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Impact of electron beam irradiation in potato starch films containing hibiscus aqueous extract

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ABSTRACT

The development of starch films containing natural antioxidants is one alternative of active packaging. Starch is a well-studied natural biopolymer that can be used for the development of biodegradable films because it presents a low cost, is easy to obtain and presents good ability to form films. *Hibiscus sabdariffa,* commonly known as roselle or red sorrel, is an annual herbaceous sub shrub that contains many types of biocompounds, including organic and phenolic acids. The aim of the present work was to determine the influence of electron beam irradiation on potato starch film containing hibiscus extract. The aqueous hibiscus solution was prepared by boiling for 3 min 1% w/ml dehydrated hibiscus flowers in 500 ml deionized water. The film forming solution was prepared by casting (5% potato starch, 3% glycerol as plasticizer and the hibiscus solution) and irradiated in a 1.5 MeV electron beam accelerator Dynamitron II (Radiation Dynamics Inc.), with doses of 0, 20, 40 and 60 kGy. After drying some mechanical properties were measured. The tensile strength of the control films and the irradiated ones was established. There were no significant differences among them. Hibiscus antioxidants were able to prevent the starch radiation degradation process caused by radiation induced free radicals.

Keywords: **hibiscus, films, electron beam.**

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1. INTRODUCTION

Natural polymers constitute an actual alternative for diminishing the use of non-degradable and non-renewable materials in the packaging industry. Among them, starch has been considered as one of the most promising candidates for future materials because of its low price, abundance, and thermoplastic behavior [1]. Moreover, several researchers have reported the good film forming properties of starches from a variety of botanical sources such as corn, wheat, cassava, rice, potato, and others [2,3]. It has been stated that the physicochemical properties of the starch films vary greatly depending upon the starch botanical origin, the content and type of the plasticizer and the processing conditions [4]. In order to obtain active food packaging, biodegradable films have been added of functional additives, like antioxidant and antimicrobial agents, which may be migrated from the packaging to the food product so as to extend the shelf life of food and to improve its safety and quality properties [5].

Antioxidants are natural or synthetic substances used to retard deterioration, rancidity and discoloration resulting from the oxidation, and that can be used to help preserving food [6]. Natural antioxidants mainly polyphenols extracted from plants and fruits and vitamins [7,8] are preferred over synthetic ones to be used as food components [9]. *Hibiscus sabdariffa* is an herbaceous plant, cultivated largely in tropical and subtropical areas of both hemispheres. The color of the hibiscus is mainly determined by the presence of anthocyanin, a flavonoid important among plant polyphenols, responsible for most of the blue, violet and all shades of red colors present in flowers [10].

The application of starch films is limited by poor mechanical properties and a strong hydrophilic character [11]. To overcome these disadvantages, further modification is usually necessary. Physical methods including dehydrothermal treatment, ultraviolet, heat and gamma irradiation [12] help to modify the polymeric network through the cross-linking of the polymer chains and also help to improve the functionality of polysaccharide [13].

The irradiation of polymeric materials with ionizing radiation can lead to the formation of very reactive intermediates, free radicals, ions and excited states. These intermediates follow several quick reaction pathways that result in disproportionation, hydrogen abstraction, arrangements and/or the formation of new bonds within polymer chains, which therefore modifies the final structure of the network [14]. Thus, the aim of this work was to determine the impact of electron beam

irradiation on potato starch films incorporated with natural antioxidants extracted from hibiscus, which electron beam irradiated and to characterize its physical properties.

2. MATERIALS AND METHODS

A commercial potato starch and dehydrated hibiscus (*Hibiscus sabdariffa)* flowers obtained at local market were employed.

2.1. Film preparation

A 3% (w/w) of potato starch, 3% (w/w) glycerol, 0.5% (w/w) calcium propionate and supplemented with 1% (w/100 ml) hibiscus decoction were heated to gelatinization. Subsequently, 60 g of gel was added to glass plates lined with plastics of 15 cm in diameter driven polyethylene. In order to produce the films, the films were dried in a ventilated climatic chamber at 25°C and 45% relative humidity (for 18–24 h).

