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Radical initiated oxidative degradation of oat-spelts xylan

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Abstract

The oxidative degradation of oat-spelts xylan (OSX) initiated by peroxydisulfate in contact with atmospheric air was investigated. The polymer degradation was assessed through the estimation of the molecular mass of the reaction mixture by size exclusion chromatography. The influence of initial OSX concentration, initiator concentration and reaction time were investigated. The results show a significant molecular mass decrease at high initiator concentration or low xylan concentration. The molecular mass decrease and the sugar analysis results indicate that the radical attack is at the glycosidic linkage. These results can be explained by a depolymerization reaction associated to the oxidative degradation. After an initiation step, the degradation is dominated by the depolymerization reaction, followed by a competition between depolymerization and recombination reactions. The recombination reaction can lead to a small increase of the molecular mass.

Keywords: Xylan; Radical initiator; Oxidative degradation; Depolymerization; Size exclusion chromatography

1. Introduction

Recently, there is an increasing interest in polysaccharide degradation reactions due to their role in many important industrial processes, like baking (Rouau, 1993) and brewing (Letters, 1969). Moreover, polysaccharide degradation products have been reported to posses useful physiological function as potential prebiotics (Gibson & Roberfroid, 1995), dietary fibres and anticarcinogenic agents (Ebringerova, Hromadkova, Alfodia, & Hribalova, 1998; Playne & Critenden, 1998). These features, combined with their desirable physicochemical characteristics, make them useful to the food industry (Crittenden & Playne, 1996; Mountzouris, Gilmour, Grandison, & Rastall, 1999).

There are two pathways to achieve polysaccharide degradation: enzymatic or chemical (Uffen, 1997). Most of the research work in the literature refers to enzymatic degradation of the polysaccharides. For the particular case of the xylan enzymatic degradation, most of the published data refers to: the relationship between xylan structure and enzymes required for hydrolysis (Li, Azadi, Collins, Tolan, Kim, & Eriksson, 2000; Puls, 1997), the mechanism of enzymatic hydrolysis (Kantelinen, Hortling, Sundquist, Linko, & Viikari, 1993; Saake, Clark, & Puls, 1995), the evaluation of enzyme activity (He, Bickerstaff, Paterson, & Busswell, 1994; Tenkanen & Poutanen, 1992) and the molecular biology of xylan degradation (Lee, Lowe, & Zeikus, 1992; Thomson, 1993).

There are very few studies on the chemical degradation of polysaccharides. The major pathways to achieve chemical degradation of polysaccharides are acid hydrolysis, thermomechanical degradation and oxidative degradation.

The acid hydrolysis of polysaccharides is known since long time, but has been used mostly for analytical purposes (Abdel-Akher, Hamilton, Montgomery, & Smith, 1952; Nakasawa, Tohira, Inoue, & Takanoura, 1971; Torget, Kim, & Lee, 2000). The main difficulty associated to the industrial application of the hydrolysis is that, this reaction normally yields only monomers or very small oligomers. Lewis acid or $CaCl_2$ catalysed hydrolysis, can result in industrial applications, as they allow a better reaction control (Aoyama & Seki, 1999). Thermomechanical degradation includes ultrasonication, twin-screw extrusion and jet cooking with high-pressure steam. All these methods reduce the polysaccharide molecular mass and solution viscosity (Cote & Willet, 1999).

For oxidative degradation, various reagents are used: periodates (Hamilton & Smith, 1956), hypochlorites

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(Teleman, Kruus, Ammalahti, Buchert, & Nurmi, 1999; Thaburet, Merbouh, Ibert, Marsais, & Queguiner, 2001), sulphites (Paterson, Hill, Mitchell, & Blanshard, 1997), ozone (Wang, Hollingsworth, & Kasper 1999; Zhang, Kang, Ni, & Van Heiningen, 1997), H_2O_2 (Sun, Tomkinson, Ma, & Liang, 2000) and the atmospheric oxygen in the presence of radical initiators (Ovalle et al., 2001).

