

# The Role of Water in Radiation-induced Fragmentation of Cellulosic Backbone Polysaccharides

Emanuela Muscolino (✉ [emanuela.muscolino@unipa.it](mailto:emanuela.muscolino@unipa.it))

Università degli Studi di Palermo

Maria Antonietta Sabatino

Università degli Studi di Palermo

Mats Jonsson

KTH Royal institute of Technology

Clelia Dispenza

Università degli Studi di Palermo

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## Research Article

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# Abstract

Xyloglucan (XG) is a cellulosic backbone polysaccharide commercially used for food applications, but also widely investigated in biomedical applications, for its gelling properties and specific biological activity. In this study, the possibility of using gamma radiation to cleave XG and generate lower molecular weight variants was explored. The impact of absorbed dose and irradiation conditions on the XG molecular weight distribution was investigated. Two other cellulosic polysaccharides, hydroxypropyl cellulose (HPC) and an oxidized variant of XG (CXG), were also studied for comparison. Before irradiation, the polymers were characterized with thermal gravimetric analysis (TGA) and, after irradiation, with gel filtration chromatography (GFC). The results showed that for XG irradiated in dilute aqueous solution, a dose of 10 Gy is sufficient to significantly reduce the polymer molecular weight, while HPC is less affected by irradiation under identical conditions. When the polymers were irradiated in the solid form, either dry or humid, the reduction in average molecular weight is much less pronounced. Interestingly, for HPC the cleavage of the chains is more pronounced for the dry than for the humid powder. A similar behavior, but less pronounced, was observed for XG and CXG. Arguably, when water was present in the system as bound water it had a protective effect. This is probably due to energy transfer from the polymer to the bound water preventing chain scission. Indeed, humid HPC has more bound water than XG and CXG. Conversely, when water was present as solvent, water radiolysis products were able to efficiently induce depolymerization.

## 1 Introduction

Polysaccharides are the most abundant nature-made polymers and thereby a very valuable resource of macromolecules and chemical compounds (e.g., mono-, di- and oligosaccharides). Unlike other biopolymers, such as proteins or DNA, which have rather unique linear primary structures, polysaccharides do not have precise structures. They can be composed of different, stochastically distributed monomers constituting the backbone and/or forming pending side-chains, each with distinct monosaccharide composition and glycosidic linkages (Aspinall, 1970). Their structural characteristics and properties often need to be modified in view of their envisaged applications, e.g., as components in food and pharmaceutical and biomedical formulations (Muscolino et al., 2022, 2021; Nayak et al., 2021; Pal et al., 2019; Sood et al., 2021; Toia et al., 2020), or as plastics substitutes in packaging (Lomartire et al., 2022; Nechita and Roman (Iana-Roman), 2020; Teixeira-Costa and Andrade, 2021).

As mentioned above, polysaccharides are a source of valuable mono- and di-saccharides. For instance, in drug delivery carbohydrate binding sites are often attractive and practical targets for defined sites at protein receptors (glyco-targeting). Mono- and disaccharides are used to exploit the carbohydrate binding activity of target cell proteins, such as lectins (Yuan et al., 2018). Mono- and di-saccharides can also have a role in keeping the stability of the protein–lipid structure of cell membranes and their organelles, acting as cryo-preserved of cellular materials, to limit the amount of ice that forms destroying their internal organization and to reduce osmotic shrinkage. These include reducing disaccharides, such as maltose, lactose, arabinose, and other and non-reducing disaccharides (for example, trehalose and sucrose),

monosaccharides (D-fructose, D-glucose, and galactose) and the carbocyclic sugar inositol (Stanishevskaya et al., 2021). Furthermore, polysaccharides, when depolymerized, represent a source of rare sugars that can replace conventional sweeteners, with reported positive health effects (Van Laar et al., 2021).

For other applications, attempts are made to reduce the molecular weight without changing the chemical functionality or, conversely, to functionalize without significantly changing the molecular weight, with the aim of modifying solubility and colloidal properties, their self-assembly or gelling behavior, or to introduce functional groups for further reactions.

