Effect of high gamma dose irradiation on the structure of waxy starch

Mariana de Oliveira Reis[1,*], Ricardo Geraldo de Sousa[2], Adriana de S. M. Batista[1,3]

[1]Department of Nuclear Engineering, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Brazil
[2]Department of Chemical Engineering, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Brazil
[3]Department of Anatomy and Image, Federal University of Minas Gerais, 30130-100, Belo Horizonte, Brazil

[*]E-mail: mor_reis@hotmail.com

Abstract

Starch is a polysaccharide formed by the union of α-glucose molecules of amylose and amylopectin, and is stored in different plant organs. It has been used as an important ingredient in the food and non-food industries, with diverse applications due to its low cost and biodegradability. To expand its use, modifications to natural starch have been studied to make it suitable for each specific application. A processing technique that can be used to modify materials is exposure to high doses of gamma radiation. Starch has already been subjected to gamma irradiation in previous studies, but usually at low doses (below 100 kGy). In order to increase knowledge about the effect of gamma radiation on starch structure, samples of natural starch (Starch), waxy starch (WX) and cationic waxy starch (WX Cat) were irradiated with 500 kGy. The samples were irradiated in the Gamma Irradiation Laboratory (LIG) of the Center for the Development of Nuclear Technology (CDTN), which has a panoramic irradiator equipped with a Cobalt-60 source. The materials were evaluated before and after irradiation through the techniques of Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) and thermal gravimetric analysis (TGA). Lugol's iodine test was performed to qualitatively evaluate the relationship between amylose and amylopectin in the samples. The FTIR made it possible to characterize and compare the starches studied. SEM analysis demonstrated the effects of irradiation on starch morphology. The TGA results indicate an increase in residue in the irradiated samples (Irrad), which may be related to the formation of radiation-induced cross-links. Lugol’s iodine test showed that the prevalence of amylopectin in the composition of waxy starches was maintained.

***Keywords**:* Waxy starch; Gamma-rays; Irradiation process.

1.- INTRODUCTION

Starch is a natural polymer widely used not only in the food industry but in other sectors such as pharmaceuticals, textiles and papermaking [Sudheesh *et al.*, 2019]. It is a polysaccharide that consists of two forms of α-glucans: amylose – which is linear with an α-1,4 linkage – and amylopectin – which has a highly branched structure with α-1,4 and α-1,6 linkages [Sunder *et al.*, 2022]. One of the main sources of starch is corn, and in its waxy form it contains more amylopectin, which has the advantage of being more stable to retrogradation [Bashir and Aggarwal 2019].

Due to some limitations in its native form (less stability to changes in temperature, pH, shear force), modification methods via chemical, physical and enzymatic processes have been developed [Rostamabadi *et al.*, 2023]. Within these methods, irradiation has the advantages of being relatively fast, requiring minimal sample preparation, safe, economically viable, and the final product obtained is free of toxic residues [Sudheesh *et al.*, 2019].

In particular, gamma irradiation (ionizing radiation) is widely used to modify starch, enabling cross-linking, grafting and degradation in the amylose and amylopectin chains of granules [Sudheesh *et al.*, 2019]. This is a physical technique that allows the alteration of functional and processing characteristics, and previous studies have already reported physicochemical changes in the irradiated material, but these properties will depend on the dose of irradiation to which the starch was subjected and the proportion between amylose and amylopectin, in addition to its origin [Chung *et al.*, 2015]. ^{60}Co gamma irradiation can induce chemical changes such as degradation of starch macromolecules accompanied by oxidation, leading to the formation of carbonyl and carboxyl derivatives, and thus modify properties, such as increasing solubility, decreasing viscosity and molecular weight [Li *et al.*, 2018].

Research on corn starch irradiation explores very low doses of irradiation, typically below 100 kGy [Rostamabadi *et al.*, 2023], but high dose irradiation is still little explored. Thus, the objective of this work was to preliminarily study the effect of gamma irradiation in high doses on the structure of corn starch, mainly waxy starch, and for this purpose different characterization analyzes were carried out to compare 3 materials before irradiation -

commercial starch, waxy starch and cationic waxy starch - with these same materials after irradiation at 500 kGy.