2.2. Electron beam Irradiation (EBI)

After drying, the films were irradiated with doses 0, 20, 40 and 60 kGy in a 1.5 MeV electron beam accelerator Dynamitron II (Radiation Dynamics Inc.), at ambient temperature (20 ± 0.5°C), using a 0.55 MeV energy electron beam at a dose rate of 22.38 kGy/s. One batch of film was kept as non-irradiated reference (0 kGy control films). The 60 kGy maximum dose was selected to achieve some crosslinking, a legitimate technological purpose established by *Codex Alimentarius* Standard [15].

2.3. Thickness measurement

Film thickness were measured with accuracy of ± 0.001 mm, at ten different regions of the film, using a micrometer (Mitutoyo, model MDC 25 M, MFG, Japan).

2.4. Water activity

Water activity was measured in triplicate by direct reading using an Aqua Lab Lite water activity meter (Decagon Devices Inc., Pullman, USA), operating at 25°C. The samples were circularly sized, 35 mm in diameter and placed in plastic capsules on the equipment.

2.5. Solubility in water

Water solubility of the films was determined according to the method proposed by Gontard *et al.* [16]. Samples of the films were cut into disks of 3 cm in diameter, in triplicate, dried at 105°C for 24 h and weighed. The dehydrated samples were individually immersed in 50 mL beakers filled with distilled water, and maintained under slow mechanical agitation (75 rpm) for 24h at 25 ± 2°C. After this period, not solubilized samples were removed and dried (105°C for 24h) to determine the final dry mass. Solubility was expressed according to equation (1):

$$Sw = \frac{mi-mf}{mi} x100 \quad (1)$$

In which 'mi' is the initial dry mass of the films (g), 'mf' is the final dry mass on-solubilized films (g).

2.6. Water absorption

The water absorption capacity of the films was measured using the method described by Chiono *et al*. [17]. Samples of the films were cut into squares (10 x 10 mm) and weighed (Mi). They were then placed in distilled water and stored at room temperature for 24 h. After, the swollen films were removed from the water, dried with absorbent paper and weighed (Mf). Thus, the calculation of film absorption (A) was performed according to the following equation (2):

$$A(\%) = \frac{Mf-Mi}{Mi} x100 \quad (2)$$

2.7. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were acquired on a Perkin Elmer Spectrum Model 1000 FTIR spectrometer. Films were directly placed on the reading surface. Sixteen scans were performed at a 4 cm^{-1} resolution. Measurements were recorded between 4000 and 400 cm^{-1}.

2.8. Texture analysis

After the films formed were cut into squares of 30x30 mm and were analyzed using a texture analyzer Stable Micro Systems TA-XT2 with capacity of compression of 50 kg. The films were measured for their compressibility, according to ASTM standard method D882 [18], using a cylindrical stainless steel probe, 35 mm in diameter (P/35). The pre-testing, testing and post-test speeds were set at 2, 1 and 10 mm/sec, respectively. Approximately 10 samples from each applied irradiation dose and non-irradiated samples were analyzed.

2.9. Color determination

Color parameters were determined using a Hunter Lab colorimeter (Color Quest XE 2819, USA). The equipment was set with D65 illuminant and calibrated with a standard white reflector plate. Three films of each treatment were evaluated. A CIE-Lab (International Commission of L'Eclairage) color scale was used to measure the degree of lightness (L*), redness (+a*) or greenness (-a*), and yellowness (+b*) or blueness (-b*) of the films. Hue angle is the arctan (b*/a*).

2.10. Total polyphenols content (TPC)

Total polyphenols content (TPC) was determined by the Folin-Ciocalteu methodology, as follows: 160 μL of Na_2CO_3 (7% w/v) were mixed with 400 μL of the sample and 200 μL of Folin-Ciocalteu reagent (1:10 v/v). After 30 min, sample absorbance was measured at 760 nm in a spectrophotometer (SpectraMax i3). The Gallic Acid Equivalent (GAE) was used as standard. Determi-

nation was performed in duplicate and expressed as milligram of Gallic Acid Equivalent per gram of dried film (GAE/g dried film (mg)).

2.11. Antioxidant capacity - DPPH assay

Antioxidant activities were tested by DPPH free radical scavenging assay [19]. The 100 mL of each sample (film extract solution) was mixed with 3.9 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanol solution (25 mg DPPH/L). The absorbance reduction was collected at 515 nm when the reaction reached a plateau. The DPPH scavenging activity of each sample was expressed as the inhibition percentage calculated with the following equation:

$$DPPH°inhibition(\%) = \left(\frac{(As-Ab)}{Ab}\right) x100 \quad (3)$$

where: *Ab* is the absorbance of the blank and *As* is the absorbance of the sample.