In the search for more environmentally friendly approaches to polysaccharide modification, the use of ionizing radiation continues to receive considerable attention from the scientific community because it does not require the use of chemicals and enzymes, or significant energy consumption. (Al-Assaf and International Atomic Energy Agency, 2017; Khandal and Coqueret, 2020). Polysaccharides can be irradiated in the solid state or as aqueous solutions/dispersions. The use of water as a solvent does not introduce any problematic contamination, but it does affect the chemical reactions that occur, shifting from mainly direct effects (i.e., direct deposition of the radiation energy in the polysaccharide), in the solid state, to mainly indirect effects (radiation energy deposited in the solvent) where the transformations of the polysaccharide are governed by the reactivity and diffusivity of water radiolysis products. The structural transformations induced by ionizing radiation are dependent on the chemical structure of the polymer, but also on its conformation and the supramolecular organization of the chains, which in turn influence chain mobility and the degree of hydration. Consequently, the utilization of ionizing radiation in modifying the structure and adjust the properties of polysaccharides has significant technical potential provided that the effects of irradiation can be controlled. (Bhat and Karim, 2009; Khandal and Coqueret, 2020).

The present study analyzes the impact of absorbed dose and irradiation conditions (aqueous solution, humid and dry powders) on the molecular weight distribution of xyloglucan. The interest in xyloglucan stems from its use as soluble dietary fibers, a food class with beneficial health effects such as blood cholesterol lowering and obesity prevention (Zhu et al., 2023) and its promising potential applications in the biomedical field, as a hydrogel precursor for tissue engineering (Dispenza et al., 2017; Muscolino et al., 2021; Nisbet et al., 2006; Todaro et al., 2016), component of wound dressing formulations (Ajovalasit et al., 2021, 2018; Picone et al., 2019), and as a source of fragments with interesting antioxidant properties (Limsangouan et al., 2019). As reference compounds, carboxylated xyloglucan and hydroxypropyl cellulose were used. CXG was obtained by Tempo-mediated oxidation of XG (Ajovalasit et al., 2021). XG and HPC were irradiated in dilute aqueous solutions at various doses in the 10-1000 Gy range. Solid XG, CXG and HPC were irradiated in the 10–40 kGy range with the same dose-rate, as dry and humid powders. The three polymers were subjected to thermogravimetric analysis (TGA) after dry and wet conditioning, before irradiation. Molecular weight distributions were determined by Gel Filtration Chromatography (GFC) and deconvolution of GFC chromatograms was performed through gaussian curves with full width at half maximum of 0.5. The experimental strategy is illustrated in Fig. 1.

## 2 Materials and Methods

### 2.1 Materials

Tamarind seed xyloglucan was purchased from Megazyme International (Ireland) (purity 95%w; sugar composition: xylose 34%w; glucose 45%w; galactose 17%w; arabinose and other sugars 4%w). Ethanol (C<sub>2</sub>H<sub>6</sub>O), 2,2,6,6-tetramethyl-1-piperindinyloxy (TEMPO), sodium bromide (NaBr), sodium borohydride (NaBH<sub>4</sub>), hydrogen chloride (HCl) 1M, sodium hydroxide (NaOH) 1M, sodium hypochlorite solution (available chlorine 10–15%) were purchased from Sigma-Aldrich and used as received. Hydroxypropyl cellulose (HPC) was purchased from Sigma Aldrich with an average molecular weight of 1.04 ± 0.33 MDa (from SLS analysis).

### 2.2 Synthesis of carboxylated Xyloglucan

0.2%w XG aqueous dispersion was reacted with 0.128 mM TEMPO, 0.81 mM NaBr and 900 µl of 15%w NaOCl solution for 4 h, at 4°C under inert atmosphere, providing continuous magnetic stirring. The pH was constantly monitored and maintained around 10 by adding small volumes of 0.1 M NaOH. After 4 h 4 mM NaBH<sub>4</sub> was added to stop the reaction maintaining the magnetic stirring for further 15 min, then ethanol was used to recover the precipitated polymer. The product was recovered after 24 h by filtration (20 µm cut-off filters), washed with ethanol to remove unreacted reagent residues, wringed and let dry for 24 h. Finally, CXG was dissolved in water and freeze dried.