2.- MATERIALS AND METHODS

Initially, three natural polymeric materials based on starch - commercial corn starch (Starch), waxy starch (WX) and cationic waxy starch (WX Cat), the latter 2 provided in partnership with a chemical industry, were subjected to 500kGy irradiation at the Gamma Irradiation Laboratory (LIG) of the Center for the Development of Nuclear Technology (CDTN), which has a panoramic irradiator equipped with a Cobalt-60 source. The materials after irradiation were called Irrad.

The precursor materials and materials after irradiation were then analyzed by Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) and thermal gravimetric analysis (TGA). SEM was conducted on a benchtop Scanning Electron Microscope model Hitachi TM4000Plus. FTIR spectra were obtained on a Thermo Scientific equipment model Nicolet 6700, with 64 scans ranging from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. TGA was conducted on a TA Instruments SDT Q600 analyzer, using a temperature ranged from 30 ºC to 600 ºC by a heating rate of 10 ºC min^{-1} under atmosphere of nitrogen (100 mL min^{-1}), and each sample mass was between 5.0 – 5.5 mg. Next, a qualitative test was performed using Lugol's iodine (according to Knutson, 1985) to evaluate the relationship between amylose and amylopectin in the samples, where 1 drop of the solution was added to 0.01g of each starch placed in a test tube with 3 mL of deionized water.

3.- RESULTS

Below are presented the results obtained by the analyzes previously mentioned in the Materials and Methods section. Firstly, the FTIR spectrum for each of the 6 materials studied is shown, in a comparative way, that is, Starch, WX and WX Cat are presented in three graphs

(Figure 1), with the non-irradiated sample and the one analyzed after irradiation being shown in the same graph.