For the degradation of polysaccharides, the free radical induced oxidation can be an interesting pathway, as it makes possible the control of the extent of the degradation. However, the mechanism of this reaction is not well established and different authors propose different interpretations. Although it was proved that in the case of cellulose the radical attack is at the glycosidic linkage (Simionescu & Rozmarin, 1972), for other polysaccharides some authors still believe that the attack is on the pyranosidic ring. For instance, Ovalle et al., studying the radical induced oxidation of laminaran by $H_2O_2/FeSO_4$ redox system, proposed that the reaction takes place in the ring. This interpretation cannot explain the accentuated decrease of the molecular mass of scleroglucan, reported by Hjerde, Stokke, Smidsrod, and Christensen (1998) using the same Fenton reagent (H_2O_2/Fe^{2+}). These authors observed that after an apparently initial stable regime, the degradation occurs with a significant decrease of the molecular mass. The radical induced depolymerization was also reported for xanthan (Hjerde, Kristiansen, Stokke, Smidsrod, & Christensen, 1994). It was also reported that in the presence of free radical generating agents (e.g. H_2O_2/peroxidase, ammonium peroxydisulfate, ferric chloride, linoleic acid/lipoxygenase) some polysaccharides, like arabinoxylans, are able to form three-dimensional networks (gels or viscous solutions) (Izydorczyk & Biliaderis, 1995), It was assumed that the ferulitic acid associated with the arabinoxylans was responsible for oxidative gelation (Geissmann & Neukom, 1973).

The present work addresses the investigation of the radical initiated oxidative degradation of the oat-spelts xylan (OSX). Polymer degradation is assessed by the molecular mass of the reaction mixture by size exclusion chromatography (SEC). The influence of the initiator concentration, reaction time and xylan concentration are investigated in order to understand the reaction mechanism.

2. Experimental

2.1. Materials

The raw material for this study was OSX (Sigma, USA).

2.2. Analytical method

The OSX and the degradation products were characterised in terms of the saccharides residues by GC analysis of alditol acetates (Albersheim, Nevis, English, & Karr, 1967), using inositol as the internal standard. The alditol acetates were separated and quantified on a Shimadzu gas chromatograph GC-14B with a J&W Scientific DB-225 capillary column (30 m × 0.25 mm). The column temperature was set at 200 °C for 11 min, and then raised to 220 °C at 20°/min and after 4 min raised to 235 °C at 10°/min for 5 min. Nitrogen was used as the carrier gas.

Total sugar content was determined by the phenol–sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the quantification achieved through calibration with glucose.

2.3. Radical induced thermooxidative degradation

Taking into account the OSX very poor solubility in water, the degradation reactions were carried out in aqueous 0.5 M NaOH. A very well known, water-soluble radical initiator was selected, potassium peroxydisulfate (PK). The reactions were performed in contact with atmospheric air.

The reactions took place in a glass flask connected in the top to a water-cooled condenser. The reaction flask was immersed in a thermostat bath and agitation was provided by means of magnetic stirring. The OSX and PK were introduced directly into the reaction flask and 10 ml of aqueous 0.5 M NaOH was added. The reaction was timed from the beginning of stirring. After preliminary tests a temperature of 80 °C was chosen for all the reactions in this study.

All percentages in this work are on a mass basis. The xylan percentages are relative to NaOH 0.5 M aqueous solution. The PK/OSX mass ratio was varied from 2 to 10%.

2.4. Size exclusion chromatography

The chemical degradation was assessed by SEC to measure the modifications of the reaction mixture molecular mass. Prior to the SEC runs, the reaction mixtures were diluted with 0.5 M NaOH to prepare solutions corresponding to 1% of initial OSX. The eluents were fed with a peristaltic pump P-1 (Pharmacia, Sweden). The sample injection volume was 200 μl. A refractive index detector, Gilson model 133, was used and the chromatograms recorded on paper using a PM 8252 (Philips) dual-pen recorder.

Two chromatographic columns with different molecular mass separation ranges were used. The first column used, named column1, was a 16 mm × 660 mm glass column with Sephacryl S-400 HR (Pharmacia, Sweden). The eluent was glycine-NaOH 0.1 M buffer, pH 9, containing 0.1 M NaCl at 1 ml/min. Column 2 consisted of a 300 mm × 16 mm-glass column with Sephadex G-100 (Pharmacia, Sweden). Two different eluents were used on this column: glycine-NaOH 0.1 M buffer, pH 9, containing 0.1 M NaCl (at 1 ml/min flow rate) and aqueous 0.2 M NaOH (at 0.5 ml/min flow rate).