The presence of carboxyl groups, after the TEMPO-catalyzed regioselective oxidation, was qualitatively confirmed by infrared spectroscopy (**Figure S1 A-B**). The peak of stretching of the carbonyl group associated with the presence of aldehyde groups, already present in the native polymer, is at 1643 cm<sup>-1</sup>. The two spectra are almost identical except in the 2000 – 1400 cm<sup>-1</sup> spectral region. Indeed, in CXG infrared spectra, a new peak is present at 1736 cm<sup>-1</sup> attributed to the stretching vibration of carbonyl groups (C = O) of carboxyl groups (-COOH) in protonated form.

The carboxyl group content of CXG was determined by acid-base titration. An average value of 1.3 mmol<sub>COOH</sub>/g<sub>CXG</sub> was calculated from the values determined from the two curves (**Figure S1 C**).

Hydrodynamic volume distributions of XG and CXG were compared with pullulan standards (**Figure S1 D**). Both the polymers are quite polydisperse even if less for CXG than for XG. The CXG chromatogram is shifted towards lower molecular weights, in comparison to XG. The reduction of the molecular weight caused by the carboxylation reaction was also confirmed by Zimm plot analysis of static light scattering measurements, that yielded a weight average molecular weight of 350 kDa (Ajovalasit et al., 2021).

#### 2.2.1 Fourier-transformed Infrared Spectroscopy

FTIR analysis was performed using a Fourier Transform Infrared Spectrometer (FTIR), Spectrum Two, Perkin Elmer on CXG and XG. The powders of unirradiated polymers were mixed with KBr and pressed into

tablets.

## Titration of Polymeric Substrate Carboxyl Groups

The quantitative analysis of carboxyl groups of CXG was performed by acid-base titration.

A volume of 15 ml of CXG 0.1%w was poured in a glass beaker containing a pH-meter probe (HI 2211 pH/ORP Meter, Hanna instrument) and kept to a constant magnetic stirring.

Titration was performed by progressive addition of a constant volume (10  $\mu$ l) of HCl (0.05 M) and monitoring the pH until a value of  $\sim 3$  was reached. Then, a constant volume (10  $\mu$ l) of NaOH (0.05 M) was added progressively to reach pH value of  $\sim 9.5$ . The pH variation with the titrant volume was plotted to determine the equivalence point.

### 2.2.3 $\gamma$ -Irradiation

All irradiations were performed using a Cs-137  $\gamma$  source (MDS Nordion 1000 Elite) with a dose rate of 0.12 Gy s<sup>-1</sup> as determined by Fricke dosimetry (Spinks and Woods, 1990).

Xyloglucan solutions (0.1%w) were prepared by dissolving xyloglucan in Millipore Milli-Q water and filtrated with 1.2  $\mu$ m syringe filters in glass containers. The solutions were prepared under ambient laboratory atmosphere and are therefore considered to be air saturated. The solutions were irradiated with doses ranging from 10 to 1000 Gy.

Prior to irradiation of solid XG, CXG and HPC, the samples were conditioned. Dry samples were prepared by conditioning the polymers in a desiccator containing silica gel for one week. Samples with adsorbed water were prepared by conditioning the polymers in large, sealed beakers also containing a smaller open beaker with pure Millipore Milli-Q water for one week. Conditioning was performed at ambient temperature. The vials containing each sample of the polymer during the conditioning were closed and sealed in the desiccator/beaker upon finalizing the conditioning. The atmosphere inside the vials is air with a humidity largely corresponding to the conditioning done. The solid polymers were irradiated with doses ranging from 10 to 40 kGy.

### 2.3 Gel Filtration Chromatography Analysis

GFC was performed with the Agilent 1260 Infinity HPLC using two Shodex SB HQ columns (804 and 806) connected in series on XG, CXG and HPC samples, before and after irradiation.

All samples were prepared as 0.1%w aqueous dispersions, filtered through 0.80-micron membrane syringe filters prior to analysis to remove contaminants, and eluted with 0.02%w NaN<sub>3</sub> solution at 0.6 ml/min.