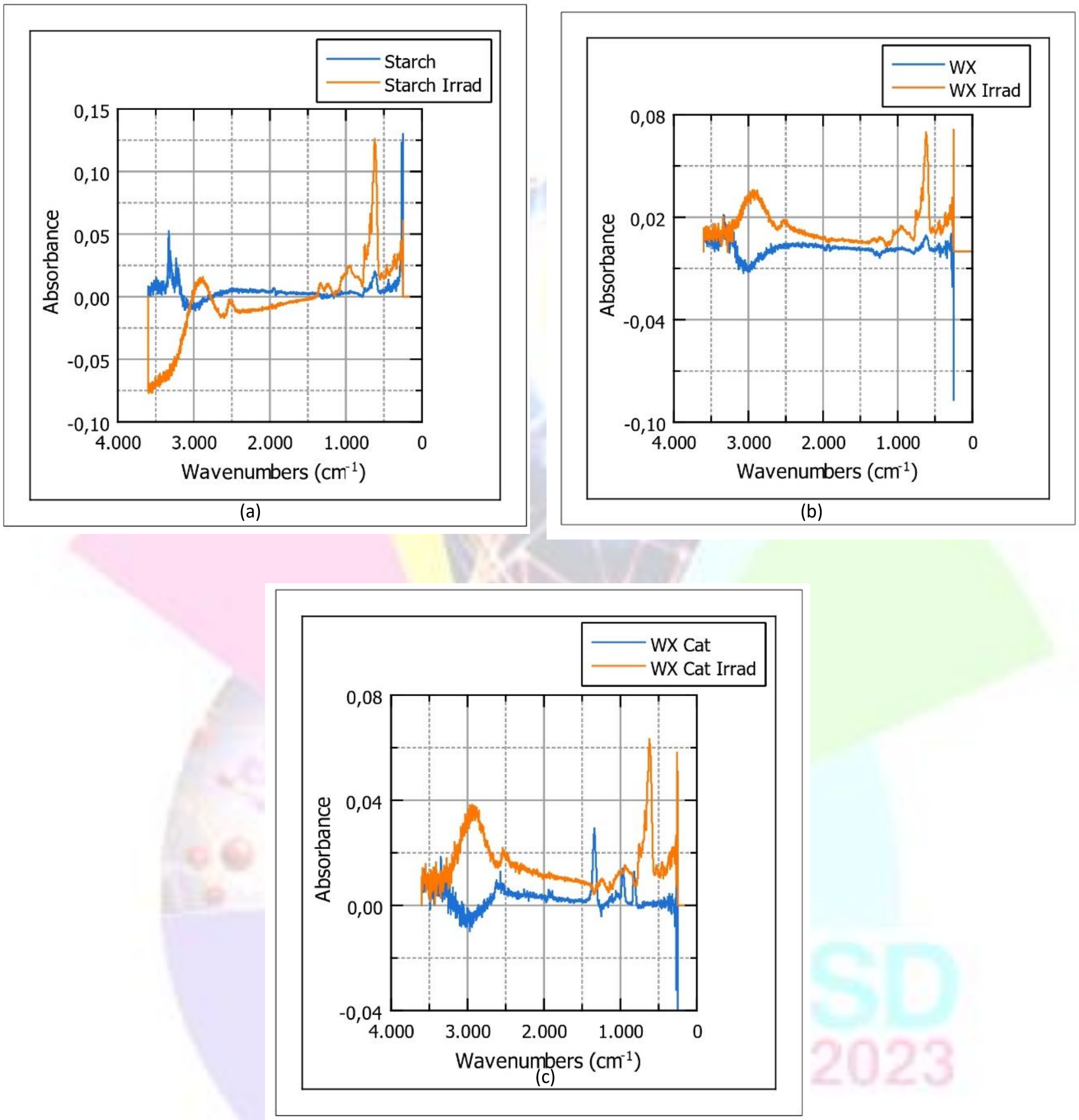

Figure 1. – FTIR analyzes for (a) Starch, (b) WX and (c) WX Cat with and without irradiation.

The images obtained by SEM are presented below, shown in Figure 2.

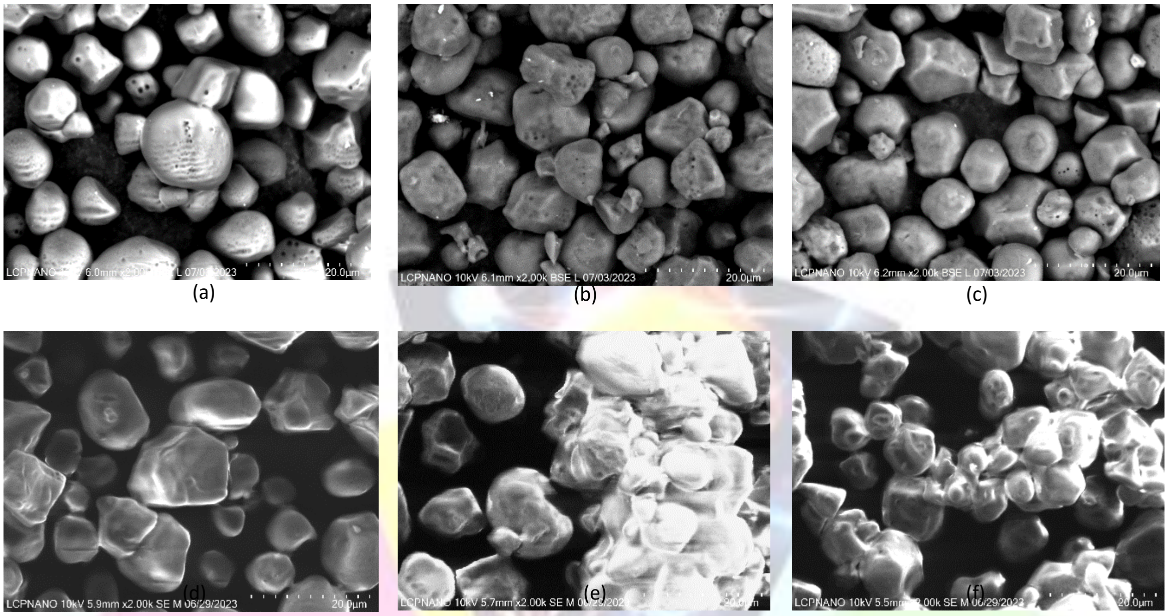

Figure 2. - SEM images for (a) Starch, (b) WX, (c) WX Cat, (d) Starch Irrad, (e) WX Irrad, (f) WX Cat Irrad.