The universal calibration (Trathnigg, 2000) could not be used because of the changing of the molecular mass distribution by the degradation. The calibration was done with dextrans T10, T40, T70, T110, T500 (Pharmacia Fine Chemicals products) and dextran 2 000 000 (Sigma). Due to the asymmetry of many peaks, the molecular mass of the products was considered to be the one corresponding to the peak maximum.

3. Results and discussion

3.1. Preliminary experiments

Preliminary assays on OSX chemical degradation were carried out with three mixtures: a suspension of OSX in water in the absence of initiator, a OSX solution in 0.5 M NaOH without initiator and a OSX solution in 0.5 M NaOH containing initiator (PK/OSX ratio 10%). After heating at 80 °C for 8 h in contact with atmospheric air, the reaction mixture was analysed by SEC to evaluate the eventual molecular mass changes. The chromatograms obtained are presented in Fig. 1 and compared with the chromatogram of unheated OSX solution in 0.5 M NaOH. The results show that the chromatogram obtained after heating the OSX suspension in water (b) is similar to the one corresponding to non-heated OSX (a), indicating that no degradation has occurred. The presence of 0.5 M NaOH without initiator caused a slight shift of OSX peak towards higher elution volume, i.e. lower molecular mass, showing a slight degradation of the polymer. As expected a huge modification in the OSX chromatographic profile is obtained when heating an OSX solution in NaOH in the presence of PK. The peak corresponding to OSX is shifted to higher elution volume, partially overlapping the peak of the salts. The chromatograms in Fig. 1 clearly show that a degradation process is occurring in the presence of radical initiator.

The chromatographic media used in the above studies (Sephacryl S-400 HR) did not allow the assessment of the low molecular masses. In order to evaluate the molecular mass of the reaction products, a different set of SEC runs was performed using another chromatographic media (Sephadex G 100, column 2) eluted with glycine-NaOH–NaCl. The chromatograms of the intact OSX and the reaction mixture obtained with a PK/OSX ratio of 10% are presented in Fig. 2. The reaction mixture shows that the peak at the exclusion limit of this column (22 ml), which corresponds to the products with high molecular mass, was strongly diminished after the reaction. After this peak, the chromatogram of the reaction products does not come back to the baseline. This plateau corresponds to the elution of lower molecular mass products of the degradation. In order to quantify and identify the products of the radical induced OSX degradation a solution of the reaction mixture in 0.5 M NaOH was loaded in column 2, and eluted with aqueous NaOH 0.2 M. The corresponding chromatogram is presented in Fig. 3. There is a peak near the column exclusion limit followed by a high increase of the refractive index overlapping the salt peak. The differences observed in the chromatograms in Figs. 2 and 3 are due to the different solvent and eluents used.

Six fractions were collected as represented in Fig. 3. The total sugar content was determined for each collected fraction. Table 1 presents the mass distribution of total sugar

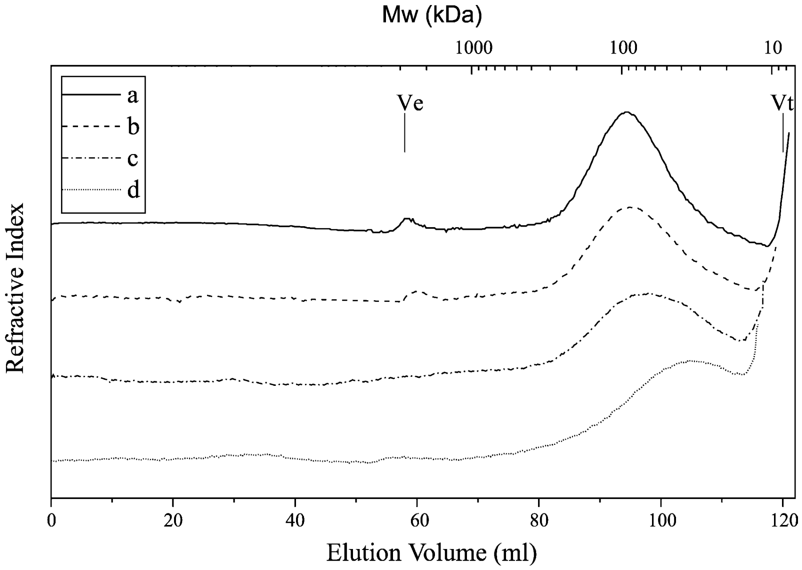


Fig. 1. Comparison of the chromatograms of the intact OSX and of the reaction products after 8 h at 80 °C on column 1. (a) Intact OSX; (b) OSX suspension in water; (c) OSX solution in NaOH 0.5 M without initiator; (d) OSX solution in NaOH 0.5 M with 10% initiator/OSX. OSX initial concentration was 4%. Ve and Vt refers to exclusion and total permeation volumes, respectively.