When the chromatograms are plotted against the molecular weight (Mw) the conversion from elution volume to Mw is performed using the following empirical equation, derived from the analysis of standard polymers (pullulans) with defined molecular weights (i.e., the calibration curve):

$$\text{Log } Mw[\text{Da}] = 12.75 - 0.49 V[\text{ml}]$$

where  $V$  is the elution volume calculated considering the elution flow of 0.6 ml/min.

All molecular masses reported in this work are based on pullulan standards and are not absolute.

## 2.4 Thermogravimetric Analysis

TGA was performed on HPC, XG and CXG after dry- and humid-conditioning for one week at 25°C in the 30–200°C range by means of a Netzsch Simultaneous Thermal Analyzer STA 449 Jupiter F1 with a nitrogen flux of ca. 20 mL min<sup>-1</sup>.

## 3 Results and discussion

### 3.1 Effect of irradiation in aqueous solution

Xyloglucan extracted from Tamarind seeds consists of a b-(1, 4)-D-glucan backbone, which is the same as in cellulose (Nishinari et al., 2007), partially substituted by a-(1, 6)-D-xylose. Additionally, a number of the xylose residues are substituted by b-(1, 2)-D-galactoxylose (Chen et al., 2012). The chemical structure of one of the possible repeating units of the polymer is reported in **Figure 2A**. The cellulose backbone promotes inter-chain association. The hydrophilic galactosylated side branches present in the structure prevent crystallization and provide the polymer with colloidal stability in water. At low concentration, XG readily dissolves in water at room temperature; the aqueous system appears clear and homogeneous, yet supramolecular, flat, ribbon-like aggregates are always present (Dispenza et al., 2020; Phillips, 2009). Therefore, any molecular weight value obtained from chromatographic analysis or static light scattering measurement should be considered an apparent molecular weight.

Xyloglucan was irradiated as air-saturated aqueous solutions to total doses ranging from 10 to 1000 Gy. The xyloglucan concentration was 0.1 %w, that is approximately the critical chain overlap concentration for this polymer (Freitas et al., 2005). Considering that water is by large the most abundant component of the system, under these irradiation conditions, any observed changes must be attributed to the reactions of polymer chains and their colloidal aggregates with aqueous radiolysis products, mainly hydroxyl radicals, hydrogen atoms and hydrated electrons. In the presence of molecular oxygen, hydrogen atoms and hydrated electrons are rapidly scavenged to produce HO<sub>2</sub><sup>•</sup> and O<sub>2</sub><sup>•-</sup>, respectively (Spinks and Woods, 1990).

Non-irradiated and irradiated XG solutions were analyzed using GFC. The chromatograms expressed as molecular weight, determined using pullulan standards, are presented in **Figure 3A**. The weight and number average Mw as function of the dose are shown in **Figure 3B**. The unirradiated XG solution presents a polydisperse profile with an apparent weight average molar mass of 2.57 MDa and a polydispersity index of 1.79. The chromatograms of the irradiated solutions show a significant shift towards lower molecular weights, indicating that chain scission is the dominating effect. Already a dose of 10 Gy caused a detectable reduction in the average molecular weight; it decreased by almost an order of magnitude at a dose of around 250 Gy, and at the highest dose, 1 kGy, it fell well below 10 kDa. This change in molecular