Table 1 shows the main results of the TGA analysis for the 6 materials, showing the mass residue of each of them, and the temperature associated with the mass loss peak.

Table 1. - TGA analysis results for the study materials.

Material	**Temperature at peak mass loss ± 1 [°C]**	**Total mass residue ± 1 [%]**
Starch	311	11.8
Starch Irrad	302	18.2
WX	306	20.6
WX Irrad	311	22.6
WX Cat	293	22.9
WX Cat Irrad	298	24.8

Finally, an image of a qualitative test using Lugol is presented, in order to compare the colors obtained.

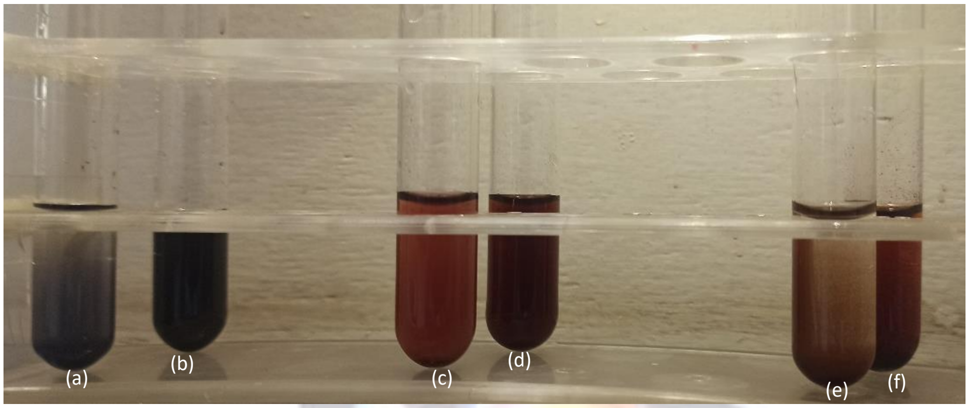

Figure 3. - Lugol's iodine test for the (a) Starch, (b) Starch Irrad, (c) WX, (d) WX Irrad, (e) WX Cat, (f) WX Cat Irrad.

4.- DISCUSSION

The spectra obtained by FTIR (Figure 1) show changes between the peaks obtained by the spectrum of Irrad and non-irradiated starches for all materials. Starch is composed of primary (-RCH2OH) and secondary (-R2CHOH) hydroxyl groups and contains carboxylate ion (COO), which are important when evaluating the variation in the chemical composition of starch after irradiation [Sunder et al., 2022]. When subjected to gamma irradiation, starch interacts with free radicals or high-energy electrons from the radiolysis of water molecules, inducing changes in its structure through fragmentation or crosslinking [Bashir and Aggarwal 2019]. An absorption band around 3300 cm^{-1} can be observed for non-irradiated starches, attributed to –OH stretching, which is correlated to the hydrophilicity of the starch [Li et al., 2018], presenting peak broadening and shift towards irradiated starches, as well as CH_2 around 3000 cm^{-1} [Sunder et al., 2022]. Bands in the region of 1000 cm^{-1} can also be highlighted for irradiated starches, which are related to C-O-H bending vibrations, much more pronounced for irradiated starches [Sudheesh et al., 2019].

The images obtained by SEM (Figure 2 (a), (b), (c)) showed that the studied starch grains have a round or polyhedral morphology, with sizes varying from approximately 5 to 20 µm. While Starch did not have its morphology greatly affected after irradiation, the WX and WX Cat starch grains appear to have agglomerated, forming small polymeric groups, as can be seen in Figure 2 (d), (e), (f), not identifying pronounced grain shear. This can be justified by the fact that waxy samples are more strongly affected by gamma irradiation, or that the starch amylopectin fraction is the most affected, as suggested by Chung et al. (2015).

