradiated samples have higher tensile strength values than samples polymerized by chemical process. At the lower dose rate, the values decrease with dose rate; at the higher dose rate, occur the opposite. These results suggests irradiation improve high mechanical resistance at processed skins compared to *in natura* skins.

Fig. 8 shows the tensile strength results for transversal cut of irradiated processed skins. Dry samples show decreasing values for tensile strength in function of increase of absorbed dose and these values are higher at low dose rate. Moist samples present low tensile strength at 20 kGy; this parameter is high at 40 kGy (lower dose rate). The opposite occurs at the high dose rate

The previous degrease process applied to skins samples removes the lipids and the remaining meat to assert high mechanical characteristics performance. The following results are only for processed skins submitted to irradiation procedures.

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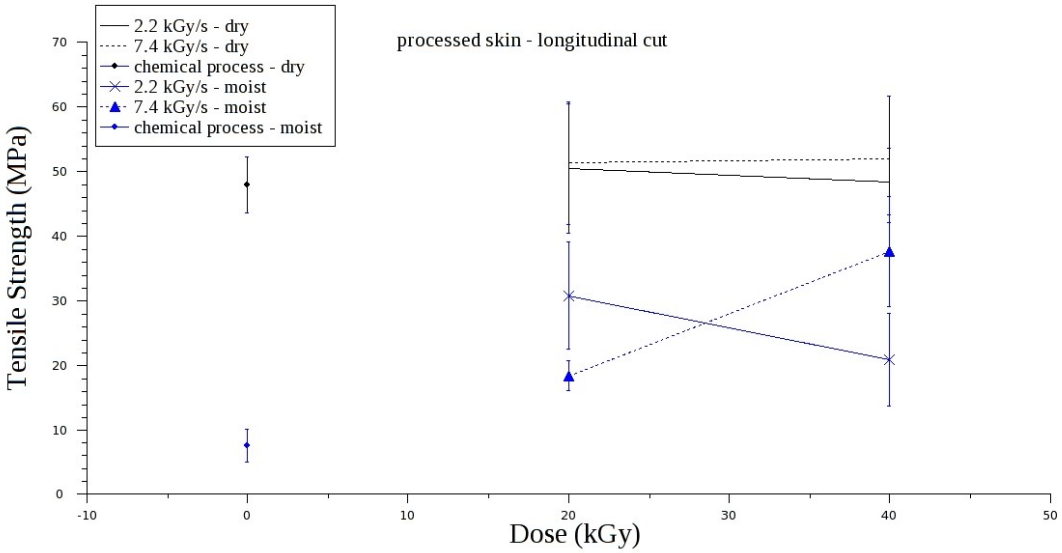

Figure 7. Tensile strength of processed skin (longitudinal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

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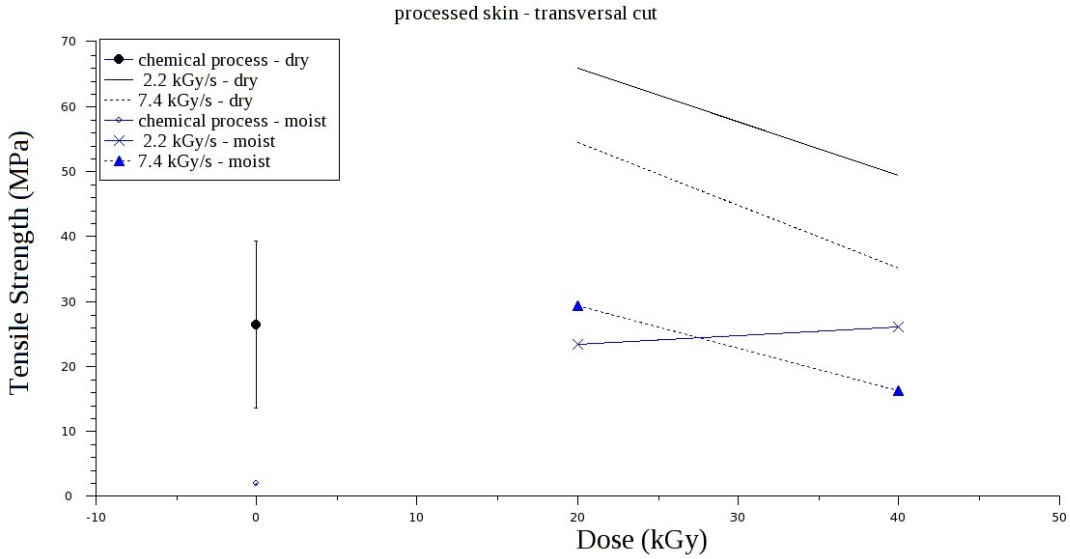