weight roughly corresponds to a G-value (radiation chemical yield) for scission in the range of  $3-6 \times 10^{-8}$  mol J<sup>-1</sup> based on the simple assumption that one scission per chain reduces the number average molecular weight by a factor of 2. This corresponds to ca 10 % of the G-value for hydroxyl radicals in the g-radiolysis of water, i.e., one hydroxyl radical out of ten produced in the aqueous solution yields scission of XG. Considering the very low molar concentration of XG in solution (< 1 mM), the scission yield is surprisingly high. Hydroxyl radicals can induce cleavage of glycosidic bonds at any location in the polymer chain, close to the middle of the macromolecule as well as very close to the end of the chain. Cleavage of small fragments will not induce significant modification of the molecular weight distribution obtained by GFC. Hence, the scission yield can actually be higher than the one roughly estimated on the basis of the measured changes in apparent molecular weight. The polydispersity index significantly increases with increasing absorbed dose. This implies that radiolysis of XG in dilute aqueous solution does not produce the same effects on all chains. Probably, chains or segments that are fully hydrated scavenge the hydroxyl radicals more effectively and undergo multiple scissions, whereas segments that are aggregated in condensed domains are less accessible to the primary radicals and, hence, less affected by fragmentation. The importance of hydration has been demonstrated in the study by Long et al. (Long et al., 2019) that investigates the degradation of insoluble  $\beta$ -Glucan irradiated in water (5 g of polymer dispersed in 100 ml of water).  $\beta$ -Glucans are linear homopolysaccharides composed of D-glucopyranosyls residues linked via a mixture of  $\beta$ -(1 $\rightarrow$ 3) and  $\beta$ -(1 $\rightarrow$ 4) linkages. Due to extensive intermolecular interactions the high molecular weight variants of these polymers are insoluble in water. Water-solubility increases in alkaline media, due to the ionization of hydroxyl groups, disrupting the ordered structures formed. In their study, much higher doses are required to achieve a significant reduction in polymer molecular weight and formation of significant amounts of soluble fractions than for XG and the observed effects are pH-dependent, hence water solubility-dependent. At pH 7, a dose of about 50 kGy is required to achieve a 50% reduction in Mw and this dose is reduced to less than 20 kGy when the pH is increased to 9.

We can compare the results for XG to those obtained upon irradiation of HPC at the same doses and concentrations. HPC is a semi-synthetic polysaccharide that shares the cellulosic backbone with XG. It is obtained by partial substitution of hydroxyl groups of cellulose with iso-hydroxypropyl groups (see **Figure 2C**). The slightly hydrophobic hydroxypropyl substituents give the polymer amphiphilic properties and a lower critical solution temperature (LCST) in water at 40°C. At temperatures below the LCST, HPC is readily soluble in water. At temperatures above the LCST, the solutions phase-separate into more and less concentrated regions. The phase separation is connected to conformational transition from random coil to compact globule (Bulone et al., 2023). HPC is known to undergo mainly degradation when exposed to  $\gamma$ -radiation, either in solid state or in relatively dilute aqueous solution at temperatures below the LCST, both in air and in absence of oxygen (Wach et al., 2002). When we compare aqueous XG to aqueous HPC irradiated at the same concentration, dose rate and dose, we see that degradation is more pronounced for XG than for HPC. The polydispersity of HPC is almost constant with the irradiation dose. We can argue that the iso-hydroxypropyl substituents of HPC offer competitive reaction sites for hydroxyl radicals. Unlike the sites on the cellulose backbone, reactions of hydroxyl radicals with sites on the iso-hydroxypropyl substituent do not induce chain scission. The radicals formed upon reaction between hydroxyl radicals and the iso-hydroxypropyl substituent can undergo radical–radical combination to form inter- or intra-

molecular crosslinks depending on the conditions. This can clearly be seen at moderate and high concentrations (10-30%w), where inter-molecular crosslinking of HPC dominates and macrogel formation is observed upon irradiation (Wach et al., 2002).

### 3.2 Effect of irradiation in the solid physical form

Preliminary studies conducted with XG in solid physical form, irradiated in air or in a nitrogen atmosphere, showed a dose-dependent reduction in molecular weight over a significantly higher dose range (0-40kGy) than for irradiation in aqueous solutions, with no appreciable influence of the gas atmosphere (data not shown). When XG was purified prior to irradiation, by extensive dialysis with double-distilled water followed by lyophilization, the purified XG was more prone to chain scission than the unpurified XG. One possible explanation was that the unpurified polymer contained some protective impurities that were successfully removed by dialysis. Another possible explanation is that the two polymers differed in their moisture content, which was assumed to be lower in the freeze-dried polymer than in the powder stored in air. The latter hypothesis prompted us to carry out a more systematic investigation of the effect of moisture content, considering two extreme cases, one obtained by conditioning the polymer for one week at 25°C in a desiccator (dry conditioning) and the other by conditioning in a water-saturated atmosphere at the same temperature, for the same time (wet conditioning). The moisture content was quantified by TGA analysis. To find out whether the state of the water, which can be strongly or weakly bound to the polymer or unbound and fill the pores in the solid, also has an effect, the same pretreatments were performed on HPC and CXG, as these two polymers, by virtue of their different substituents, should have different moisture adsorption characteristics from XG. The water adsorption properties are a result of their adsorption sites, the exposed OH groups present in all three polymers, and carboxyl groups for CXG, but also of their specific surface area and porous structure.