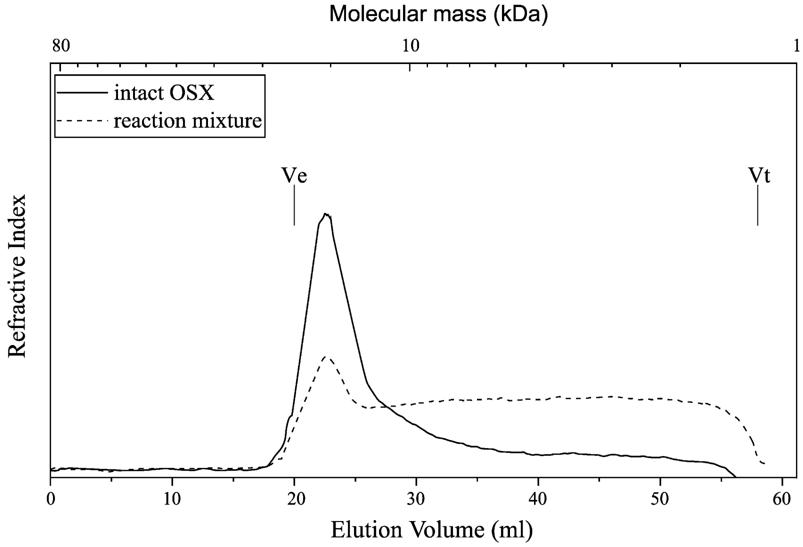


Fig. 2. Comparison of the chromatograms for the intact OSX and the one of the reaction mixture of OSX solution in NaOH 0.5 M, after 8 h at 80 °C with PK/OSX ratio of 10%. The chromatograms were obtained using the column 2 with glycine-NaOH–NaCl 0.1 M eluent. Ve and Vt refers to exclusion and total permeation volumes, respectively.

content in the six fractions collected. The results show that carbohydrates are present in all the fractions collected with maximum concentration in the fractions corresponding to the peak at the exclusion limit. The presence of sugars in the fractions corresponding to low molecular mass shows that radical induced OSX degradation can produce oligomers in a wide range of molecular mass.

The saccharide residues composition of the whole reaction mixture and the collected fractions is presented in Table 2. Due to the low sugar content of fraction 6, the neutral sugar residues composition of this fraction was not determined. The results show no significant differences between the intact OSX and the reaction mixture, showing that no sugar degradation occurred and the degradation reaction took place at the glycosidic linkage. The sugar residues analysis shows that the xylose content is higher for the fractions corresponding to the lowest molecular mass degradation products.

3.2. Influence of initiator concentration

In order to investigate the influence of initiator concentration, several reactions were performed with different PK/OSX ratios keeping the OSX concentration at