Figure 8. Tensile strength of processed skin (transversal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

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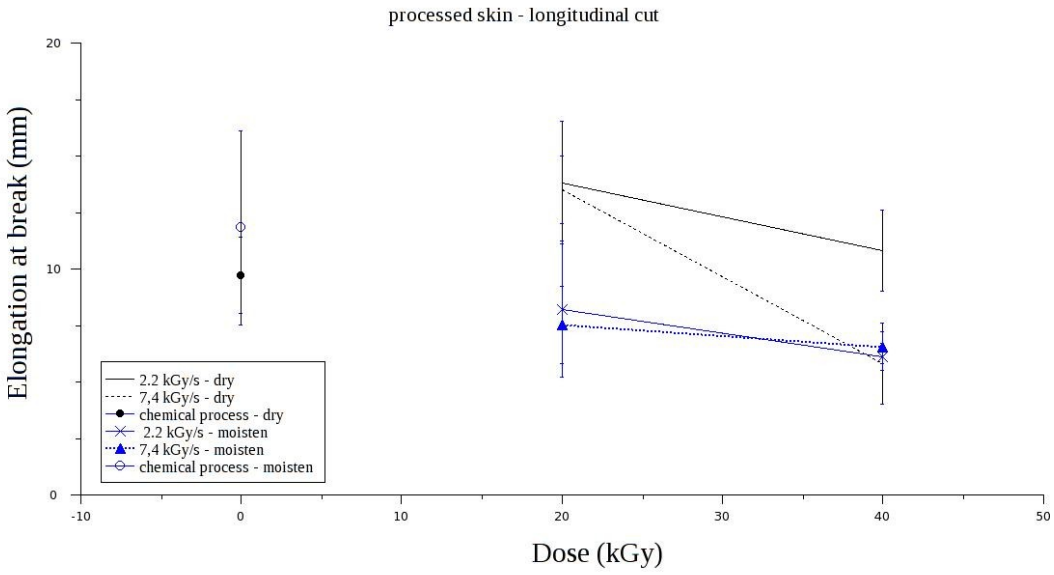

Figure 9. Elongation at break of processed skin (longitudinal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

The elongation at break for longitudinal cut of dry processed skin samples (Fig. 9) shows at a done dose rate, high values at 20 kGy and the values decrease at 40 kGy; high values are observed at the lower dose rate and all values of this parameter are higher than dry skins polymerized by chemical process. Moist irradiated samples presented the same behavior that dry skins (considering all average and standard deviation values), however, the irradiated samples do not elongate at the level that skins polymerized by chemical process and they present always the lower values.

The polymerization by chemical process have produced skins impregnated with chromium ions and it is observed by the green color in all samples obtained by chemical process. By the literature, high chromium concentration ensure the collagen crosslinking, but the final product contains these ions at a non-removable level, even, it can influence at water uptake characteristics.

In this study, the samples obtained by chemical process have high water swelling compared to irradiated samples. it influences at the increase of elongation and is a drawback because it influences to the decrease of mechanical resistance.