The results of the TGA analysis are illustrated in Figure 4. Interestingly, XG and CXG conditioned to be dry appear to have a water content of about 5 %w and 8 %w, respectively, and cannot be considered dry in the strict sense. HPC has a much lower water content after dry conditioning (1-2 %w). After wet conditioning, the water content is fairly high for all three polymers. For XG and HPC, it is around 40 %w, while for CXG it is above 60 %w. The higher water uptake of CXG may reflect the presence of carboxyl groups in addition to hydroxyl groups and the reduction of molecular weight and probably intermolecular association, favoring the water molecules to diffuse from the surface into the bulk polymer structure. Although XG and HPC have more or less the same water content, the state of the adsorbed water differs significantly. For XG, desorption occurs at temperatures around 80°C, while for HPC we observe several desorption phenomena, starting from about 40°C to well above 100°C. The wider temperature interval points to a variety of water release mechanisms acting and possible different states of water. At the lower end of the temperature range, water desorption can be associated with the typical coil-to-globule transition of HPC that releases water molecules and is prodromal to condensation and eventually phase separation. At the higher end of the temperature range, the largest fraction of water is released. Considering that temperatures are above 100°C, this water is strongly bound to the polymer. At the intermediate temperatures, the water released can be considered "free" or only loosely bound, i.e. water filling the pores of the solid powder.



The three polymers, preconditioned in dry and wet atmospheres, were subjected to the same irradiation doses (10 kGy, 20 kGy and 40 kGy) in air. After irradiation, the powders were dissolved in water and the solutions were analyzed using GFC. The chromatograms were further analyzed through deconvolution analysis. As for the irradiation in aqueous solutions, there is no indication of radiation-induced crosslinking in the irradiated samples.

The chromatograms, the relative occurrence of the different size fractions used for the deconvolution of the chromatograms as a function of dose and the Mn, Mw and PDI plots as function of dose, for both dry and humid XG powders are presented in **Figure 5**. The molecular weight fractions used in the deconvolutions were determined from the best fit to the chromatograms of the corresponding unirradiated polymers. The same fractions were used for the analysis of all the irradiated samples. At higher doses, additional lower molecular weight fractions were added if deemed necessary. In general, the fits were of high quality, as shown in **Figure S2** for dry powders and **Figure S3** for wet powders, where the original chromatograms, in green, and the curves resulted from gaussian deconvolutions, in yellow, mostly overlap. The chromatograms (**Figure 5A, A'**) show an evident shift of the peak towards the lower molecular weights with increasing dose. For each system, we also observe that Mw and Mn decrease almost to the same extent; in other words, the polydispersity increases to a much smaller extent as compared to irradiations in solution, especially for the irradiated dry powder, which points to random backbone scission as a result of the direct effect of irradiation (Kempner, 2011). The deconvolutions of the chromatograms (**Figure 5B, B'**) show that the two largest size fractions (dark and light blue lines) are progressively consumed, while the intermediate size fractions first increase, being fed by fractionation of the longer chains, then decrease and/or reach a plateau, while the abundance of smaller size fractions steadily increase. The largest size fraction is consumed faster in the dry conditioned XG compared to the humid sample. This would imply that adsorbed water has a slight protective effect. For the other size fractions, a comparison is more difficult to make. However, the conditioning has an insignificant impact on the radiation-induced change in Mw and Mn (**Figure 5C**).

When comparing the XG results to the other two polymers, CXG (**Figure 6**) and HPC (**Figure 7**), we observe the same general trend, with molecular degradation increasing with dose.

Judging from the chromatograms of CXG (**Figure 6**), the difference between the dry and humid conditions is not significant. However, a slight protective effect of adsorbed water can be observed at the highest dose. From the chromatograms of HPC (**Figure 7**), the protective effect of moisture is clear.