Table 1 shows the data obtained by TGA. According to the results, the main mass loss for all samples occurs around 300 °C, and the residue obtained for all irradiated samples was higher than the residue of the same type of material not yet irradiated, with the largest difference identified for Starch (from 11.2 to 18.2%), while for WX and WX Cat starches this difference is similar. The fact that there was an increase in the residue mass for the irradiated samples may be related to cross-linking processes that may have occurred between the polymer chains, leading to greater stability of the materials to temperature changes. It is also important to highlight that the increasing order of total waste mass occurred from Starch to WX Cat Irrad, as reported.

Finally, a qualitative iodine test was carried out using Lugol, which is capable of indicating the content of amylose or amylopectin by the formation of complexes of different colors depending on the predominant type of polysaccharide – blue for amylose and brown for amylopectin. The test showed that even after irradiation, the samples that contained a greater amount of amylopectin (waxy starches) remained that way, becoming brownish-red in the presence of Lugol, changing only in the intensity of the color obtained, which can be attributed to a change in the solubility of starch after irradiation, the same happening for starch with a lower amylose content. Between WX and WX Cat starches, a variation in the color tone obtained was noted, but both turned brown in the presence of Lugol.

5.- CONCLUSIONS

The effect of a high dose of gamma irradiation on the structure of starches, particularly waxy starch, was studied, according to the objective of the work. It is noted through the FTIR spectra that there were changes between the spectra of all materials and their respective irradiations, indicating changes in the functional groups present. Using scanning electron microscopy, it was possible to identify changes in morphology for starch grains, mainly for WX and WX Cat starches, all three of which were similar before irradiation. The TGA analysis shows that the residue increased for all irradiated samples compared to the non-irradiated ones, with commercial starch being the sample that presents the least residue and the cationic waxy the one that presents the most among the non-irradiated ones, indicating a possible greater crosslinking for starches under the influence of radiation. Finally, the Lugol iodine test showed that Starch is blue in color as expected, WX is brown and WX Cat is also darker brown; the irradiated samples always presented a more intense color than the non-irradiated samples of the same material, but maintaining the respective amylose/amylopectin indicator.

For future work, new investigations must be carried out through other analyzes such as XRD and UV-Vis spectroscopy in order to improve knowledge of the effect of high dose radiation on natural polymers such as starch, which has application advantages in its waxy form.

Acknowledgments

We would like to thank the financial support of the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), of the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and of the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq). We also would like to thank the Center for the Development of Nuclear Technology (CDTN) and the LCPNano - Nanomaterials Characterization and Processing Laboratory at UFMG by the analyzes, and *IQR Indústria e Comércio de Produtos Químicos LTDA*, which provided the waxy starch samples.

REFERENCES

Bashir K; Aggarwal1 M. (2019). *Physicochemical, structural and functional properties of native and irradiated starch: a review.* J Food Sci Technol **56(2)**: 513–523.

Chung K; Othman Z; Lee J. (2015). *Gamma irradiation of corn starches with different amylose-to-amylopectin ratio.* J Food Sci Technol **52(10)**: 6218–6229.

Knutson CA. (1985). *A Simplified Colorimetric Procedure for Determination of Amylose in Maize Starches.* Cereal Chem. **63(2)**: 89-92.

Li L; Chen H; Wang M; Lv X; Zhao Y; Xia L. (2018). *Development and characterization of irradiated-corn-starch films*. Carbohydrate Polymers **194**: 395–400.

Rostamabadi H; Demirkesen I; Taze BH; Karaca AC; Habib M; Jan K; Bashir K; Nemțanu MR; Colussi R; Falsafi SR. (2023). *Ionizing and nonionizing radiations can change physicochemical, technofunctional, and nutritional attributes of starch*. Food Chemistry: X **19**: 100771.

Sudheesh C; Sunooj KV; George J; Kumar S; Vikas; Sajeevkumar VA. (2019). *Impact of γ-irradiation on the physico-chemical, rheological properties and* in vitro *digestibility of kithul (Caryota urens) starch; a new source of nonconventional stem starch.* Radiation Physics and Chemistry **162**: 54–65.

Sunder M; Mumbrekar KD; Mazumder N. (2022). *Gamma radiation as a modifier of starch – Physicochemical perspective*. Current Research in Food Science **5**: 141–149.