2.12. Statistical analysis

Significant differences between average results were evaluated by analysis of variance (ANOVA) and Tukey test at 5% of significance level.

3. RESULTS AND DISCUSSION

3.1 Thickness measurement, Water activity, Solubility in water and Water absorption

Table 1 displays the results of thickness, water activity, solubility and water absorption measurements of starch potato films prepared with aqueous hibiscus extract and irradiated at doses from 0 to 60 kGy.

Table 1: Thickness measurement, Water activity, Water absorption and Solubility.

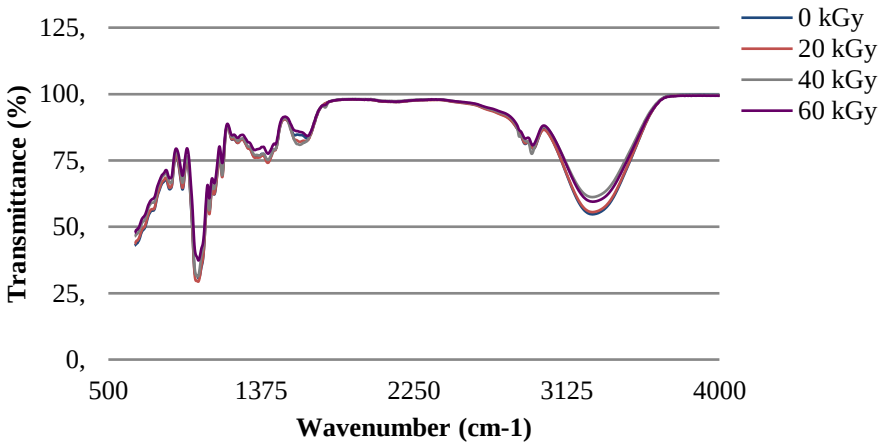
	Dose			
Parameter	**0 kGy**	**20 kGy**	**40 kGy**	**60 kGy**
Thickness	0.33±0.03[a]	0.32±0.02[a]	0.29±0.01[a]	0.33±0.31[a]
Water activity	0.62±0.01[a]	0.58±0.01[b]	0.58±0.01[b]	0.55±0.04[b]
Water absorption	42.38±1.93[a]	21.45±1.08[b]	12.81±0.69[c]	12.53±0.40[d]
Water solubility	57.15±0.54[a]	51.28±0.34[b]	46.75±0.23[c]	51.12±0.16[b]

Irradiation did not significantly affect the results of films characterization, with the exception of water absorption and to a less extend, water solubility. The mass uptake of moisture into the films in the present work did not correlate with film thickness.

3.2. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used in our study to assess interactions between potato starch films containing hibiscus aqueous extract before and after electron beam irradiation. FTIR spectra of irradiated and control films are displayed in Fig.1, and band assignments from these spectra are listed in Table 2.

Figure 1: *FTIR spectra of starch film containing hibiscus aqueous extract*



The spectrum of samples of starch films showed characteristic peaks at 3300 and 3360 cm^{-1} assigned to the OH stretching of free water, 1550 and 1680 cm^{-1} assigned to the C=C [20-21]. The peak observed at 1035 cm^{-1} is related to possible interactions arising between the plasticizer (OH group of glycerol) and the polymer structure via hydrogen bonds [22]. Irradiation did not seem to induce differences in the spectra.

Table 2: Main attributions for absorption bands of the studied starch films

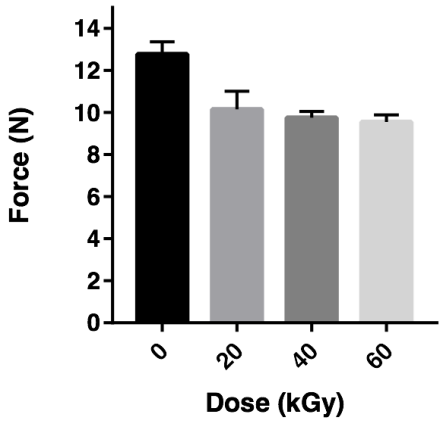
Dose (kGy)	Frequency (cm^{-1})	Attribution
0	3340	Axial deformation of O-H
20	3341	
40	3341	
60	3341	
0	2950	Axial deformation of C-H
20	2849	
40	2949	
60	2948	
0	1680	Angular deformation of O-H (H_2O)

20	1678	
40	1678	
60	1681	
0	1376-1328	Angular deformation of C-H
20	1336	
40	1325	
60	1327	
0	1163-866	Axial deformation of ether linkage
20	1000	
40	1035	
60	997	

3.3 Maximum breaking force of films

The maximum breaking force properties of edible films are of great importance due to their influence on product performance. Fig. 2 shows that electron beam irradiation treatment caused a little decrease in Force (N). The decrease in resistance with increasing of irradiation dose can be a direct consequence of the depolymerization of film constitutive macromolecules into shorter molecules induces by ionizing radiation.