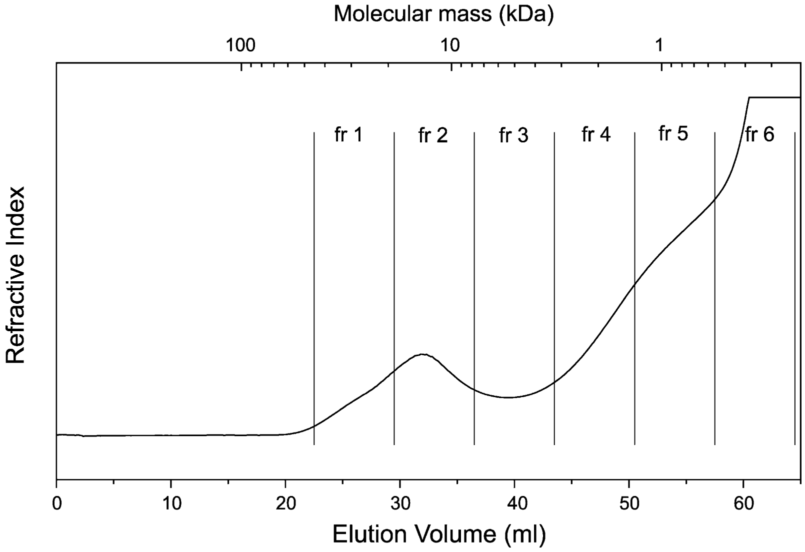


Fig. 3. Chromatogram of the reaction mixture of OSX solution (4%) in NaOH 0.5 M, after 8 h at 80 °C with PK/OSX ratio of 10% The chromatograms were obtained using the column 2 with NaOH 0.2 M eluent. Six fractions of 7 ml were collected, indicated as fr 1 to fr 6.

Table 1
Mass distribution of the total sugar content on the fractions fr1 to fr 6

Fraction	fr 1	fr 2	fr 3	fr 4	fr 5	fr 6
% of total sugars	13.02	33.65	21.94	15.95	11.24	4.20

Table 2
Neutral sugar residues composition of the fractions fr 1 to fr 5, the whole reaction mixture before the fractionation and initial OSX

Fraction	Neutral sugars %				
	Arabinose	Xylose	Mannose	Galactose	Glucose
fr 1	11.0	76.0	0.2	3.4	9.4
fr 2	11.7	77.0	0.3	2.9	8.3
fr 3	11.9	81.2	0.2	1.2	5.6
fr 4	10.6	81.9	0.2	0.9	6.5
fr 5	9.4	83.2	0.3	0.8	6.4
Whole reaction mixture	10.4	80.7	0.5	1.5	6.9
Initial OSX	10.1	81.1	0.2	1.5	7.2

Note. The values are averages of three determinations.

4%. The chromatograms of the reaction mixture, after 8 h of reaction, are presented in Fig. 4. A small shift of OSX peak maximum is observed both in the absence of initiator or for a small PK/OSX ratio of 2%. For higher amounts of initiator, 5 and 10% PK/OSX ratio, the peaks are displaced towards higher elution volumes, indicating a strong decrease in the molecular mass. The plot of average molecular mass after reaction versus the PK/OSX ratio is presented in Fig. 5. As stated above, even in the absence of initiator some degradation occurs, with a molecular mass decrease of about 20 kDa. This can be explained by alkaline hydrolysis or by an oxidative process promoted by in situ formed hydroperoxides (Simionescu & Rozmarin, 1972). In the presence of low concentration of initiator, PK/OSX ratio of 2%, there is a small decrease in the molecular mass that is comparable with the one observed without initiator. Higher concentrations of initiator cause a significant decrease of molecular mass of ca. 35 kDa. The decrease of molecular mass is similar for both 5 and 10% PK/OSX ratio.

3.3. Influence of the reaction time

The OSX radical oxidative degradation kinetics was investigated for two PK/OSX ratios of 2 and 10%. The variation of the molecular mass with the reaction time is displayed in Fig. 6. For an initiator/OSX ratio of 2%, there is a negligible molecular mass variation during the first 2 h. After 4 h of reaction, a significant decrease of the molecular masses (about 20 kDa) was observed. At 10% PK/OSX, a drastic reduction of the molecular mass is observed after the first hour of reaction, followed by a slight increase during the subsequent 7 h.

The behaviour shown in Fig. 6 cannot be explained by alkaline hydrolysis, because, in this case, a continuous decrease of the molecular mass during the reaction should be observed. This behaviour can be explained taking into account a radical reaction. Assuming a similar mechanism to that proposed for the thermooxidative destruction of cellulose with radical initiation (Simionescu & Rozmarin, 1972), the first two reaction hours at 2% PK correspond to the initiation step. At 10% PK/OSX ratio, the higher amount of initiator decreases the duration of the initiation step to less than 1 h. After this step, the degradation reaction leads to the smaller molecular mass products by an autocatalytic process. Simultaneously, a recombination reaction takes