We can also compare the results of the dry irradiations in more absolute terms. For XG and HPC the average molecular weight decreases by a factor of 4.5-5 at 40 kGy while for CXG the average molecular weight only decreases by a factor of 2 at 40 kGy. XG and HPC have similar initial molecular weights, ca. 3 times higher than that of CXG. The difference in initial average molecular weight can to a large extent explain the apparent difference in degradation between CXG and the other polymers. For lower molecular weights, a higher number of scissions are required for a given reduction in molecular weight compared to higher molecular weights. This is simply due to the fact that a given mass of longer chains requires less scissions than the same mass composed of shorter chains in order to reduce the average molecular

weight by a factor of 2. Accounting for the initial average molecular weight and the overall change in molecular weight upon irradiation we can estimate the G-values for scission using a very simplified approach (based on the assumption that one scission per chain reduces the average molecular weight by a factor of 2 and that for every additional reduction in molecular weight by the same factor  $2^n$  scissions are required, where  $n$  is the number of scissions leading to a reduction by a factor of 2 in the previous step). The resulting G-values for scission are in the order of  $6-9 \times 10^{-8} \text{ mol J}^{-1}$  for all three polymers implying that the differences in structure play a very minor role in dry-irradiation of XG, CXG and HPC.

A protective effect has been observed before for other macromolecules and is often attributed to energy transfer from the macromolecule to the adsorbed water molecules (or in some cases other small molecules adsorbed to the macromolecule) (Ehrenberg et al., 1957). This would apply mainly to excited states originating from the absorption of  $\gamma$ -radiation. By transferring the excess energy to the adsorbed water molecules, scission of the excited states is avoided. From the TGA, it is clear that HPC has the most strongly bound water. It is also evident that pre-conditioning in humid atmosphere has the largest effect on radiation induced fragmentation in the case of HPC as compared to XG and CXG. This would imply that energy transfer is more efficient to strongly bound water.

## 4 Conclusions

Aqueous solutions of XG and HPC and XG, CXG and HPC solid powders conditioned to be dry and in humid atmosphere at  $25^\circ\text{C}$  were exposed to  $\gamma$ -radiation to explore the possibility of using ionizing radiation as a tool to tailor the polymer molecular weight. Indeed,  $\gamma$ -irradiation induces noticeable changes in the polymer molecular weight distributions in all cases. The dominating effect is a reduction in molecular weight with increasing absorbed dose as revealed by the GFC-chromatograms. When XG is irradiated in dilute aqueous solution, a very low dose (10 Gy) is sufficient to significantly affect the polymer molecular weight while HPC results less affected than XG when irradiated under the same conditions.

For dry powders of XG, CXG and HPC, higher doses are needed (at least 10 kGy) to significantly affect the molecular weight. For dry conditioned XG and HPC the average molecular weight decreases by a factor of 4.5-5 at 40 kGy, while for CXG the weight average molecular weight decreases by a factor of 2 at the same dose. The apparent discrepancy is attributed to differences in molecular weight and, in fact, the G-values for scission is the same for all three polymers. When irradiated after conditioning in humid atmosphere, the polymer powders are protected to some extent from the radiation-induced effects. From the TGA results, there is qualitative evidence that the state of water differs from polymer to polymer. This can probably be attributed to energy transfer from the excited polymer to the bound water competing with fragmentation of excited polymer chains. Considering that the protecting effect is more pronounced in HPC where water is more strongly bound, we conclude that energy transfer from the irradiated polymer to the bound water is more efficient when water is strongly bound. In conclusion, irradiation of aqueous dispersions of polysaccharides can be a viable green methodology to produce oligomers from polysaccharides. When trying to downsize the molecular weight rather than depolymerize, one can conveniently irradiate the polymer in the solid state, although this requires higher radiation doses and humidity control.

# Declarations

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**Author contributions** *All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Emanuela Muscolino and Maria Antonietta Sabatino. All the irradiations were performed by Mats Jonsson. The first draft of the manuscript was written by Emanuela Muscolino. The supervision, review & editing writing and funding acquisition were performed by Clelia Dispenza and Mats Jonsson. All authors read and approved the final manuscript.*

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