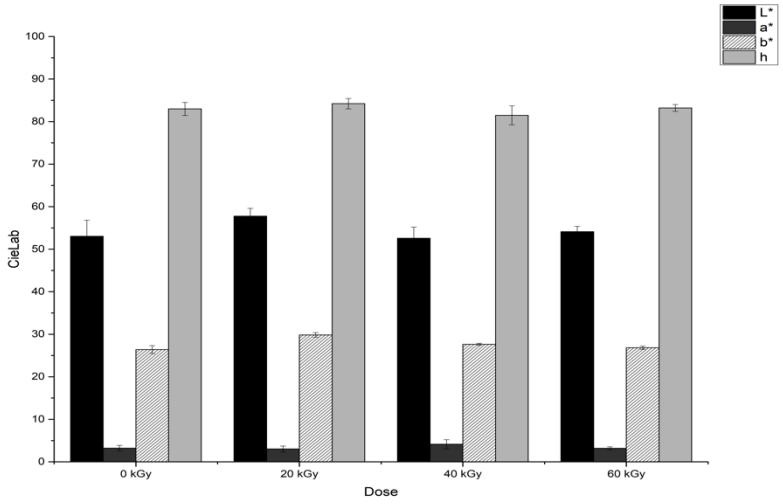
Figure 2: *Relationship between Maximum Breaking Force (N) and dose (kGy) of films*



3.4 Color determination

Color parameter average results (Figure 3) obtained for potato starch films incorporated with hibiscus extract irradiated at 0, 20, 40 and 60 kGy show that whiteness (L*value) were 53, 58, 53 and 54, respectively, while a*-value (red/green) were 3.2, 3.0, 4.1 and 3.1 respectively, and b*-values (yellow/blue) were 26.4, 29.8, 27.6 and 26.8 respectively. Similarly, angle Hue readings were 82.9, 84.2, 81.4 and 83.2 respectively. There was no significant differences from the unirradiated and irradiated samples related to parameters L*, a*, b* and angle Hue when EBI was used in the applied doses.

Figure 3: *Relationship between Cielab scala and dose (kGy) of films*



Color is the attribute of reflected and transmitted light that, while based on certain arbitrarily agreed upon mathematical notation, is nevertheless scientifically derived from a systematic description of the spectral response of the human eye. The study of color changes in irradiated polymers is very important and has been used to assess physical changes, as changes in film color are related to physic-chemical changes in the molecule. Then, considering present measurements, the physical structures of the starch films was no affected by the EBI. Teixeira *et al.* [23] showed that potato starch films prepared with irradiated starch did change color as a result of the increase of the ionizing radiation dose. In present work, the lack of change can denote a radioprotection of the films derived by the presence of hibiscus.

3.5 Antioxidant capacity

DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit oxidation, so scavenging of DPPH radical, and therefore determinate free radical scavenging capacity. Table 3 presents the total polyphenols content and DPPH activity of the starch films. Total phenolic compounds presented a slight tendency of decrease and DPPH decreased significantly with the increase of the EBI dose.

Table 3: Total polyphenols content and DPPH activity of starch films

Irradiation Dose (kGy)	GAE/g dried film (mg)	DPPH (inhibition%)
0	8.43±0.04	62.78±0.04
20	8.18±0.02	60.73±0.13
40	8.04±0.02	59.39±0.01
60	8.01±0.02	58.45±0.02

From present results, starch films with the addition of natural edible antioxidants can be proposed for the development of active packaging.

4. CONCLUSION

Present results show that electron beam irradiation of starch films incorporated with hibiscus extract had impact in some properties as absorption and solubility in water and maximum force of rupture, but had irrelevant impact on other parameters. The lack of change in other parameter could denote a radioprotection coming from the antioxidant presence of the hibiscus into the films.

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