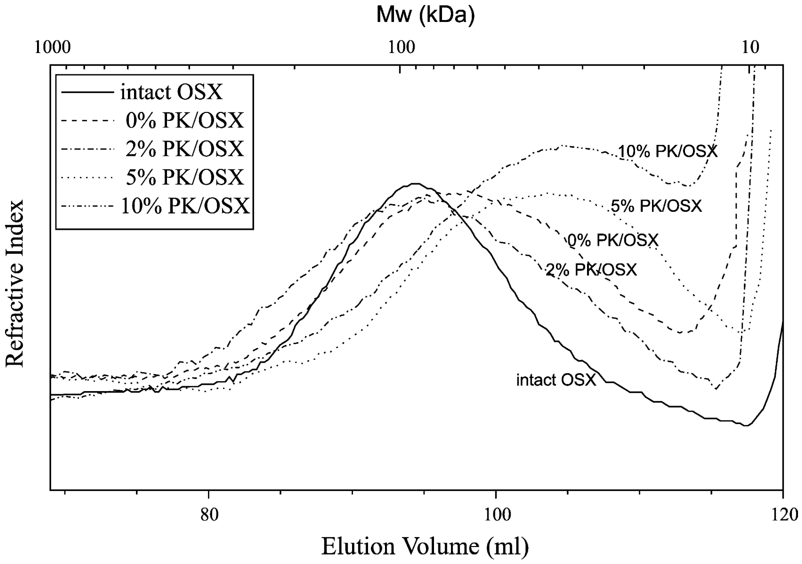


Fig. 4. Chromatograms of the intact OSX and of the reaction mixture of OSX solution (4%) in NaOH 0.5 M after 8 h at 80 °C with various PK/OSX ratios: 0, 2, 5 and 10%.

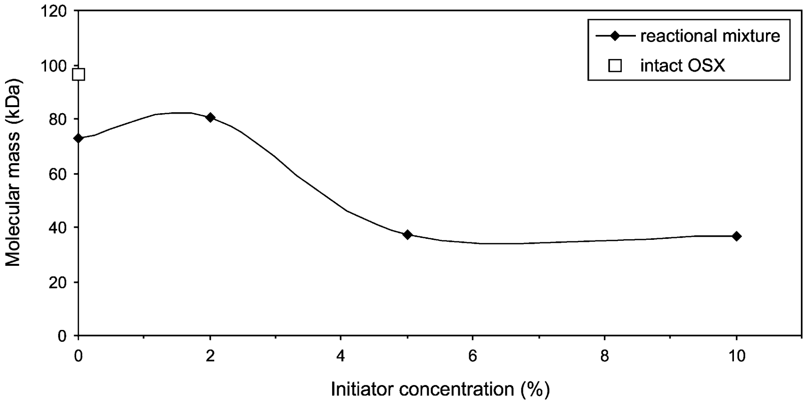


Fig. 5. The influence of initiator concentration (PK/OSX ratio) on the molecular masses of the products obtained after 8 h, at 80 °C, in aqueous NaOH 0.5 M, at initial OSX concentration of 4%.

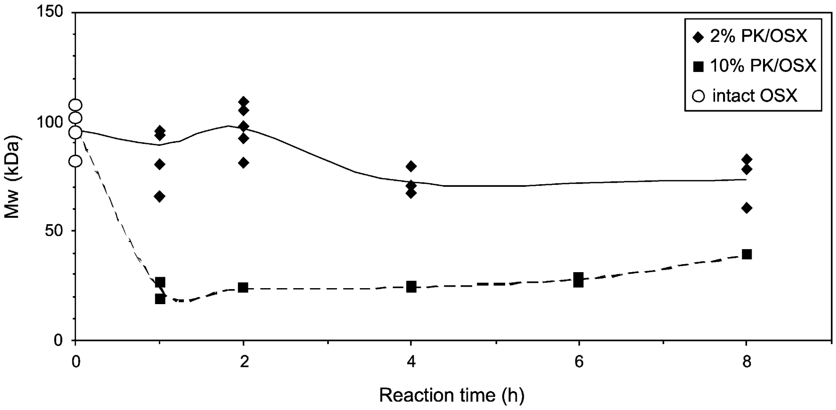


Fig. 6. Reaction time influence on the molecular mass of the reaction mixture for OSX solutions (4%) in NaOH 0.5 M, after heating at 80 °C, with PK/OSX ratios of 2 and 10%.

place and a slight molecular mass increase is observed at high reaction times.