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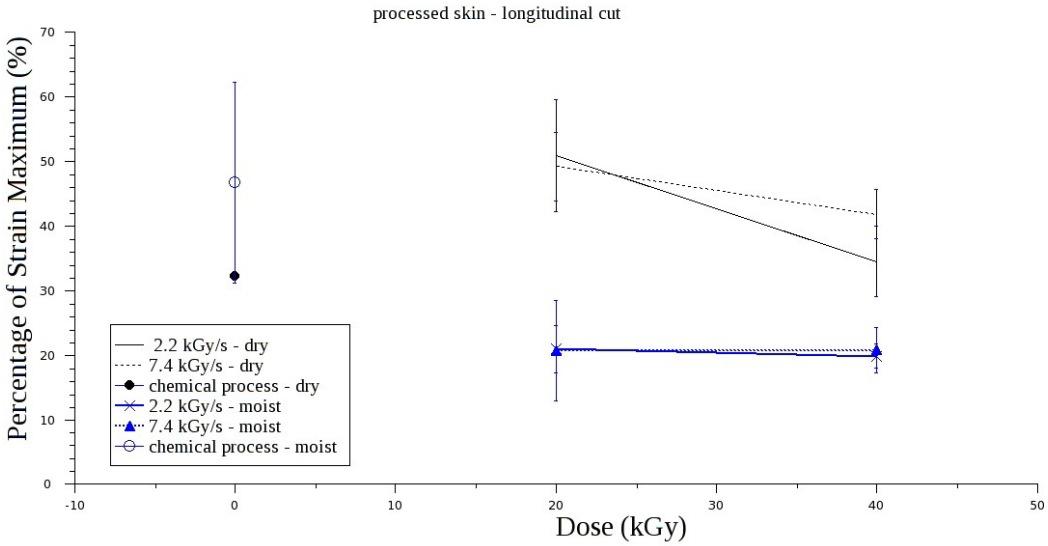

Figure 10. Percentage of strain maximum of processed skin (longitudinal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

The percentage of strain maximum (Fig.10) for longitudinal cut of irradiated processed skin samples confirms the behavior observed and discussed to Fig. 9.

Finally, the Fig. 10 shows the percentage of strain maximum for transversal cut of irradiated processed skins. Independently of the absorbed dose, the percentage of strain maximum values are high at the lower dose rate for dry samples; these values are high comparing that obtained for skins polymerized by chemical process. At moist irradiated samples, 40 kGy seems the absorbed dose where the values for this parameter are great and 7.4 kGy/s is the dose rate that express this results in this way.

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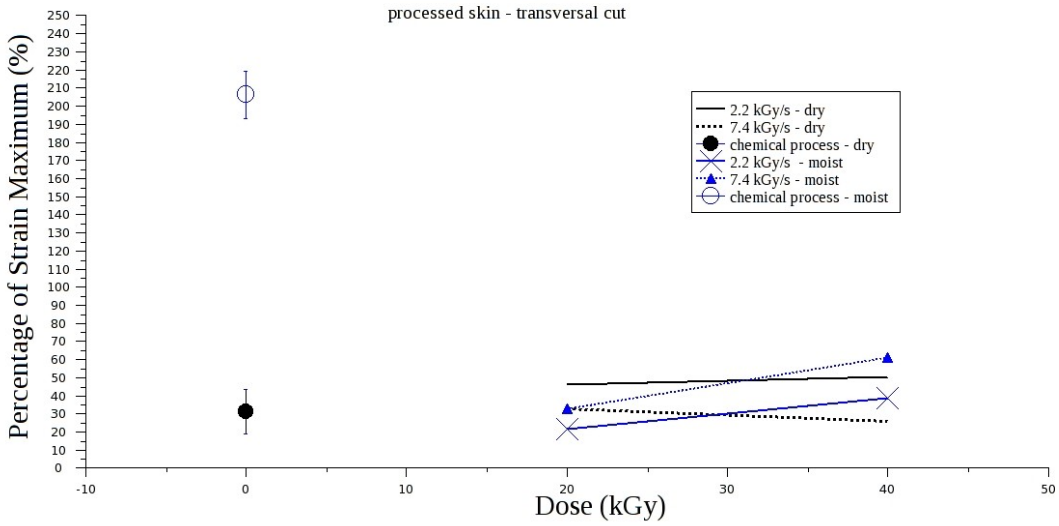

Figure 11. Percentage of strain maximum of processed skin (transversal cut) of chemical processed and irradiated samples. • chemical process (dry sample); ∘ chemical process (moist sample); ___ 2.2 kGy/s (dry sample); ..... 7.4 kGy/s (dry sample); × 2.2 kGy/s (moist sample) and ▲ 7.4 kGy/s (moist sample).

4. CONCLUSIONS

It is possible polymerize the tilapia skins by electron beam irradiation. This process is performed in lower time, with lower waste volume and without heavy metals presence compared to the chemical process.

The removal of grease and residual meat at the biological substrate studied allows good mechanical resistance to the new material obtained. The longitudinal and transversal cut have distinct behaviors and its characteristics must be considering in mechanical tests.

New approaches can be performed at other dose values to obtain a tendency curve of these samples.

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