In very few experiments the molecular mass of the reaction mixture was much higher than the molecular mass of the intact OSX. For instance, at 10% PK/OSX ratio two high molecular mass products of 175 kDa at 2 h and 130 kDa at 8 h were obtained (not shown in Fig. 6). These results prove that the recombination occurs simultaneously with depolymerization. For this reason, the reproducibility of the experiments is sometimes variable.

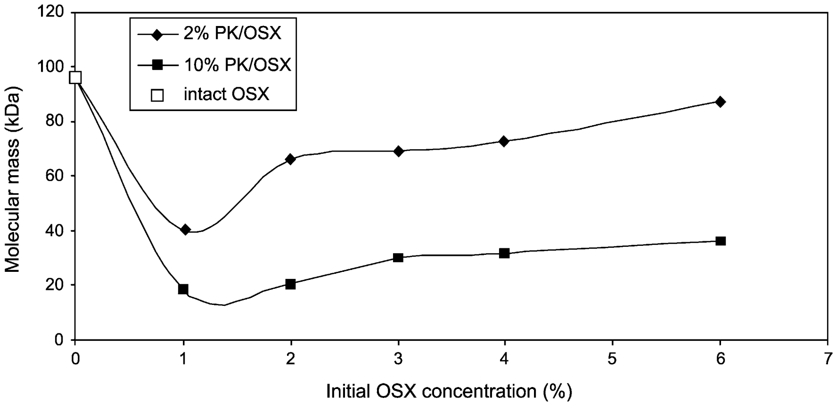
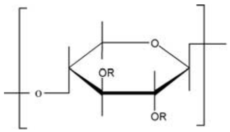


Fig. 7. Initial OSX concentration influence on the molecular masse of the degradation products obtained after heating NaOH 0.5 M solutions 8 h at 80 °C with 2% (a) and 10% (b) PK/OSX ratios.

Taking into account the decrease in molecular mass observed, the mechanism of the radical induced oxidative degradation of the OSX can be considered similar to the one of cellulose (Simionescu & Rozmarin, 1972) but including a depolymerization reaction. Such a reaction could be:

$$\text{H-(xyl)}_n\text{-OH} + \text{HO}^{\bullet} \rightarrow \text{H-(xyl)}_{n1}\text{-OH} + {}^{\bullet}\text{(xyl)}_{n2}\text{-OH}$$

(depolymerization)

where, xyl =  , n, $n1$ and $n2$ represent degrees of polymerization, $n = n1 + n2$; R represents an H or a saccharide substituent. $\text{HO}^{\bullet}$ is formed in situ by the oxidation (Simionescu & Rozmarin, 1972).

The depolymerization probably occurs by a heterolytic radical attack to the β-glycosidic bond. The new radical could have the active centre to the other end of the pyranosic ring than the initial xylan. If the radical attack occurs in the pyranosic ring the chain length will remain unaltered with negligible changes in the molecular mass.

The results confirm the observations of Izydorczyk and Biliaderis (1995), which explain, the formation of gels and viscous solutions, on addition of initiators (H_2O_2/peroxydise or ammonium peroxydisulfate) to OSX solutions by a radical process. These features could be understood as a result of a recombination reaction.

3.4. Influence of OSX concentration

The influence of initial OSX concentration was investigated at two different PK/OSX ratios: 2 and 10%. The variation of the mean molecular mass after the reaction upon the initial OSX concentration is presented in Fig. 7. The results show that for an initial OSX concentration of 1% there is a high decrease of the molecular mass, corresponding to a high level of depolymerization. For higher initial OSX concentrations the molecular mass decrease is lower. This sustains the assumption that the recombination is occurring simultaneously with depolymerization, as the recombination is more probable at higher polymer concentrations.

4. Conclusions

The results clearly show that oat spelt xylan degradation occurs in the presence of a radical initiator such as PK. The molecular mass decrease observed and the sugar residues composition of the final reaction mixture show that the radical attack is at the glycosidic linkage. As expected larger concentrations of initiator lead to a higher extent of the degradation reaction and to a significant decrease of the molecular mass. The variation of the molecular mass with time is well understood if one considers the mechanism of free radical induced thermo-oxidative degradation of cellulose, but including a depolymerization reaction. After an initiation step, the process is controlled by the depolymerization reaction, that reduces the polymer molecular mass. Simultaneously with depolymerization a recombination reaction occurs leading to a slight increase in the molecular mass for high reaction times.

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